GENETIC MAPPING IN CITRULLUS LANATUS

by

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(Under the Direction of Cecilia McGregor)

ABSTRACT

The development of a genetic linkage map for watermelon (*Citrullus lanatus* var. *lanatus* (Thunb.) Matsum. & Nakai) has been restricted by the low levels of genotypic diversity previously measurable. Earlier mapping studies have overcome this limitation by using intersubspecific and multispecies crosses to produce genetic maps, but through the development of SNP markers for watermelon the level of measurable genotypic diversity has been increased and allowed the production of the first known intrasubspecific and intervarietal genetic maps. The three maps produced for this study used an elite F_7 recombinant inbred line (RIL), an elite by egusi F_2, and an elite by citron F_2 population. The first objective of this study was to analyze the three maps to form a consensus order and develop a universal nomenclature for watermelon. The second objective of this study was to analyze the elite by elite population for horticulturally important quantitative trait loci (QTL).

INDEX WORDS: *Citrullus lanatus*, linkage map, consensus map, QTL, SNP, elite, egusi, citron, RIL, weight, length, diameter, shape, rind thickness, furrowing, hollow heart, first female flower, Brix

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DEDICATION

I would like to dedicate this thesis to my family and friends for all of the help, patience and support they have given me in the process of completing my Masters degree.

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

REVIEW OF CITRULLUS LANATUS

The genus *Citrullus* is a member of the *Cucurbitaceae* family, and consists of four diploid (2n=22) species classified as *C. lanatus* (Thunb.) Matsum. & Nakai), *C. colocynthis* (L.) Schrad., *C. ecirrhosus* (Cogn.) Chakrav., and *C. rehmii* De Winter. All members of the *Citrullus* genus are native to desert areas in either Northern (*C. colocynthis*) or Southern Africa (*C. lanatus*, *C. ecirrhosus*, and *C. rehmii*). *C. lanatus* and *C. rehmii* are both annual species while *C. colocynthis* and *C. ecirrhosus* are perennials (Jarret and Newman, 2000; Jeffrey, 1975; Robinson and Decker-Walters, 1997). Of the four species within the *Citrullus* genus, *C. lanatus* exhibits the greatest level of phenotypic diversity (Robinson and Decker-Walters, 1997). This species consists of two subspecies, *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* (Jarret and Newman, 2000; Levi et al., 2001a).

The watermelon grown for commercial fruit production, also referred to as elite cultivars, belongs to the variety *C. lanatus* var. *lanatus*. Watermelons have been cultivated for at least 5000 years (Wasylikowa and van der Veen, 2004) and since the 16th century, when C. *lanatus* var. *lanatus* was introduced to North America (Sauer, 1993), over 500 cultivars have been developed in the United States alone. Little information is known concerning the ancestries of many of these cultivars and cultivar identification and evaluation of line purity are reliant on fruit characteristics (Levi et al., 2001b). Global production of watermelon has risen by approximately 31.5% in just the last ten years, to almost 100.7 million metric tons produced on 3.8 million hectares in 2009 (FAOSTAT, 2009). In 2009, China was the number one producer of watermelon, at 68.2 million metric tons (FAOSTAT, 2009). Nationally, watermelon was grown on 126,300 acres with a production value of 460 million U.S. dollars, or 4.4% of the value of vegetables and melons produced in 2009 (USDA, 2010). On the local level, Georgia was the third largest producer of watermelon in the United States, behind Florida and California (USDA, 2010).

Watermelon is the highest ranked vegetable in both area of production and crop value in the state of Georgia with a production area of 24,238 acres and a value of 139 million U.S. dollars or 15% of both the production area and value of the all vegetables produced in Georgia (Boatright and McKissick, 2010).

Fruits with a special egusi seed trait are also classified as *C. lanatus* var. *lanatus* (Gusmini et al., 2004). This fruit cultivated solely for their seed as the flesh of these fruits is hard, bitter, and inedible while the seeds of these fruits have fleshy pericarp that is rich in proteins and carbohydrates (Gusmini et al., 2004). Production and consumption of this fruit is largely limited to West Africa, where it is an important source of food security for subsistence farmers (Achigan-Dako et al., 2008).

The other subspecies of *C. lanatus*, *C. lanatus* var. *citroides*, are commonly labeled as citron types. The variety consists of a group of cultigens found in Southern Africa. This fruit is also commonly called a preserving melon as the rind of this hard and sometimes bitter fruit can be used for making a variety of preserves. It is also a source of water and animal fodder. It can be found growing in the wild in Africa and in other watermelon production areas in the world as an escape (Robinson and Decker-Walters, 1997).

Although the global cultivation of watermelons has increased by 31.5% (FAOSTAT, 2009) in the last ten years, the development of molecular tools for watermelon has lagged behind those of other commercially important Cucurbits (Levi et al., 2006). Current genetic linkage maps are not based on populations derived from hybrids of the elite, fresh market species of watermelon (*Citrullus lanatus* var. *lanatus)*. This is due in part to the low level of genetic diversity found within the cultivated *C. lanatus* var. *lanatus* subspecies (Dane and Lang, 2004; Levi et al., 2001b; Navot and Zamir, 1987). The narrow genetic base may be due to a breeding bottleneck, which might have been created during the domestication and dispersal from the fruit's center of origin (Dane and Lang, 2004; Levi et al., 2001b). The Navot & Zamir (1987) screened several samples of the two subspecies of *C. lanatus* as well as other members of the *Citrullus* species for genetic diversity. The low level of genetic variation with in *C. lanatus* var. *lanatus* was clearly illustrated by the very limited amount of polymorphic isozymes produced (Navot and Zamir, 1987; Zamir et al., 1984). Subsequent mapping studies have circumvented this lack of

genetic variability between cultivars by using intervarital hybrids for their mapping populations, and in some cases interspecific testcrosses (Hashizume et al., 2003; Hawkins et al., 2001; Levi et al., 2001c; Levi et al., 2006). Although this increased the amount of genetic polymorphisms available for mapping, several studies experienced significant levels of marker segregation distortion, from (11-48%) (Hashizume et al., 2003; Hawkins et al., 2001; Levi et al., 2006; Zhang et al., 2004). Segregation distortion occurs when one of a pair of heterozygous alleles or heteromorphic chromosomes is exhibited by the progeny at a statistically higher rate (Lyttle, 1991). This skewed representation is caused genetic elements that exhibit genic or chromosomal meiotic drive (Lyttle, 1991). In these cases, marker segregation distortion is hypothesized to be due to a difference between the subspecies in the genes controlling their reproductive processes (Zamir and Tadmor, 1986).. As these distorted markers were thrown out for some of these linkage maps, areas of the genome were not mapped.

These unmapped regions in the linkage maps for *Citrullus* hinder the development of molecular breeding tools and the ability for breeders to use forward genetics for crop improvement. The citron variety (C. *lanatus* var. *citroides*) and wild watermelon species (*C. colocynthis*, *C. rehmii*, and *C. ecirrhosus*) contain valuable pest and disease resistance qualities which could be introgressed into elite cultivars (Hashizume et al., 2003; Hawkins et al., 2001; Lin et al., 2009; Robinson and Decker-Walters, 1997). These other species also contain several highly undesirable qualities, including hard and bitter flesh. A saturated genetic linkage map for *C. lanatus* would be a valuable tool for understanding the nature of the gene flow between the subspecies, and may help explain the high levels of segregation distortion occurring in the intervarietal or interspecific hybrid crosses. Such a map would also be the basis for developing tools for improving traits already valued in elite cultivars and for introducing new traits from egusi and citron types while excluding the extremely undesirable ones.

Review of Mapping Studies of *Citrullus lanatus*

Several genetic maps have been created for *Citrullus lanatus*, but progress towards the development of a saturated linkage map has been slow in comparison to other members of the *Cucurbitaceae* family. Not only have high density maps been created for *Cucumis melo* L. (melon)

(Deleu et al., 2009; Perin et al., 2002; Silberstein et al., 2003), *Cucumis sativus* L. (cucumber) (Fazio et al., 2003; Weng et al., 2010), and *Cucurbita pepo* L. (gourd, squash and pumpkin), but consensus maps have been created for *Cucurbita pepo* (Zraidi et al., 2007)and *Cucumis melo* (Gonzalo et al., 2005) and the cucumber genome was sequenced and is publically available (Huang et al., 2009). Watermelon is considered a distant relative to these three crops, as it belongs to separate genera then melon (2n=24) and cucumber (2n=14) or pumpkin (2n=40) (Huang et al., 2009; Sauer, 1993).

The first comprehensive *Citrullus* linkage map was published by Haschizume et al. (2003). This study described two maps, one of a F_2 population derived from an intersubspecific cross between a cultivated inbred *C. lanatus* var. *lanatus* and a *Citrullus lanatus* var. *citroides*, and a BC_1 using the elite variety as the recurrent parent. The F_2 population produced a map with 11 linkage groups (corresponding to the watermelon haploid chromosome number), with a length of 2,384 cM, and an average interval length of 4.3 cM, although the distance between markers in some areas were greater than 30 cM. The map produced for the BC_1 population was constructed using markers previously shown to segregate in the F_2 population. It had a length of 1,729 cM with an average marker distance of 7.2 cM. Hawkins et al. (2001) mapped an F_2 and F_3 population derived from a wide cross between a wild *C. lanatus* var. *citroides* species and a cultivated inbred *C. lanatus* var. *lanatus*. The maps consisted of two and five linkage groups consisting of 26 and 13 RAPD markers respectively. Zhang et al (2004) also used a cross between *C. lanatus* var. *citroides* and a *C. lanatus* var. *lanatus*. This study used the wide cross to develop a recombinant inbred line (RIL) population and their map was 1,027 cM in length with an average distance of 11.7cM between markers on 15 linkage groups. Levi et al (2002) developed a population from a test cross of (*C. lanatus* var. *citroides* x *C. lanatus* var. *lanatus*) x *C. colocynthis* which they used in an attempt to control some of the segregation distortion encountered by other studies when mapping wide interspecific crosses. The use of a testcross did produce a lower rate of segregation distortion then what was seen some previous studies (Hawkins et al., 2001), but not as low as the level present in the Haschizume et al. (2003) study (Levi et al., 2002). The testcross population map was extended in 2006 by adding AFLP, SRAP and SSR markers to the RAPD and ISSR markers originally used to construct this

linkage map (Levi et al., 2006). The additional markers increased the map length to 1,976 cM with a distance of 5.8 cM between markers from the previous length of 1,166.2 cM with an average genetic distance of 8.1 cM between markers. The number of linkage groups produced was also reduced from 25 to 19.

Traits of Interest

What is considered a desirable trait for commercial production of watermelon is heavily affected by public opinion. A minimum level of expression for several traits must be maintained to be commercially acceptable (Wehner, 2008a). Several genes have been described and studied for watermelon (Wehner, 2008b). Traits constantly desired by producers and consumers include a high concentration of sugars and disease resistance. Many others like flesh color, shape, and weight are important but the desirable level of expression of each trait may vary depending on consumer taste and the fruits end use (Wehner, 2008a). Several different flesh colors exist for watermelon beyond red, including orange, yellow, and white (Wehner, 2008b) which may be considered novel to consumers. Typically, consumers prefer large (8-11 kg) seeded fruit to be blocky in shape, medium (5-8 kg) seedless types to be oval, and small icebox (4-5.5 kg) or mini (<4.0 kg) types to be round (Wehner, 2008a). Rind thickness is also an important trait, cosmetically as well as practically. A thick rind is thought to be aesthetically pleasing for the large fruit types as they are typically served as slices. The thick rinds of these fruits also play a more practical role in protecting the fruit from damage while shipping (Wehner, 2008a). Other traits that are important to watermelon producers include fruit yield, lack of the flesh deformation called hollow heart, early fruit production, and a higher ratio of female to male flowers (Maynard and Hopkins, 1999; Wehner, 2008a). Hollow heart is an internal crack or separation of the flesh and its presence is grounds for rejection by distributors (Maynard and Hopkins, 1999). As watermelon is monecious, with most cultivars having a ratio of 1 female flower for every 7 male flowers, a higher female to male ratio may translate into a higher amount of fruit produced per plant (Wehner, 2008a).

While the fruit of the wild and citron *Citrullus* species exhibit several undesirable horticultural traits, these species do contain other advantageous characteristics. These traits include disease resistance,

rind strength, and a higher percent of female flower production (Hashizume et al., 2003; Hawkins et al., 2001; Lin et al., 2009; Robinson and Decker-Walters, 1997). Navot and Zamir (1987) were the first to analyze a quantitative trait locus (QTL) for watermelon. They used isozymes to analyze a QTL for flesh color. Haschizume et al. (2003) also analyzed QTL for flesh color as well as for rind color, rind hardness, and soluble solids (in degrees Brix). These have been the only two studies so far to map QTL in watermelon, and neither was able to do so using an elite population. Current use of *C. lanatus* var. *citroides* germplasm would be a slow process, as extensive backcrossing is to remove unwanted citron traits. The creation of molecular markers for desirable traits would allow for molecular screening methods, such as marker assisted selection. Screening and selection using markers can be done in young plants, significantly reducing the time and resources needed for crop improvement. A better understanding regarding the segregation distortion which occurs when crossing *C. lanatus* subspecies would also help make the desirable traits in the citron varieties more accessible or useable to breeders.

Although genus screening efforts using isozymes alone were able to discern the loci for flesh color (Navot and Zamir, 1987) and The Haschizume et al. 2003 study was able to analyze quantitative trait loci (QTL) for both flesh and rind color loci through with their linkage map, none of the other mapping studies analyzed for anything more than disease segregation (Hawkins et al., 2001).

SNP markers

One of the most common and one of the most basic sources of variation found within a species are single nucleotide polymorphisms (SNPs) (Henry, 2008; Kole and Abbott, 2008). SNP markers do not need to be separated by size, so are not limited by some of the issues other types of DNA based markers have concerning size variance and homoplasy. They are also easier to locate, especially in single copy regions, and the assay process is typically automated (Rafalski, 2002). This automated process has many advantages over the use of PCR-based high-throughput markers, as gel profiles have to be compared to identify co-migrating bands for PCR based markers. SNP markers have routinely been used in agricultural breeding programs; in plant and animal variation studies, genome mapping, and association mapping (Deleu et al., 2009; Kole and Abbott, 2008). SNP markers have already proven useful in

increasing marker resolution in melon (Deleu et al., 2009; Gonzalo et al., 2005) cucumber (Fazio et al., 2003), and *Cucurbita pepo* (Zraidi et al, 2007).

Consensus Mapping

Consensus or integrated linkage maps are created by combining linkage maps of multiple populations representative of a species. Typically, linkage maps are limited in their usefulness to the genetic background which they represent, but this can be overcome by combining maps with varying genetic backgrounds. This also allows for the comparison of QTL positions and genes that may exist between the different subspecific populations. As previously mentioned, consensus mapping has already been performed in *Cucumis melo* (Perin et al., 2002) and *Cucurbita pepo* (Zraidi et al., 2007). Perin et al. (2002) produced a composite map with over 668 loci covering the majority of the melon genome. Zraidi et al. (2007) combined two maps developed from phenotypically diverse *Cucurbita pepo* mapping populations, an Austrian oil seed pumpkin x zucchini population and a U.S. oil seed pumpkin x crookneck squash population. This mapping process would be valuable in the *Citrullus lanatus* species, both to overcome the lack of coverage due to distortion in marker segregation and as a starting place for the comparison of QTL present in elite and citron populations.

Summary and Goals

The lack of a high density genetic map for *Citrullus lanatus* has greatly hindered the development of molecular tools for the species. The reduced level of nucleotide polymorphisms has previously made mapping using an elite x elite cross infeasible. Although previous mapping attempts were able to take advantage of the added polymorphism garnered from interspecific crosses, they were hampered by the skewed segregation of a large percentage of the developed markers. These maps would also be less useful to watermelon breeders without a map representative of commercially grown cultivars. The goals of this research were to (i) create a consensus SNP map for *C. lanatus* and (ii) to map horticulturally important traits in an elite by elite watermelon population.

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CHAPTER 2

COMPARITVE MAPPING WITHIN THE SPECIES *CITRULLUS LANATUS*[1]

[1]Katherine C. Sandlin, Jason Prothro, Cecilia McGregor, Adam F. Heesacker, Nelly Khalilian, Rebecca Okashah, Wenwen Xiang, Eleni Bachlava, David Caldwell, Danelle Seymour, Victoria White, Eva Chan, Greg Tolla, Cathy White, Dolores Safran, Elaine Graham, Steven J. Knapp. To be submitted to Theoretical and Applied Genetics

Abstract

In this study, a consensus marker order for the species *Citrullus lanatus* (Thumb.) Matusm. and Nakai was created from two intrasubspecific and one intersubspecific linkage maps. The linkage maps in this study were created from an F_6 elite x elite (Klondike Black Seeded (PI 635609) by New Hampshire Midget (PI 635617)) recombinant inbred line (RIL) population, an elite x egusi (Strain II (PI 279461) by Egusi (PI 560023)) F_2 population, and an elite x citron (ZWRM50 (PI 593359) by Delagoa (PI 244019)) F_2 population using single nucleotide polymorphism (SNP) markers. The elite x elite linkage map consisted of 379 markers with a length of 1,438.05 cM and an average marker distance of 3.79 cM. The elite x egusi linkage map consisted of 357 markers with a length of 1,514.26 cM and an average distance between markers of 4.24 cM. The elite x citron population linkage map consisted of 338 markers with a length of 1,114.06 cM and an average marker distance of 3.38 cM. These maps were then combined to form a consensus marker order consisting of 706 markers, which reduced the number of linkage groups to 11, the haploid number of watermelon (2n=22) . This consensus marker order will be useful for future efforts to use marker assisted selection in watermelon breeding.

