ABSTRACT

KINCZYK, JONATHAN PAUL. Discovery and Analysis of QTL for Important Processing Traits and Resistance to *Fusarium oxysporum* f.sp. *batatas* in the NCDM04-0001 x Covington Sweetpotato Mapping Population. (Under the direction of Dr. G. Craig Yencho).

Sweetpotato is an important staple food that feeds millions, but despite our understanding of its genetic architecture and the inheritance of important traits has lagged behind that of most other major crops. Recent advances in polyploid quantitative genetics and bioinformatics have facilitated entry into a new era where quantitative trait loci (QTL) and candidate genes for specific traits in polyploid crops such as sweetpotato are becoming more feasible. These new tools have become critical components for advancing cultivar development by helping us to understand the genetic factors underlying both simple and complex traits in sweetpotato. In this study, a biparental mapping population (DC) was created by crossing the light yellow-fleshed, high dry matter breeding line NCDM04-0001 (female) with the orange-fleshed, low dry matter cultivar Covington (male). Genotyping by sequencing (GBS), via a modified protocol called GBSpoly, was used to identify high-quality single nucleotide polymorphisms (SNPs) in the DC population from Illumina sequence data. The SNPs were analyzed utilizing novel genetic linkage mapping and QTL analysis algorithms (MAPpoly and QTLpoly respectively) for complex polyploids to develop a high-density phased and integrated genetic linkage map of the DC population made up of 15 linkage groups that was phenotyped for important sweetpotato traits including the disease *Fusarium oxysporum* f.sp. *batatas* (*Fob*), and the storage root quality traits β-carotene, sucrose, glucose, and fructose.

Analyses of the mapping population revealed two significant QTL for *Fob* on linkage groups 10 and 3, responsible for ~34% and ~10% of total phenotypic variation within the progeny.

Based on the combined molecular and phenotypic data it is hypothesized that resistance to *Fob* is a polygenic trait in sweetpotato, a finding common in plant disease resistance studies.

For β-carotene, sucrose, and fructose we identified three QTL each on linkage groups 2, 3, and 12: 1) On linkage groups 3, 4, and 12 for β-carotene; 2) On linkage groups 1 and 3 (two QTL) for sucrose; and 3) On linkage groups 1 and 3 for fructose. For glucose four QTL were identified on linkage groups 1, 3 (two QTL), and 12. In total, these linkage groups accounted for 82.1% (β-carotene), 69.2% (fructose), 71.8% (sucrose), and 71.0% (glucose) of total phenotypic variation. Simultaneously, we attempted to ascertain candidate genes responsible for these four traits utilizing recently developed reference genomes of sweetpotato relatives *Ipomoea trifida* and *I. triloba*. Using this information, we hope to develop methods for genomic-assisted breeding in the sweetpotato breeding program.

Discovery and Analysis of QTL for Important Processing Traits and Resistance to *Fusarium oxysporum* f.sp. *batatas* in the NCDM04-0001 x Covington Sweetpotato Mapping Population.

by
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DEDICATION

“To my amazing and hardworking wife who supported me in this endeavor while pursuing her own Ph.D. You have no idea how strong you are.”

BIOGRAPHY

Jonathan Paul Kinczyk was born on April 23, 1988, in Winfield, IL. The youngest of three boys from the parental crossing of Cheryl and Ronald Kinczyk. He spent most of his life in the town of Glen Ellyn, IL, where he attended school from K-12. He participated in many different sports such as baseball, tennis, ultimate frisbee and soccer. Outside of school he participated in the Boy Scouts of America, achieving the rank of Eagle Scout. He also was a member of his high school's drumline and maintains his passion for music to this day.

It was during his second year of college that he was first exposed to plant science through seeing a graft that his friend had completed during their horticulture class. The process intrigued Jonathan and, upon further reading, he discovered the fascinating research being performed around the subject of plants. He also developed a passion for growing cacti and succulents at home, which blossomed into vegetable gardening, and more. His experience working as a teaching assistant for an agronomy course at Wheaton College was particularly pivotal in helping him see the wider world of plants and the possible career paths. His additional experiences as an intern in the Shedd Aquarium's horticulture department, a plant propagator, as a greenhouse manager/head grower, and a high school horticulture teacher all contributed to his fiery desire to work with plants. Somewhere along the way he heard about plant breeding and became particularly intrigued by it.

He and his wife then moved to Maryland where Jonathan worked at the USDA's Beltsville, MD location as a contributing member of the Floral and Nursery Research Unit. This opportunity was his first real foray into plant breeding work dealing with ornamental shrubs and trees. During this time, and in the two years prior, he continued to hone his skills and apply to graduate programs for plant breeding. Upon acceptance into the NCSU Horticultural Science graduate program, he began his work with the Potato and Sweetpotato Breeding Program under the advisement of Dr.

G. Craig Yencho. Throughout his tenure he volunteered in various organizations as a leader or contributing member, captained and coached a mixed gender adult ultimate frisbee team, ate a lot of sweetpotatoes, bought a house, got a dog, and learned many valuable lessons. After graduation he hopes to use the skills he has learned during his graduate program to move into the entrepreneurial realm to tackle some of the most pressing questions in the plant breeding industry.

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I would like to thank the numerous friends I have made during my time here in Raleigh, NC, as they have been a great support system for a person who never quite knows what direction to go in at times. These include Marlee Trandel, Jesse Crouch, Seth Gold, and so many of my teammates on my ultimate frisbee team which kept my mind at ease during stressful times.

Most importantly, I want to thank my parents for believing in me and always being there to support me as well as my wife who seems to think that I can accomplish anything I set my mind to. Your support means more than I can even say.

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Literature Review

Introduction

Sweetpotato, *Ipomoea batatas* (L.) Lam. is an increasingly favored commodity around the world with production in the United States growing during the last decade (FAOSTAT, 2018). In portions of the world it is a critical food staple and is consistently one of the top 10 crops produced globally, most recently with over 91.9 million tons (FAOSTAT, 2018). In the USA and Europe increasing consumer demand has been driven by awareness of its high nutritional value (Grüneberg et al., 2015). Due to its increase in popularity, growers must now store storage roots for up to a year after harvest to meet the demand in the United States alone (da Silva and Clark, 2013). Storage roots are used for various processed products including chips, fries, and syrups, frozen foods, and fresh use (Truong et al., 2018). In the developing world consisting mostly of tropical and warmer temperature areas it serves as a major source of starch and other nutrients for millions of people (Padmaja, 2009). The crop has played a role in famine relief and food security in areas of Sub-Saharan Africa and in Japan if other crops fail in a given year (Loebenstein, 2009). In Africa, however, it is considered a 'poor man's crop' with most production directed towards subsistence or small-scale farming (Loebenstein, 2009). Although China is the largest producer of sweetpotatoes (57.6% of global production), less than half of the harvest (~40%) goes to human consumption and industrial uses (FAOSTAT, 2018; Padmaja, 2009). The remaining portion is used as animal food. While roots are primarily eaten after boiling, baking, or frying, many secondary products include starch, flour, or puree (Padmaja, 2009) in addition to those mentioned earlier. These different products typically have varying specifications as to the amounts of starch, β-carotene, reducing sugars (glucose and fructose) that are preferred for processing (Truong et al.,

2018). Work of researchers to understand the trade-offs of these components of sweetpotato is important for developing a wide range of value-added products.

A brief review of *Ipomoea batatas*

The sweetpotato is a member of the plant family Convulvulaceae, genus *Ipomoea*, section Batatas. The leaf and storage root morphologies among *Ipomoea* vary and the wild species are geographically distributed across the Americas, though *I. littoralis* is believed to be native to Australia and Asia (Huamán, 1999). Sweetpotato's wild relatives include multiple ploidy levels such as *I. trifida* (2x, 3x, and 4x) and *I. cordatotriloba* (2x and 4x) (Huamán and Zhang, 1997). *Ipomoea batatas* itself is a hexaploid with 15 somatic chromosomes (2n = 6x = 90), is highly heterozygous, and exhibits high levels of self- and cross-incompatibility (Cervantes-Flores et al., 2011; Gurmu et al., 2013; Grüneberg et al., 2015). This, along with its large genome size of 1.5Mbp, has made research into its genetic and cytological origins difficult as the tools necessary to elucidate this information have only recently been developed (Wu et al., 2018; Mollinari et al., 2019).

Research has resulted in multiple origin theories for sweetpotato. Studies by Roullier et al. (2011, 2013) declared evidence for two separate origins of domestication in Central and South America through autopolyploidization. Several hypotheses exist as it pertains to sweetpotato evolution: 1) *I. batatas* is the result of interspecific hybridization between the *I. trifida* and *I. triloba* (Austin, 1988; Grüneberg et al., 2015; Wu et al., 2018); 2) Polyploidization of *I. trifida* is responsible for the current species (Kobayashi, 1984); 3) Generation via allo-autopolyploid means wherein either two unique populations of *I. trifida* or a close relative were the progenitor species

(Schafleitner et al., 2010). The most widely accepted hypothesis is the first and is described in detail in Wu et al. (2018).

Polyploids and their breeding implications

Polyploids such as sweetpotato are not uncommon in the plant kingdom. Virtually all angiosperms and vertebrates we know of today have experienced at least one round of polyploidization (Glover et al., 2016; Otto, 2007). For sweetpotato, however, it is still unknown to this point whether polyploidization (increase in genome size via inheritance of an additional set or sets of chromosomes) occurred prior to domestication (Roullier et al., 2013). Before humans began domesticating plants, polyploidization in nature created vast diversification among plants (Otto, 2007). The addition of chromosomes can occur via two pathways. In one case (autopolyploidy) the additional chromosomes arise from the duplication of a single species' genome, while the combination of genomes from two different, but related species, is called allopolyploidy. Breeders commonly induce polyploidization, using the nature of polyploids to their advantage. By replicating this process they can increase genetic diversity and, according to Otto (2007), "might release gene duplicates from the constraints of having to perform all the functions of a gene (pleiotropy), providing extra "degrees of freedom" upon which selection can act to favor new functions." Genome redundancy in polyploids also provides backup functionality of genes when a copy is lost but remains on another chromosome and also promotes protection from deleterious alleles by other wild-type alleles (Sattler et al., 2016).

Despite the many benefits of polyploidy, some drawbacks exist. Favorable alleles may be left behind if not present in the duplicated genome, and the introgression of those alleles from relatives of lower ploidy levels generally results in lower fitness (Otto, 2007). The same

duplication process, however, can provide a polyploid with opportunities for altered gene pathways for pathogen resistance or drought tolerance that lower ploidy level relatives lack as increased genome size allows for more mutations in future generations (Ramsey and Schemske, 2002; Baduel et al., 2018). This may give the polyploid an evolutionary advantage or disadvantage within a specific environment (Otto, 2007) and may explain some of the issues encountered in sweetpotato breeding.

Two of the biggest limitations with sweetpotatoes are seed production (Grüneberg, 2015) and self-incompatibility (Gurmu et al., 2013). Limited seed leaves breeders with a smaller gene pool of possible plants to select from while self-incompatibility presents the inability to develop true inbreds which have a proven track record in various crops. Therefore, when a superior line is selected asexual reproduction and tissue culture to clean up generations of virus accumulation is a necessity to keep promising lines productive (Gaba and Singer, 2009).

More recently, various programs, both private and public, have worked to develop molecular markers, linkage maps (Cervantes-Flores et al., 2008, Shirasawa et al., 2017), and a general understanding of the germplasm diversity present in the USDA's collections (Wadl et al., 2018). *Ipomoea batatas* being a hexaploid presents several challenges to working with the crop in a breeding setting as the ploidy level adds layers to its genetic complexity. The work herein is intended to build off a small, but growing body of information on the genome of *I. batatas* as it relates to traits important for processing and disease/pest resistance qualities.

Molecular markers in sweetpotato

Despite their usefulness in improving our understanding of the origin of sweetpotato, there has been little development of molecular markers for applied sweetpotato breeding. This is due in

part to the lack of powerful funding that backs other staple crops and the complex genetics of sweetpotato (Chang et al., 2009). Early marker-assisted studies of sweetpotato involved RAPD (Ukoskit and Thompson, 1997) and AFLP markers (Cervantes-Flores et al., 2008) which were easy to score. More recent studies have used SSR markers for characterization of parental materials (David, 2012) and to search for potential heterotic gene pools among Asian (China, Korea, & Japan) sweetpotato accessions held at CIP (Maria David, unpublished). Two studies of Ugandan sweetpotato germplasm used SSR markers to determine genetic relationships. Yada et al. (2010) assessed genetic relationships among 192 selected superior landraces for various traits while Tumwegarmire et al. (2011) used 75 cultivars from that same collection to determine relationships with cultivars from China, the USA, Papua New Guinea, and Peru. A 2015 study by Zawedde et al. also studied the genetic diversity and structure of 260 crop cultivars of Ugandan sweetpotatoes using 93 SSR markers.

Modern studies have begun to utilize next-generation sequencing (NGS) technologies and rapidly improving bioinformatic pipelines to enable the development of SNP markers for use in sweetpotato (Mwanga et al., 2017, Shirasawa et al., 2017, Gemenet et al., 2020). SNPs, consisting of the nucleotides of an organism's genome, are the most abundant markers available to researchers. These new methods produce vast numbers of SNP markers for downstream applications such as high-density linkage map construction and quantitative trait loci (QTL) mapping, genetic diversity analyses, association mapping, and genomic assisted breeding. With reduced costs of NGS, these valuable markers are much more feasible for crops that have traditionally been less endowed with funding.

Genetic linkage mapping

Though few existed for many years, there has been a recent increase in genetic linkage maps for sweetpotato. Ukoskit and Thompson (1997) reported the first linkage map developed from a mapping population of 76 genotypes. The map consisted of 167 RAPD markers derived from the cross of the cultivar Vardaman, known for its drought tolerance, RKN susceptibility, and early root yield, and Regal, an RKN resistant cultivar. The second was two separate maps produced by Kriegner et al. (2003) using the cultivars Tanzania and Bikilamaliya which were mapped using 632 and 435 AFLPs, respectively. These were ordered into 90 (Tanzania) and 80 (Bikilamaliya) linkage groups which integrated simplex and multiplex markers in order to analyze the genomic makeup of sweetpotato. A subset of 94 randomly selected plants of these two African landraces were used, one susceptible to sweetpotato virus disease (SPVD) (Bikilamaliya) and the other resistant (Tanzania).

In 2008, Cervantes-Flores et al. released a third map which also utilized AFLP markers. A total of 1944 and 1751 markers from parents Tanzania and Beauregard were scored, allowing them to build maps of 86 (Tanzania) and 90 (Beauregard) linkage groups. Another group used sequence-related amplified polymorphism (SRAP) markers to create a linkage map from a cross between the Chinese cultivars Luoxushu 8 and Zhengshu 20 (Li et al., 2010). In total 770 (Luoxushu 8) and 523 (Zhengshu 20) SRAP markers were used to identify 81 and 66 linkage groups, respectively. A fifth map, developed by Zhao et al. (2013) utilized a combination of AFLP and SSR markers from a cross between Xushu 18 and Xu 781, with 202 individuals. Both parents' maps contained 90 linkage groups with Xushu 18's utilizing 2077 markers (1936 AFLP and 141 SSR) while Xu 781's had 1954 markers (1824 AFLP and 130 SSR). This map was the first to have the 90 complete linkage groups, in agreement with the actual number of sweetpotato chromosomes.

In 2017 the first genetic linkage map of sweetpotato using SNP markers was developed (Shirasawa et al., 2017). The SNPs were generated from a self-pollinated S1 population of the cultivar Xushu 18 using a double digest RADseq method, which resulted in 28,307 double-simplex SNPs that were mapped into 96 linkage groups with a total genome distance of 33,020.4 cM. The size of their map is much larger than others reported, which they admitted could be due to residual genotyping errors, each of which can add ~2 cM to the final map per every 1% error rate. Since then, Gemenet et al. (2020) produced a genetic linkage map made up of 15 linkage groups. The total length was 2708.4 cM, which is consistent with previously reported maps. It contained 30,684 GBSpoly-generated and phased SNPs.

Oloka (2019) and Amankwaah (2019) both recently published two maps each utilizing the same methods as Gemenet et al. (2020). The two maps by Oloka (2019) included: 1) A population of 287 individuals from the New Kawogo x Beauregard cross to study SPVD, sweetpotato weevil resistance, storage root dry matter content, and β-carotene content; 2) A population of 240 individuals from the Tanzania x Beauregard (TB) cross which was phenotyped for resistance to root knot nematode. The maps by Amankwaah (2019) included: 1) The same TB population as Oloka (2019) studied for storage root flesh color, β-carotene, dry matter, starch, glucose, fructose, sucrose content, and α- and β-amylase activity; 2) The reciprocal cross of the TB population studied for the same properties as the prior map minus the α- and β-amylase activity.

The development of the sweetpotato reference genomes of *I. trifida* and *I. triloba* (Wu et al., 2018) has been a crucial resource for these recent maps and for mapping projects going forward. While the lack of a whole genome sequence for sweetpotato has hindered the validation of mapped QTL regions, this recent development will serve as a means for the identification of candidate genes in the meantime.

Processing qualities

The processing quality of sweetpotato is determined by the desired traits of different geographical locations, and various characteristics (sugars, starch, etc.) determine their processing uses (Truong et al., 2018). Research into the processes that determine the sizes, shapes, and number of storage roots (SRs) has helped growers better understand how to produce quality roots for processors (Pardales and Yamauchi, 2003; Villordon et al., 2009; Villordon et al., 2012). Other research investigated pre- and post-harvest storage conditions and the genetic/environmental components that combine with various cultural treatments to determine the final product (Walter, 1987; Amankwaah, 2019). Additionally, studies into the outcomes from how sweetpotatoes are cooked or otherwise processed have been performed, as this can create different tastes and textures, leading to differences in the overall enjoyment of the crop (Picha, 1985; Picha, 1986, La Bonte and Picha, 2000; Chan et al., 2014).

Within this thesis I report findings on four important processing characteristics: β-carotene, glucose, fructose, and sucrose. β-carotene is a member of the carotenoid family, which is widely represented in algae, bacteria, fungi, and plants (Ladygin, 2000). Members of the carotenoids are natural tetraterpenoid pigments that provide flowers, fruits, and roots with vivid hues of orange, yellow, and red (Sun et al., 2018). β-carotene is the most prolific carotenoid found in orange-fleshed sweetpotato (OFSP) and is a precursor to vitamin A (Takahata et al., 1993; Hagenimana et al., 1998). β-carotene in sweetpotato and many other crops is touted for its involvement in a healthy diet as vitamin A promotes healthy vision, healthy immune systems, and cell growth (Blomhoff and Blomhoff, 2006).