Introduction

The species *Citrullus lanatus* (Thunb.) Matsum. & Nakai is composed of two varieties, *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*. *C. lanatus* var. *lanatus* consists of two subvarietal types, the cultivated (elite) watermelon which is grown commercially for its sweet flesh and the egusi types which have a hard and bitter flesh, and are grown in areas of West Africa. Egusi types are an important source of food security for subsistence farmers as the gelatinous pericarp coating the seeds which can either be eaten raw, cooked, mashed into a paste, or ground into a powder to be added to soups and stews (Achigan-Dako et al., 2008; Djè et al., 2010; Gusmini et al., 2004; Idehen et al., 2006). Although the egusi types have been described in previous literature as *C. lanatus* subsp. *mucosospermus* or *C. lanatus* var. *vulgaris* (Achigan-Dako et al., 2008; Idehen et al., 2006), the currently accepted nomenclature for this group of cultigens is *C. lanatus* var. *lanatus* (USDA). The other variety of *C. lanatus* is *C. lanatus* var. *citroides*, commonly called a citron or preserving melon. This species is grown in sub-Saharan Africa but is also present as an escape in other watermelon production areas. It is primarily used as a source of water, animal fodder, and as a food source when the hard bitter flesh is processed into fruit preserves (Robinson and Decker-Walters, 1997). Egusi and especially citron watermelon types contain potentiality valuable traits, such as disease resistance, which would be useful for improving elite watermelon varieties (Hashizume et al., 2003; Levi et al., 2001a).

The development of a genetic linkage map is a prerequisite for the construction of many molecular breeding tools which would aid in integrating beneficial traits from egusi and citron types, while maintaining the quality factors expected for elite types. Linkage maps have been developed for other major and minor crops and have proved to be an important tool for defining major genes, allowing for quantitative trait loci analysis and as backbone for potential genome sequencing. Several other studies have developed linkage maps for *Citrullus lanatus* (Hashizume et al., 2003; Hawkins et al., 2001; Levi et al., 2006; Zhang et al., 2004). Although the maps produced in these studies were constructed using a wide variety of marker technologies, the level of genotypic diversity present between elite varieties (Levi et al., 2001b) was prohibitively low for the production of an elite x elite linkage map. To this effect, these

studies used populations derived from intersubspecific crosses, and so no other known study was able to produce an elite x elite population linkage map.

The first study to develop comprehensive linkage maps for this species was published by Haschizume et al. (2003). The two maps were created using an F_2 and a BC_1 population derived from an intersubspecific cross using isozymes, RAPD, RFLP, and ISSR markers. The F_2 population was developed from a cross between *C. lanatus* var. *lanatus* and a *Citrullus lanatus* var. *citroides*, while the BC_1 population was developed was using the elite parent as the recurrent parent. The F_2 population produced a map of 11 linkage groups (LG) with a length of 2,384 cM with an average interval length of 4.3 cM, but with some areas with distances greater than 30cM. This was the only study to find a number of LGs which corresponded to the haploid chromosome number in watermelon, (2n=22) (Wehner, 2008). The map produced for the BC_1 population was constructed using markers shown to segregate in the F_2 population. It had a length of 1,729 cM with an average marker distance of 7.2 cM. The BC_1 population was phenotyped, and four QTL were successfully mapped. This was the only comprehensive mapping study to do so. Hawkins et al. (2001) used an F_2 and an F_3 population, also derived from a cross between a wild *C. lanatus* var. *citroides* species and a *C. lanatus* var. *lanatus* species. This study constructed two and five linkage groups consisting of 26 and 13 RAPD markers respectively from a population known to be segregating for resistance to fusarium wilt, an economically significant disease in watermelon production (Wehner, 2008). The Zhang et al (2004) study also used a cross between *C. lanatus* var. *citroides* and a *C. lanatus* var. *lanatus*. This was the only study to develop recombinant inbred lines (RILs). The development of a population in to RILs provides a higher amount of homozygosity within each line of a population and increases the likelihood of separation of tightly linked alleles. The separation increases the ability to analyze the population for QTL, although this was not attempted in this study. This study used RAPD, ISSR, and SCAR markers to develop a map with a length of 1,027cM and an average distance of 11.7cM between markers, on 15 linkage groups. The latest study, published by Levi et al. (2006) is an extension of a map published by the group in 2002 (Levi et al., 2002). The population mapped in this study was a test cross using (*C. lanatus* var. *citroides* x *C. lanatus* var. *lanatus*)

crossed with a *C. colocynthis* species, in an effort to control some of the segregation distortion encountered when mapping a wide interspecific cross. Segregation distortion is a factor which has hampered map construction in all of mapping studies of this species to date. The use of a testcross in this study did not completely eradicate the segregation distortion, but the resulting percentage of distortion (18%) was significantly lower than some levels seen in previous studies (Hawkins et al., 2001). The 12 linkage group map produced by these two studies began with the use of RAPD, ISSR, and SCAR markers (Levi et al., 2002) and was extended using AFLP, SRAP and SSR markers (Levi et al., 2006), as it increased the length to 1,976 cM from 1,166.2 cM and reducing the distance between markers to 5.8 cM from 8.1 cM. Although all of these studies have produced genetic maps, their usefulness was limited by the populations and marker types used.

The first objective of this study was to develop three linkage maps for the *C. lanatus* species, using single nucleotide polymorphisms (SNP) markers. SNPs are present at a much higher level than those measured by other marker types as they are the most elementary type of variation found within a species (Henry, 2008; Kole and Abbott, 2008). The second objective was to form a consensus order from these three populations.

Materials and Methods

Plant materials

Six different varieties obtained from the Germplasm Resource Information Networks (GRIN) Southern Regional PI Station in Griffin Georgia were used to create the different populations for this study. The parents used to create the elite x elite population were Klondike Black Seeded (PI 635609) and New Hampshire Midget (PI 635617). These two cultivars were chosen on the basis of the levels of genotypic and phenotypic diversity they provided for linkage and QTL mapping. The parents for the elite x egusi cross were the elite variety Strain II (PI 279461) from Japan, and a wild egusi type (PI 560023) from Nigeria. The parents used for the elite x citron cross were the Chinese elite cultivar ZWRM50 (PI 593359) and the wild *C. lanatus* var. *citroides* accession Delagoa (PI 244019) from South Africa.

The Klondike Black Seeded by New Hampshire Midget (elite x elite) cross was advanced to the F_6 generation through single seed descent and controlled self pollinations in the greenhouse. Tissue was collected from the parents and F_1 hybrid, frozen, then ground and lyophilized for storage. The Strain II by Egusi (elite x egusi) and the ZWRM by Delagoa (elite x citron) populations were advanced to the F_2 generation through the controlled self pollination of a single plant grown from each of the F_1 hybrid seeds. Tissue was collected from the F_1 hybrids and the F_2 generation for DNA extraction, but bulks were used for the parents. Leaf tissue from these populations was also frozen, ground, and lyophilized for storage. DNA was extracted from all samples, with the exception of the elite x elite F_6 RIL population, using a modified CTAB procedure (Murray and Thompson, 1980). The DNA was quantified using a Quant-iT Picogreen DNA reagents kit (Invitrogen, Ltd. Paisley, PA) then diluted to a concentration of 50 ng/ml. These samples, along with fresh leaf tissue collected from the elite x elite F_6 population, were sent to Monsanto's (Monsanto Company, St. Lewis, Missouri) facilities in St. Lewis, Missouri for SNP analysis.

SNP analysis and linkage map development

All SNP markers used for mapping in this study were created in collaboration with Monsanto, using proprietary methods. These SNP markers were used to form a 1,536 SNP Illumina GoldenGate (Illumina Inc., San Diego, CA) array for genotyping the three populations. The mapping data was provided, including the genotypes of the samples sent and the resulting mapping distances from the markers analyzed. Linkage groups for each population were drawn using MapChart (Voorrips, 1999-2006) (Figure 2.1).

Consensus order

The markers and their mapping distances were manually arranged in order and used to create scatter plots comparing the homologies of the different mapping populations (Figure 2.2). The

directionality assigned to the linkage groups was corrected for when needed, and the linkage groups aligned to form the homology scatter plots. From these graphs, relationships between the three sets of linkage groups could be visualized, and corresponding pairs of linkage groups could be matched up and relabeled (Table 2.1). The marker data was further analyzed for segregation distortion using a chi-squared test with a P=0.05 level of significance. Distorted markers were not considered for the consensus order.

From the three linkage maps, a compiled list of all the mapped markers, arranged in their homologous linkage groups based on the numberings of the elite x elite linkage groups was formed. This process began with the elite x elite map and shared markers from the other two maps were paired with the markers found on the elite x elite map. Between these markers those shared between the elite x egusi and elite x citron markers, but not the elite x elite map, were placed based on their mapping distances. Lastly, any unmatched markers were placed within the order relative to the shared markers (Table 2.1).

Results and Discussion

A total of 737 different markers were mapped to any of the three populations (Table 2.2 and Figure 2.1). Of the three populations, the elite x elite linkage map consisted of the highest number of markers with 12.13% more than the amount mapped in elite x citron, the population with the fewest markers mapped. This was most likely due to the SNP design process, and not a reflection on the level of diversity of these three populations, as the markers on the genotyping array used were designed based for use with an elite population. The elite x elite map had a total distance of 1,357.74 cM with an average marker distance of 3.58 cM on 13 LGs. The elite x egusi map has a total distance of 1,514.26 cM with an average marker distance of 4.24 cM on 14 LGs. The elite x citron map had a length of 1,144.06 cM and an average marker distance of 3.39 cM on 16 LGs. The level of segregating distortion was low for the elite x elite population (3.7%) and the elite x egusi population (2.8%), but occurred at a higher level in the elite x citron (12.7%) (Table 2.2 and Figure 2.1). The distorted markers were included in maps for the individual populations, but were excluded from the consensus order (Table 2.3). When excluding both the

markers exhibiting segregation distortion and ones with positional conflicts, a total of 43 markers mapped in all three populations. The elite x elite population had a total of 119 (20.07%) markers in common with the elite x egusi population and 111 (20.26%) in common with the elite x citron population, while the elite x egusi and the elite x citron populations had a total of 104 (19.22%) markers in common (Figure 2.2). There were 6 markers of the 729 total that had conflicting positions in one of the three populations. The conflicts these markers present were discrepancies of 1-3 cM. There is the possibility that these could be due to scoring errors, in which case they would represent a relatively low rate at approximately 0.8%.

Although the markers exhibiting segregation distortion were excluded from the final consensus marker order, areas where they coalesced could be informative (Figure 2.1). The distorted markers found in the elite x elite population were evenly distributed across the linkage groups, with no more than three distorted markers per linkage group. However, in the other two populations there were several areas where the distorted markers were found in higher numbers. The elite x egusi population had the lowest amount of distorted markers (10), but had a section on LG 3 (consensus group 6) where half of the distorted markers are found consecutively. There were several areas on the elite x citron linkage groups that exhibited this phenomena. All of the markers on the elite x citron LG16 were distorted, the last 7 markers on LG 2, 5 markers at the end of LG 7, and 6 markers on the end of LG 8. These areas of distorted segregation could be due to differences between the sequence in the elite and egusi or citron genomes that control the reproductive process (Zamir and Tadmor, 1986). It has been proposed (Hashizume et al., 2003) that the decrease in hybrid fertility encountered in some elite x citron crosses could be associated with the distorted marker segregation. However, the pollen viability for this elite x citron population was tested and did not show a significant amount of pollen abortion (Prothro, 2010). The marker distortion could also be caused by meiotic drive, which has been shown to be a problem intersubspecific crosses (Zamir and Tadmor, 1986).

The consensus mapping allowed for several of the linkage groups to be combined. The original number of linkage groups was 13 for the elite x elite, 14 for the elite x egusi, and 16 in the elite x citron. The number of linkage groups in the elite x elite and elite x egusi populations were reduced to 11, which

is the haploid chromosome count of *Citrullus* (2n=22), as some linkage groups coalesced within the consensus order. The number of linkage groups for the elite x citron population was only reduced from 16 to 13, as LGs 3 and 6 were not able to be placed in the consensus order. Markers from LG 16 from this population were excluded from the consensus marker order, as they all markers showed skewed segregation.

The combination of these three linkage maps into a consensus order also filled large gaps existing in the single maps with markers from the other two. Of all the markers from the three populations, only 10 were unable to be placed relative to the other markers. These 10 markers make up LGs 3 and 6 of the elite x citron population. These small linkage groups consisted of only 8 markers with a distance of 27.23 cM and 2 markers with a distance of 17.79 cM, respectively. The consensus order also illustrates areas of the genome that are not represented in some of the linkage maps, as these areas are covered by the maps from other populations. Several of these gaps exist in the elite x citron map, which were then covered by both the elite x elite and the elite x egusi maps.

Of the three linkage maps the map for the elite x citron population consisted of the lowest amount of mapped markers, the highest amount of markers with segregation distortion (12.7%), and the largest amount of gaps when compared to the consensus order. It also had the largest number of linkage groups, some of which were unable to be aligned in the consensus order. This may be because the population was developed from the wider cross, as the egusi type watermelons are subvariety of *C. lanatus* var. *lanatus* whereas citron as a separate variety watermelons are further removed within the species (Levi et al., 2001b). Considering the design of the SNP markers used in this study, a better understanding of these regions could be gained using additional citron specific markers as they might potentially contain some of the desirable traits exclusively found in citron cultigens.

This is the first known study to establish linkage maps developed from elite x elite and elite x egusi populations. This study is also the first to develop linkage maps for *Citrullus* species using SNP markers and the to establish a consensus marker order for this species. Further development of this consensus marker order into a consensus map with cM distances would enhance the data from this study

as a tool for molecular plant breeding. Future studies might also align this consensus order with the sequenced cucumber genome to potentially align these areas with known genes within *Cucurbitaceae* (Huang et al., 2009). By studying the relationship of the linkage groups found for these three different populations, a greater understanding of the gene flow between these three types of watermelon can potentially be gained.

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Table 2.1 Corresponding linkage group labels for *Citrullus* SNP linkage maps. *Citrullus* linkage groups as they are aligned in the consensus order. Linkage group numbers within individual populations correspond to those originally assigned to them.

	Linkage Groups		
Consensus group	elite x elite	elite x egusi	elite x citron
1	1, 12	8,7	10, 16
2	2	2	9
3	3	9	2
4	4	13	4
5	5	14	15
6	6	3	5, 14
7	7, 13	4	7
8	8	1	1
9	9	6, 5	11
10	10	12	8
11	11	10, 11	12,13
remaining			3, 6

Table 2.2 Summery of linkage maps and consensus order of the three watermelon populations.

Population	elite x elite	elite x egusi	elite x citron	consensus order
Population Size	164	187	182	534
Markers total	379	357	338	706
Map length (cM)	1,438.05	1,514.26	1,144.06	-
Distance between markers (cM)	3.79	4.24	3.38	-
Largest gap (cM)	22.47	27.3	33.04	-
Linkage groups	13	14	16	11
Markers with distorted segregation	14	10	43	-

Table 2.3 Consensus order of SNP markers by linkage group for the three watermelon populations. Markers were placed in order based on combined map positions. Bold faced markers were mapped in all three populations and bold italicized markers were mapped in two populations. Grayed markers indicate where an additional (conflicting) marker existed.

Consensus Linkage Groups										
1	2	3	4	5	6	7	8	9	10	11
NW0249792	NW0251236	NW0248772	NW0249742	NW0249862	NW0248569	***NW0251293***	NW0250390	NW0248382	NW0248866	***NW0248120***
NW0249951	NW0248521	***NW0248920***	***NW0249245***	***NW0249154***	***NW0250974***	NW0250418	***NW0250663***	NW0249041	NW0251382	NW0250945
NW0250062	NW0251035	***NW0249571***	NW0248427	NW0249315	NW0248523	***NW0248267***	NW0248871	***NW0248190***	NW0249466	**NW0248776**
NW0250097	***NW0248105***	***NW0251373***	NW0248167	NW0249284	***NW0249556***	NW0247963	***NW0248647***	NW0249421	***NW0249096***	**NW0248599**
NW0247973	***NW0251311***	NW0248156	***NW0248412***	***NW0252531***	NW0251124	NW0248684	**NW0248966**	***NW0250285***	***NW0249191***	***NW0249247***
NW0249637	NW0251009	**NW0251029**	***NW0251071***	NW0251274	***NW0250684***	NW0252073	NW0247998	***NW0249984***	NW0251270	NW0249016
NW0249289	NW0249048	NW0248094	NW0249741	NW0249610	NW0249824	NW0250430	**NW0248314**	***NW0248184***	NW0249882	NW0250479
NW0251262	NW0249251	**NW0248857**	NW0249521	***NW0248277***	**NW0248917**	***NW0248722***	**NW0250095**	***NW0250651***	NW0248392	***NW0248107***
NW0249411	NW0249396	***NW0248481***	NW0250300	***NW0248714***	***NW0248436***	NW0248773	**NW0250331**	NW0249597	NW0248998	NW0249365
NW0251122	***NW0248967***	NW0248639	***NW0249088***	***NW0252251***	***NW0248912***	NW0248534	**NW0248066**	NW0249195	NW0248443	***NW0250956***
NW0248176	NW0251455	NW0251077	NW0251309	***NW0248349***	NW0248604	NW0249430	***NW0249011***	NW0248168	NW0249773	***NW0250499***
NW0251017	NW0250500	NW0248269	NW0249216	***NW0249290***	***NW0248053***	***NW0248789***	NW0250660	NW0248635	NW0252106	NW0247961
NW0248361	NW0250854	NW0249364	NW0250849	***NW0250738***	***NW0248590***	***NW0247924***	***NW0251301***	NW0248703	NW0248502	NW0249583
NW0251075	NW0249591	NW0248922	NW0249249	NW0248157	**NW0250541**	NW0249400	***NW0250012***	NW0248892	NW0249449	NW0250112
NW0251241	***NW0250242***	NW0249255	***NW0250697***	NW0248421	NW0248212	NW0250369	***NW0250318***	NW0248809	NW0249367	***NW0249140***
NW0248622	NW0248325	NW0250435	NW0249873	***NW0250080***	NW0250483	NW0249869	NW0250281	NW0251187	***NW0252082***	***NW0249412***
NW0249444	NW0248118	***NW0249108***	NW0249239	NW0249945	NW0250460	***NW0248479***	***NW0248260***	NW0251383	NW0251475	NW0248306
NW0251086	NW0248583	NW0247970	***NW0249225***	NW0250472	NW0249344	NW0251128	NW0248975	***NW0249789***	***NW0251480***	NW0248283
NW0249179	NW0249599	NW0249127	NW0248233	***NW0251438***	NW0251285	***NW0248299***	***NW0248042***	NW0248758	NW0251189	***NW0251331***
NW0249401	***NW0249314***	***NW0249630***	NW0248890	NW0251254	NW0248739	***NW0250703***	NW0249345	**NW0248650**	NW0248085	***NW0248957***
NW0249541	NW0250496	***NW0247962***	NW0250229	NW0248814	NW0249078	***NW0249408***	***NW0249253***	**NW0248571**	NW0248073	NW0247960
NW0248450	NW0249312	NW0250261	NW0248810	NW0249949	NW0252146	NW0249369	***NW0248410***	NW0250665	***NW0248662***	***NW0251372***
NW0250274	NW0251153	***NW0248518***	***NW0249026***	NW0250793	***NW0248236***	NW0249112	NW0248959	***NW0249518***	NW0249087	**NW0248648**

NW0249380	NW0250325	NW0251468	***NW0249297***	NW0250894	***NW0248874***	NW0250083	**NW0249872**	NW0250470	NW0250299	NW0250092
NW0252078	NW0250837	NW0248132	***NW0248379***	NW0250677	NW0250824	***NW0249151***	NW0250232	***NW0249012***	***NW0250598***	***NW0248748***
NW0251369	***NW0248249***	NW0251381	NW0252421	***NW0250791***	NW0249371	***NW0250100***	NW0249183	***NW0251205***	NW0248181	NW0247990
NW0248223	***NW0248815***	NW0250902	NW0249395	NW0249294	NW0248477	***NW0250333***	NW0249262	NW0248819	***NW0248334***	NW0251028
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Figure 2.1 SNP linkage maps for (A) elite x elite, (B) elite x egusi, and (C) elite x citron populations. Markers with segregation distortion are labeled (***).

A.

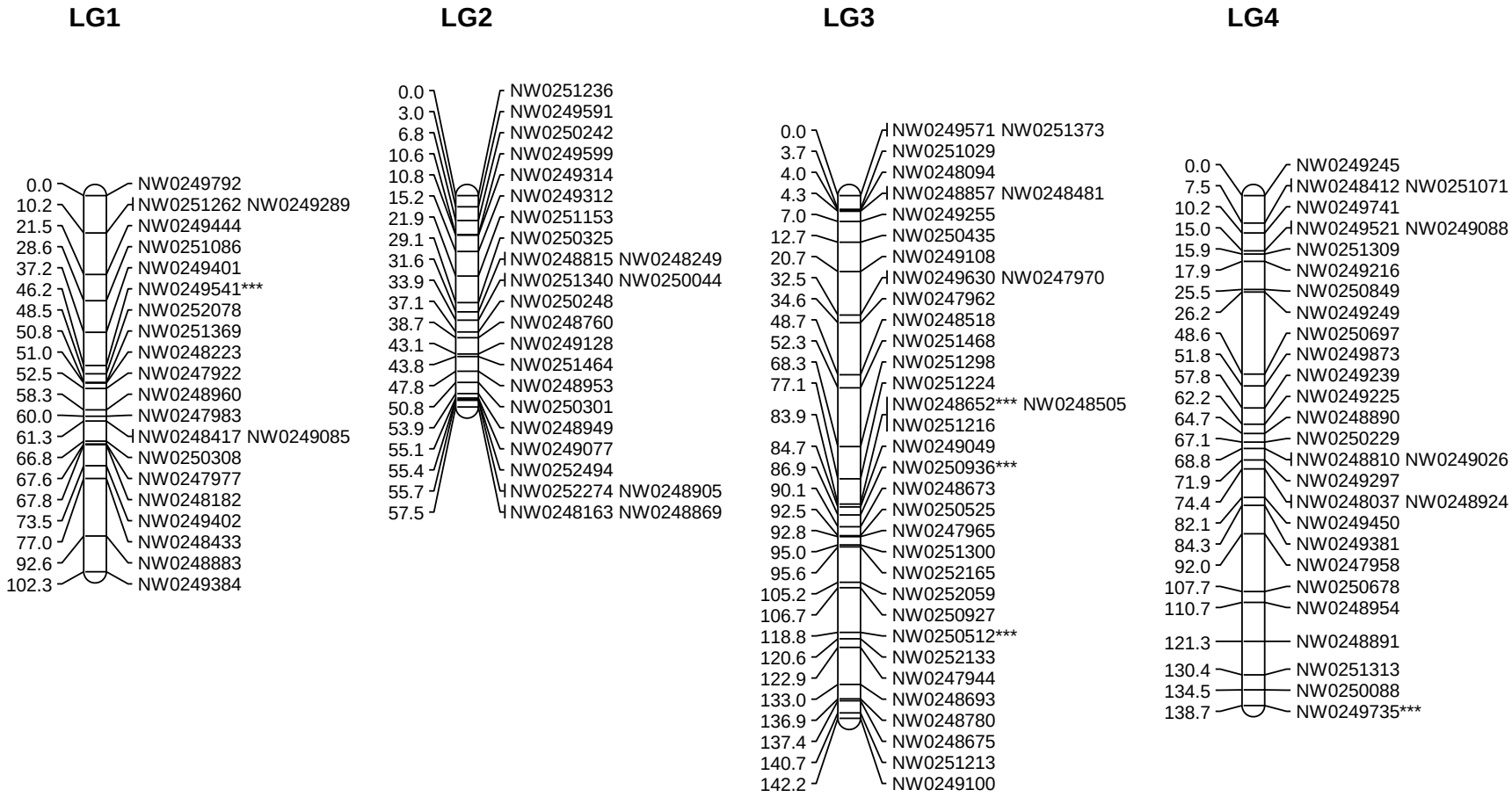


Figure 2.1 continued

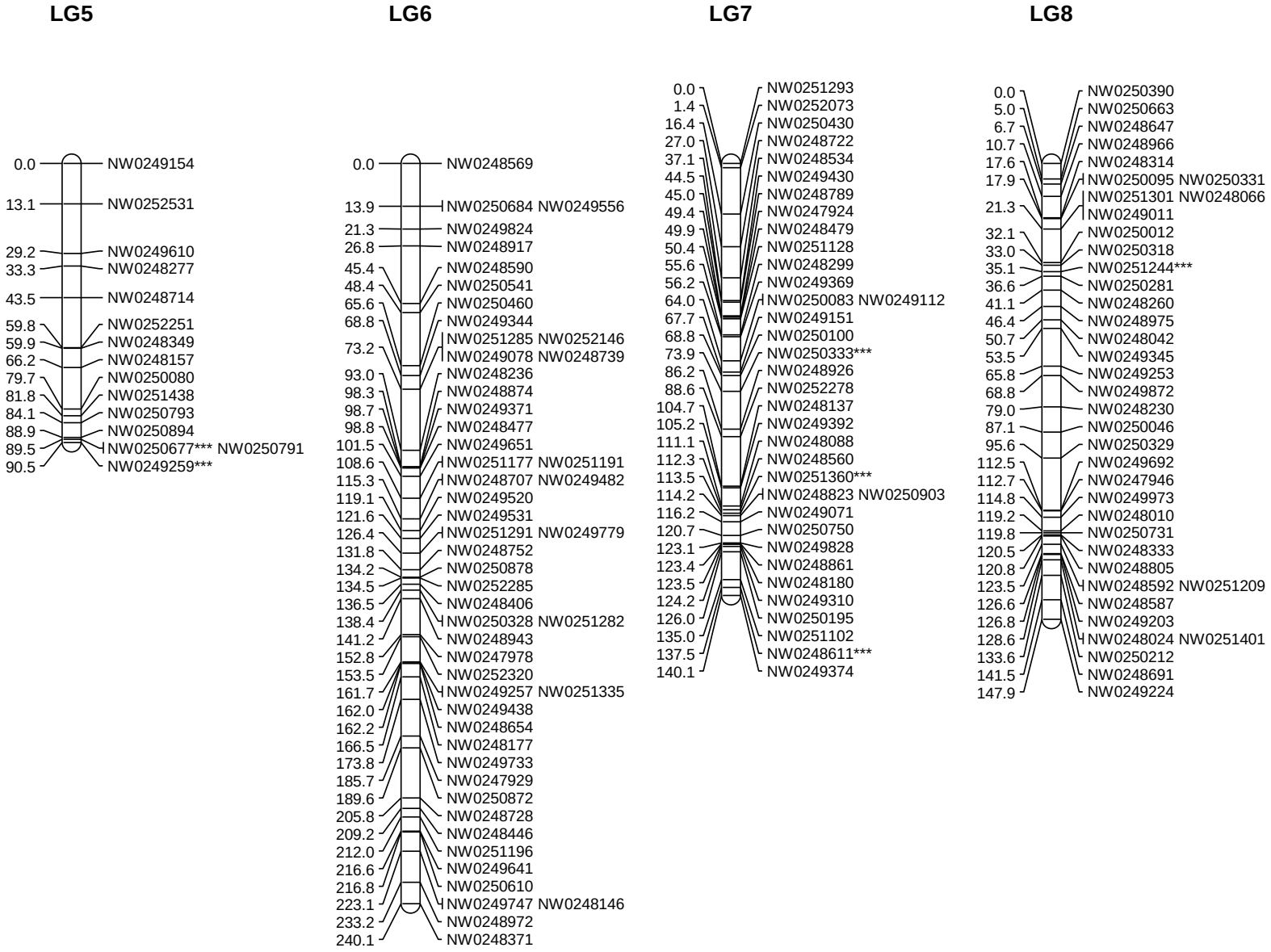
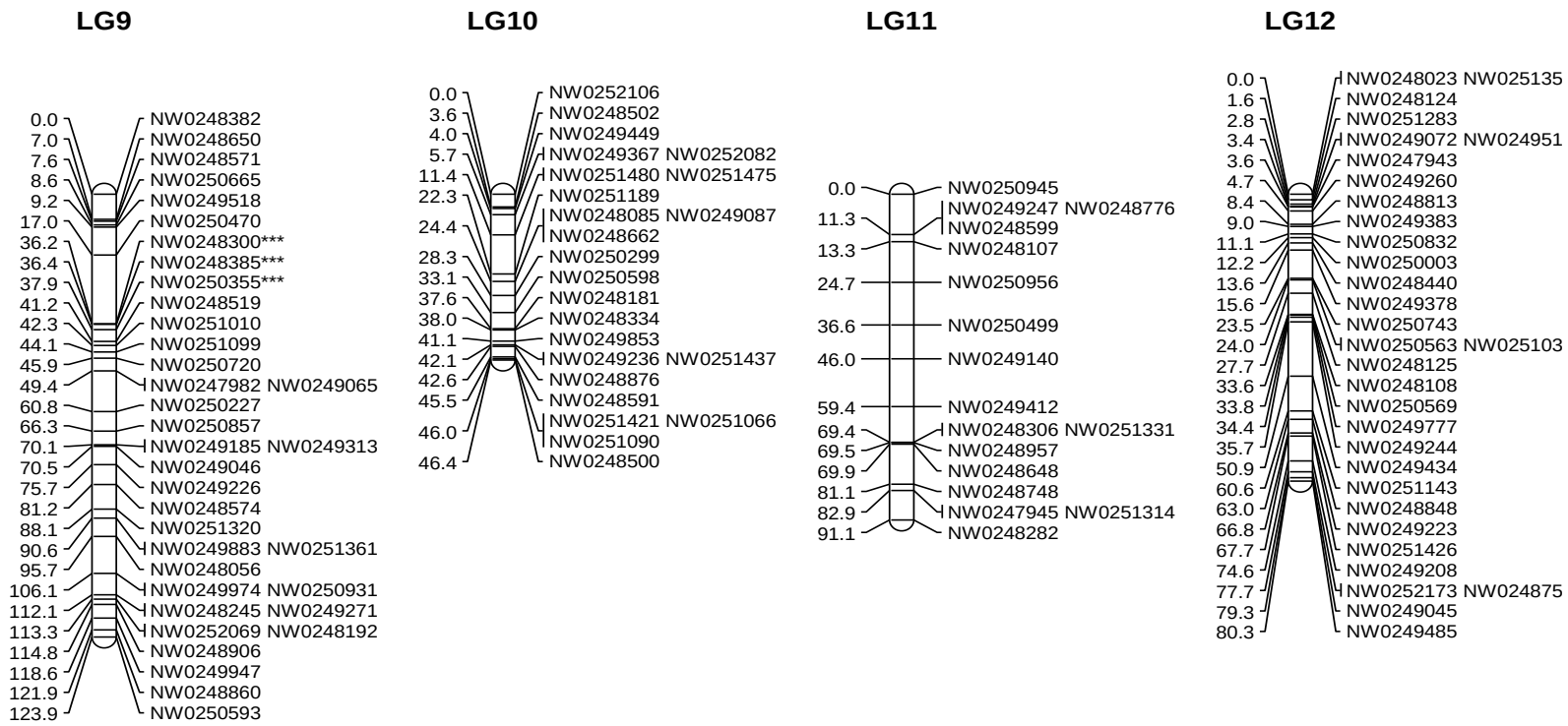


Figure 2.1 continued



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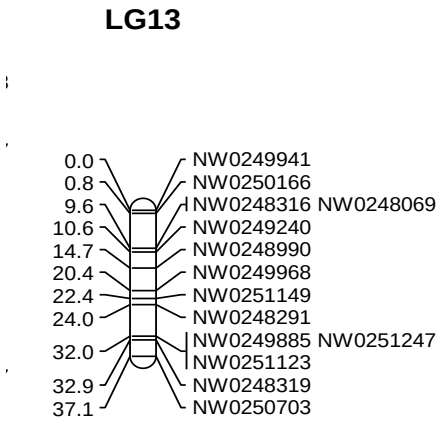

Figure 2.1 continued
B.