Sweetpotato storage roots are predominantly made up of fructose, glucose, and sucrose (Picha, 1985), though maltose may be detected in trace amounts, if at all, prior to cooking. The

reducing sugars glucose and fructose contribute some of the sweetness to sweetpotatoes, but are generally studied for their impacts on the final appearance of fried and chip products due to the Maillard reaction (Picha, 1986; Truong, 2013) as they are in other crops such as potato and cereals (Mottram et al., 2002). Higher levels of reducing sugars and asparagine have been implicated as being partially responsible for browning and the accumulation of acrylamide (a known carcinogen) during the frying and chipping process (Picha, 1986; Mottram et al., 2002). Sweetness in cooked sweetpotatoes comes from combination of sucrose, glucose, and fructose prior to cooking along with maltose formed via starch hydrolysis during the cooking process (Kays and Horvat, 1984; Sistrunk et al., 1954). After cooking, maltose becomes the predominant sugar present (Picha, 1985). Sucrose plays a central role in plant growth and development, supplying sink organs of most plant species with carbon and energy (Winter and Huber, 2000; Sturm and Tang, 1999). Once inside cells it can be broken down as required by the plant. The enzyme invertase breaks down sucrose into glucose and fructose while sucrose synthase begins the process of starch production by cleaving sucrose into fructose and either UDP- or ADP-glucose (Coleman et al., 2006; Kleczkowski et al., 2010). Gemenet et al. (2020) recently confirmed the presence of sucrose synthase near a QTL for starch and dry matter which makes sense based on the aforementioned pathway. This would explain the high negative correlation observed between starch and sucrose both in unpublished data (Yencho, personal observations) and in a study by Tumwegamire et al. (2011).

Carbohydrate accumulation during storage root development

Several different endogenous hormones and enzymes work together to translocate photosynthates to storage roots (Liu et al., 2013). Despite this information, not a lot is known about

how quantities of starches and different sugars (monosaccharides and disaccharides) are determined or how they change in storage roots during preharvest development. A 19-week study by La Bonte et al. (2000) measuring these carbohydrate levels at two-week intervals from 7 to 19 weeks shed some light on this. In the experiment, staple type sweetpotatoes (little to no sweetness, generally high starch) were compared to dessert types (sweet, generally less starch and more sucrose). La Bonte et al. noted that the staple types (Rojo Blanco and White Star), although sometimes starting out with higher fructose levels than some dessert types, ended up with lower levels 19 weeks after transplanting. Similar results were witnessed with glucose levels as they gradually decreased in staple types while remaining static, or increasing substantially, in most dessert types (La Bonte et al. 2000). According to La Bonte et al., the dessert type cultivar Travis, in particular, demonstrated wide ranges in its levels of both of these reducing sugars but ultimately ended up with the highest amounts. La Bonte et al. also reported that higher starch levels (correlated to dry weight %) were demonstrated in both staple types, but from weeks 7-11 starch development in dessert type (Heart-o-Gold) was higher than in the staple type White Star. Further, cultivars producing higher levels of monosaccharides were shown to compensate with lower sucrose content while the reverse is true in cultivars with high sucrose content (La Bonte et al., 2000). Whether this is a barrier that can be overcome is still being determined.

Disease screenings

Sweetpotato, like most other crops, is affected by a wide range of diseases caused or vectored by bacteria, fungi, nematodes, insects, and/or viruses. Most of these diseases can be controlled through proper cultivation practices which, when combined with good shipping and postharvest conditions, leads to rare incidents of costly losses (Clark et al., 2013). In most cases

diseases are spread via entry into wounds incurred during harvest and spread from systemically infected storage roots to uninfected storage roots (Clark et al., 2013). In combination with improper handling and other factors, more than one-half of harvested sweetpotatoes can be lost in a given year before they reach the consumer (Edmunds et al., 2008).

Fusarium solani, *Rhizopus stolonifera*, and *Fusarium oxysporum* f.sp. *batatas* (*Fob*) are three of the most economically devastating diseases observed in sweetpotato. The first two diseases are primarily a postharvest concern. Resistance to *Fob* is a mandatory trait in all released sweetpotato varieties because there are no effective control strategies for *Fob* in sweetpotato other than genetic resistance (Clark et al., 2013). Over time sweetpotato breeding programs have bred for resistance to these and other diseases via traditional methods (K. Pecota, personal communication). As molecular breeding techniques have become more ubiquitous and economically feasible, researchers in numerous other crops have begun to incorporate these practices into their programs (Moose and Mumm, 2008). Molecular techniques give researchers opportunities to associate sequences of DNA or RNA with specific traits in the crops they work on. In particular, if a program has the opportunity to use this to define what diseases the lines they produce are resistant to, it can speed up breeding times tremendously (Moose and Mumm, 2008). In sweetpotato, currently very little is known about the genetic component of disease resistance, a mechanism that may have major implications for the future of this crop.

Fusarium wilt

Fusarium oxysporum f.sp. batatas (*Fob*) is classified in the Kingdom Fungi; Phylum Ascomycota; Class Sordariomycetes; Order Hypocreales; Family Nectriaceae; Genus Fusarium; Species oxysporum; Forma Specialis batatas (Michielse and Rep, 2009).

Fusarium species are broadly distributed throughout the world, existing in soils and organic substrates. The *Fusarium* genus includes many plant pathogens of agricultural crops, some of which produce deadly mycotoxins. As of 2014, ~300 phylogenetically distinct species of *Fusarium* have been discovered (Aoki et al., 2014). Among them, *Fusarium oxysporum* is the most widely dispersed (Agrios, 2005) and regularly threatens many economically important crops (e.g., tomato, pea, sweetpotato, and cucumber). Banana, in particular, has received a lot of attention recently because of its ubiquitous consumption and because over 40% of production belongs to one cultivar, Cavendish (Ploetz, 2015; Garcia-Bastidas et al., 2019). *Fusarium oxysporum* is categorized as a species complex (because of its predominantly asexual reproduction) and is known primarily for causing vascular wilts. Host range is very broad at the species level, while over 120 different formae speciales (host-plant specific) have been identified (Michielse and Rep, 2009), of which *Fob* is one. Some, however, are pathogenic on multiple species (Clark et al., 1998). Within each forma specialis there is typically multiple races (currently two in *I. batatas*), commonly defined by the *R* gene spectrum effective against them in crops where *R* genes are employed.

Fusarium wilt (FW) of sweetpotato is caused by *Fob*. There are no morphologically distinguishing characteristics between *Fob* and *Fusarium oxysporum* (Clark and Moyer, 1988), with the former causing surface rot in sweetpotato. Early on, fusarium wilt was attributed to *Fusarium batatis* and *Fusarium hyperoxysporum* Wollenw., but has since been condensed into *Fob* as we know it today. As Clark and Moyer (1988) state, three spore forms have been discovered: microconidia, macroconidia, and chlamydospores. They add that the microconidia are hyaline (2-3.5 X 5-12 μm), develop on phialides, and are largely aseptate, though some are one-septate. Gathering on the ends of the phialides in drops of liquid, they disperse readily. Clark and Moyer also report that macroconidia are similarly hyaline (3-4 X 25-45 μm) and bountiful, being

produced on false heads of the phialides. Compared to the smaller microconidia they are predominantly three-septate, though some will have as many as four or five septa (Clark and Moyer, 1988). Chlamydospores, the long-term/unfavorable condition spores, can be produced by two means (Clark and Moyer, 1988). Clark and Moyer observed that one means is through the differentiation of an internal cell of macroconidia while the other is produced in the mycelia itself. They are spherical in shape with a diameter ranging from 7-10 μm, with a golden-brown color at maturity (Clark and Moyer, 1988).

Infection can occur in a number of ways including soilborne inoculum entering vascular wounds and via previously infected storage roots while specialized spores can survive in the soil for extended periods of time (Clark and Moyer., 1988). The earliest cited account of *Fob* is from 1890, when Halsted (1890) described the disease. Two years later he officially named it "eggplant stem-rot" (Halsted, 1892), claiming the causal agent was a *Nectria sp*. already known for plaguing eggplant. Interestingly, the *Fusarium* genus is the anamorph (asexually reproducing) form of *Nectria*, though *Fusarium oxysporum* stands out from other *Fusarium* species in its long history of predominantly, if not exclusive, asexual reproduction (Michielse and Rep, 2009). In the United States the disease has been reported in all growing regions over the course of time but has had little impact on growers since the development of resistant cultivars beginning in the 1950s.

Cross protection from nonpathogenic strains of *Fusarium oxysporum* present on plants was observed by Ogawa and Komada (1985) but has yet to be developed as a pathogen retardant. This does not mean that these isolates couldn't be used to protect crops, but no meaningful study has been presented to quantify this effect. Other researchers have found cross protective effects against FW caused by *Fob* via the chlorotic leaf distortion pathogen, *Fusarium lateritium* (Clark, 1994).

The mechanism of protection has not been uncovered at this point, however, and commercialization of a protective product has not come to fruition.

Soil type and previous infection levels have been shown to have a significant impact on infection rate in greenhouse settings (Smith and Snyder, 1971; Smith and Snyder, 1972). They found that soils taken from growing regions susceptible to wilts in other crops had increased disease development. This lines up with research performed on other formae speciales of *Fusarium oxsyporum* (Amir and Alabouvette, 1993; Stotzky and Martin, 1963) and other pathogens (Gill et al., 2000; Bonanomi et al., 2010). Therefore, it would be prudent to avoid jumping to conclusions regarding environmental stability of QTL for *Fob* resistance in lieu of studies looking at different soil types.

Recent genetic research on sweetpotatoes proposed a relationship between a plasma membrane-localized sucrose transporter named IbSWEET10 and resistance to *Fusarium oxysporum* (Li et al., 2017). SWEET (Sugars Will Eventually be Exported Transporter) proteins have been identified in rice, maize, and *Arabidopsis* and were discovered by Chen et al. (2010). In animals they maintain blood glucose levels, while in plants they have previously been implicated in nectar production, as well as seed and pollen development (Chen et al., 2010) and are exploited by bacterial and fungal pathogens alike. Many soil pathogens' success or failure is largely determined by the nutrients provided by the host plant (Hancock and Huisman, 1981). Chen et al. noted that SWEET transporters are probably targeted by pathogens for nutritional gain, as many pathogens acquire glucose from their hosts (Hancock and Huisman, 1981). Li et al. (2017) showed that when IbSWEET10 was overexpressed in sweetpotato it reduced sugar content and consequently *Fob* infection was decreased.

Unpublished (Wells, 1963) and published data (Collins, 1976) indicate that *Fob* is at least partially controlled by the concentration/production of tyloses in the vascular tissue of infected plants. Their studies were performed on one of the initial major sources of *Fob* resistance, Tinian (Clark et al., 2013). This research also indicated that no significant levels of fungitoxic substances were found in either inoculated or uninoculated 'Tinian' plants, nor were there any noticeable levels of fungal metabolites that may have adversely affected the susceptible versus the resistant lines. Later research by Harrison et al. (2001) concluded that periderm components from sweetpotato were at least a contributor to inhibition of *Fob* in vitro. Their ultimate conclusion, in conjunction with similar thoughts from Collins (1976) and Wells (1963) was that neither tyloses nor fungistatic metabolites were solely responsible for resistance. Our current data lends credence to this as will be discussed later in this thesis.

Conclusions

There is a tremendous amount of work to be done as it relates to identifying the genetic makeup of specific traits in sweetpotato. Research to date has revealed some information regarding starch and β-carotene production, and recent studies by Gemenet et al. (2020), Oloka (2019), and Amankwaah (2019) have begun building a better understanding of other characteristics, but continued work and verification of these studies is necessary. What we have begun to learn from our study of the DC population verifies some of these previous studies and will hopefully lead to additional advances in the understanding of *I. batatas* genetics while potentially providing us with parental material for future lines of sweetpotatoes for growers.

Herein, we discuss a disease screening for *Fob* resistance in sweetpotato along with a study on various processing characteristic in sweetpotato. The former provides us with new knowledge

on *Fob* resistance and tells us which lines from the DC population are susceptible, resistant, or somewhere in between. The latter confirms and bolsters previous studies on sweetpotato processing characteristics in the unique DC mapping population. Based upon this information we can dig deeper to begin the process of teasing out the genes responsible for encoding these traits. We hope this research will be invaluable to sweetpotato breeding programs around the world and will hopefully lead to further studies on other characteristics not covered here.

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CHAPTER II

Discovery of quantitative trait loci for resistance to *Fusarium oxysporum* f.sp. *batatas* in sweetpotato

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ABSTRACT

Breeding for disease resistance in sweetpotato (*Ipomoea batatas*) has traditionally been performed using greenhouse and field bioassays to determine susceptibility or resistance to various pathogens. Though sweetpotato is generally known for its tolerance to many abiotic and biotic stresses, these pressures are continually in the minds of breeders and farmers. One of the biotic pressures that new lines are tested against is *Fusarium oxysporum* f.sp. *batatas* (*Fob*), a wilt inducing fungus capable of drastically reducing yields in highly susceptible varieties. While the current impact of *Fob* has been relegated to a small nuisance via development of resistant sweetpotato varieties, this scenario could quickly change if the pathogen overcomes current sources of resistance. To better understand present-day resistance mechanisms, we used

genotyping by sequencing (GBS) to identify single nucleotide polymorphisms that were associated with quantitative trait loci (QTL) for resistance to *Fob* in a mapping population consisting of 454 genotypes from a cross between a susceptible breeding line NCDM04-0001 (female) and the resistant cultivar Covington (male). Seven experiments were conducted over two growing seasons (2017 and 2018) under controlled greenhouse conditions using a detached leaf bioassay routinely used by the breeding program to ascertain resistance to *Fob*. The broad sense heritability of *Fob* resistance was estimated at 84.9%. QTL analysis revealed two QTL located on linkage groups 3 and 10, that explained 9.6% and 33.7% of observed phenotypic variation in *Fob*, respectively. Estimated effects of the QTL alleles from both parents were also estimated and important alleles were noted. Further research needs to be done in other sweetpotato genetic backgrounds to determine whether these QTL and their effects are consistent across different populations or whether there are additional QTL contributing major or minor effects to *Fob* resistance in sweetpotato.

INTRODUCTION

Sweetpotato, [*Ipomoea batatas* (L.) Lam. (2n = 6x = 90)], is an economically important crop providing sustenance to people around the globe (FAO, 2016). It belongs to the morning glory family (*Convolvulaceae*) and is subject to diseases caused by a broad range of factors (Clark and Moyer, 1988). In the past, crop rotation has been suggested as a means to keep disease pressure down (Harter and Weimer, 1929; Guertal et al., 1997). Weedy *Convolvulaceae* species prevalent in agricultural fields harbor known sweetpotato pathogens, however, making breeding for resistant lines particularly important (Clark and Watson, 1983).

Disease resistance is one of the most important characteristics that a crop needs for commercial release as an estimated 20-40% of global harvest is lost to plant diseases (Hogenboom, 1993; Oerke, 2006; Savary et al., 2012). An outstanding cultivar that produces high yields during ideal conditions can quickly fail under stress conditions (Velásquez et al., 2018). North Carolina can be a particularly difficult growing region with its combination of excessive heat and variable rainfall patterns, including hurricanes and high humidity (Shelby et al., 2005), all of which lead to favorable environments for many types of pathogens and the emergence of new challenges almost yearly (Velásquez et al., 2018).

Fusarium oxysporum f. sp. *batatas* (*Fob*), which causes fusarium wilt (FW), is a fungus that belongs to the Ascoymcetes division. *Fob* is one of the major pathogens of sweetpotato (Clark and Moyer, 1988) and, as is the case with other formae speciales of *Fusarium oxysporum*, there are little to no means of chemical or physical prevention of the fungus, leaving farmers to rely on resistant lines developed by breeders (Michielse and Rep, 2009). Fusarium wilt is prevalent and costly in many economically important crops (Michielse and Rep, 2009). Tomato, banana, cotton,

melon, sweetpotatoes, and more have all been devastated by the disease recently or in the past (Michielse and Rep, 2009; Clark and Moyer, 1988).

The disease caused significant crop losses in sweetpotato fields for decades with some states reporting 10 to 50 percent yield losses and, in some cases, upward of 95 percent loss (Hildebrand and Cook, 1959). Beginning in the 1950s researchers began developing and releasing resistant lines with the introgression of resistance from the cultivar Tinian (Clark and Moyer, 1988). This cultivar served as the beginning of a breeding effort for resistance to *Fob* and its progeny have served as one of the major sources of *Fob* resistance in the United States since its discovery during World War II on Tinian Island (Clark and Moyer, 1988). Researchers have also screened germplasm from around the globe searching for additional sources of resistance (Lin et al., 2017). One of the more successful *Fob* resistant cultivars, Beauregard, dominated the market after its release in 1987 and typically shows above average resistance in *Fob* trials (K. Pecota, personal communication). Despite this success, the number of cultivars used in sweetpotato production is limited, and very little is known about germplasm diversity and the genetic basis of resistance to *Fob* (K. Pecota, personal communication).

Past experiments identifying QTL in sweetpotato have often focused on yield and other processing traits (Cervantes-Flores et al., 2008a; Cervantes-Flores et al., 2011; Zhao et al., 2013; Kim et al., 2017; Amankwaah, 2019; Oloka, 2019; Gemenet et al., 2020). Other QTL studies for sweetpotato diseases such as *Sweet potato chlorotic stunt virus* and *Sweet potato feathery mottle virus* (Mwanga et al., 2002), root-knot nematode (Cervantes-Flores et al., 2008b; Oloka, 2019), sweetpotato virus disease (Oloka, 2019), and sweetpotato weevil resistance (Oloka, 2019) have also taken place. Despite earlier pathology studies by Wells (1963), Collins (1976), and Clark et al. (1998), no QTL study on *Fob* resistance has been published before this one.

Resistant cultivars such as Beauregard and Covington have demonstrated that breeders can develop materials resistant to *Fob*, but this screening process is labor and time intensive. The objectives of this study were to use our advancing knowledge of the sweetpotato genome and next-generation-sequencing to develop molecular markers associated with resistance to *Fob* that can be used to increase breeding efficiency and consequently speed up the breeding process. Based on the results of our greenhouse trials we discuss the QTL we found, their estimated effects, and the steps for moving forward with further research.

MATERIALS AND METHODS

Plant material and experimental design: Resistance to *Fob* was studied in the DC population, which represents a biparental cross between the NCSU breeding line NCDM04-0001 (female) and Covington (male), and consisted of a total of 454 progeny. NCDM04-0001 is a light yellow-fleshed, purple/red-skinned, high dry matter (ca. 32%) breeding line. It was developed in 2004 as part of a high dry matter breeding program and is considered moderately susceptible to *Fob*. Covington has orange flesh, rose skin, lower dry matter (ca. 20%), and is considered resistant to *Fob*. Seven experiments for *Fob* resistance were conducted during the summers of 2017 and 2018. Each experiment was set up in a randomized complete block design with four replications.