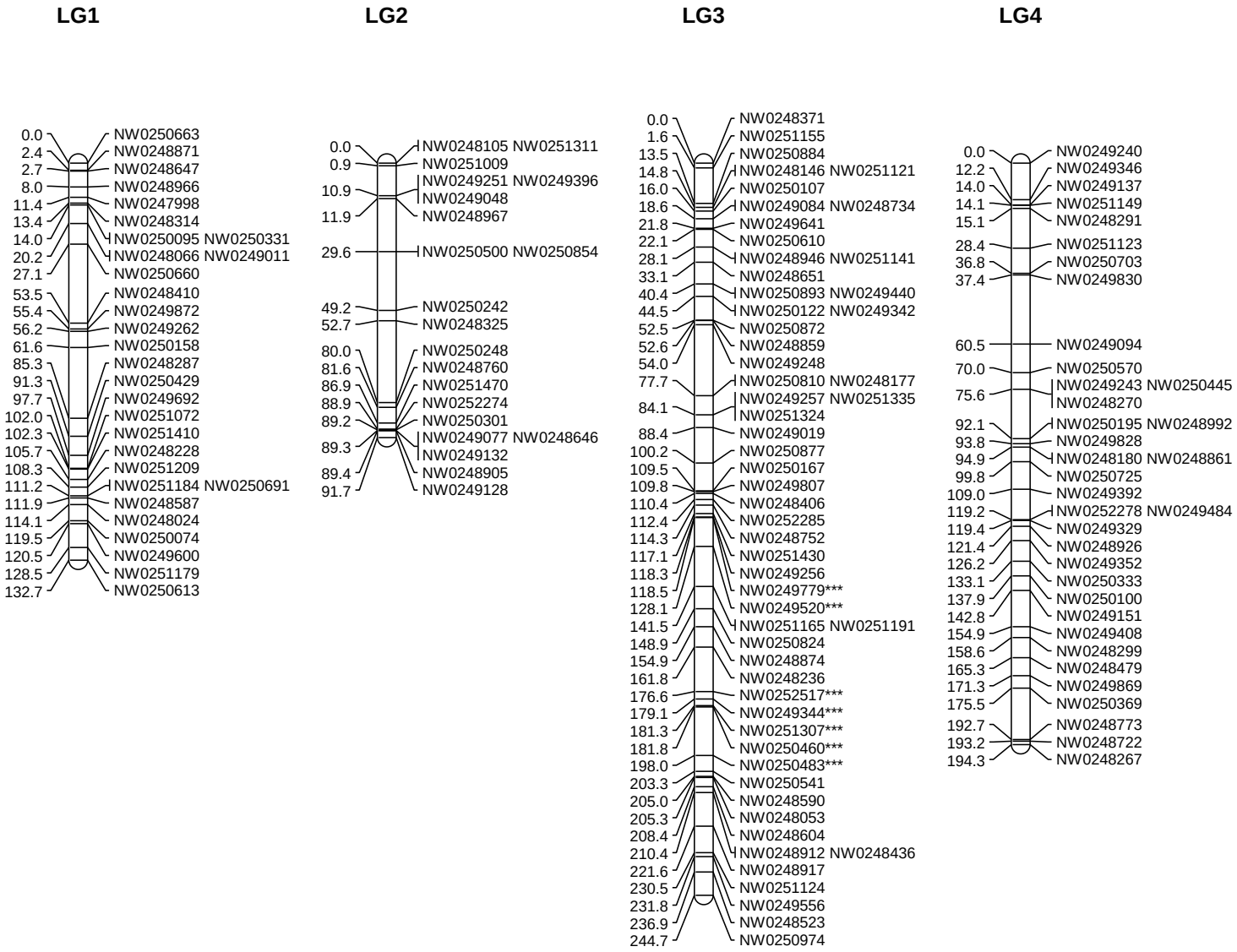

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Figure 2.1 continued

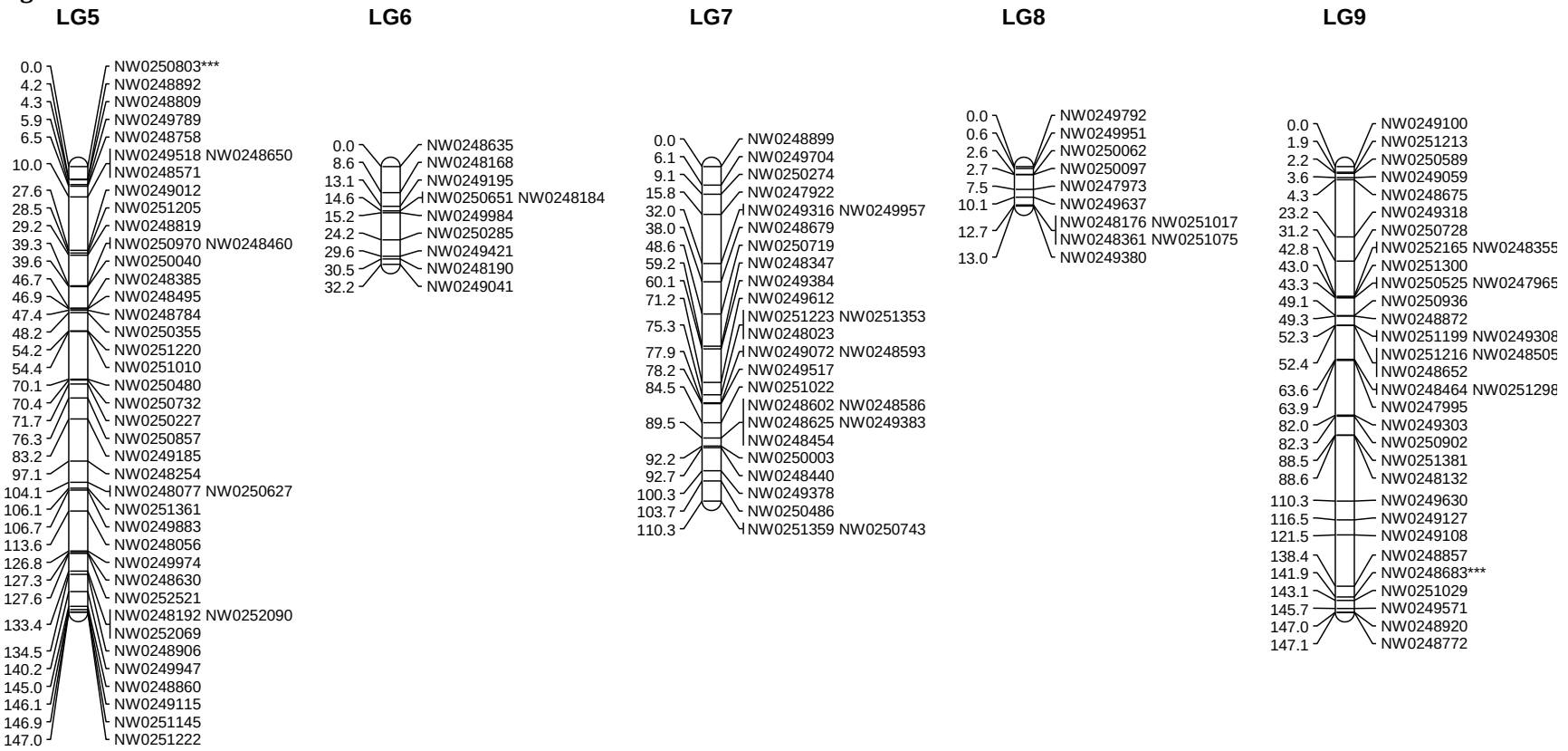


Figure 2.1 continued

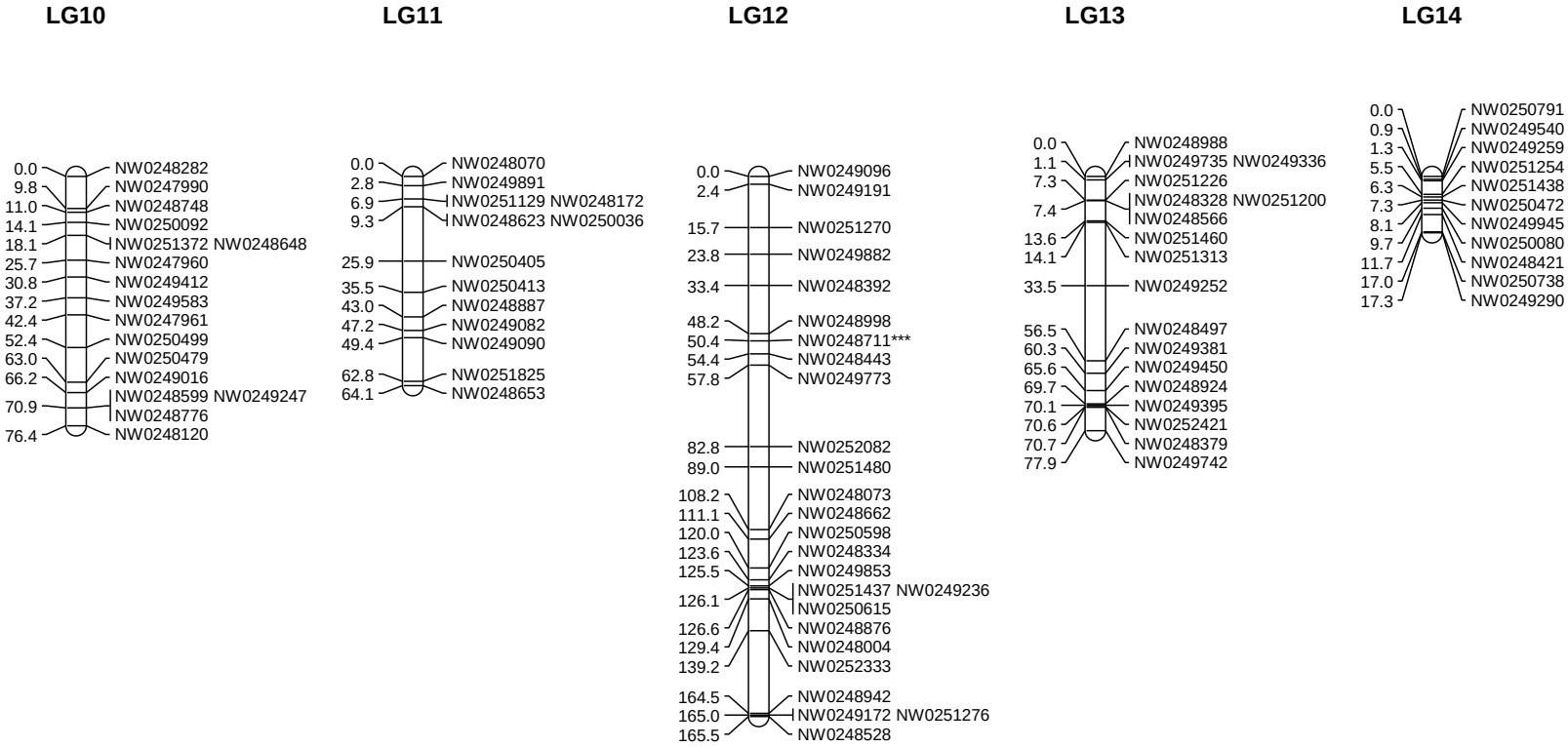


Figure 2.1 continued
C. ZxD

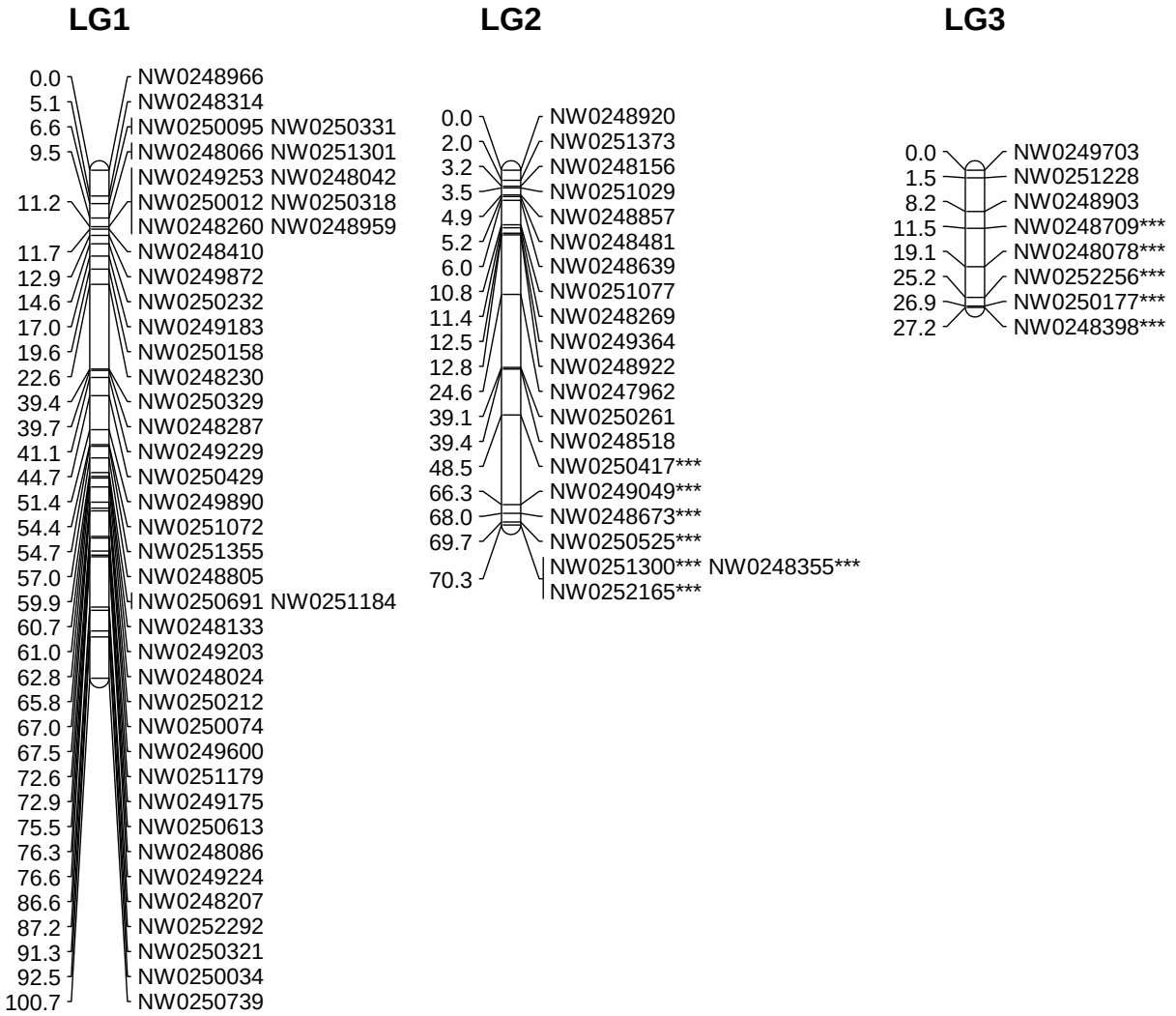
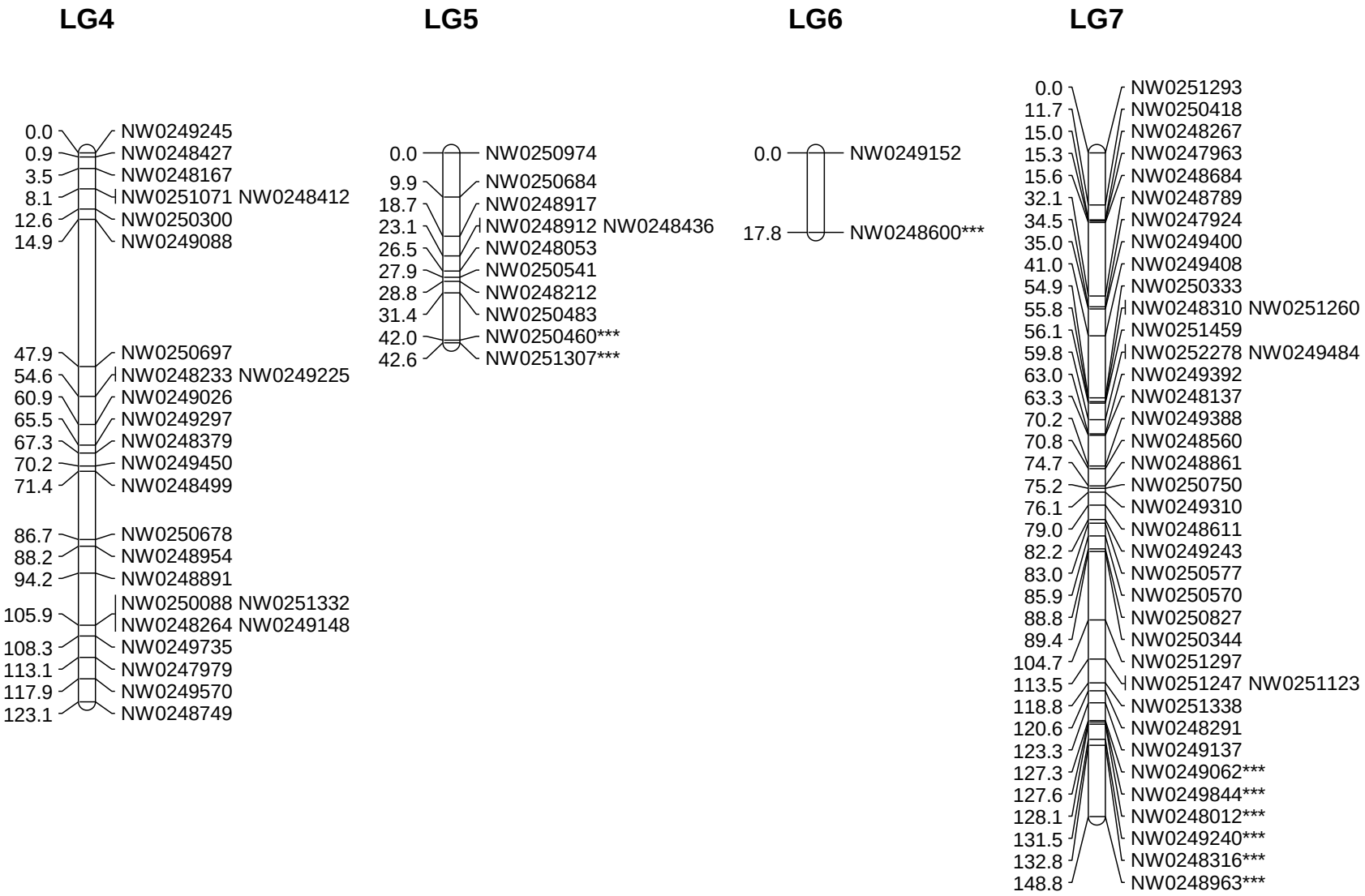


Figure 2.1 continued



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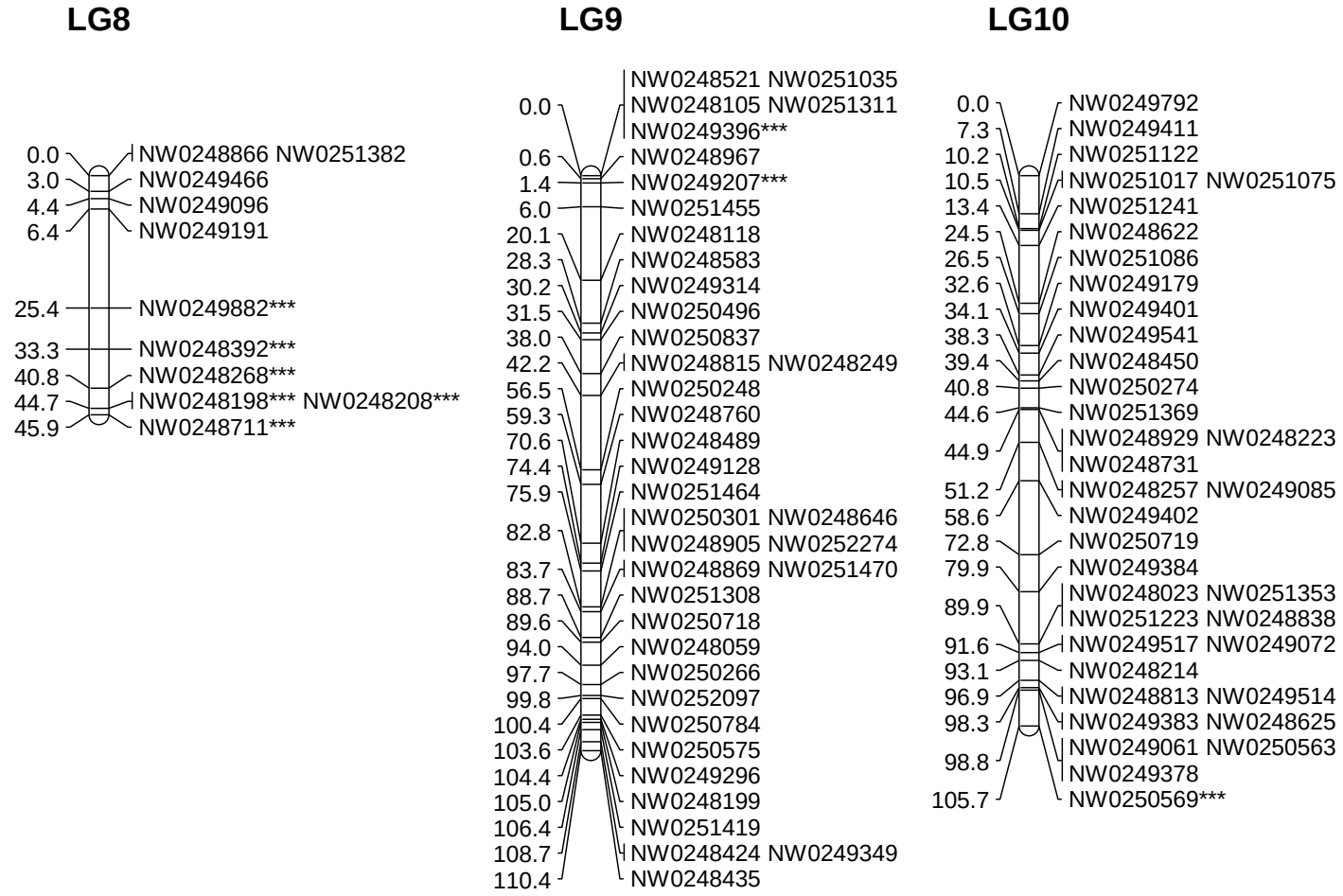
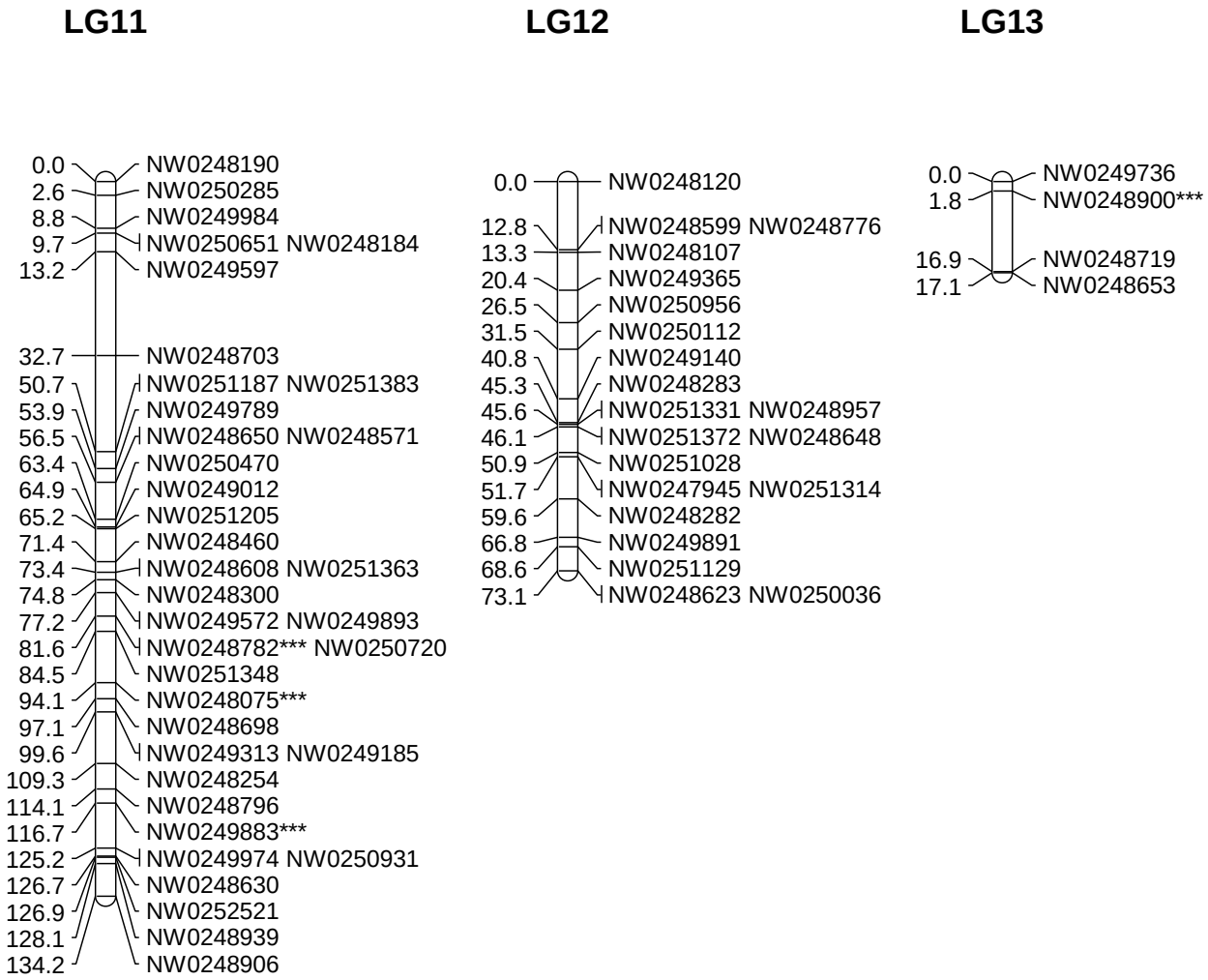


Figure 2.1 continued



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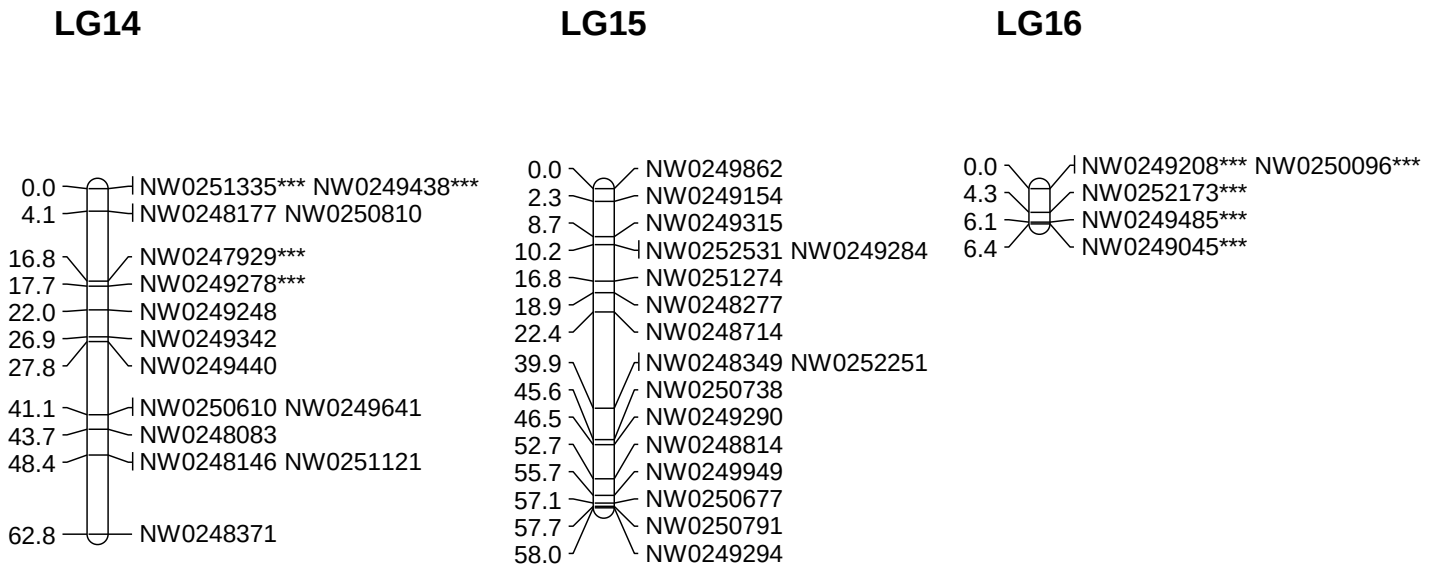
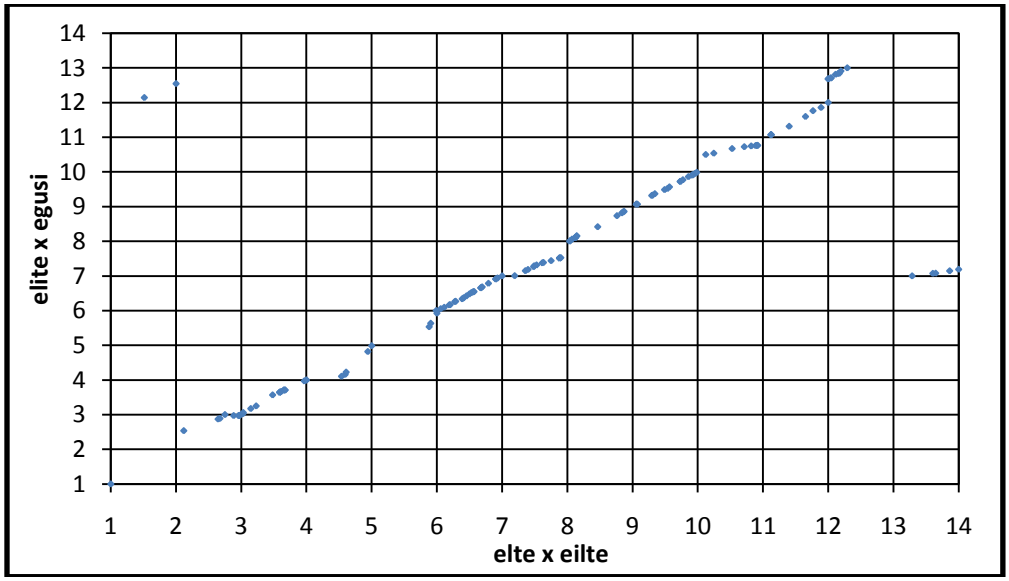


Figure 2.2 Homology scatter plots of the three *Citrullus lanatus* populations.
Linkage groups are not plotted to scale.



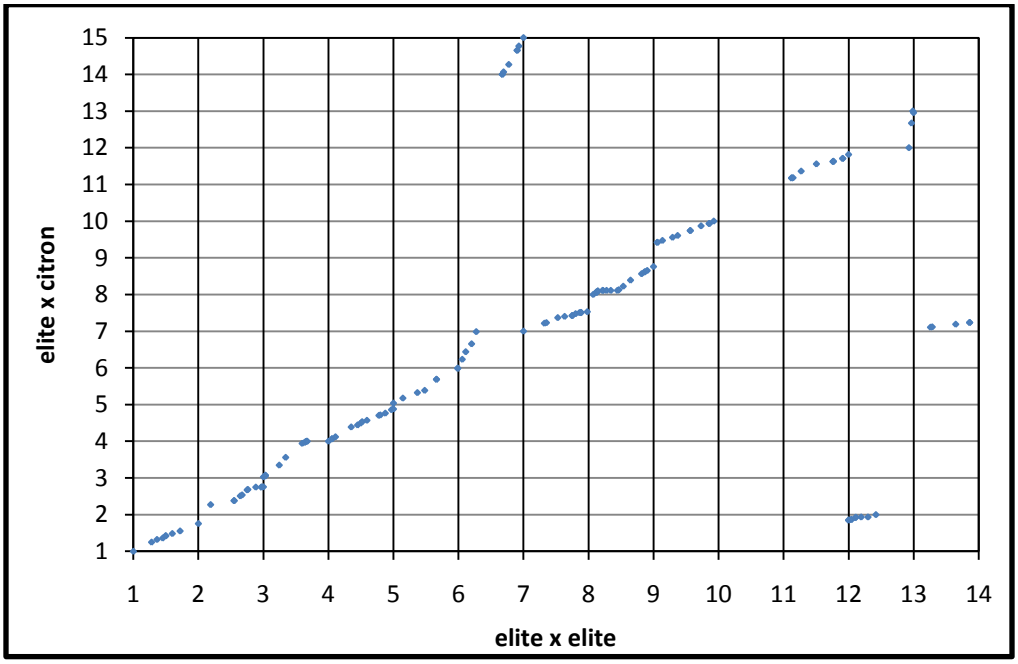
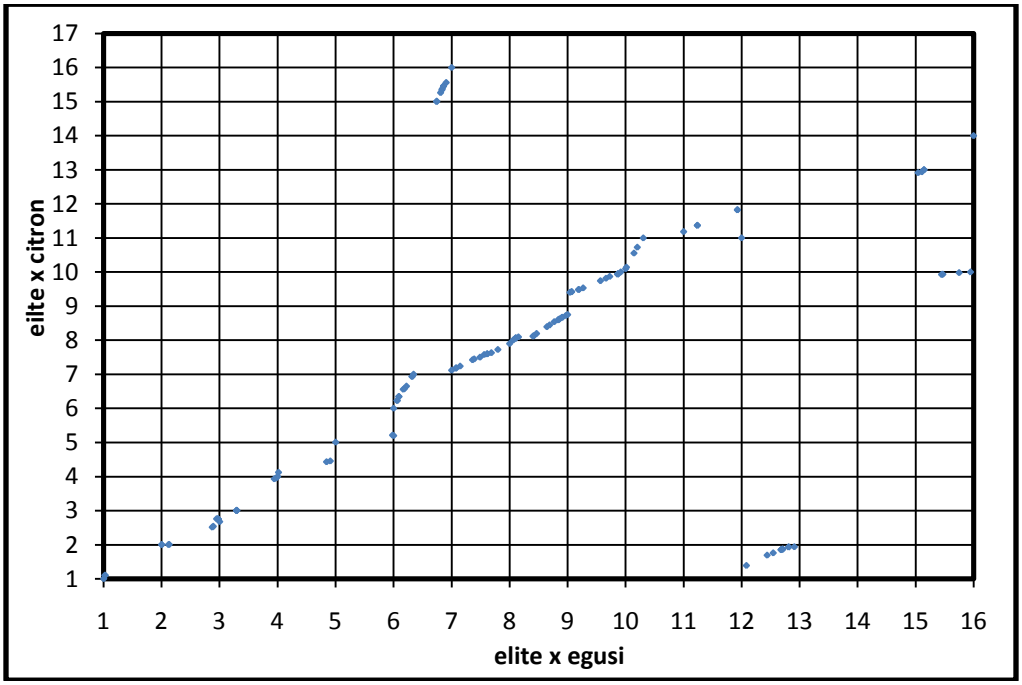


Figure 2.2 continued



CHAPTER 3

QTL ANALYSIS OF HORTICULTURAL TRAITS IN ELITE WATERMELON RECOMBINANT INBRED LINES[2]

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Abstract

A genetic linkage map was created for cultivated watermelon (*Citrullus lanatus* var. *lanatus* (Thumb.) Matsum. and Nakai) using a population of F_6 recombinant inbred lines (RILs) developed from a cross between the two elite cultivars Klondike Black Seeded (PI 635609) and New Hampshire Midget (PI 635617). The linkage map was constructed using 379 single nucleotide polymorphism (SNP) markers and consists of 13 linkage groups (LGs) with a total distance of 1,438.05 cM and an average distance of 3.79 cM between markers. This map was used to map 33 quantitative trait loci (QTL) for nine horticulturally important traits. Phenotypic data was measured for QTL mapping in the F_7 RIL population grown at two locations; at the University of Georgia's Plant Sciences farm in Watkinsville, Georgia and in Woodland, California. QTL were analyzed for fruit length, fruit diameter, fruit shape, thickness of the rind, Brix, the presence of hollow heart, fruit weight, degree of fruit furrowing, and the number of days from sowing to the first female flower. Several QTL for important morphological traits were found to be co-localized. This linkage map and the QTL analyzed are the first of such for elite x elite cross, and may provide a useful tool for plant breeders working on cultivar improvement within the variety *Citrullus lanatus* var. *lanatus*.

Introduction

Global production of watermelon (*Citrullus lanatus* ((Thunb.) Matsum. & Nakai) var. *lanatus)* has risen by approximately 31.5% in the last ten years to almost 100.7 million metric tons produced on over 3.8 million hectares in 2009 (FAOSTAT, 2009), with the United States as the fourth largest producer. Domestic production of watermelons was valued at 460 million U.S. dollars, which was 4.4% of the total value of vegetables and melons produced nationally in 2009 (USDA, 2010). Desired attributes for commercial (elite) cultivars include smaller size, early maturation, high sugar content, and improved disease resistance (Wehner, 2008a). Popular elite varieties can either be seeded or seedless, are typically red fleshed, and vary in size and shape based on their use. Seeded varieties are large (8-11kg) and blocky in shape, while seedless varieties tend to be medium in size (5-8kg) and oval in shape. Mini and icebox types that have been bred to be very small (<4.0 kg and 4-5.5kg) have recently gained in popularity (Wehner, 2008a). Several genes have been described for these and other horticulturally important traits (Wehner, 2008b). The designation of quantitative trait loci (QTL) for these important traits would be the next step in order to develop molecular tools for watermelon breeding programs.

However, there is a limited amount of genetic diversity between elite watermelon varieties (Levi et al., 2001b), which limits the level of polymorphisms available for molecular marker development. This makes the construction of a saturated map, with less than 2cM mean distance between markers, much more difficult. Several genetic maps have been constructed for watermelon and QTL have been designated for some horticulturally important traits (Hashizume et al., 2003; Navot and Zamir, 1987). However, compared to fellow members of the *Cucurbitaceae* family, *Cucumis melo* L. (melon) and *Cucumis sativus* (cucumber), watermelon QTL mapping lags far behind. Saturated maps have already been created and significant QTL designated for horticulturally important traits in both cucumber an melon (Deleu et al., 2009; Fazio et al., 2003; Perin et al., 2002; Silberstein et al., 2003). The cucumber genome has also been sequenced (Huang et al., 2009). In order to increase the genotypic diversity available for marker development the genetic maps developed for watermelon used wider crosses with *Citrullus lanatus* var. *citroides* , the hard bitter citron type which is generally limited to sub-Saharan

Africa, to increase the genotypic diversity available for marker development. As a result, these studies have encountered segregation distortion which left areas of the genome unmapped (Hawkins et al., 2001; Levi et al., 2006; Zhang et al., 2004). The first comprehensive linkage map was published by Haschizume et al. (2003) and describes two maps, one constructed from an F_2 population derived from an intersubspecific cross between a cultivated inbred *C. lanatus* var. *lanatus* and a *Citrullus lanatus* var. *citroides*, and a BC_1 using the elite parent as the recurrent parent. The F_2 population produced a map with a length of 2,384 cM, and an average interval length of 4.3 cM and 11 linkage groups (LGs), corresponding to the haploid chromosome number in watermelon (2n=22) (Wehner, 2008a). However, the distances between markers in some areas were greater than 30 cM. The map produced for the BC_1 population was constructed using markers shown to segregate in the F_2 population and had a length of 1,729 cM with an average marker distance of 7.2 cM. This BC_1 population was phenotyped and QTL for four horticulturally significant traits, rind hardness, flesh color, rind color and Brix were identified. Hawkins et al. (2001) used an F_2 and an F_3 population, derived from a wide cross between a wild *C. lanatus* var. *citroides* accessions and the *C. lanatus* var. *lanatus* cultivar New Hampshire Midget, to construct maps of two and five linkage groups consisting of 26 and 13 RAPD markers respectively. Zhang et al (2004) also used a cross between *C. lanatus* var. *citroides* and a *C. lanatus* var. *lanatus*, but to develop a recombinant inbred line (RIL) population. The map produced for this population has a length of 1,027 cM with an average distance of 11.7cM between markers, on 15 linkage groups. Levi et al (2002) used a population developed from a test cross of (*C. lanatus* var. *citroides* x *C. lanatus* var. *lanatus*) x *C. colocynthis* which they used in an attempt to control some of the segregation distortion encountered by other studies when mapping wide interspecific crosses. Considering Hawkins et al. (2001) experienced marker segregation distortion at rates of 47.5% and 48% with an F_2 and BC_1 population using the same *C. lanatus* var. *lanatus* cultivar for the elite parent, the use of a test cross aided in producing a lower rate of marker segregation distortion (18%) (Levi et al., 2002). The testcross population map was later enhanced in the Levi et al. (2006) study using AFLP, SRAP and SSR markers (Levi et al., 2006) . This increased the map to a length of 1,976 cM with an average distance of 5.8 cM between markers from the previous

length of 1,166.2 cM with an average genetic distance of 8.1 cM between markers and reduced the number of linkage groups from 25 to 19. The only one of these studies to phenotype horticulturally significant traits and analyze QTL was Haschizume et al (2003). The use of different genetic backgrounds in these studies makes the transfer of markers from one population map to another difficult and limits their usefulness in elite x elite crosses. A map based on a population derived from two cultivated varieties would also be more useful for mapping QTL of the horticulturally significant traits targeted in most breeding programs.