The DC population was maintained in two forms: 1) in a disease-free greenhouse as plants; and 2) as storage roots harvested from vegetative cuttings planted in the field. The population has been maintained in both forms (plants and storage roots) since the creation of the mapping population in 2017. In 2017, plants for the *Fob* disease screenings were obtained from disease-free (Elite Plants) maintained in 72-cell seedling trays filled with Pro-Line C/P Growing Mix (Jolly Gardener) in the greenhouse at the NC State, Method Road greenhouses (Raleigh, NC). In 2018, the cuttings for the *Fob* experiments were produced from plants obtained from G2 (Generation 2) storage roots produced by storage roots bedded in 40.0 cm x 40.0 cm x 12.7 cm (AFLAT5, 0.48cm bottom mesh, Anderson Pots, Portland, OR) nursery flats in a plastic tunnel house.

Isolates used: The *Fob* isolates used were WJM-7, 85-27, and 84-46. WJM-7 was a 1977 Louisiana isolate provided by W.J. Martin, Louisiana Agricultural Experiment Station, Baton Rouge. 85-27 was a 1985 isolate originating from North Carolina provided by J.W. Moyer, North Carolina State University, Raleigh, while 84-46 was a 1984 isolate originating from North Carolina

and provided by P.D. Dukes, the United States Department of Agriculture, U. S. Vegetable Laboratory Charleston, SC. They are currently stored on silica gel at -20^{o}C in Kilgore Hall at North Carolina State University.

Inoculum preparation: Before inoculation, the isolates were taken out of the freezer and transferred onto potato dextrose agar (PDA) plates. The plates were incubated at 28^{o}C in an incubator (Thermo Fisher Scientific, model PR505755L, Waltham, MA) for ~1 week. When sufficient mycelial growth was seen, four to five plugs of isolate 84-46 and WJM-7 and eight plugs of isolate 85-27 (it produces fewer conidia) were transferred from the PDA plates into four 500 ml flasks per isolate. Each flask contained 100 ml of Czapek's-Dox broth, 35g/L.

Five to six days before inoculation, the 12 flasks were placed in a Nalgene tub and the tub was placed on a rotary shaker set at 105 rpm. After shaking, mycelium was removed from each flask by straining through two layers of cheesecloth. A hemocytometer was used to adjust the solutions to 1 x 10^6 spores/ml ($C_1V_1 = C_2V_2$). The three culture solutions were then combined to create a "cocktail" which was used to inoculate the plants in the greenhouse.

Harvest, sample collection, and processing: In 2017, ~6 inch (15.24 cm) vegetative tip cuttings of each full-sib were collected on July 11th from our disease-free greenhouse at the NCSU Method Road facility (35°47'15.6"N 78°41'43.9"W). The plants were transported to our greenhouses located at the Horticultural Field Laboratory (HFL) (35^{o}47'28.0"N 78^{o}41'52.6"W). Four cuttings were taken from each clone for use in the experimental *Fob* bioassay. After the tip cuttings were obtained, and immediately prior to treatment with inoculum, they were stripped of their lower

leaves to provide ease in planting, cut to an equal length (~12.7 cm), and the distal ends were immediately placed in the prepared *Fob* inoculum "cocktail" for 1 minute. During this time the *Fob* spores were imbibed by the cuttings to artificially initiate infection. Both inoculated and un-inoculated controls of check lines Beauregard, Covington, Hernandez, Jewel, NCDM04-0001, and Porto Rico were included for comparison purposes.

Cuttings were planted in separate seedling tray cells, but kept together in their respective groups of four, and planted randomly across twenty-five 72-cell trays in a randomized complete block design (RCBD). After planting, the plants were irrigated per normal procedures with a breaker nozzle to allow for rooting and watered as needed thereafter. Upon established infection and death of susceptible check lines (cultivar Porto Rico) the entire population was rated for degree of resistance or susceptibility. The plants were rated on a 0-4 scale, with: 0 = dead; 1 = very severe symptoms; 2 = vascular browning, wilt, chlorosis, loss of leaves observed; 3 = slight wilting and/or chlorosis, slight vascular browning observed; and 4 = healthy plant with no symptoms.

In 2017, three sets of experiments were performed. Cuttings from the first experimental trial in 2017 were planted on July 11th and evaluated 13 days later on July 24th. The second and third trials of 2017 were planted on August 17th and evaluated 11 days later on August 28th.

In 2018, an additional four experiments were completed. In contrast to 2017, ten cuttings were taken from storage roots planted in 40.0 cm x 40.0 cm x 12.7 cm (AFLAT5, 0.48cm bottom mesh, Anderson Pots, Portland, OR) nursery flats at the Horticultural Crops Research Station (35°01'28.5"N 78°16'58.9"W). The cuttings were then transported to our sweetpotato greenhouses at HFL where the lower leaves were stripped off, cuttings were dipped in Bifenthrin (Bifen I/T (22 ml/gal), Control Solutions Inc., Pasadena, TX) to kill possible insects from the field and placed in 50 cell trays to root. These plants were grown for several weeks until new shoots of at least 6

inches (~15.24 cm) were available for cutting. The fourth and fifth trials were inoculated and planted on July 18th and rated 14 days later on August 1st, while the sixth and seventh trials were inoculated and planted on August 23rd and rated 13 days later on September 5th.

Phenotypic model and broad sense heritability analyses: In order to obtain adjusted means for QTL mapping, restricted maximum likelihoods (REML) variance components analysis of the phenotypic data was performed using GenStat (v. 17.1) and the following model:

$$y_{ijk} = \mu + h_k + b_{j(k)} + t_i + \varepsilon_{ijk}$$

Where y_{ijk} = was the mean score of clone i in replicate j of year k, h_k was the fixed effect of year k ($k = 1, \ldots, K$; $K = 2$), $b_{j(k)}$ was the fixed effect of block j in year k ($j = 1, \ldots, J$; $J = 7$), t_i was the fixed effects of clone i ($i = 1, \ldots, I_g + I_c$); $I_g = 410$ full-sib genotypes and $I_c = 6$ checks), and ε_{ijk} was the residual random effect, with $\boldsymbol{\varepsilon} \sim N(\mathbf{0}, \mathbf{R})$, where $\boldsymbol{\varepsilon}$ was the vector of residuals and $\mathbf{R} = \mathbf{I}_I \otimes \mathbf{R}_J$ was the variance-covariance (VCOV) residual matrix and $\otimes$ was the direct product. The $\mathbf{R}_J$ matrix was selected by looking at the Akaike (AIC) and Bayesian Information criteria (BIC) of seven alternative models, which accounted for variance and covariance heterogeneity among blocks.

We fit the same model above with g_i as the random effect of full-sib genotype i ($i = 1, \ldots, I_g$) with $g_i \sim N(0, \sigma^2_g)$, and c_i as the fixed effect of checks ($i = I_g + 1, \ldots, I_g + I_c$), and the identity residual matrix, so that $\varepsilon_{ijk} \sim N(0, \sigma^2)$. The broad-sense heritability, H^2, was then computed as shown below:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma^2}{J}}$$

An alternative analysis, modeling the individual plant scores (rather than their mean scores) was carried out (data not shown), but the fixed effect of plant was not significant (Wald test, $p = 1.71$), therefore the mean score responses are reported.

Optimized genotyping-by-sequencing for polyploids (GBSpoly): GBSpoly, a modified GBS protocol, was utilized according to Wadl, et al. (2018) and is described in greater detail by Mollinari et al. (2020). Briefly, it is a library preparation and next-generation-sequencing protocol that is optimized for polyploids and highly heterozygous genomes. By re-designing barcoded adapters (to ensure accurate demultiplexing and base calling), GBSpoly produces uniform coverage across samples and loci for the targeted population. The barcodes used varied in length between 6-9 base pairs, which minimizes phasing error during sequencing-by-synthesis and preserves nucleotide diversity, accounts for both substitution and indel errors (based on edit/levenshtein distance) while also minimizing phasing error. Upstream of the barcodes, buffer sequences ensure that barcodes lie within high-quality base regions since the beginning and ends of reads tends to have higher base calling errors. *Tse*I and *Cvi*AII double digests produce fragments for adapters to ligate to which are subsequently chosen according to size in order to minimize PCR bias. Chimeric ligations were eliminated by performing a secondary digest with the same enzymes. Digestion at the adapter-insert junction are completely avoided because the restriction site is not reconstituted at the adapter-insert junction. Next-generation sequencing was performed on the Illumina HiSeq 2500 platform (Illumina, Inc., San Diego, CA) (Mollinari et al., 2020).

Genotype calling: The software SuperMASSA (Serang et al., 2012) was utilized to perform the genotype calling of parents and offspring in the DC full-sib population. SNPs with read depth <20

were eliminated for quality control. Additionally, SNPs with more than 25% of missing data and with segregation distortion (Bonferroni $P < 2.9 \times 10^{-6}$) were filtered out. Five individuals with less than 100 reads on average were also removed. There is currently no hexaploid *I. batatas* reference genome, therefore we used the diploid *I. trifida* and *I. triloba* reference genomes developed by Wu et al. (2018). Since there is a high collinearity between the *I. trifida* and *I. triloba* genomes (Wu et al., 2018; Mollinari et al., 2020), we opted to use exclusively the genomic information provided by the *I. trifida* reference genome (Wu et al., 2018).

Linkage map construction: Pairwise recombination fraction and linkage phase estimation produced 15 distinct linkage groups that were aligned with the 15 *I. trifida* chromosomes present in the reference genome (Wu et al., 2018). We used two procedures to order the markers within each linkage group. First, we used the MDS algorithm (Preedy and Hackett, 2016) with the recombination fractions converted into genetic distances using the Haldane map function and the squared linkage LOD Scores to construct the stress criterion. Second, we used the SNP order proposed in the Beauregard x Tanzania (BT) map (Mollinari et al., 2020) using the *I. trifida* reference genome to connect both maps. The former yielded the highest likelihood and, therefore, was chosen as the final map. In both cases, SNP phasing and multipoint recombination fraction estimation was performed in accordance with Mollinari and Garcia (2019). The final map was re-estimated using the probability distribution of the genotypes through HMM (Mollinari et al., 2020).

QTL mapping: Mapping of QTL was performed using QTLpoly software (https://github.com/guilherme-pereira/QTLpoly) by using the QTL genotype conditional

probabilities obtained from the genetic map and the joint adjusted means from the phenotypic analysis as well as the raw scores from each replication. QTLpoly is based on a random-effect multiple interval mapping model (Pereira et al., 2019) where the effect of g_q of a putative QTL q ($q = 1, \dots, Q$) is treated as random with $g_q \sim N(0, \sigma_q^2)$. The variance component associated with the QTL (σ_q^2) was tested every 1 cM along the genetic map using score statistics. First, QTL detection took place in a stepwise procedure: 1) Forward search which added on QTL at a time under a less conservative threshold ($p < 0.01$), 2) Backward elimination where each QTL was tested again under a more conservative threshold ($p < 0.001$) where QTL may be dropped if they fall below the threshold or their position is refined if changes occur. Then, a final multiple QTL model was fitted in order to estimate: (1) the final set of variance components associated with each detected QTL, σ_q^2, (2) the QTL heritability, h_q^2, as the proportion of the total variance explained by QTL q, and (3) the allele and allele-combination effects for each QTL. To complete this we compared our QTL peaks and support intervals to an anchored reference genome of one of the diploid relatives of sweetpotato, *I. trifida* (Wu et al., 2018), in order to find which genes of interest are in our QTL regions.

RESULTS

Phenotypic analyses: In 2017 and 2018 the un-inoculated check lines all had averages of 4.00 (= no symptoms) on the rating scale, except for one cutting from Beauregard in trial 3, which was likely due to either accidental contamination or damage prior to or during planting. The inoculated check lines, averaged across all bioassay experiments, were rated as follows in 2017: 2.2 – Beauregard; 2.4 – Covington; 1.6 – Hernandez; 1.6 – Jewel; 0.5 – Porto Rico; and 2.3 – NCDM04-0001. In 2018 the positive check lines, averaged across all experiments, had average scores of: 2.1 – Beauregard; 2.8 – Covington; 0.7 – Hernandez; 1.9 – Jewel; 1.1 – Porto Rico; and 1.0 – NCDM04-0001.

After combining all seven experiments together from the two years the positive check lines had average scores of 2.2 – Beauregard, 2.7 – Covington, 1.1 – Hernandez, 1.8 – Jewel, 0.8 – Porto Rico, and 1.5 – NCDM04-0001. The mean disease scores for parents NCDM04-0001 and Covington across all seven experiments were 1.5 and 2.7, and disease score ratings of the whole population were normally distributed (Figure 1). Some progeny exhibited transgressive segregation in both directions, which possibly indicates the inheritance of resistance was quantitative. Two in particular, NCDC_20 (3.7) and NCDC_413 (3.5) had scores ~0.7 or greater than the high parent, Covington (2.7).

The fairly wide range of scores observed between clones (0.2 to 3.8) after adjusted means were calculated is indicative of broad genetic diversity among the segregating progeny (Figure 1). Covington, the resistant parent, exhibited the greatest resistance (E1 – 3.4, E4 – 3.8, E5 – 1.9, E6 – 2.7, E7 – 2.9) among the check lines in all tests (except E2 and E3) which is not surprising based on previous years of screening (K. Pecota, personal communication).

The broad-sense heritability of resistance to *Fob* in the DC population was estimated to be 85.0%, suggesting that resistance to *Fob* is highly heritable and likely controlled by a few genes. The correlation coefficients (*r*) between individual tests were all significant ($p < 0.001$) and ranged from 0.28 to 0.67 (Figure 2). When comparing the individual tests to the overall adjusted means, correlations were typically strongest.

SNP and linkage map development: The GBSpoly genotyping-by-sequencing protocol, which was developed specifically for highly heterozygous and polyploid genomes (Wadl et al., 2018) such as the DC population, was very effective for SNP development. Tassel-GBS (Glaubitz et al., 2014) was used to collapse the ca. 13.2 billion barcoded reads into 31,938,636 sequence tags by eliminating non-redundant barcoded reads. BOWTIE 2 (Langmead and Salzberg, 2012) was used to align the tags to the two *I. batatas* progenitor species reference genomes (*I. trifida* and *I. triloba*) developed by the Genomic Tools for Sweetpotato Improvement Project (Wu et al., 2018). In total, 1,331,267 and 1,281,190 polymorphisms (SNPs and indels) from *I. trifida* and *I. triloba* were identified, respectively. Dosage calling was conducted using SuperMASSA (Serang et al., 2012) resulting in 15,966 markers markers distributed among 15 linkage groups (LG), covering 2,727.1 cM which is consistent with the 2,708.4 cM estimated for the Beauregard x Tanzania (BT) biparental sweetpotato population described by Mollinari et al. (2020). No visible gaps were observed with an average of 5.36 cM between markers.

Filtering of the markers for an average read depth >20 yielded 467,691 markers aligned to the *I. trifida* genome. Of these, 20.8% (97,628) were classified as monomorphic while 44.4% were classified as hexaploids. After filtering for missing data, 42,705 markers were tested for segregation distortion and redundant markers were also removed, resulting in 15,966 high-quality

SNPs that were used to build the genetic map. Additional filtering was performed using MAPpoly, resulting in the 14,367 markers seen in Table 1 (from an original 15,966 markers). Table 2 summarizes the distribution of dosage combinations in the parents NCDM04-0001 and Covington. Simplex markers (0-1, 1-0) were the most abundant marker type, accounting for 51.5% of the markers. The average length of the linkage groups was 181.8 cM with the longest being linkage group 1 at 243.0 cM and the shortest being linkage group 8 at 120.5 cM. The ordered markers were all phased to the expected number of homologues based on parental dosages, leading to a fully phased linkage map (Figure 3).

QTL detection: Adjusted means calculated from the raw phenotypic data for each replication were used for the QTL analyses of all experiments and we identified two QTL accounting for 9.6% and 33.7% of total variation in *Fob* resistance on linkage groups 3 and 10, respectively (Table 3). The QTL on linkage group 3 (*Fob1*) was located at 8.11 cM (marker S3_764435) with a support interval of upper marker (S3_1179682) and lower marker (S3_152020), while the second and more significant QTL (*Fob2*) was located on linkage group 10 at 130.29 cM (S10_21534291) with a support interval of upper marker (S10_23067775) and lower marker (S10_19457866) (Table 3). We compared these two to the *I. trifida* reference genome and found these regions are full of leucine-rich repeat receptor kinases (*itf10g02620, itf10g02660*, and *itf10g02730*) while *Fob2* was located within a gene annotated as Transcriptional factor B3 family protein / auxin-responsive factor AUX/IAA-related (*itf10g19820*) located between 21,532,579 bp and 21,533,187 bp. The QTL on linkage group 10 was consistent across all seven experiments and the overall mean indicating stability of the QTL (Figure 4). The QTL on linkage group 3 was detected in only one individual experiment (E4) but was detected when the scores of all seven experiments were

averaged together. A QTL on linkage group 8 was detected in one individual experiment (E1) but was not detected when all seven experiments were average together.

Estimation of allelic effects: By averaging the QTL-based genotypic values of individual clones we found that both parents had alleles that either positively or negatively impacted the phenotypic mean of *Fob* resistance. For example, clones with alleles g and h from NCDM04-0001 at *Fob1* (Figure 5) would be expected to have an approximate phenotypic mean decrease of -0.03 and -0.08, respectively. However, individual clones with *Fob1* alleles d and e from Covington would expect a resistance score increase of +0.05 and +0.07, respectively, compared to the population mean of *Fob* resistance. For *Fob2*, an individual with alleles a and c from Covington would expect a resistance score increase of ca. +0.18 and +0.15, respectively, compared to the population mean of *Fob* resistance (Figure 6).

DISCUSSION

We report here the first QTL mapping study for resistance to *Fob*. As far as we know, Jones (1969) and Collins (1977) are the only other studies to investigate quantitative resistance to *Fob* in sweetpotato. The two QTL we report here explain roughly half of the total phenotypic variation (43.3%) for resistance to *Fob* with one QTL (*Fob2*) responsible for 33.7%. Since *Fob2* was consistent across all seven experiments it should be a good candidate for future QTL validation studies in other sweetpotato populations. The frequency distribution from the phenotypic analyses suggests additional genes being responsible to differing degrees (Figure 2). Polygenic resistance is common in plant-pathogen interactions as the two organisms evolve in response to changes in each other's genomes (Perchepied and Pitrat, 2004; Dracatos et al., 2016).

Covington's contribution of two major resistance alleles from both QTL could serve as a starting point for future breeding efforts. The presence of three alleles (*Fob1*) and four alleles (*Fob2*) contributing negative effects to Covington suggests that several of the progeny in this population received positive alleles from NCDM04-0001 in addition to positive alleles from Covington. Research by Rieseberg et al. (1999) points out that while overdominance and epistasis can contribute to transgression, the main cause is usually complementary genes. By focusing on these alleles in the full-sib population we could 1) select more resistant individuals (and transgressive segregants) based on their breeding values, and 2) use these alleles in marker-assisted selection (MAS) upon validation in a breeding population with a broader genetic basis. Since this population is limited by the alleles of the two parents, upcoming analyses of other training populations within our program could help us to obtain a broader genetic basis for resistance to *Fob*.