Each of the previously described studies has had to circumvent the lack of diversity between watermelon cultivars (Levi et al., 2001b) by using intersubspecific crosses to develop mapping populations. SNPs are a basic and bountiful source of variation found within a species, existing in a higher abundance than the previously implemented polymorphisms used in marker development (Henry, 2008; Kole and Abbott, 2008). Previous maps created for watermelon have been based on older marker technology including isozymes (Navot and Zamir, 1987; Zamir et al., 1984), RAPD (Hawkins et al., 2001), ISSR, SCAR (Hashizume et al., 2003; Levi et al., 2002; Zhang et al., 2004), AFLP, SRAP, and SSR markers (Levi et al., 2006) while maps for melon and cucumber have already made use of SNP marker technology for map extension and cultivar identification (Deleu et al., 2009; Fazio et al., 2003). With the exception of Zhang et al. (2004), the previous studies used F_2, BC_1, and testcross mapping populations. Through the development of a RIL population, the progeny become homozygous for most alleles, increasing the likelihood of separating tightly linked alleles which allows for increased segregation and QTL mapping potential. Another main advantage a RIL population provides is the ability to perform replicated trials, as every line is represented by many homozygous individuals. Genetic mapping and QTL analysis has been successfully performed in many other crops using RILs, including several horticulturally important cucurbits including pumpkin and squash, melon, and cucumber (Barchi et al., 2009; Causse et al., 2002; Fazio et al., 2003; Perin et al., 2002). The goal of this study was to utilize the extensive reservoir of genotypic diversity provided by the development of SNP markers for watermelon to map QTL for horticulturally important traits in elite x elite RIL population.

Materials and Methods

Plant material and trait evaluation

The mapping population was developed from a cross between two elite cultivars, Klondike Black Seeded (KBS) and New Hampshire Midget (NHM) (Figure 3.1). Seed for this study was provided by the Germplasm Resource Information Networks (GRIN) Southern Regional PI Station in Griffin, GA. The parents used in the cross were self-pollinated to produce parental seed for use in the field trial. The two parental cultivars were chosen based on a comparison of genotypic polymorphisms to other potential parental lines screened by our lab (unpublished data) and the significant phenotypic differences they presented. KBS cultivar is a later maturing (45 days from pollination) (Wehner, 2008a), smooth surfaced, medium sized, elongated, dark green fruit with a higher Brix. NHM is an early maturing (29 days from pollination) (Wehner, 2008a) cultivar slightly furrowed, mini sized, round, gray fruit with an lower Brix. The phenotyped population was composed of F_7 RILs, derived from repetitive self- pollination and single seed descent from the F_2 population created by selfing a single F_1 KBS x NHM hybrid plant. Tissue was collected for genotyping from the F_6 generation, parents, and the F_1 hybrid. The horticultural traits of the parents and the F_7 RILs were measured in a single year experiment in two locations, the University of Georgia's Plant Science farm in Watkinsville, Georgia and the Monsanto's field trial facilities in Woodland, California. The two locations are from this point on distinguished as the Georgia (UGA) and California (CA) locations. At both locations each RIL was represented by a row of at least 8 plants. The parental checks were replicated twice at the UGA location and were un-replicated at the CA location. At the UGA location traits were measured using fruit from 1-8 plants per RIL and at the CA location data was taken from 1 representative fruit per RIL. Data from the fruit of the same RIL were averaged for the UGA location to obtain a single value for each RIL. The horticultural traits phenotyped at both locations were fruit length (FL) measured as the distance (in cm) from the blossom-end scar to the pedicel attachment, fruit diameter (FD) measured as the maximum fruit width (in cm), rind thickness (in mm, using an OEM 6" electronic digital calipers) (RindT) , the percentage of soluble solids measured as the degree Brix (Brix) of the fruit's juice using a Master handheld refractometer (ATAGO Co., LTD, Tokyo,

Japan), and hollow heart (a void or cracked separation of the fruit flesh) (Maynard and Hopkins, 1999). HH was calculated at the UGA location from measurements (cm) taken of the length, width, and depth of the separation divided by the corresponding fruit dimension measurement. The three measurements were then averaged to calculate the area affected (HH). At the CA location, HH was rated on a scale of 1 to 9 with 9 being the greatest effect. The data from FL and FD were used to calculate fruit shape (FL:FD). Additional traits were phenotyped at the UGA location including the fruit weight (FW), the degree deformation by fruit surface furrowing (Fur) on a scale of 0-3 (0 being completely smooth and 3 being severely furrowed), and the number of days from sowing to the appearance of the first female flower (FFlower). The FW was transformed using log10 (TFW) to give a normal distribution when used for QTL analysis. Measurements for flesh firmness were also taken with a 1000g/ 10g penetrometer with a 8 mm probe (QA Supplies, LLC. Norfolk, Virginia).

Linkage map construction

Parental and F_1 DNA was extracted from lyophilized tissue using a modified CTAB extraction method (Murray and Thompson, 1980). Samples were quantified using a Quant-iT Picogreen DNA reagents kit (Invitrogen, Ltd. Paisley, PA), and diluted to a concentration of 50 ng/ml. The DNA samples were sent along with fresh leaf tissue samples collected from the F_6 plants to Monsanto's facilities in St. Lewis Missouri. There, DNA was extracted from the fresh tissue, and the DNA produced was run along with the parental and F_1DNA samples on an Illumina Golden Gate array (Illumina Inc., San Diego, CA). The 1,536 SNP markers used on this array were developed by the Monsanto Company, in collaboration with the University of Georgia. The results were scored and mapped at Monsanto, and the genotypes and mapping distances were provided for use in QTL analysis.

QTL analysis

QTL detection and analysis was performed using Windows QTL Cartographer (WinQTLCart) (Wang et al., 2010). The data collected at the two locations were maintained as separate sets of data for QTL analysis and were analyzed using the single marker analysis and composite interval mapping (CIM) functions. The threshold value for each trait was set empirically by running 1,000 permutations to

determine a LOD significance threshold at 5%. After the threshold was generated, the CIM analysis was run at a walk speed of 2cM. QTL were designated using the automatic QTL locator tool in WinQTLCart, with set parameters of a minimum of 5 cM separation and a minimum difference (from top to valley) of LOD3 between peaks. The location of the QTL borders at a confidence lever of LOD1 (0.05%) and the additive effect and proportion of the observed phenotypic variation attributable to each QTL (R^2) were provided in the WinQTLCart CIM results file.

Results and Discussion

Trait analysis

The parental values for the flesh firmness measurements were not significantly different, so were not useable for mapping. The distribution of the other traits measured in this RIL population generally exceeded the values for the parents, at both the low and high ends of the phenotypic spread (Table 3.1 and Figure 3.2). This is indicative of transgressive segregation and was observed for all but one trait at both locations (RindT at the CA location). Transgressive segregation is generally found at a higher level of incidence in intraspecific crosses then interspecific crosses (Rieseberg et al., 2003), so higher levels of incidence in this type of population is expected. However, what was not expected was that only 7 of the 13 transgressively segregating traits had antagonistic additive effects, the expected cause of trait transgression (Rieseberg et al. 2003). Of the 6 remaining traits at the two locations 2 (FL:FD and TFW) had only one analyzed QTL, and the remaining four had 2-3 analyzed QTL.

Trait correlations

The Pearson's coefficients of correlations were calculated for the phenotyping data between the two locations and between the traits at each individual location (Table 3.2). When correlations were calculated between the locations, the all traits except for Brix correlated strongly with each other. This supports our findings as none of the QTL analyzed for Brix at the different locations are in agreement. At both locations the FL, FD, and FL:FD were strongly correlated to one another. RindT, Brix, and HH were also found to correlate with FL and FD but not with FL:FD at both locations. The only other trait to correlate with FL:FD was Fur, which was only rated at the UGA location. RindT, Brix and HH were

found to correlate in at the UGA location but not at the CA location. These correlations support our findings of several co-localizing QTL for these traits.

QTL analysis

The elite x elite map consists of 379 markers arranged into 13 linkage groups (LG), with a total map distance of 1,357.74 cM and an average distance of 3.58 cM between markers (Figure 3.2). A total of 33 QTL were found for the six traits measured in both locations and the three traits measured only at UGA (Table 3.3, Figure 3.3, and Figure 3.4). The QTL were spread across ten of the thirteen linkage groups, with the majority found on LG 9 (27%) and LG 11 (39%). The range of the phenotypic variation described by the QTL is between 5.02% (CA FL:FD) and 69.57% (UGA FL:FD). The greatest number of QTL found for a trait was 4 (FFlower). Three traits, CA FL:FD , CA RindT and UGA TFW only had one analyzed QTL, however the most common number of QTL per trait was 3 (42.9%). While this is certainly higher than previous QTL analysis with this species (Hashizume et al., 2003), it is far fewer QTL per trait found for other crops, including cucumber (Barchi et al., 2009; Fazio et al., 2003).

Fruit length, Fruit Diameter, Fruit Weight and Rind Thickness

At both the UGA and CA locations a QTL for fruit length was found at the same location on LG 11 (Table 3.3, Figure 3.3). QTL for FL were also identified on LG 9, one for the UGA location and two for the CA location. The QTL for the UGA location and the second one from the CA location also map to the same area, the smaller QTL from the UGA population exists inside the area covered by the CA QTL. QTL mapped on LG 11 for both locations are flanked by the same markers. As expected, the additive effect in each QTL on LG 9 and LG 11 for length was contributed by the longer KBS parent. The QTL for FD were also located on LG 9 and LG 11 in both locations. The QTL on both LGs for FD from the UGA location exist within the flanking markers of the LGs for the FD QTL for the CA population. The additive effects of the QTL on LG 9 are contributed by the KBS parent and additive effect for the QTL on LG 11 were contributed by the NHM parent. The QTL found for both FL and FD from both locations are co-localized with the QTL found for TFW measured at the UGA location. This is to be expected as the traits had such a high correlation between locations (Table 3.2), and it lends confidence to our results.

The QTL for RindT on LG 9 for both locations is flanked by the same markers as the QTL for FL, FD, and TFW are located on LG 9. This co-localization of fruit characteristics could explain the correlation found between the FL, FD, TFW, and RindT as it suggest that the development of these fruit characteristics influence each other. The greater the length and width, the heavier the fruit and the thicker the rind as RindT was a measurement of rind thickness without considering its proportion to overall fruit size. A more representative method for measuring this trait may reduce the significance of the correlation between FL, FD, and TFW with RindT. The percentage of the phenotypic variation explained by the QTL on LG 9 is 26.95% at UGA and 13.47% at CA for FL, 57.50% at UGA and 46.63% at CA for FD, and 44.41% for TFW. While slightly larger amounts of the variation for length is explained by the QTL on LG 11, 30.72% at UGA and 38.72% at CA, a much lower percentage is explained for width, 9.67% at UGA and 6.90% at CA. This would mean that breeding programs focusing on changing the FL or FD would want to focus on the QTL found on LG 9 for modifications to FL and LG 11 for modifications of FD. The QTL on LG 9 for TFW is located within the QTL for the CA location, but ends at the same point as the QTL for the UGA population. This QTL may be useful for developing heaver fruit, but if the goal were to develop heaver round fruit the affect the co-localized QTL for FL could have would need to be considered.

Fruit Shape

Fruit shape was defined in this study as FL:FD. Although this ratio does not translate well into the standard shapes used by watermelon breeders (Wehner, 2008b) it does describe the larger difference between round and elongated shapes. A more rounded shape is usually desired by consumers, which may be due in part to an increase in the edible and seedless area of consumable fruit tissue. The largest value for FL:FD was 2.73 and the smallest value was 1.00, both found at the CA location. Higher ratio values correspond to an elongated fruit, as its length is much greater than its width, and the smaller the difference between the length and the width the more rounded the fruit was. The QTL found for FL:FD for the UGA location was on LG 11, while the three QTL found for the California location are on LG 10 and LG 11. The QTL on LG 11 explains more than 69.75% and 62.01% of the phenotypic variation in the UGA and

CA locations respectively. The additional QTL on LG 10 and LG 11 from the CA location was contributed by an allele from the NHM parent and explains 5.02% and 13.66% of the phenotypic variation. The QTL on LG 11 for UGA and the second QTL on LG 11 for CA overlap, having the same flanking markers. In cucumber it was found that environment can have a negative effect on the ability to detect QTL found for fruit shape (Fazio et al., 2003). This can be seen in the present study by the greater variation in fruit FL:FD and higher number of QTL analyzed for the trait (Table 3.1) at the CA location. The FL:FD ratio could potentially be mitigated by environmental stress by suppressing fruit enlargement in watermelon as was the case with cucumber (Fazio et al., 2003). The FL:FD QTL could be used in selecting for shape specifically as it is independent of weight in correlation and QTL location (Table 3.2, Figure 3.3), where FL and FD are not.

The region LG 11 QTL for FL:FD is associated with is also where QTL for FL on LG 11 is located. These two QTL overlap with the QTL on LG 11 for FD at both locations and the QTL for Fur spans across this area, overlapping all seven of the other QTLs on this arm of LG 11. Of the three other traits found in this region, Fur is negatively correlated with FL and FL:FD and the additive effects of these QTL are from alleles from different parents. The Fur QTL on LG 11 only explains 15.8% of the variance and there are several other QTL associates with this trait (Table 3.3). As some subjectivity from the method used for classifying the degree of Fur could have an effect on the QTL analyzed, but the fruit fell distinctly into the four categories used. Both the areas on LG 9 and LG 11 where several traits have co-localized could be analyzed with finer mapping to determine if the co-localization is due to linkage or a pleiotropic effect.

Brix, First Female Flower, Hollow Heart

Two and three QTL were found to be associated with Brix at the UGA and CA locations respectively (Table 3.3, Figure 3.2). None of the QTL were common across locations. Brix is the standard method for analyzing total sugar content, as reports state that almost 90% of the soluble solids measured in watermelon juice consist of sugars (Kurata, 1971; Wehner, 2008a). While Haschizume et al. (2003) found one significant QTL for the trait, it is thought to be polygenic and heavily affected by growing

conditions (Hashizume et al., 2003), which is supported by the lack of correlation between the two locations for this trait. The plants evaluated by Haschizume et al. (2003) were grown in the greenhouse under controlled environmental conditions, which may not have allowed for the detection of QTL contributing to Brix under different environmental conditions. This trait is also heavily dependent on fruit maturity, and any variation between the ripeness evaluation between the UGA and CA populations may have altered the results. The development of a more standardized method for harvesting ripe fruit may decrease the difference between the two locations. The type of population used could also affect the number of QTLs to be discovered. Haschizume et al (2003) used a BC_1 population from a cross between a cultivated variety and a citron type, where citron type watermelons are characterized by their extreme bitterness and low (2-4) Brix, while the present study used an elite x elite population. The relative difference in the degrees Brix between the parents used in this study was not as extreme as that found in the Haschizume et al. (2003) study.

The phenotypic data for Brix is correlated with TFW, HH and FFlower at the UGA location. Both HH and FFlower are economically significant traits to growers. HH is greatly affected by environment, but has occurred in all growing areas (Maynard and Hopkins, 1999) and is selected against in breeding programs since it produces a lower quality fruit. FFlower is thought to affect earliness. Earliness is a desirable trait because pricing for watermelon tends to be the best at the beginning of the season (Wehner, 2008a) and is measured as the number of days from pollination. The length of time from seeding to the appearance of a female flower also effects the time from seeding to harvestable fruit, and could possibly be combined with the earliness trait to shorten the growing season. The Brix QTL on LG 9 is in the same area that is overlapped by TFW, and is also overlapping with the HH trait. The HH QTL on LG 9 is located between the same flanking markers as TFW and the Brix QTL is flanked with the same starting marker ends with a more distant flanking marker. Brix is also correlated with the FFlower trait, and the two have overlapping QTL regions on LG 11 for TFW. The environmental effect of this trait is clearly illustrated as the data for HH collected from the CA location did not produce a significant QTL in contrast to the 3 found for the UGA location. As HH and sugar content are two of the more important attributes

breeders select for, a study encompassing multiple years and locations is probably needed to confidently map these traits.

Several of the QTL found in this study have potential to produce valuable tools for a watermelon molecular breeding program. QTL in sections of LG 9 and LG 11 could be used to select for fruit length, width, shape, and weight. These areas would be valuable for the development of molecular tools for multiple trait breeding strategies. Although more research would be needed to confirm and further explore QTL for FL:FD, Brix, FFlower, and HH, this study has shown that it is possible to map these traits in an elite x elite population. Since these are all economically important for production they would be valuable areas in which to continue research efforts.

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Table 3.1 Trait means and standard deviations for the horticultural traits measured in the parental lines and F_7 RILs at the Georgia (UGA) and California (CA) locations.

	UGA			CA		
Trait	*KBS*	*NHM*	F_7	*KBS*	*NHM*	F_7
Fruit Length	32.19 ± 3.83	16.16 ± 1.0	13.44-43.46 ± 6.19	31.24	16.51	13.97-41.91± 5.75
Fruit Diameter	20.65 ± 0.81	13.3 ± 0.92	9.00-37.20 ± 3.04	19.05	14.73	8.38-26.67± 2.83
Shape (FL:FD)	1.56 ± 0.18	1.22 ± 0.07	1.05-2.44 ± 0.38	1.64	1.12	1-2.72± 0.40
Rind thickness	12.43 ± 2.29	4.26 ± 0.8	2.47-19.48 ± 4.07	25.40	7.62	2.54-50.8 ± 1.21
BRIX	10.19 ± 0.94	8.53 ± 0.85	6-11.5 ± 1.06	10.50	8.90	7.5-12.8± 1.2
Hollow Heart	0 ±0	0.022 ± 0.08	0-1.16 ± 0.19	1.00	1.00	1-9 ± 1.31
Weight	16.9 ± 2.85	3.75 ± 0.64	2.0-20.67 ± 4.08	-	-	-
Furrowing	1.33 ± 0.63	0.59 ± 0.51	0-2.67 ± 0.56	-	-	-
First female flower	61.33 ± 4.24	46.73 ± 7.63	38.67-65.75 ± 5.87	-	-	-

Table 3.2 Pearson's correlations between traits assessed in the KBX x NHM F_7 population between the two locations (A), at the Georgia (B), and at the California (C). Traits analyzed were log-transformed fruit weight (TFW), fruit length (FL), fruit diameter (FD), ratio of FL:FD (FL:FD), degree of furrowing (Fur), rind thickness (RindT) , number of days to the first female flower (FFlower), degrees Brix (Brix), and presence hollow heart (HH). Grayed boxes indicate a significant (P<0.05) correlation.