In our study, a high broad-sense heritability estimate of 85.0% indicates that *Fob* resistance in this population is highly heritable. This is consistent with research by Jones (1969) which calculated the heritability of *Fob* resistance as 86.0%. Based on this and what we have seen in sweetpotato polycross nurseries over the years, maintaining resistance to *Fob* is relatively easy so long as good parental materials are selected (K. Pecota, personal communication). For other sweetpotato diseases and pathogens research, broad-sense heritability has been reported as 49.0% for sweetpotato weevil resistance (Oloka, 2019), 89.0% and 96.0% for root knot nematode resistance (Cervantes-Flores et al., 2008b; Oloka, 2019), and 73-98% for resistance to sweet potato virus disease. These studies suggest that rapid genetic gains should be possible when the correct parental phenotypes are used in crosses.

The most commonly cited mechanism of *Fob* resistance in sweetpotato is the production of tyloses by resistant lines (Wells, 1963; Collins and Nielsen, 1976). This response has been noted in other crops responding to FW, most notably banana and tomato (Beckman, 1964; Beckman et al., 1961). Wells (1963) found that 12 days post inoculation, tyloses had completely occluded 75-88% of the xylem vessels 22-32 mm above the invasion site in the resistant line, Tinian, while uninoculated control plants had between 0-3% of vessels filled. Through the combination of tyloses, gels, and gums clogging the xylem, resistant plants seem to, at the very least, slow the rate of fungal hyphae growth in the vascular tissue (Talboys, 1958; Talboys, 1972; Fradin and Thomma, 2006; Yadeta and Thomma, 2013). The production of inhibitory chemicals has also been uncovered in other crops (Braun, 1942; Gottlieb, 1943; Langcake et al., 1972), but despite looking for such chemicals in sweetpotato (Wells, 1963), nothing has been found to date. Other studies have attempted to elucidate genes responsible for *Fob* resistance (Li et al., 2017; Lin et al., 2017), with one implicating sucrose sugar transporters as part of the resistance mechanism. However, no

studies have sought to explore marker assisted breeding for the introgression of resistance to *Fob* in sweetpotato.

A search for candidate genes revealed that the major QTL on linkage group 10 for *Fob* resistance was located within *itf10g19820* (marker S10_21534291), which was annotated as Transcriptional factor B3 family protein / auxin-responsive factor AUX/IAA-related. Traditionally, research on plant defenses against pathogens focused on systemic acquired resistance (SAR) and induced systemic resistance (ISR), which result in resistance against subsequent pathogen attack. Wang et al. (2007) concluded that downregulation of auxin-related genes could be a component of the SAR response in plants after finding that 18 of 21 auxin-related genes in *Arabidopsis thaliana* were repressed after induction of SAR. Auxin-responsive genes have been shown to be globally downregulated during *Botrytis cinerea* infection in Arabidopsis (Llorente et al., 2008), while differential expression was seen in cotton in response to *Fusarium oxysporum* f. sp. *vasinfectum* (Dowd et al., 2004). Other research has suggested that exogenous auxin application may trick plants into thinking they are being attacked by a pathogen, thus inducing systemic resistance via ISR (Petti et al., 2012). Furthermore, there is evidence showing that auxin signaling enhances plant resistance to necrotrophs while subsequently increasing susceptibility to biotrophs, potentially by serving as a negative regulator of the salicylic acid pathway which is required for SAR (Robert-Seilaniantz et al., 2007). Interestingly, *Fob* is a hemibiotroph, and therefore may be able to take advantage of plants during its biotrophic stage before switching to its necrotrophic life stage.

Major QTL have been found for resistance to other formae speciales of *Fusarium oxysporum* in other crops. For example, in chickpea (*Cicer arietinum* L.), where favorable conditions for *Fusarium oxysporum* f.sp. *ciceris* can cause 100% yield losses, Sabbavarapu et al.

(2013) found two QTL responsible for 10.4% and 18.8% of phenotypic variation. Wang et al. (2009) found four QTL explaining from 15.9% to 60.9% of phenotypic variation in upland cotton (*Gossypium hirsutum*). Additionally, they found that a resistance gene for root knot nematode (RKN) in upland cotton was located within the same interval as one of their *Fusarium* genes. Taking this finding into consideration, it may be worth comparing our work to past RKN research by Oloka (2019) and Cervantes-Flores et al., (2008b) later to see if any similarities exist within sweetpotato.

It is possible that the results obtained in this study may have been influenced by the fact that plant cuttings for our *Fob* assays were taken from different locations between the two growing seasons. The difference potentially introduced between cuttings from the two environments is that those from the field station were most likely exposed to other strains of *Fusarium* such as *Fusarium lateritium* which have been shown in previous studies to provide a protective effect against *Fob* (Clark, 1994). It is also possible that the use of plant cuttings from different generations of the asexual breeding cycle could have had an impact on disease severity among years. Previous research noted differences in storage root quality between G1 virus-indexed and G2-5 generations infected with *Sweet potato feathery mottle virus* (Bryan et al., 2003a; Bryan et al., 2003b). Research by Okpul et al. (2011) on additional sweetpotato viruses concluded that effects are possibly cultivar dependent, but generally negative, while Lewthwaite et al. (2011) noted cultivar decline after subsequent generations from material untested for pathogens. We do not believe this was a confounding effect, however, since the worst and best performing clones tended to maintain their rankings over the course of the two separate years of testing regardless of their source (data not shown).

Further studies could be useful to determine the efficacy of the resistant lines against other races of *Fob*. Clark et al. (1998) described at least two distinct pathogenic races, but they have differential effects on both traditionally resistant and susceptible varieties. Being from geographically isolated regions could have led to divergent forms of pathogenicity and additional research would enable us to determine the efficacy of *Fob1* and *Fob2* to other *Fob* isolates. Additionally, Clark et al. (1998) showed that isolates of *F. oxysporum* f. sp. *nicotianae* can be weak to highly aggressive on sweetpotato cultivars, which is cause for concern as many farmers in North Carolina rotate the two crops with shared equipment, allowing for disease inoculum to spread.

In recent years additional funding sources, decreased sequencing costs, and advances in bioinformatics have allowed for an expanded role and focus on genomic studies in sweetpotato and its relatives. With these new resources we studied the genetic makeup of the DC mapping population in order to rapidly detect resistance to *Fob*. This research is the first report of QTL for resistance to *Fob,* and we have identified two QTL responsible for 43.3% of phenotypic variation for the trait. Further investigation is warranted into the candidate gene discussed on linkage group 10. These results will be help us to design methods for genomic-assisted breeding in our sweetpotato breeding program. Additional studies on other mapping populations are necessary first, however, to determine to what degree we captured diversity of *Fob* resistance within sweetpotato germplasm around the world and within our breeding program.

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Tables

Table 1. Summary of the NCDM04-0001 x Covington genetic mapping population integrated linkage map created using MAPpoly.

Linkage Group (LG)	Length (cM)	No. of SNPs	SNPs/cM
LG_1	243	1357	5.6
LG_2	200.6	1052	5.2
LG_3	216.1	1230	5.7
LG_4	220.5	1311	5.9
LG_5	182	1048	5.8
LG_6	148.5	825	5.6
LG_7	159.2	838	5.3
LG_8	120.5	468	3.9
LG_9	207.6	1183	5.7
LG_10	178.5	965	5.4
LG_11	169.6	546	3.2
LG_12	205.7	1137	5.5
LG_13	160.1	777	4.9
LG_14	145.9	709	4.9
LG_15	169.3	921	5.4
Total	**2727.1**	**14367**	**5.2**

Table 2. Allelic marker dosage in the NCDM04-0001 and Covington mapping population parents. The dosage groups 0, 1, 2, 3, 4, and 5 correspond to nulliplex, simplex, duplex, triplex, quadruplex, and quintuplex marker types, respectively.

Dosage		No. SNP	% of Total
NCDM04-0001	Covington		
0	1	3778	23.7
0	2	810	5.1
0	3	139	0.9
0	4	12	0.1
0	5	1	0.1
1	0	4449	27.9
1	1	2113	13.2
1	2	878	5.5
1	3	220	1.4
1	4	79	0.5
1	5	10	0.1
2	0	931	5.8
2	1	845	5.3
2	2	476	3.0
2	3	305	1.9
2	4	64	0.4
3	0	131	0.8
3	1	201	1.3
3	2	262	1.6
3	3	83	0.5
4	0	17	0.1
4	1	70	0.4
4	2	84	0.5
5	0	2	0.1
5	1	6	0.1
Total		15966	100

Table 3. Summary statistics of QTLs *Fob1* and *Fob2* for *Fob* resistance in the DC mapping population from the years 2017 and 2018 combined. h^2 is the broad sense heritability of the QTL and σ^2_{QTL} is the estimated variance.

Trait	QTL	LG.	Position (cM)	Support Interval (cM)	Score	Marker	p-value	σ^2_{QTL}	h^2(%)
Fusarium	*Fob1*	3	8.11	2.12 - 14.00	388.67	S3_764435	3.33E-07	0.052	9.6
	Fob2	10	130.29	113.32 - 149.05	875.89	S10_21534291	<2.22E-16	0.182	33.7
								Total	**43.3**

Figures

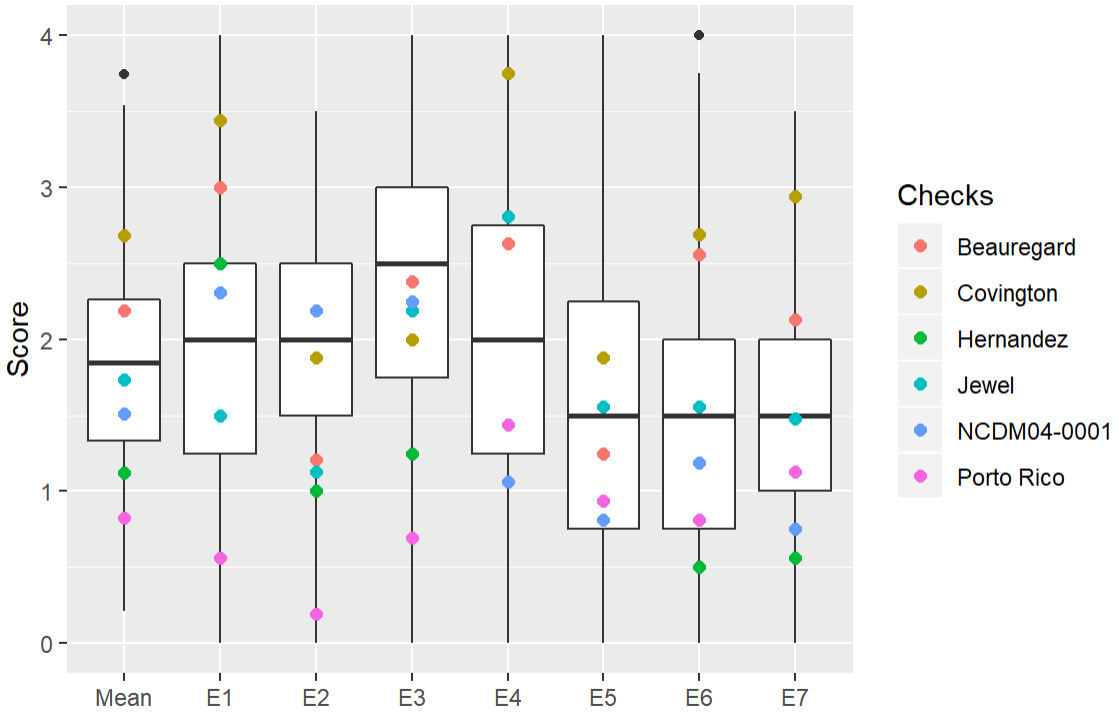

Figure 1. Boxplot with distribution of jointly adjusted means (Mean) and raw data per block (E1 through E7) for *Fob* resistance in the DC mapping population. Y-axis is score based on 0-4 severity scale with: 0 = dead; 1 = very severe symptoms; 2 = vascular browning, wilt, chlorosis, loss of leaves observed; 3 = slight wilting and/or chlorosis, slight vascular browning observed; and 4 = healthy plant with no symptoms. Check lines include highly susceptible cultivars Porto Rico and Hernandez, moderately susceptible cultivar Jewel, moderately susceptible breeding line NCDM04-0001, and resistant cultivars Beauregard and Covington.

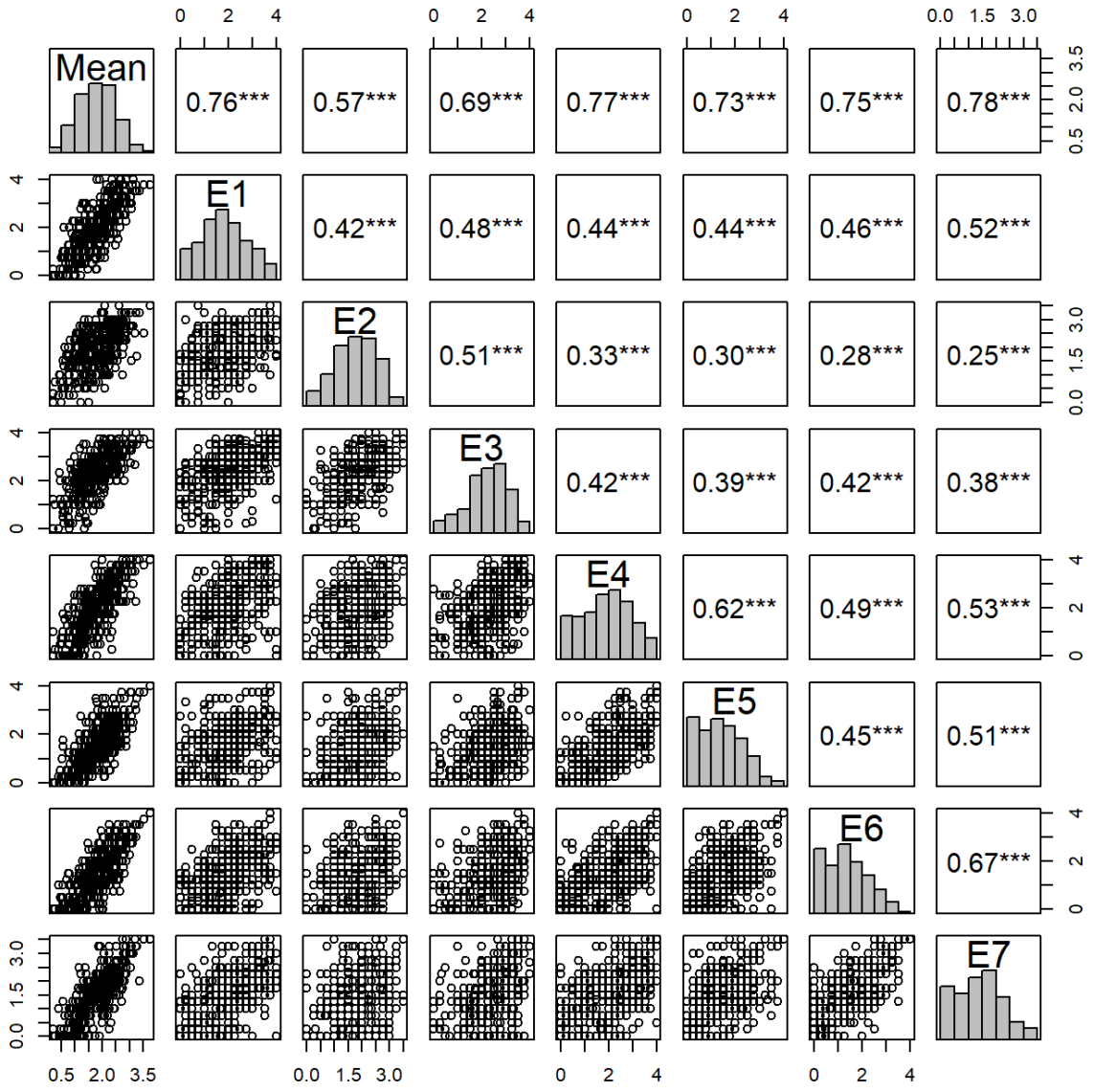

Figure 2. Correlation coefficients (***$p < 0.001$) for *Fob* resistance across all 7 experiments (E1-E7) and joint adjusted means in the DC sweetpotato mapping population. Frequency histograms for joint adjusted means and all 7 experiments (E1-E7) are also provided.

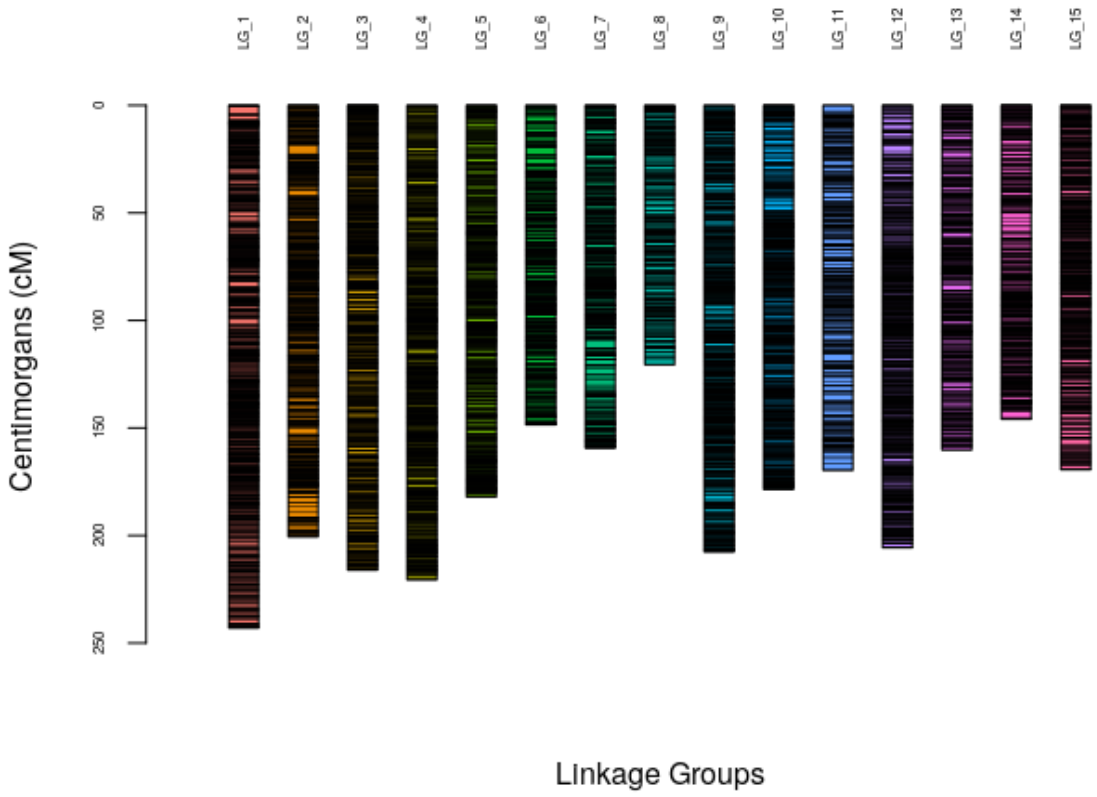

Figure 3. Phased linkage map of the DC mapping population.

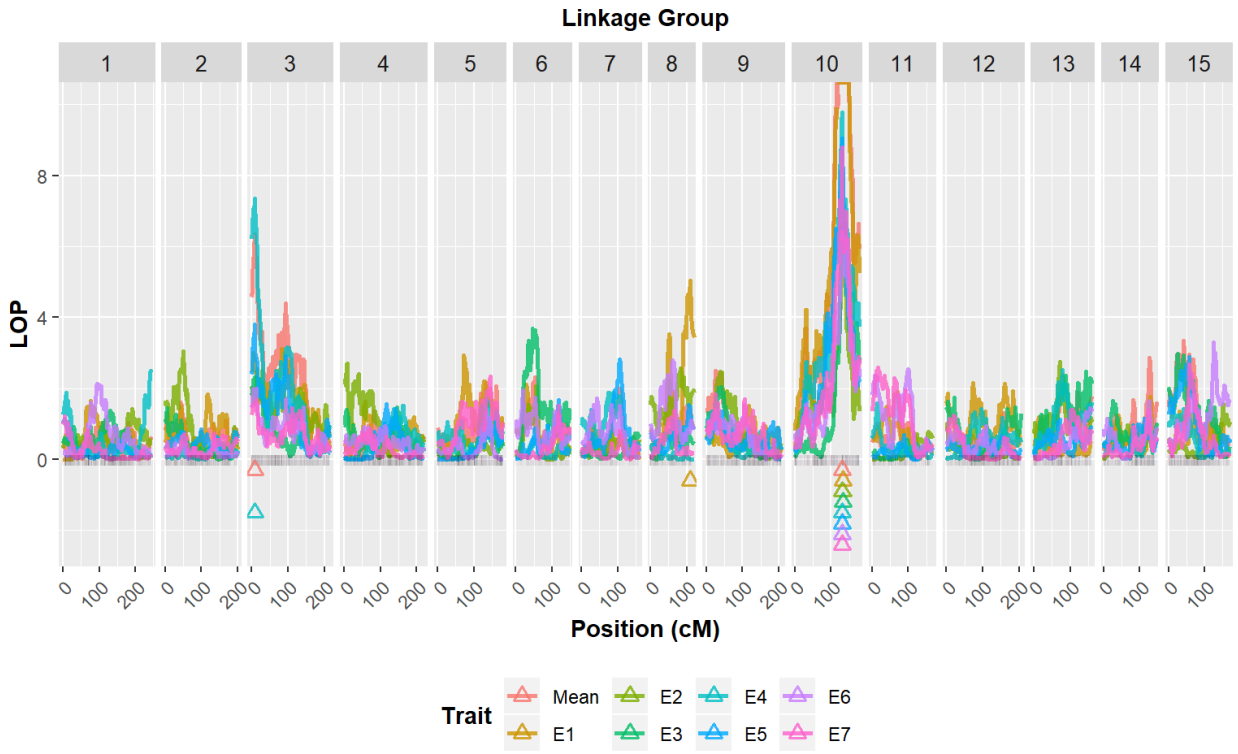

Figure 4. LOP (-$\log_{10}$ of the p-value) profiles from random-effect multiple interval mapping (REMIM) of fusarium resistance for each experiment (E1-7) along with the adjusted mean in the DC mapping population. Triangles show the QTL peak locations.

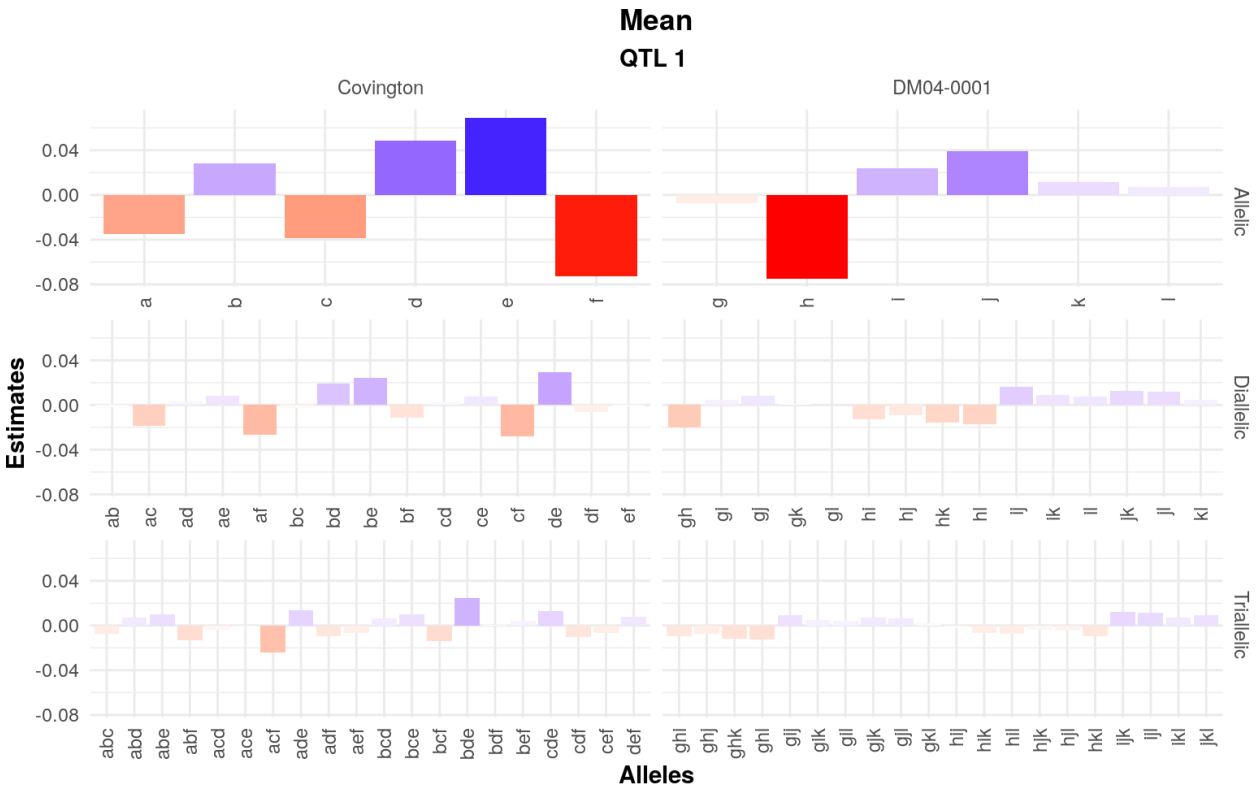

Figure 5. Allele and allelic-combination additive effects for the decomposed best linear unbiased predictions (BLUPs) for QTL 1 (*Fob1)* identified on linkage group 3 at 8.11 cM with major effects for fusarium resistance.

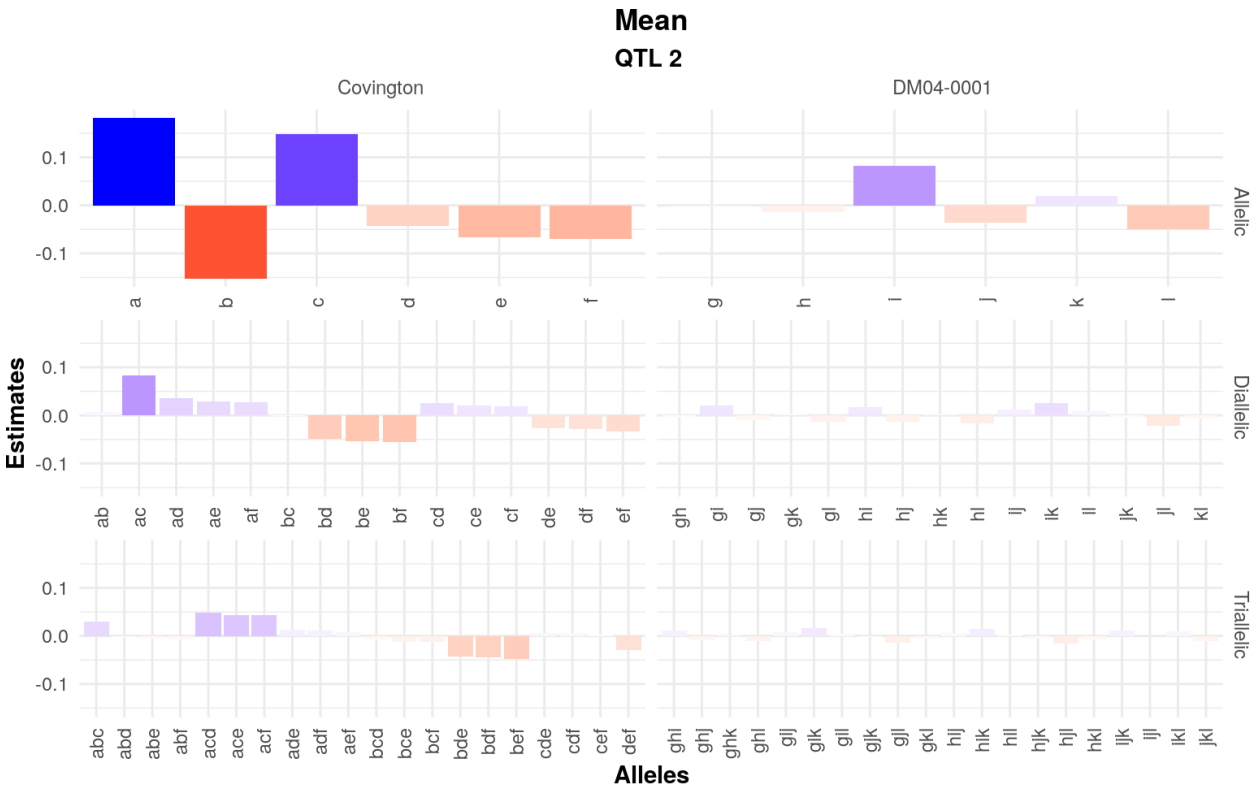

Figure 6. Allele and allelic-combination additive effects for the decomposed best linear unbiased predictions (BLUPs) for QTL 2 (*Fob2*) on linkage group 10 at 121.09 cM with major effects for fusarium resistance.

CHAPTER III

Quantitative trait loci for key processing traits in sweetpotato

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ABSTRACT

Sweetpotato (*Ipomoea batatas*) is one of the fastest growing commodities in the agricultural marketplace, with acreage and interest increasing in the United States as consumers become more aware of the nutritional benefits of sweetpotato. Partly due to its status as a specialty crop in American and European markets, there have been little investments in developing genomic resources for crop improvement in sweetpotato. Here we report on quantitative trait loci (QTL) mapping results for four key sweetpotato processing traits, β-carotene, sucrose, glucose, and fructose in the NCDM04-0001 x Covington (DC) genetic mapping population. Our mapping population consisted of 454 genotypes, and we utilized SNPs developed by genotyping by sequencing (GBS) along with high-throughput storage root chemistry phenotyping using near-infrared spectroscopy (NIRS) to identify QTL for β-carotene, sucrose, glucose, and fructose. The linkage map for this research spanned 2,727.1 cM and consisted of 15 linkage groups. We

identified 3 QTL each for β-carotene, fructose, and sucrose, and 4 QTL for glucose, accounting for 82.1%, 69.2%, 71.8%, and 71.0% of total phenotypic variation, respectively. Future work must include QTL validation studies and implementation of a marker-assisted breeding program for these important processing quality traits in different materials. In addition, based on the identified QTL and reference maps recently developed for *I. trifida* and *I. triloba* we have identified potential candidate genes responsible for these four traits.

INTRODUCTION

Sweetpotato, *Ipomoea batatas* L. (Lam.), is a highly diverse crop, not only genetically, but in the multiple ways it is utilized around the world. It fills many niches in the global food system, helping to meet nutritional requirements, reduce poverty in developing countries, and increase food security (Grünenberg et al., 2015; El-Sheikha and Ray, 2017; Low et al., 2017). It is a highly nutritious crop, serving as a source of carbohydrates, vitamins A and C, fiber, iron, copper, calcium, and folic acid (Woolfe, 1992). While the majority of sweetpotatoes consumed in the United States are of the orange-fleshed sweetpotato (OFSP) type due to their β-carotene (BC) content, there is great variation internationally in terms of tastes, textures, and flesh colors (cream, orange, purple, yellow, and white) (Truong et al., 2018). This has resulted in a potential for many value-added food products, including dehydrated chips and flours, canning, fried products, purees, noodles, sugar syrups, and more (Padmaja, 2009; Truong et al., 2018).

The market for sweetpotato products in the United States has been growing at a rapid pace, increasing by nearly 80% between 2000 and 2014 (Truong et al., 2018). Despite the general perception of sweetpotato as a holiday food served during Thanksgiving and Christmas in the United States, this perception is quickly changing as the health benefits have become increasingly known. In response, several food companies have begun developing methods for processing sweetpotato chips in response to this growing demand for healthy foods (Truong et al., 2018). With sweetpotato fry and chip demand increasing (ConAgra Foods, 2010; U.S. Sweetpotato Council, 2013), breeders have begun to develop cultivars better suited for these purposes.

Much like the more commonly consumed potato (*Solanum tuberosum*) there are various processing standards that must be met for different product categories, but studies on the chemical and textural properties of sweetpotato varieties are limited (Walter et al., 2002; Oner and Wall,

2012; Sato et al., 2018). Starch is a crucial component of white potato and sweetpotato French fries and chips (Miranda & Aguilera, 2006; Sato et al., 2018). High dry matter, which primarily consists of starch, leads to a firm and mealy texture, while low dry matter produces a soggy texture (Truong et al., 2011). In white potatoes, lower levels of dry matter content (thus lower starch) in French fries of 21-23% produces acceptable textures for sensory panels (Pavlista and Ojala, 1997), while the same levels in sweetpotato result in an unpleasant soft and moist texture (Sato et al., 2018). This presents a dilemma as the predominant OFSP varieties for processing (Beauregard and Covington) have low dry matter content (21-22%), resulting in soggy and moist French fries. To overcome this current deficiency some processors will use a coating with starch-based materials which has been noted to improve textural properties (Truong et al., 2018).

Beyond the textural properties, however, other chemical constituents are important for quality French fries and chips. The reducing sugars glucose and fructose are known for inducing browning in fried potato and sweetpotato products via the Maillard reaction (Picha, 1986). Recently, though, research on sweetpotato genotypes with a wide range of reducing sugar content has indicated the browning may be the result of the content of asparagine and similar amino acids in the raw product (Lim et al., 2014; Xiao, 2019). Equally important, reducing sugars and free asparagine are precursors to acrylamide formation. Acrylamide has been classified as “probably carcinogenic to humans” by the World Health Organization and the International Agency for Research on Cancer (Friedman, 2003).

Additional components that set sweetpotato French fries and chips apart from their white potato counterparts are their sweet taste and orange color. The sweet flavor of OFSP sweetpotato products results mainly from the high levels of sucrose present, though maltose produced from starch hydrolysis by β-amylase during the cooking process is also a contributing factor (Walter et

al., 1975; Takahata et al., 1994). The orange color, as noted earlier, is due to the BC content and other carotenoids in OFSP, which can vary from one cultivar to another (Grüneberg et al., 2015). β-carotene makes up over 80% of the carotenoids in sweetpotatoes and is an important precursor to vitamin A (Takahata et al., 1993; Hagenimana et al., 1998) that promotes healthy vision, healthy immune systems, and cell growth in humans (Blomhoff and Blomhoff, 2006). In fried sweetpotato products BC retention has been reported between 72-86% (Vimala et al., 2011).

While some of these properties can be controlled via specialized processing treatments (Baba and Yamamura; Oner and Wall, 2012; Truong et al., 2014), eliminating these extra processing costs is ideal. For breeders, however, this presents a dilemma as a strong negative association between BC and starch/dry matter content has been established in sweetpotato (Grünenberg, et al., 2005; Cervantes-Flores et al., 2011; Yada et al., 2017; Gemenet et al., 2020). In addition, a positive association between BC and sugar content exists (Baafi et al., 2015). These two factors combined means that varieties with higher starch content tend to have lower sugars (thus less taste) and reduced to no BC content. In order to develop crispy fries and crunchy chips that are orange, and taste sweet, researchers need to better understand how to control for these traits at a genetic level. Sensory panels and processor data have given breeders a sense of consumer preference when it comes to various sweetpotato products (Walter et al., 2002; Afuape et al., 2013; Sato et al., 2018). In order to develop crispy fries and crunchy chips that are orange, and taste sweet, we need to better understand these traits.

In this study, we utilized single nucleotide polymorphisms (SNPs) developed by genotyping by sequencing (GBS) and high-throughput storage root chemistry phenotyping using near-infrared spectroscopy (NIRS) to identify quantitative trait loci (QTL) for BC, sucrose, glucose, and fructose in the NCDM04-0001 x Covington (DC) genetic mapping population. In

doing so, we have begun to understand the genetic architecture for these four traits. Using this information, and our advancing knowledge of the sweetpotato genome, we aim to increase breeding efficiency so we can develop superior sweetpotato varieties targeted for the US consumer's preferences. Herein we discuss the QTL we found, their estimated effects on the individual traits, and the next steps for continuing this research.

MATERIALS AND METHODS

Plant material: A cross between NCDM04-0001 (female) x Covington (male) hereafter referred to as DC, was performed to develop the full-sib family used for this study. NCDM04-0001 is a high dry matter (ca. 32%), low BC, low reducing sugar, and low sucrose breeding line with purple/red skin and light-yellow flesh from the NC State Sweetpotato Breeding Program selected in 2004. Covington is a low dry matter (ca. 20%), high BC, moderate reducing sugars, and sucrose cultivar with rose skin and orange flesh. The population consisted of 454 full-sibs and has been maintained: 1) through vegetative propagation in the breeding program's virus-free greenhouses at NC State's Method Road greenhouse complex in Raleigh, NC; and 2) in the form of harvested storage roots used as a source of vegetative plants maintained at the North Carolina Department of Agriculture and Consumer Services (NCDA&CS) Horticultural Crops Research Station (HCRS) in Clinton, NC.