A.

Trait	FL	FD	L:W	RindT	Brix	HH
FL	0.86	-				
FD	0.22	0.80	-			
FL:FD	0.59	-0.31	0.87	-		
RindT	0.33	0.54	-0.06	0.51	-	
Brix	0.10	0.04	0.05	-0.06	0.13	-
HH	0.14	0.02	0.13	0.06	0.02	0.32

B.

Trait	FL	FD	FL:FD	RindT	Brix	HH	TFW	Fur
FD	0.44	-						
FL:FD	0.70	-0.31	-					
RindT	0.54	0.81	-0.06	-				
Brix	0.33	0.47	0.00	0.38	-			
HH	0.32	0.35	0.07	0.41	0.17	-		
TFW	0.77	0.89	0.12	0.80	0.48	0.37	-	
Fur	-0.24	0.13	-0.35	0.11	-0.07	-0.03	-0.05	-
FFlower	0.07	0.10	0.00	0.05	0.22	0.05	0.10	-0.01

C.

Trait	FL	FD	L:W	RindT	Brix
FD	0.18				
FL:FD	0.75	-0.49			
RindT	0.29	0.47	-0.05		
Brix	0.05	-0.06	0.06	0.00	
HH	0.22	0.17	0.02	0.06	-0.06

Table 3.3 QTL analized for KBS xNHM F_7 RIL populationc grown in Georgia and California

Trait	Location	Linkage group	Flanking markers	cM	location	distance	LOD	additive effect*	R^2
Length	UGA	9	NW0249226-NW0248056	77.7	75.6-92.6	3.9	19.91	-3.32	26.95
		11	NW0248107-NW0250956	15.3	13.3-17.7	6.7	20.58	-3.48	30.72
Length	CA	9	NW0249065-NW0250857	62.8	60.5-66.3	13.4	3.98	-1.41	5.96
		9	NW0249046-NW0248574	79.7	66.3-79.7	9.2	10.05	-2.16	13.47
		11	NW0248107-NW0250956	13.3	13.3-19.5	7.9	21.08	-3.58	38.72
Width	UGA	9	NW0249226-NW0251320	79.7	77.2-88.5	5.5	31.05	-2.34	57.50
		11	NW0250945-NW0249247	8	2.3-10.6	12.4	7.76	0.96	9.67
Width	CA	9	NW0249046-NW0249974	81.2	79.6-100.7	7.7	25.24	-1.94	46.63
		11	NW0250945-NW0249247	11.3	7.0-13.3	9.9	5.58	0.77	6.90
L:W	UGA	11	NW0248107-NW0250956	15.3	13.3-18.7	5.4	39.80	-0.32	69.57
L:W	CA	10	NW0250229-NW0249853	35.1	30.3-40.5	12.3	4.30	0.89	5.02
		11	NW0250945-NW0249247	10	8.0-11.0	5.3	9.09	-0.19	13.66
		11	NW0248107-NW0250956	15.3	13.3-18.2	6.8	29.96	-0.32	62.01
Rind thickness	UGA	6	NW0249733-NW0247929	183.8	177.5-184.2	1.1	7.86	1.22	8.82
		6	NW0250872-NW0248728	191.6	190.7-199.7	17	4.63	1.01	5.15
		9	NW0249046-NW0248574	75.7	74.6-77.9	3.9	35.91	-3.28	59.18
Rind thickness	CA	9	NW0249226-NW0249883	81.2	79.7-89.6	3.8	14.18	-3.35	34.43
BRIX	UGA	8	NW0248647-NW0250012	14.7	9.9-22.1	20.7	3.30	0.30	7.85
		9	NW0249226-NW0251320	81.2	77.3-85.8	13.5	3.72	-0.30	8.09
		11	NW0248648-NW0248282	79.9	74.9-87.0	9.8	4.11	-0.46	11.13
BRIX	CA	12	NW0250563-NW0249244	33.6	24.6-34.9	12.1	2.78	-0.36	6.55
		13	NW0249941-NW0248316	0.8	0.0-9.4	14.7	3.16	-0.32	7.17
Hollow Heart	UGA	3	NW0248693-NW0251213	136.9	133.9-138.4	8.2	3.60	0.05	36.79
		4	NW0251313-NW0249735	134.4	132.1-136.5	5.9	2.92	0.05	6.31
		9	NW0249046-NW0248574	75.7	74.6-78.3	6.5	8.77	-0.09	19.61

Table 3.3 continued

Weight	UGA	9	NW0249046-NW0251320	77.7	75.7-84.7	4.1	22.39	-0.19	44.41
Furrowing	UGA	9	NW0248382-NW0250470	8.6	4.8-13.2	15.9	3.97	-0.17	8.52
		10	NW0248662-NW0248181	32.3	27.2-35.6	12.7	4.49	-0.18	10.22
		10	NW0248876-NW0248591	44.6	44.1-45.1	4.5	3.37	-0.16	7.60
		11	NW0250945-NW0250956	11.3	9.1-16.7	15.8	5.04	0.19	11.16
Days to First Female Flower	UGA	6	NW0248146-NW0248371	231.1	225.0-237.2	14.2	5.10	1.75	8.72
		7	NW0249310-NW0251102	130	124.6-134.5	13.5	4.10	-1.57	67.60
		11	NW0248648-NW0248282	81.1	79.9-89.5	6.2	16.84	-3.35	31.57

*A positive additive effect is from a NHM allele and an negetive (-) additive effect is from a KBS allele

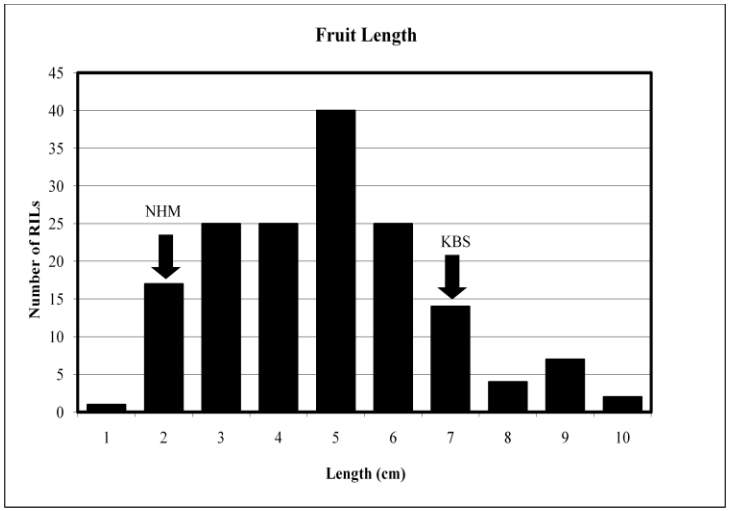
Figure 3.1 Fruit from the elite x elite parents. Klondike Black Seeded (A) and New Hampshire Midget (B).

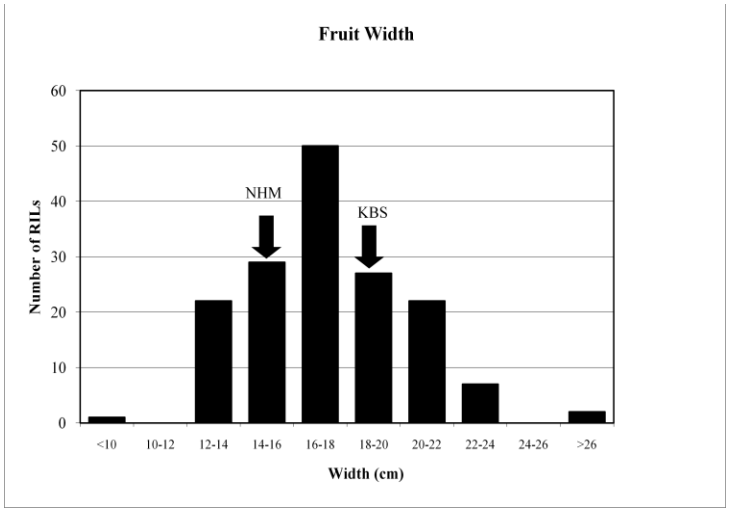
A.

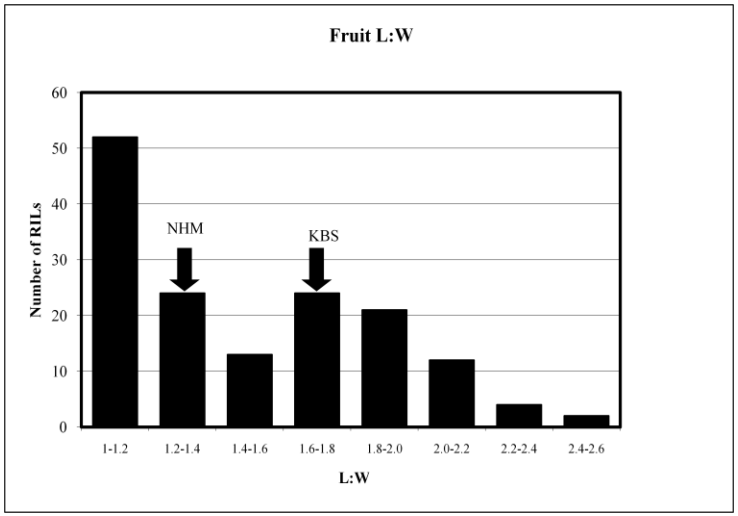
B.

Figure 3.2 Frequency distributions for phenotyped traits at the CA (A) and UGA (B) locations.

A. California trial







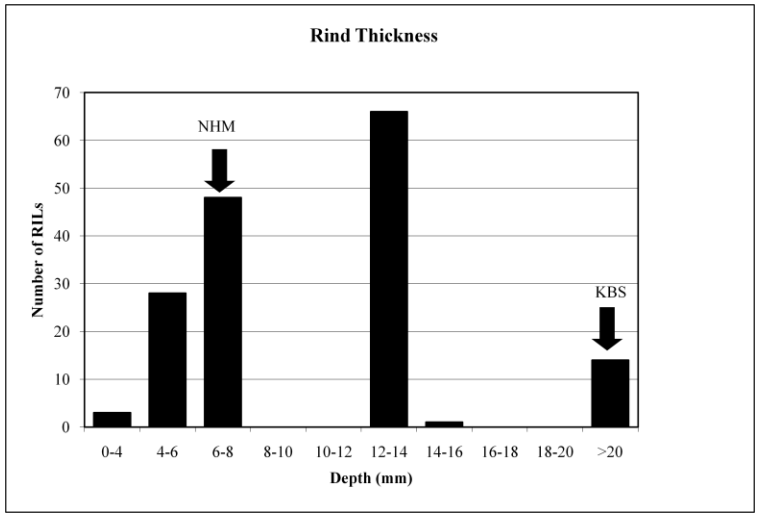
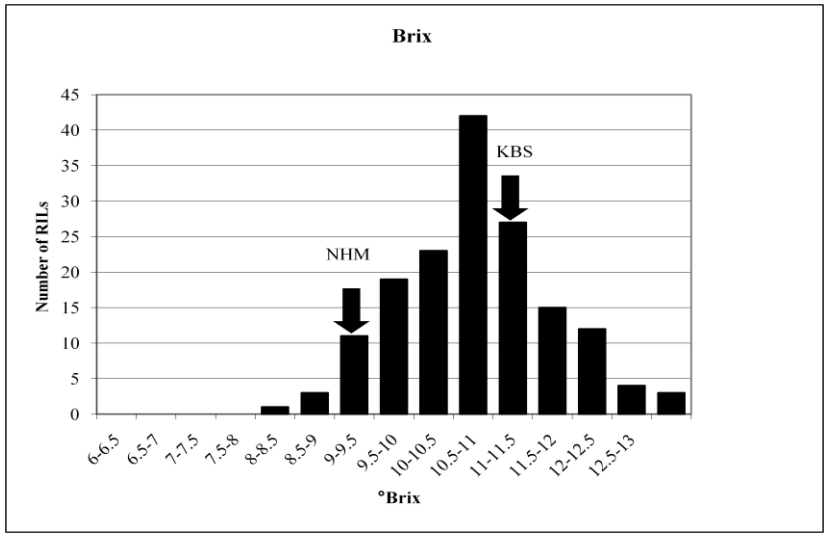
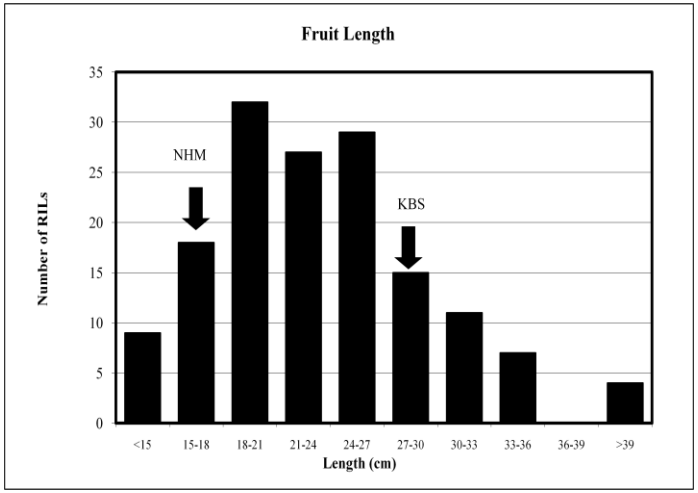


Figure 3.2 continued



B. UGA trial



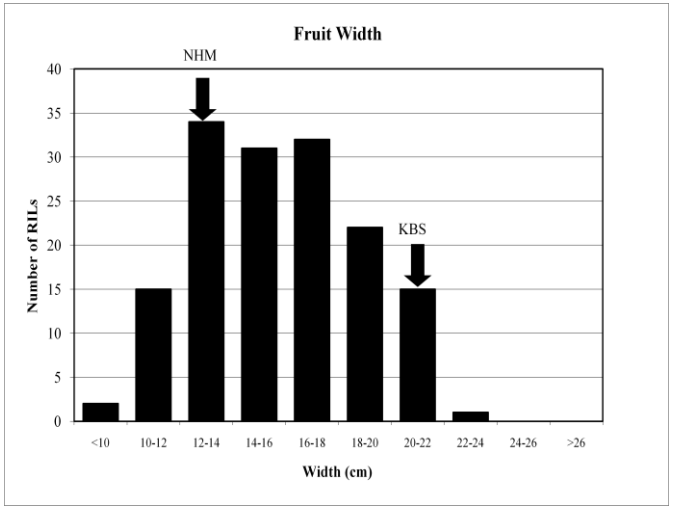
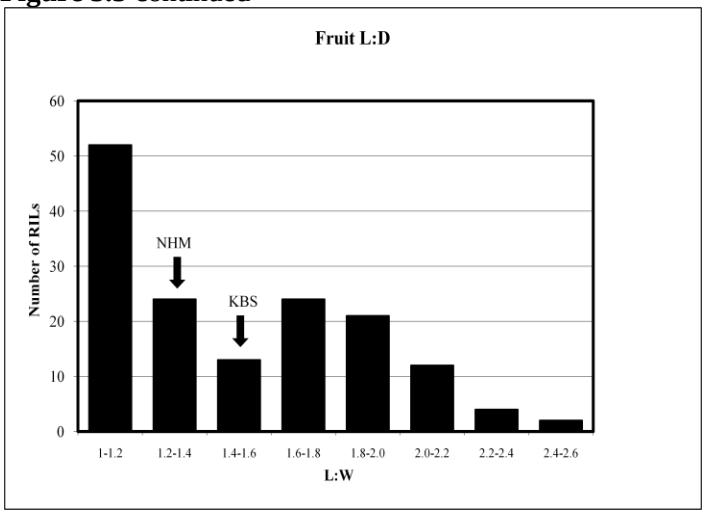
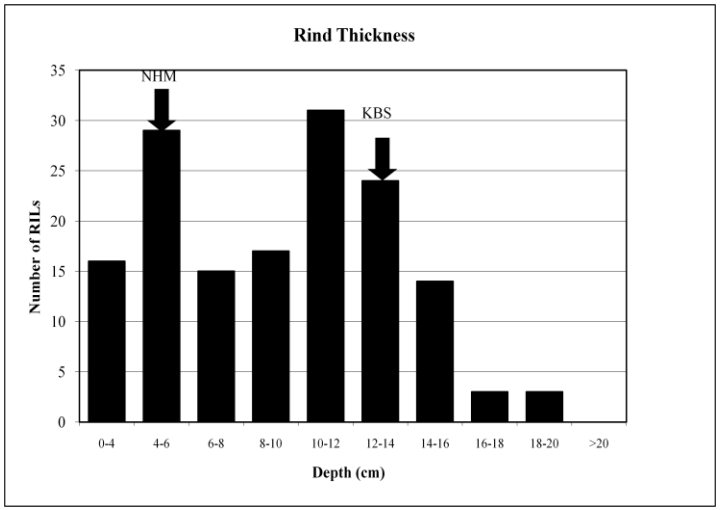
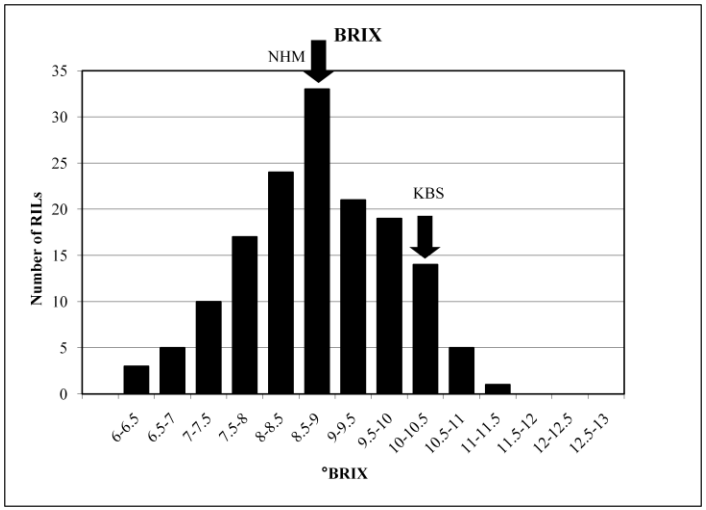