Growing seasons, harvest, sample collection, and processing: The DC population was initially planted in 2016 in the field at the HCRS from Method Road greenhouse-derived 25-30 cm long vegetative plant tip cuttings in order to develop storage roots for the subsequent growing seasons. During the summers of 2017 and 2018, the population was planted from vegetative 25-30 cm long tip cuttings obtained from storage roots planted (bedded) in 40.0 cm x 40.0 cm x 12.7 cm (AFLAT5, 0.48cm bottom mesh, Anderson Pots, Portland, OR) plant band trays in a plastic tunnel house. Experimental plots consisted of five plant plots with 28 cm between plants and 1.06 m between ridged rows. A line that produced different colored storage roots was planted between plots to maintain consistent growth and enable easy plot identification.

There were three field trials. The first one was planted at the HCRS in Clinton, NC (35°01'30" N, 78°16'35" W) in 2016, and the other two trials were planted at Caswell Research Farm in Kinston, NC (35°16'28" N, 77°36'56" W) in 2017 and 2018. In all three years, storage roots were harvested and 2-3 roots from each clone were kept for storage root chemistry determination using near-infrared spectroscopy (NIRS) analysis. The storage roots were placed in labeled bags for curing at ~29^{o}C and 85% relative humidity (RH) for one week and then stored at ~13^{o}C and 85% RH in the HCRS sweetpotato storage rooms. After curing, the storage roots were peeled, food processed, and ground into 75 g samples using material from the proximal, distal, and center portions of the separate roots. These samples were placed in labeled bags and stored at -4^{o}C until freeze-drying. During the freeze-drying process, sample moisture was brought to between <3-4% moisture content using a Virtis SP scientific vacuum freeze drier (24DX48 GPFD 35L EL -85, NY, USA) and then milled using a Cyclotec 1093 sample mill (FOSS 123 Hillerød, Denmark) fitted with a 0.1mm screen.

NIRS phenotyping: Quality trait data for BC (mg/100g), glucose (% dry matter), fructose (% dry matter), and sucrose (% dry matter) content were determined in the milled samples via NIRS using a FOSS XDS Rapid Content Analyzer (FOSS NIRSystems, Inc, Laurel, MD) connected to ISIScan software (Infrasoft International LLC, State College, PA). Estimates of chemistry traits were obtained using calibration equations that were established, validated, and improved over multiple years via NIRS scanning and wet chemistry determination in collaboration with the USDA-ARS Food Science Research Unit in the Department of Food, Bioprocessing, and Nutrition Sciences (Yencho, personal communication). All traits were evaluated on a dry weight basis (DWB).

Phenotypic model and broad sense heritability estimation: Restricted maximum likelihoods (REML) variance components analysis of the phenotypic data was performed using GenStat (v. 17.1) and the following model:

$$y_{ij} = \mu + t_j + c_i + \varepsilon_{ij}$$

where y_{ij} is the phenotypic observation of clone *i* in trial *j*, t_j is the fixed effect of trial *j* (*j* = 1, . . . , *J*; *J* = 3), c_i is the fixed effects of clone *i* (*I* = 1, . . . , $I_g + I_r$; $I_g = 447$ full-sib genotypes and $I_r = 4$ checks), and ε_{ij} is the random residual effect, with $\boldsymbol{\varepsilon} \sim N(\mathbf{0}, \mathbf{R})$, where $\boldsymbol{\varepsilon}$ is the vector of residuals and $\boldsymbol{R} = \mathbf{I}_I \otimes \mathbf{R}_J$ is the variance-covariance (VCOV) residual matrix and $\otimes$ is the direct product. The $\boldsymbol{R}_J$ matrix was selected by looking at the Akaike (AIC) and Bayesian Information criteria (BIC) of seven alternative models, accounting for variance and covariance heterogeneity among trials (data not shown).

We fitted the same model above replacing c_i with g_i as the random effect of full-sib genotype *i* (*i* = 1, . . . , I_g), with $g_i \sim N(0, \sigma_g^2)$, and the identity residual matrix, so that $\varepsilon_{ij} \sim N(0, \sigma^2)$. The broad-sense heritability, H^2, was then computed as:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma^2}{J}}$$

Optimized genotyping-by-sequencing for polyploids (GBSpoly): GBSpoly (Wadl et al., 2018), a library preparation and next-generation-sequencing protocol optimized for polyploids and highly heterozygous genomes, was used for genotyping the DC population. By re-engineering barcoded adapters (to ensure accurate demultiplexing and base calling), GBSpoly produces uniform

coverage across samples and loci for the targeted population. Specialized 6-9 base pair barcodes were designed to minimize error, preserve nucleotide diversity, and account for both substitution and indel errors (based on edit/levenshtein distance). Upstream of the barcodes, unique buffer sequences ensured that barcodes lie within high-quality base regions, since the beginning and ends of reads tend to have higher base calling errors. *Tse*I and *Cvi*AII double digests produce fragments for adapters to ligate to which were subsequently chosen according to size in order to minimize PCR bias. Chimeric ligations were eliminated by performing a secondary digest with the same enzymes. Digestion at the adapter-insert junction were avoided because the restriction site was not reconstituted at the adapter-insert junction. Next-generation sequencing was performed on the Illumina HiSeq 2500 platform (Mollinari et al., 2020).

Genotype calling: GBSpoly raw sequence data was analyzed with Tassel4-Poly software (https://github.com/guilherme-pereira/tassel4-poly; Pereira et al. 2018) modified from Tassel-GBS (Glaubitz et al., 2014) to store actual read counts for polyploid individuals. Bowtie2 (Langmead and Salzberg, 2012) was used to align the reads to the two *I. batatas* progenitor species reference genomes (*I. trifida* and *I. triloba*) developed by the Genomic Tools for Sweetpotato Improvement Project (Wu et al., 2018). The software SuperMASSA was utilized to perform the genotype calling of parents and offspring in the DC full-sib population. SNPs with read depth <20 were eliminated for quality control. Additionally, SNPs with more than 25% of missing data and with segregation distortion (Bonferroni $P < 2.9 \times 10^{-6}$) were filtered out. Five individuals with less than 100 reads on average were also removed. There is currently no hexaploid *I. batatas* reference genome, therefore we used the diploid *I. trifida* and *I. triloba* reference genomes developed by Wu et al. (2018). Since there is a high collinearity between *I. trifida* and *I. triloba* genomes (Wu et al.,

2018; Mollinari et al., 2020), we opted to use exclusively the genomic information provided by the *I. trifida* reference genome (Wu et al., 2018).

Linkage map construction: Pairwise recombination fraction and linkage phase estimation were calculated for ~12.5 million combinations. This produced 15 distinct linkage groups corresponding to the 15 *I. trifida* chromosomes available in the reference genome (Wu et al., 2018). We used two procedures to order the markers within each linkage group. First, we used the MDS algorithm (Preedy and Hackett, 2016) with the recombination fractions converted into genetic distances using the Haldane map function and the squared linkage LOD Scores to construct the stress criterion. Second, we used the SNP order proposed in the Beauregard x Tanzania (BT) map (Mollinari et al., 2020) using the *I. trifida* reference genome to connect both maps. The former yielded the highest likelihood and, therefore, was chosen as the final map. In both cases, SNP phasing and multipoint recombination fraction estimation was performed in accordance with Mollinari and Garcia (2019). The final map was re-estimated using the probability distribution of the genotypes through HMM (Mollinari et al, 2020).

QTL mapping: Mapping of QTL was performed using QTLpoly software (https://github.com/guilherme-pereira/QTLpoly) by using the QTL genotype conditional probabilities obtained from the genetic map and the joint adjusted means from the phenotypic analysis as well as the raw phenotypic data from each trial. QTLpoly is based on a random-effect multiple interval mapping model (Pereira et al., 2019) where the effect of g_q of a putative QTL q ($q = 1, \dots, Q$) is treated as random with $g_q \sim N(0, \sigma_q^2)$. The variance components associated with

the QTL (σ_q^2) were tested every 1 cM along the genetic map using score statistics. To begin, QTL detection took place in a stepwise procedure. First, a forward search added one QTL at a time under a less conservative threshold ($p < 0.01$). Second, a backward elimination then tested each QTL against a more conservative threshold ($p < 0.001$) where QTL were dropped if they fell below the threshold or their position was refined if changes occurred. Last, a final multiple QTL model was fitted in order to estimate: (1) the final set of variance components associated with each detected QTL, σ_q^2, (2) the QTL heritability, h_q^2, as the proportion of the total variance explained by QTL q, and (3) the allele and allele-combination effects for each QTL. To complete this, we compared our QTL peaks and support intervals to an anchored reference genome of one of the diploid relatives of sweetpotato, *I. trifida* (Wu et al., 2018), in order to find which genes of interest are in our QTL regions.

RESULTS

Phenotypic analyses: This study measured sucrose, fructose, glucose, and BC in the DC mapping population over three field seasons in three separate environments. Phenotypic correlations of individual traits between each of the three years (2016, 2017, and 2018) were all high, ranging from r = 0.62 to r = 0.98 ($p < 0.001$), with BC exhibiting correlations of r = 0.81 or greater in all three environments (Figure 1). For glucose, the positive linear association between each of the three years was strong (r = 0.78, 084, 0.79, $p < 0.001$, for 2016-2017, 2016-2018, and 2017-2018, respectively). Fructose had a strong positive linear association as well (r = 0.78, 0.84, 0.0.78, $p < 0.001$, for 2016-2017, 2016-2018, and 2017-2018, respectively). Sucrose had a strong positive linear association (r = 0.75, 0.79, 0.86, $p < 0.001$, for 2016-2017, 2016-2018, and 2017-2018, respectively). Lastly, BC had a strong positive linear association, as well (r = 0.80, 0.84, 0.81, $p < 0.001$, for 2016-2017, 2016-2018, and 2017-2018, respectively).

When comparing three-year jointly adjusted means, we found a strong positive association between fructose and glucose (r = 0.98, $p < 0.001$), a weak positive association between fructose and sucrose (r = 0.16, $p < 0.001$), a weak positive association between glucose and sucrose (r = 0.18, $p < 0.001$), and a strong positive association between sucrose and BC (r = 0.83, $p < 0.001$). Correlations between the jointly predicted means for fructose and BC (r = 0.22, $p < 0.001$), as well as glucose and BC (r = 0.23, $p < 0.001$) also showed weak positive associations (Figure 1).

Broad sense heritability (H^2) was also very high for all traits: Fructose = 91.5%, glucose = 91.3%, sucrose = 92.6%, and BC = 93.2% (Table 1). Based on the jointly adjusted means (Figure 2), which took environmental variability into account, 124 progeny had higher BC values than the high parent Covington (0.32 mg/100g), with many examples of transgressive segregation (e.g. NCDC_0086 = 0.91 mg/100g, NCDC_0126 = 0.64 mg/100g, NCDC_0442 = 0.63 mg/100g,

NCDC_0203 = 0.61 mg/100g). All of the sugars also had many examples of progeny with higher and lower values than the respective high and low parent. The majority of the progeny had glucose (429 genotypes) and fructose (437 genotypes) levels lower than Covington (0.68 % and 0.49% dry matter for glucose and fructose, respectively) and comparable to NCDM04-0001 (0.16% and 0.10% dry matter for glucose and fructose, respectively) in every individual year and in the three-year jointly adjusted means, with a distribution skewed to the left (Figure 2). The distribution of sucrose was normally distributed with high parent Covington averaging 2.41 % dry matter versus the population mean of 2.24 % dry matter which left 172 progeny with higher sucrose levels than Covington. The two parents diverged strongly for all trait values as expected, and transgressive segregants were common.

SNP and linkage map development: The GBSpoly protocol, which was developed specifically for highly heterozygous and polyploid genomes (Wadl et al., 2018) such as the DC population, was very effective for SNP development. GBSpoly yielded ca. 13.2 billion barcoded reads, which were collapsed into 31,938,636 sequence tags. In total, 1,331,267 and 1,281,190 polymorphisms (SNPs and indels) from *I. trifida* and *I. triloba* were identified, respectively. Filtering of the markers for an average read depth >20 yielded 467,691markers aligned to the *I. trifida* genome. SNP and dosage calling conducted using SuperMASSA (Serang et al., 2012) resulted in 97,628 (20.8%) monomorphic loci. After filtering for missing data, 42,705 markers were tested for segregation distortion and redundant markers were also removed, resulting in 15,966 high-quality SNPs that were used to build the genetic map. The final map was composed by 14,367 markers distributed among 15 linkage groups (LG), covering 2,727.1 cM. The average length of the linkage groups was 181.8 cM with the longest being linkage group 1 at 243.0 cM and the shortest being

linkage group 8 at 120.5 cM. No visible gaps were observed with an average of 5.2 cM between markers. Table 3 summarizes the distribution of dosage combinations in the parents NCDM04-0001 and Covington. Simplex markers (0-1, 1-0) were the most abundant marker type, accounting for 51.5% of the markers. The ordered markers were all phased to the expected number of homologues based on parental dosages, leading to a fully phased linkage map (Figure 3).

QTL detection: We identified significant QTL for BC, fructose, glucose, and sucrose. When jointly adjusted means were considered, several major co-localized QTL were observed on LG3 and LG12 along with two minor QTL on LG1 for fructose and glucose, and one minor QTL on LG4 for sucrose (Figure 4).

For 2016, we identified two QTL for BC (LG3 and LG12), two QTL for fructose (LG1 and LG3), three QTL for glucose (LG1, LG3, and LG12), and four QTL for sucrose (LG2, LG3 (two QTL), and LG12) (Table 4). These QTL explained 76.9%, 66.4%, 67.7%, and 63.1% of the total phenotypic variation, respectively.

For 2017, we identified two QTL for BC (LG3 and LG12), two QTL for fructose (LG1 and LG3), one QTL for glucose (LG3), and three QTL for sucrose (LG1, LG3, and LG12) (Table 5). These QTL explained 72.4%, 58.2%, 52.4%, and 71.8% of the total phenotypic variation, respectively.

For 2018, we identified four QTL for BC (LG1, LG2, LG3, and LG12), two QTL for fructose (LG3 and LG 12), two QTL for glucose (LG3 and LG12), and three QTL for sucrose (LG3, LG4, and LG12) (Table 6). These QTL explained 82.0%, 59.0%, 58.6%, and 71.2% of the total phenotypic variation, respectively.

Combining all years, co-localized QTL on LG3 with peaks for all traits between 33.02 (marker S3_3261239) and 34.07 cM (marker S3_3318347) explained 49.1%, 6.1%, 7.5%, and 43.8% of the observed variation in BC, fructose, glucose, and sucrose, respectively (Table 7). Additional co-localized QTL on LG3 with a peak at 213.07 cM (marker S3_11338993) explained 57.6% and 53.8% of observed variation in fructose and glucose (Table 7). Similar co-localized QTL on LG12 for BC and sucrose between 168.00 (marker S12_22117539) and 171.13 cM (marker S12_22337107) explained 30.7% and 23.8% of the variation of the traits, respectively. BC and sucrose shared the co-localized QTL on LG12 for all years. A minor QTL on LG12 varying between 184.15 cM (marker S12_23076980) and 188.01 cM (marker S12_23194313) was shared by glucose and fructose during 2018, absent in 2017, present in 2016 for glucose, and then was observed for glucose when all years were combined. (Tables 4-7). It explained 4.8-6.3% and 6.0% of observed variation for glucose and fructose, respectively. The two additional minor QTL for fructose and glucose were observed on LG1, explaining 5.5% and 5.0% of the observed variation, respectively, while a minor QTL on LG4 explained 4.3% of the observed variation for sucrose.

Several of the QTL overlapped across all three years indicating they are stable (Tables 4-7). These include: 1) the BC QTL on LG3 occurring between 30.02 cM (marker S3_2945631) and 33.02 cM (marker S3_3224972); 2) the BC QTL on LG12 occurring between 168.00 cM (marker S12_22117539) and 170.12 cM (marker S12_22305356); 3) the fructose and glucose QTL on LG3 with a peak at 213.07 cM (marker S3_11338993); and 4) the sucrose QTL on LG3 occurring between 33.02 cM (marker S3_3261239) and 34.04 cM (marker S3_33318347) and LG12 occurring between 169.00 cM (marker S12_22117539) and 171.13 cM (marker S12_22337107).

Estimation of allelic effects: The contribution of each parental haplotype was examined for the major QTL on LG3 and LG12 (Figures 5 & 6). We observed that while both parents contributed alleles for increasing the means of all four traits on LG3, allele *d* from Covington at the QTL on LG3 contributed a major positive allelic effect for all traits (approximately +0.124, +0.020, +0.028, and +0.300 for BC, fructose, glucose, and sucrose respectively). In contrast, both parents contributed between two and three negative alleles for all traits on LG3. On LG12, both parents contributed positive alleles (*c* and *f* from Covington and *i* from NCDM04-0001 for BC). NCDM04-0001 contributed one positive allele for BC (~+0.067), two positive alleles for glucose (approximately +0.008 and +0.022), and one positive allele for sucrose (~+0.150). Covington contributed four negative alleles for BC and sucrose alike.

DISCUSSION

The β-carotene, glucose, fructose and sucrose contents of fresh and stored sweetpotato storage roots are some of the most important biochemical traits affecting the processing quality characteristics of sweetpotato and these chemistries directly impact chip and French fry quality. Previous research has suggested that sweetpotato germplasm varies widely in these chemicals, and this research was focused on studying the genetic basis of these traits.

Previous studies in sweetpotato have reported QTL for dry matter content (Cervantes-Flores et al., 2011; Zhao et al., 2013; Amankwaah, 2019; Oloka, 2019; Gemenet et al., 2020), starch (Cervantes-Flores et al., 2011; Amankwaah, 2019; Gemenet et al., 2020), β-carotene content (Cervantes-Flores et al., 2011; Amankwaah, 2019; Oloka, 2019; Gemenet et al., 2019), the sugars glucose, fructose, and sucrose, and α- and β-amylase activity (Amankwaah, 2019).

Amankwaah (2019) and Gemenet et al., (2020) studied the inheritance of the same traits in the Beauregard x Tanzania (BT) and Tanzania x Beauregard (TB) mapping populations. In the current study of the DC mapping population, we observed QTL for β-carotene, dry matter, glucose, fructose, and sucrose that were co-localized on LG3 between 3,261,239 bp and 3,318,347 bp (markers marker S3_3261239 cM and S3_3318347, respectively) responsible for 49.1% (BC), 6.1% (fructose), 7.5% (glucose), and 43.8% (sucrose) of total phenotypic variation explained. These compared favorably to peak locations reported by Amankwaah (2019), though the reported phenotypic variation explained at the LG3 QTL contrasted, which may be due to unique gene interplay in the different genetic background. Glucose and fructose had a co-localized QTL on LG3 with peak position at 11,338,993 bp (marker S3_11338993) accounting for 53.8% and 57.6% of explained variation. For the TB mapping population, Amankwaah (2019) reported co-localized peaks for glucose at 11,196,223 bp (marker S3_11196223) and fructose at 11,050,542 bp (marker

S3_11050542), responsible for 37.7% and 28.3% of explained variation, respectively. In both the TB population and its reciprocal cross BT (Beauregard x Tanzania), Amankwaah (2019) reported co-localized peaks for glucose between 3,520,755 bp (marker S3_3520755) and 3,185,578 bp (marker S3_3185578) and for fructose between 3,377,242 bp (marker S3_3377242) and 3,185,578 bp (marker S3_3185578) responsible for 6.4-10.4% and 6.3-9.3% of explained variation, respectively. These values align similarly with the QTL we observed on LG3 for glucose and fructose at 3,261,239 bp (marker S3_3261239) responsible for 7.5% and 6.11% of explained variation. Finally, both Amankwaah (2019) and Gemenet et al. (2020) reported QTL peaks on LG3 and LG12 similar to our study. Using the same population (BT) their peaks ranged from 2,994,719 bp (marker S3_2994719) to 3,185,578 bp (marker S3_3185578) on LG3 while they shared the same peak at 22,131,994 (marker S12_22131994) on LG12. Our values for total phenotypic value explained more closely matched Gemenet et al. (2020). The relatively close proximity of QTL between the DC, BT, and TB mapping populations observed between this study and that of Amankwaah (2019) and Gemenet et al. (2020) give us increased confidence in the stability of these traits across different genetic backgrounds, and should allow us to target the proper regions for marker-assisted selection.

In the current study, two QTL for BC, one QTL for fructose and glucose, and two QTL for sucrose were consistent across the different environments over the three-year study. This was not surprising as all the traits were stable with strong positive correlations when compared to like-traits across all years. Of the comparisons between traits only the correlation between sucrose and BC had a strong positive correlation across all years. Therefore, it is possible that sucrose and BC exist within a pleotropic relationship and will likely be inherited together while there is no particular relationship (positive or negative) between these two traits and the reducing sugars.

Together, this is consistent with previous studies showing that quality traits are relatively stable with small variations in phenotypic values from one environment to another (Grüneberg et al., 2005; Grüneberg et al., 2009; Cervantes-Flores, et al., 2011; Yada et al., 2017). Cervantes-Flores et al. (2011) reported no significant differences between starch and dry matter content grown in different locations and years. Grüneberg et al. (2005) observed a relatively small amount of genotype x environment (GxE) interactions for dry matter, starch, and BC across multiple sites in Peru. No GxE interaction for BC was also observed by Vimala et al. (2011) as well. Additionally, Grüneberg et al. (2009) reported low GxE interaction for all nutritional quality traits including BC, starch, sucrose, and total sugar content across three environments in Peru. These observations are important since identifying stable and consistent QTL across multiple environments and populations is essential for marker-assisted selection.

Interestingly, NCDM04-0001 (light yellow flesh) carried an allele (*i*) responsible for the biggest positive allelic effect (+0.067) for BC content on LG12 (Figure 6). At the same time, Covington had two distinct alleles (*c* - + 0.049 and *f* - +0.054) contributing positive effects for BC on LG12, as well as the allele (*d*) with a positive effect (+0.124) for BC on LG3 with almost double the effect versus allele *i*. Considering NCDM04-0001 is not orange-fleshed the results suggest some biochemical blockage is preventing the creation of BC since at least some of the requisite machinery is present.

It has recently been suggested that the *IbOr* (*ORANGE*) gene on LG12 and the *PSY* (phytoene synthase) gene on LG3 act pleiotropically (Park et al., 2016; Gemenet et al., 2020), though the connection is only speculative at this point. Much like in the study by Gemenet et al. (2020), our BC QTL 2 (marker S3_3261329) on LG3 is somewhat distant from the *PSY* gene (*itf03g05110*) which is located between 3,117,946 and 3,122,156 bp on LG3 in the *I. trifida*

reference genome. This puts the *PSY* gene location well-within the 95% support interval (0-99.01 cM) (Table 7) for BC QTL2. The BC QTL 3 on LG12 (marker S12_22117539) is within ~4.5 kb of the *IbOr* gene (*itf12g24270*) located between 22,122,009 kb and 22,124,719 kb with the support interval of 125.25-205.67 cM. According to Gemenet et al. (2020), *IbOr* acts as a molecular switch for chromoplast biogenesis (carotenoid accumulation). This works through the modification of starch-storing amyloplasts into BC-storing chromoplasts (Lu et al., 2006). In other words, the idea is that a sweetpotato like NCDM04-0001 can potentially produce BC, but cannot store it since this pathway is not functioning. This helps to explain transgressive segregation in this and other populations. The allelic effects are additive, so an individual will likely have more BC if it inherits alleles *c* and *d* from Covington along with allele *i* from NCDM04-0001 for QTL on LG12, and allele *d* from Covington on LG3. Considering the major contributions of BC QTL 2 and 3 on LGs 3 and 12 to the explained phenotypic variation (79.8%) this may be the case, but it is currently only speculation until additional studies are done.

Additionally, Gemenet et al. (2020) suggested that starch content is likely negatively impacted by a direct linkage between *PSY* and *SuSy* (located within 12.2 kb of *PSY* on LG3), as the aforementioned amyloplast modification interaction of *PSY* and *IbOr* likely inhibits the normal production of ADP-glucose, thus reducing starch synthesis in the amyloplasts. This may also explain part of the negative correlation between BC and starch previously noted in sweetpotato research (Cervantes-Flores et al., 2011; Gemenet et al., 2020).

The results of our study indicate beneficial sugar and BC profiles for chip and fry processing. By establishing that glucose and sucrose are weakly correlated with sucrose, we believe that progeny from this material can and will have high amounts of sucrose while keeping reducing sugars down. This research will help processors move closer to the production of highly

sweet sweetpotato French fries and chips with lower browning. Additionally, the strong positive relationship between BC and sucrose indicates that they do not have to lose the health benefits of BC during this process. However, further work is necessary to gain better insight into the controlling factors of other traits. For example, the previously noted strong negative correlation between BC and starch/dry matter (Grünenberg, et al., 2005; Cervantes-Flores et al., 2011; Yada et al., 2017; Gemenet et al., 2020) suggests that starch and sucrose may also be negatively correlated.

In conclusion, we have shown the potential to develop sweetpotato genotypes with superior processing characteristics for chip and fry processing. Using the DC mapping population, we were able to analyze the genetic architecture surrounding several important processing traits in sweetpotato. The development of the sweetpotato reference genomes of *I. trifida* and *I. triloba* (Wu et al., 2018) has been a crucial resource for these recent maps and will continue to be for mapping projects going forward. While the lack of a whole genome sequence for sweetpotato has hindered the validation of mapped QTL regions, this recent development will serve as a temporary means for the identification of candidate genes. Results from other mapping populations due to these reference genomes on traits like starch, dry matter, and more is helping researchers learn the genetic architecture of these traits and to design better methods for genomic-assisted breeding in sweetpotato on a global scale.

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Tables

Table 1. Phenotypic analysis summary of storage root β-carotene content (BC), fructose, glucose, and sucrose. Parental (Covington and NCDM04-0001) and progeny ($\bar{F}_1$) means (minimum, and maximum) F_1 adjusted means for 2016, 2017, and 2018, variance components and broad sense heritability shown for all four traits.

	BC (mg/100g)				Fructose (% dry matter)				Glucose (% dry matter)				Sucrose (% dry matter)			
	2016	2017	2018	Joint	2016	2017	2018	Joint	2016	2017	2018	Joint	2016	2017	2018	Joint
Covington	0.44	0.20	0.36	0.32	0.55	0.46	0.54	0.49	0.78	0.62	0.76	0.68	2.93	2.49	2.19	2.41
NCDM04-0001	-0.02	0.06	-0.04	0.00	0.07	0.11	0.11	0.10	0.15	0.18	0.17	0.16	1.91	1.25	1.29	1.39
Min ($\bar{F}_1$)	-0.30	-0.44	-0.33	-0.31	-0.05	-0.02	0.02	0.02	-0.07	0.03	0.05	0.02	0.79	0.32	0.80	0.82
Max ($\bar{F}_1$)	0.93	0.72	0.62	0.91	1.03	0.85	1.18	1.00	1.53	1.31	1.62	1.45	4.01	4.39	4.25	4.22
$\delta^2 g$	-	-	-	0.04	-	-	-	0.02	-	-	-	0.03	-	-	-	0.34
δ^2	-	-	-	0.01	-	-	-	0.00	-	-	-	0.01	-	-	-	0.08
H^2(%)	-	-	-	**93.20**	-	-	-	**91.48**	-	-	-	**91.33**	-	-	-	**92.60**

Table 2. Summary of the NCDM04-0001 x Covington genetic mapping population integrated linkage map created using MAPpoly.

Linkage Group (LG)	Length (cM)	No. of SNPs	SNPs/cM
LG_1	243	1357	5.6
LG_2	200.6	1052	5.2
LG_3	216.1	1230	5.7
LG_4	220.5	1311	5.9
LG_5	182	1048	5.8
LG_6	148.5	825	5.6
LG_7	159.2	838	5.3
LG_8	120.5	468	3.9
LG_9	207.6	1183	5.7
LG_10	178.5	965	5.4
LG_11	169.6	546	3.2
LG_12	205.7	1137	5.5
LG_13	160.1	777	4.9
LG_14	145.9	709	4.9
LG_15	169.3	921	5.4
Total	**2727.1**	**14367**	**5.2**

Table 3. Allelic marker dosage in the NCDM04-0001 and Covington mapping population parents. The dosage groups 0, 1, 2, 3, 4, and 5 correspond to nulliplex, simplex, duplex, triplex, quadruplex, and quintuplex marker types, respectively.

Dosage		No. SNP	% of Total
NCDM04-0001	Covington		
0	1	3778	23.66
0	2	810	5.07
0	3	139	0.87
0	4	12	0.08
0	5	1	0.01
1	0	4449	27.87
1	1	2113	13.23
1	2	878	5.50
1	3	220	1.38
1	4	79	0.49
1	5	10	0.06
2	0	931	5.83
2	1	845	5.29
2	2	476	2.98
2	3	305	1.91
2	4	64	0.40
3	0	131	0.82
3	1	201	1.26
3	2	262	1.64
3	3	83	0.52
4	0	17	0.11
4	1	70	0.44
4	2	84	0.53
5	0	2	0.01
5	1	6	0.04
Total		15966	100

Table 4. Summary statistics of quantitative trait loci (QTL) for β-carotene, fructose, glucose, and sucrose for 2016. h^2 is the heritability of the QTL and σ^2_{QTL} is the variance, LG = linkage group, cM = centimorgans.

Trait	QTL	LG.	Position (cM)	Support Interval (cM)	Score	Marker	*p*-value	σ^2_{QTL}	h^2_{QTL}
β-carotene	1	3	32.26	0-75.14	1988.13	S3_3224972	<2.22e-16	0.0411	47.4
	2	12	168.00	127.02-205.67	1749.89	S12_22117539	<2.22e-16	0.0256	29.5
								Total	**76.9**
Fructose	1	1	82.00	68.38-229.03	332.30	S1_13417677	1.03e-05	0.0024	7.0
	2	3	213.07	156.14-216.07	2348.55	S3_11338993	<2.22e-16	0.0204	59.4
								Total	**66.4**
Glucose	1	1	75.15	68.38-229.03	299.43	S1_14642435	3.33e-05	0.0052	6.9
	2	3	213.07	160.44-216.07	2284.36	S3_11338993	<2.22e-16	0.0425	56.0
	3	12	186.21	176.82-205.67	380.25	S12_23148629	1.10e-06	0.0036	4.8
								Total	**67.7**
Sucrose	1	2	25.52	22.61-43.12	361.17	S2_7035420	1.85e-06	0.0381	6.8
	2	3	33.02	10.02-58.08	1045.86	S3_3261239	<2.22e-16	0.1644	29.3
	3	3	213.07	209.02-216.07	450.19	S3_11338993	4.07e-08	0.046	8.2
	4	12	168.00	156.03-188.01	829.68	S12_22117539	<2.22e-16	0.1058	18.8
								Total	**63.1**

Table 5. Summary statistics of quantitative trait loci (QTL) for β-carotene, fructose, glucose, and sucrose for 2017. h^2 is the heritability of the QTL and σ^2_{QTL} is the variance, LG = linkage group, cM = centimorgans.

Trait	QTL	LG.	Position (cM)	Support Interval (cM)	Score	Marker	*p*-value	σ^2_{QTL}	h^2_{QTL}
β-carotene	1	3	30.02	0-66.02	1288.27	S3_2945631	<2.22e-16	0.0317	45.3
	2	12	170.12	134-205.67	1102.61	S12_22305356	<2.22e-16	0.019	27.1
								Total	**72.4**
Fructose	1	1	133.20	71.01-220.24	259.64	S1_23368698	5.80e-05	0.0015	6.6
	2	3	213.07	160.44-216.07	1449.35	S3_11338993	<2.22e-16	0.0116	51.5
								Total	**58.2**
Glucose	1	3	213.07	166.26-216.07	1466.78	S3_11338993	<2.22e-16	0.0209	52.4
								Total	**52.4**
Sucrose	1	1	44.07	25.17-75.15	253.90	S1_3445136	6.71e-05	0.0346	4.2
	2	3	34.07	0-69.09	1308.14	S3_3318347	<2.22e-16	0.372	44.6
	3	12	170.12	154.28-205.67	1001.14	S12_22305356	<2.22e-16	0.1921	23.0
								Total	**71.8**

Table 6. Summary statistics of of quantitative trait loci (QTL) for β-carotene, fructose, glucose, and sucrose for 2018. h^2 is the heritability of the QTL and σ^2_{QTL} is the variance, LG = linkage group, cM = centimorgans.

Trait	QTL	LG.	Position (cM)	Support Interval (cM)	Score	Marker	*p*-value	σ^2_{QTL}	h^2_{QTL}
β-carotene	1	1	84.52	30.37-90.01	271.08	S1_16349287	1.68e-05	0.0031	3.8
	2	2	84.03	70.21-162.63	274.74	S2_11117424	1.91e-05	0.0029	3.6
	3	3	33.02	0-99.01	1578.61	S3_3261239	<2.22e-16	0.0376	46.4
	4	12	168.00	135.13-205.67	1374.28	S12_22117539	<2.22e-16	0.0229	28.2
								Total	**82.0**
Fructose	1	3	213.07	168.28-216.07	1572.11	S3_11338993	<2.22e-16	0.0157	53.0
	2	12	184.15	97.1-205.67	286.96	S12_23076980	1.54e-05	0.0018	6.0
								Total	**59.0**
Glucose	1	3	213.07	165.64-216.07	1564.48	S3_11338993	<2.22e-16	0.0325	52.3
	2	12	188.01	100.25-205.67	288.10	S12_23194313	1.38e-05	0.0039	6.3
								Total	**58.6**
Sucrose	1	3	34.07	0-66.02	1219.85	S3_3318347	<2.22e-16	0.3033	43.4
	2	4	138.04	126.54-159.48	249.42	S4_26150349	7.05e-05	0.0334	4.8
	3	12	170.12	150.23-205.67	930.28	S12_22305356	<2.22e-16	0.1604	23.0
								Total	**71.2**

Table 7. Summary statistics of quantitative trait loci (QTL) for β-carotene, fructose, glucose, and sucrose for adjusted means for all three years combined (2016-2018). h^2 is the heritability of the QTL and σ^2_{QTL} is the variance, LG = linkage group, cM = centimorgans.

Trait	QTL	LG.	Position (cM)	Support Interval (cM)	Score	Marker	*p*-value	σ^2_{QTL}	h^2_{QTL}
β-carotene	1	2	84.03	14.24-105.64	276.70	S2_11117424	8.28e-05	0.0017	2.3
	2	3	33.02	0-99.01	2262.90	S3_3261239	<2.22e-16	0.0377	49.1
	3	12	168.00	125.25-205.67	2044.86	S12_22117539	<2.22e-16	0.0236	30.7
								Total	**82.1**
Fructose	1	1	128.05	74.09-239.05	368.21	S1_22882891	2.11e-06	0.0015	5.5
	2	3	33.02	12.11-43.08	285.17	S3_3261239	5.12e-05	0.0017	6.1
	3	3	213.07	157.16-216.07	2340.93	S3_11338993	<2.22e-16	0.0156	57.6
								Total	**69.2**
Glucose	1	1	229.03	128.05-239.05	390.57	S1_31000013	6.32e-07	0.0029	5.0
	2	3	33.02	14-41.03	330.58	S3_3261239	7.74e-06	0.0043	7.5
	3	3	213.07	160.44-216.07	2351.22	S3_11338993	<2.22e-16	0.0312	53.8
	4	12	184.15	155.48-205.67	366.45	S12_23076980	1.78e-06	0.0028	4.8
								Total	**71.0**
Sucrose	1	3	34.07	0-75.14	1735.39	S3_3318347	<2.22e-16	0.2793	43.8
	2	4	155.20	83.01-173.95	288.29	S4_28048087	4.11e-05	0.0271	4.3
	3	12	171.13	141.18-205.67	1273.67	S12_22337107	<2.22e-16	0.1518	23.8
								Total	**71.8**

Figures

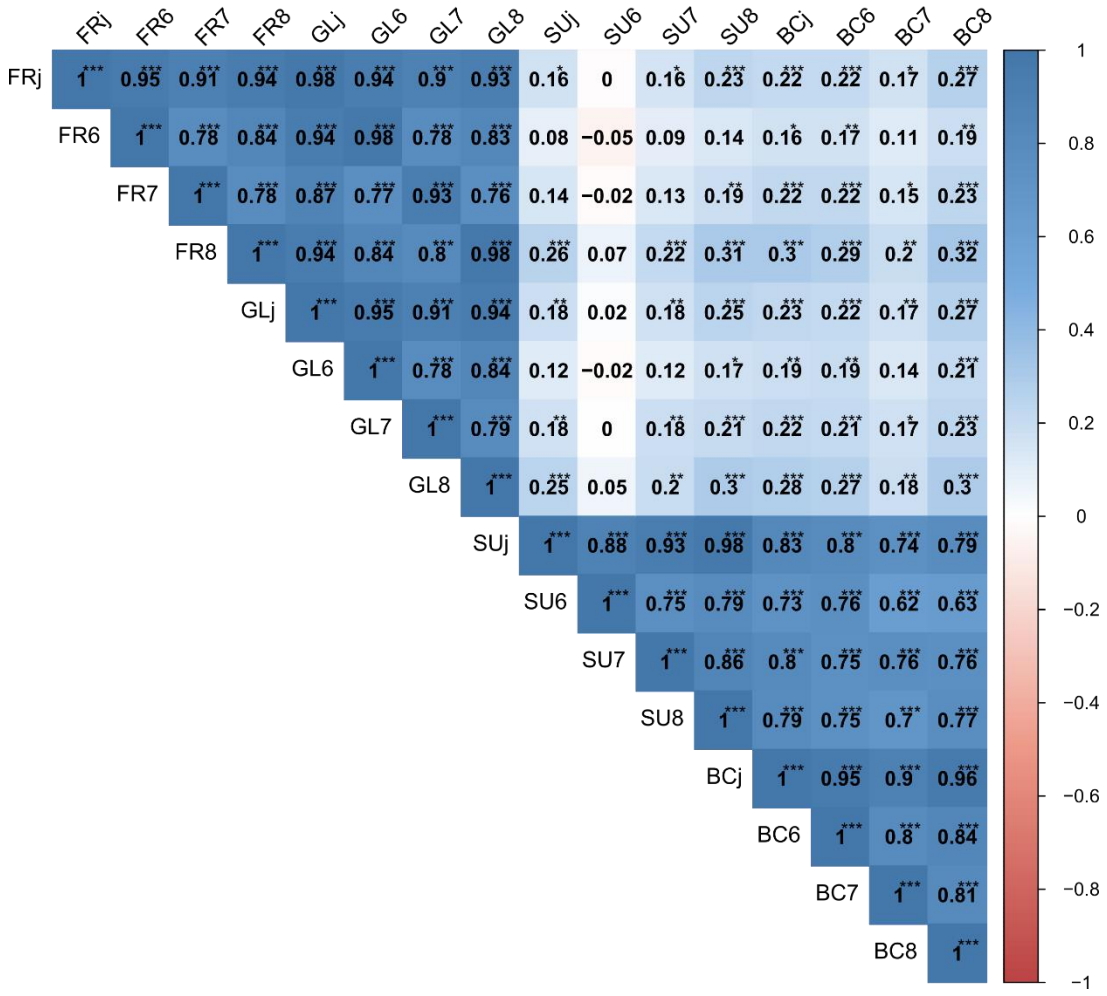

Figure 1. Correlation coefficients (*** p-value ≤ 0.001, ** p-value ≤ 0.01, * p-value ≤ 0.05) for β-carotene, fructose, glucose, and sucrose across all years (2016-2018). Heat map indice indicates degree of positive (blue) or negative correlation (red) between years. FRj = fructose joint means, FR6 = fructose 2016 means, FR7 = fructose 2017 means, FR8 = fructose 2018 means, GLj = glucose joint means, GL6 = glucose 2016 means, GL7 = glucose 2017 means, GL8 = glucose 2018 means, SUj = sucrose joint means, SU6 = sucrose 2016 means, SU7 = sucrose 2017 means, SU8 = sucrose 2018 means, BCj = β-carotene joint means, BC6 = β-carotene 2016 means, BC7 = β-carotene 2017 means, BC8 = β-carotene 2018 means.

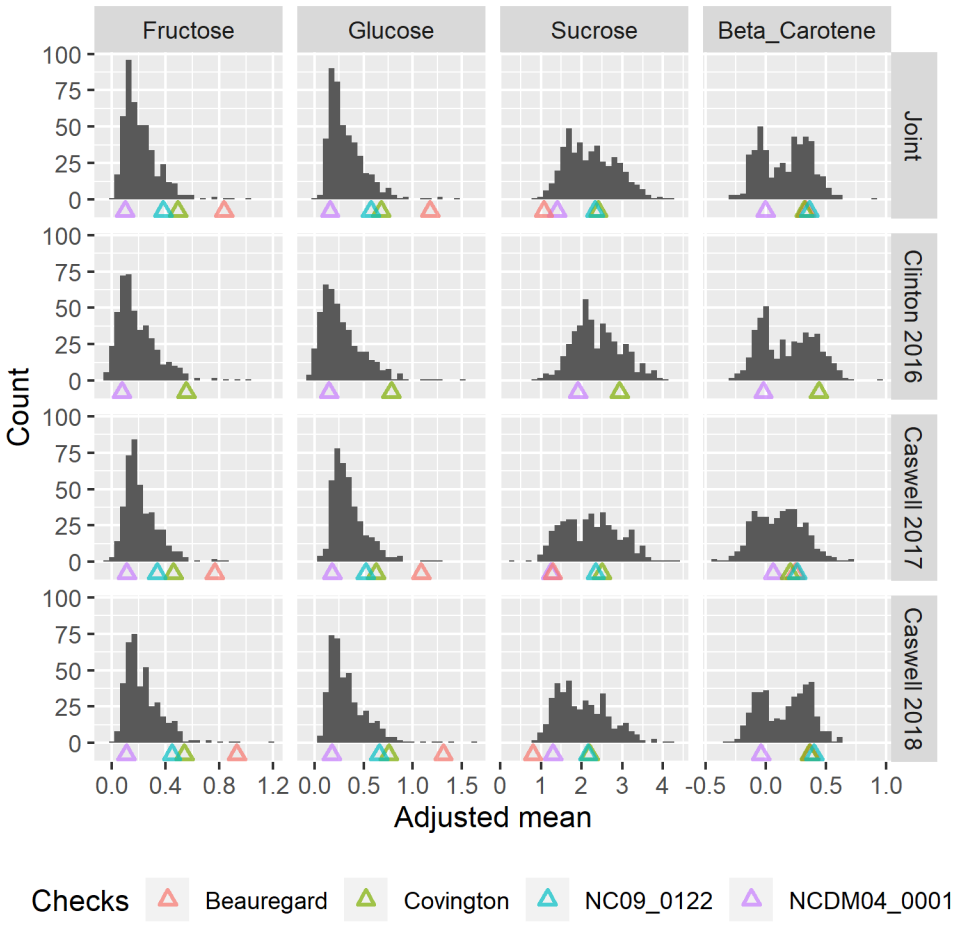

Figure 2. Histograms of adjusted means distribution for fructose, glucose, sucrose, and β-carotene in the NCDM04-0001 (D) x Covington (C) mapping population for all years combined (joint), 2016 in Clinton, NC, 2017 in Caswell, NC, and 2018 in Caswell, NC. Colored triangles represent adjusted means for check lines Beauregard, Covington, NC09_0122, and NCDM04-0001.

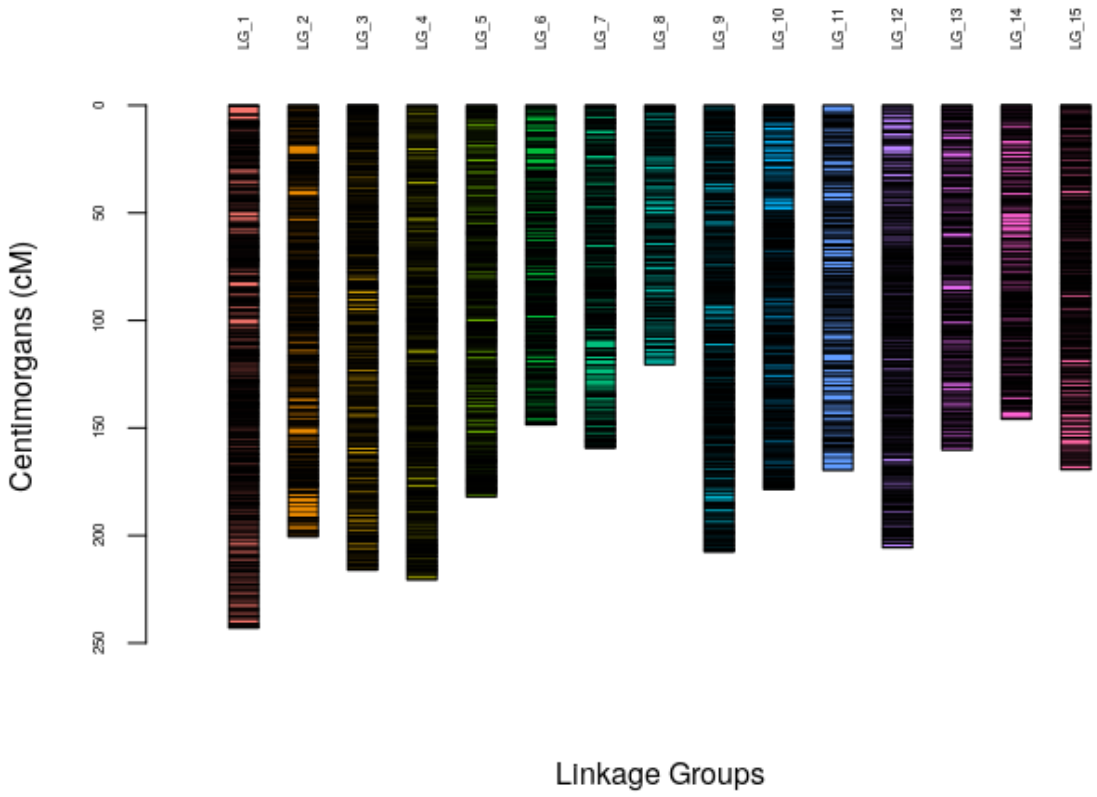

Figure 3. Phased linkage map of the DC mapping population.

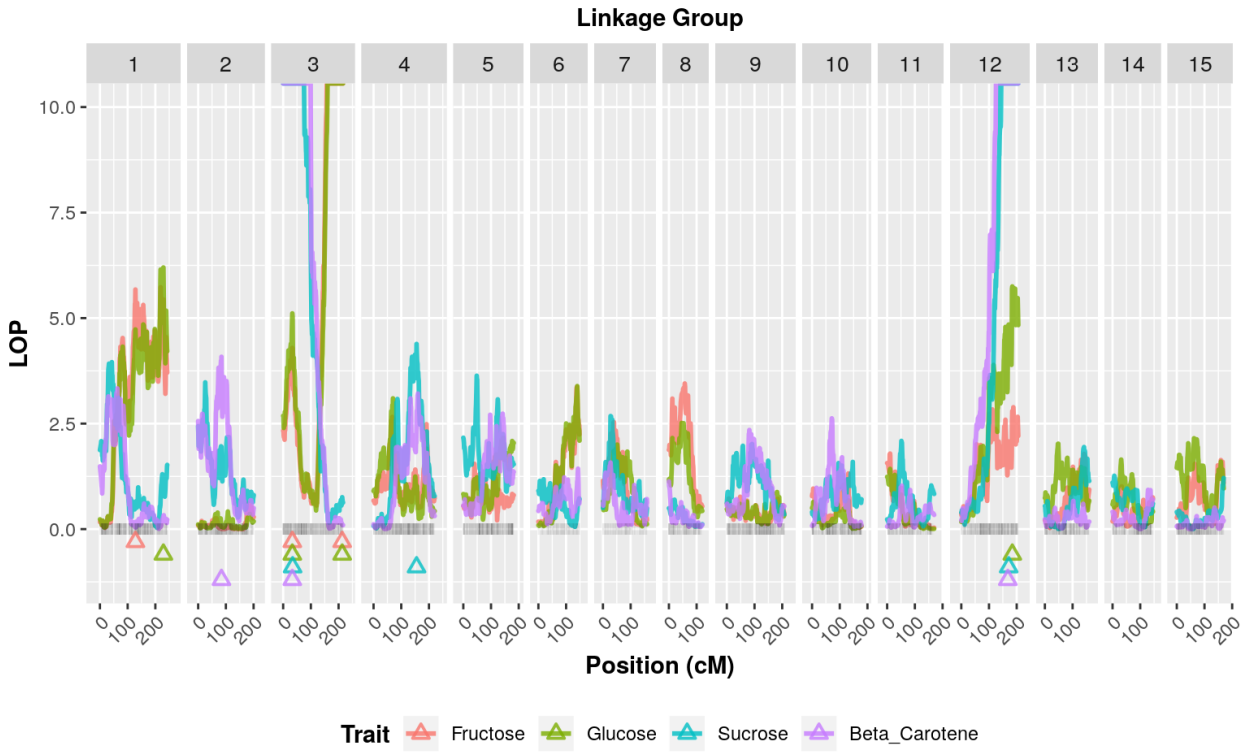

Figure 4. LOP (-$\log_{10}$ of the p-value) profiles from random-effect multiple interval mapping (REMIM) of β-carotene, fructose, glucose, and sucrose for joint adjusted means across all years (2016-2018) in the DC mapping population. Triangles show the QTL peak locations.

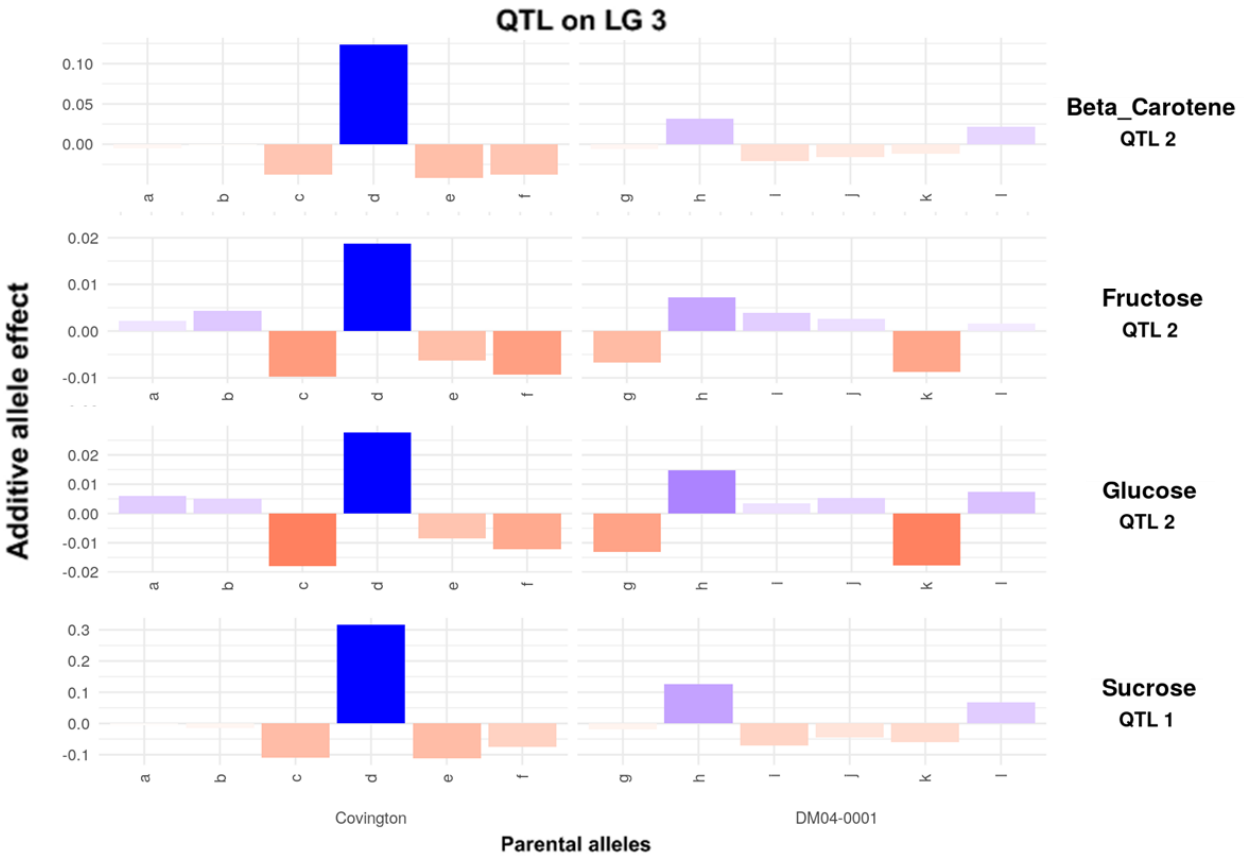

Figure 5. Additive allele effect estimates for co-localized QTL on LG3 (33.02-24.07 cM) for β-carotene, fructose, glucose, and sucrose. Letters a through f and g through l represent the six haplotypes for the specific QTL for Covington and NCDM04-0001, respectively.

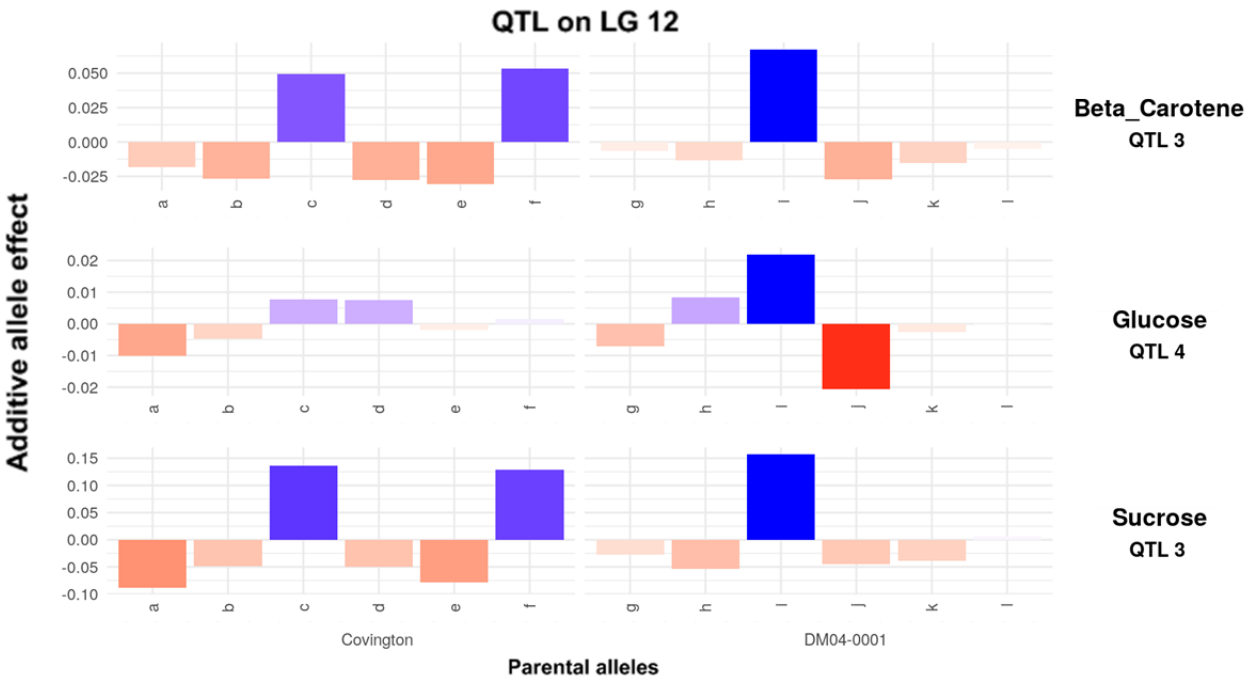

Figure 6. Additive allele effect estimates for co-localized QTL on LG12 (168.00-184.15 cM) for β-carotene, glucose, and sucrose. Letters a through f and g through l represent the six haplotypes for the specific QTL for Covington and NCDM04-0001, respectively.