Figure 3.3 continued







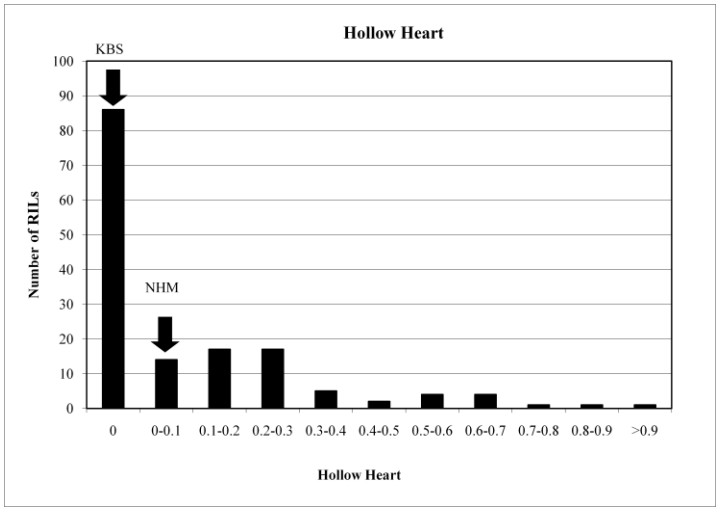
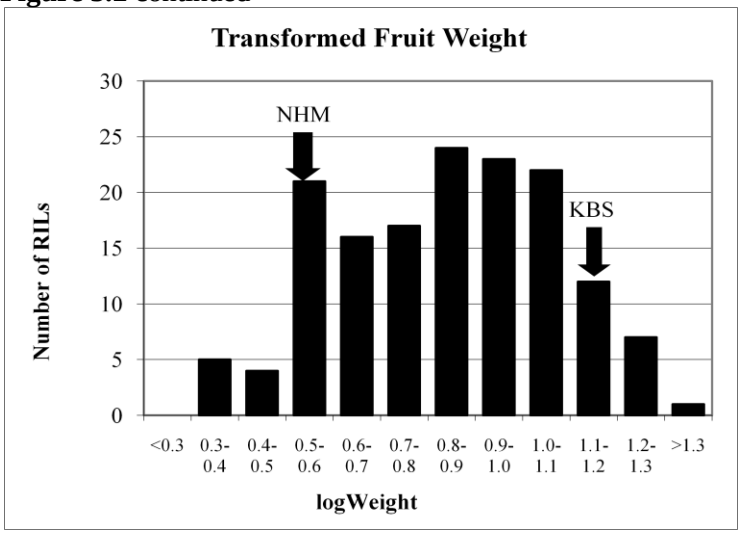
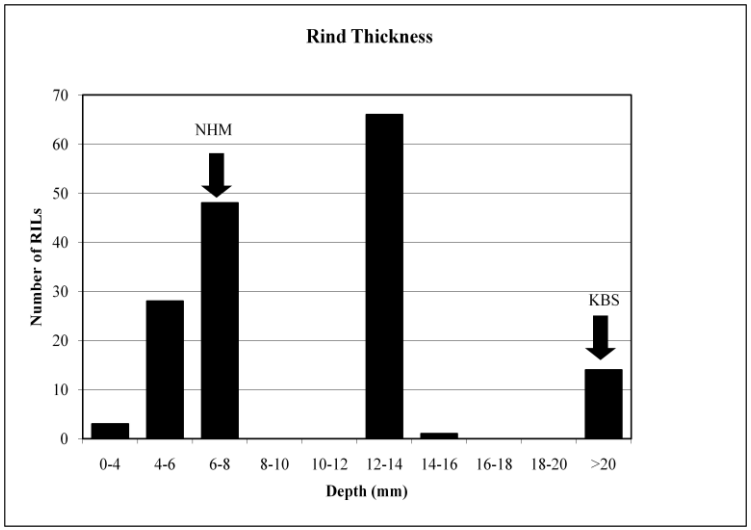
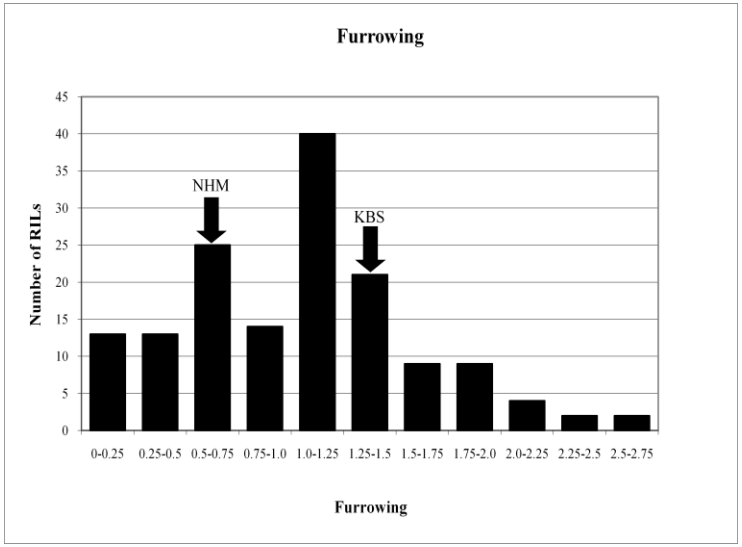


Figure 3.2 continued







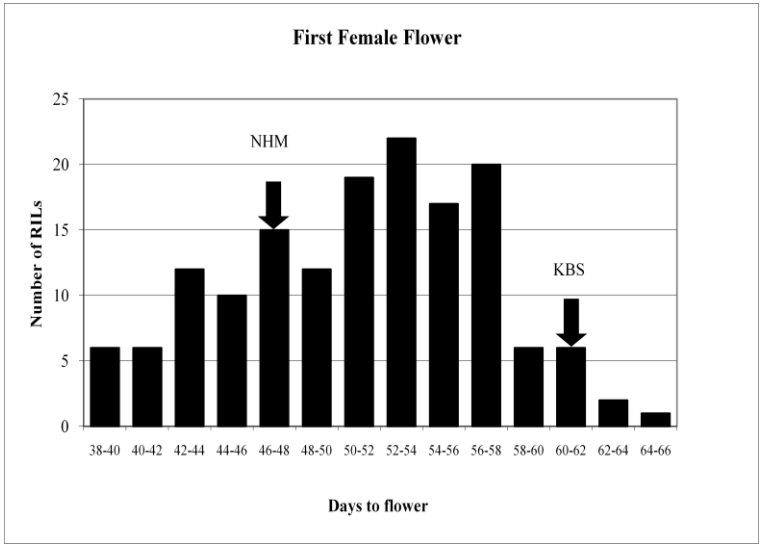
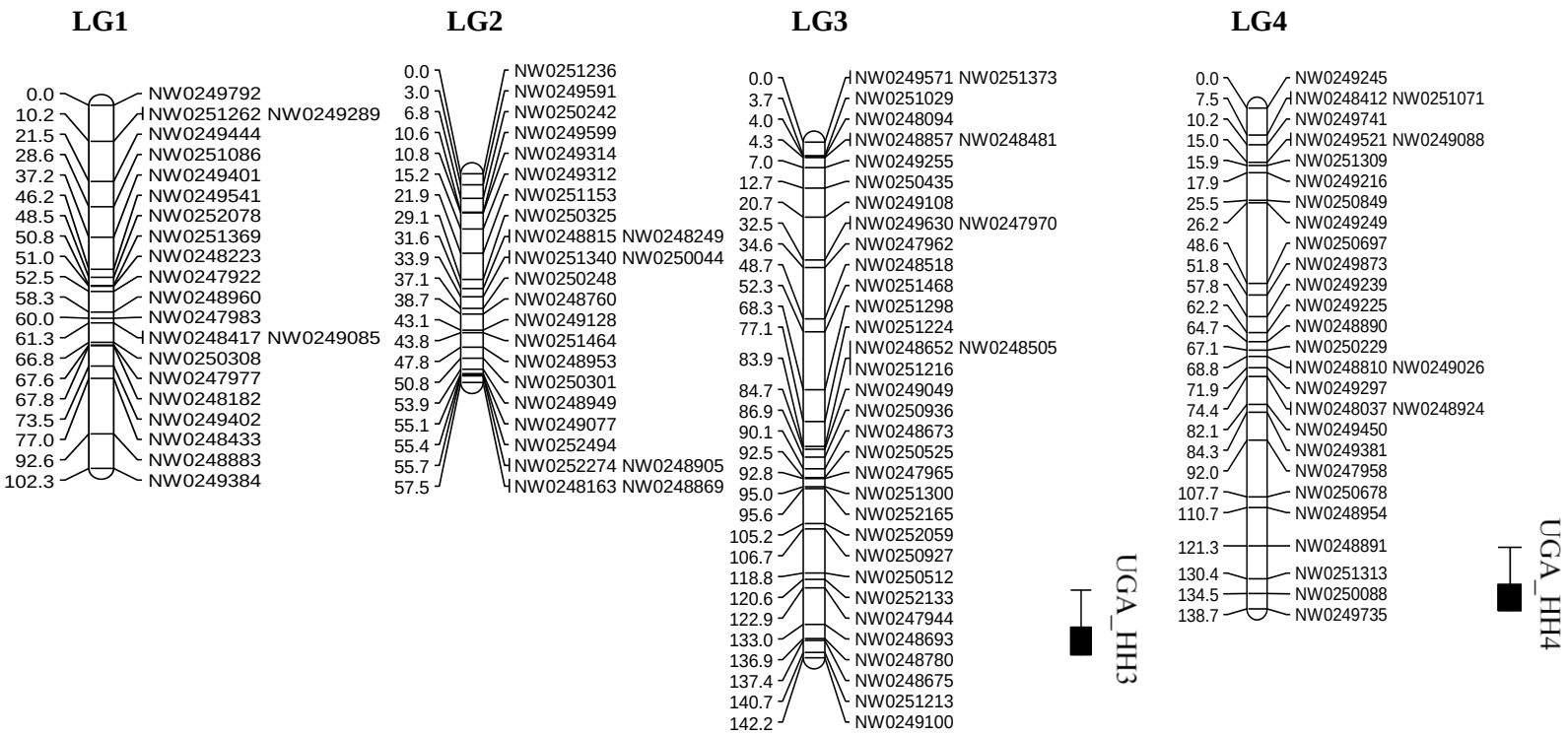


Figure 3.3 KBS x NHM F_7 SNP linkage map and positions of significant QTL



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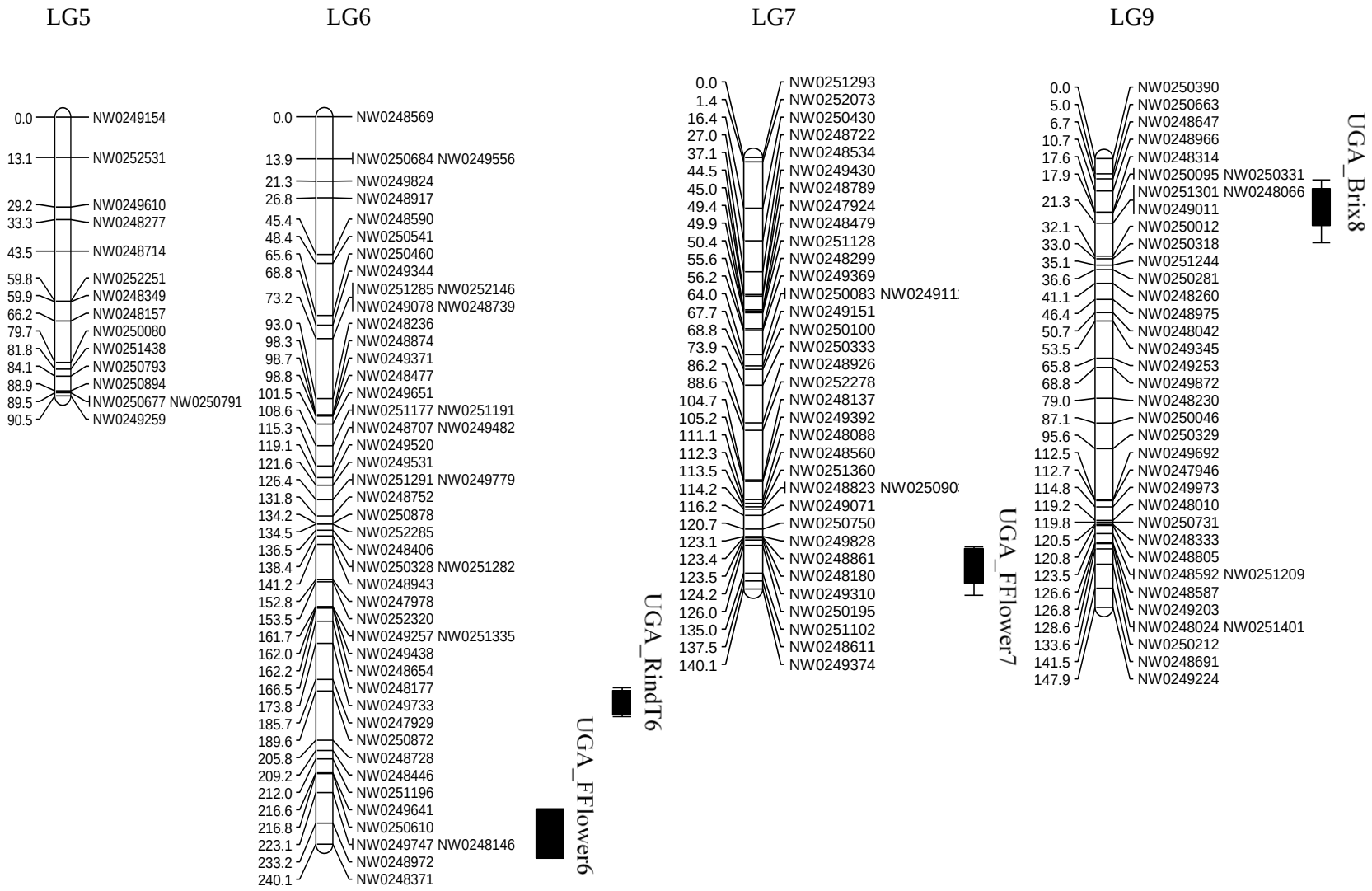

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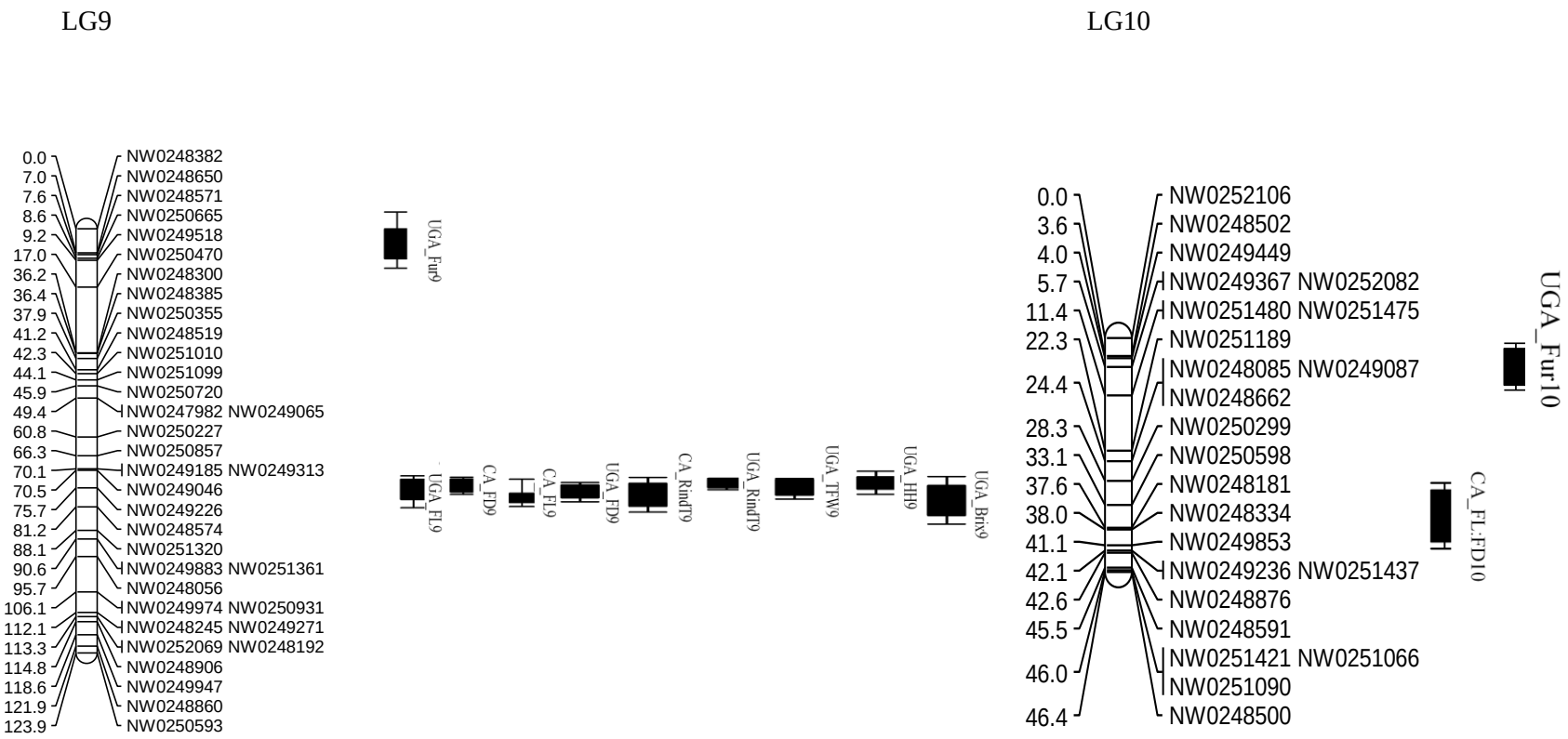
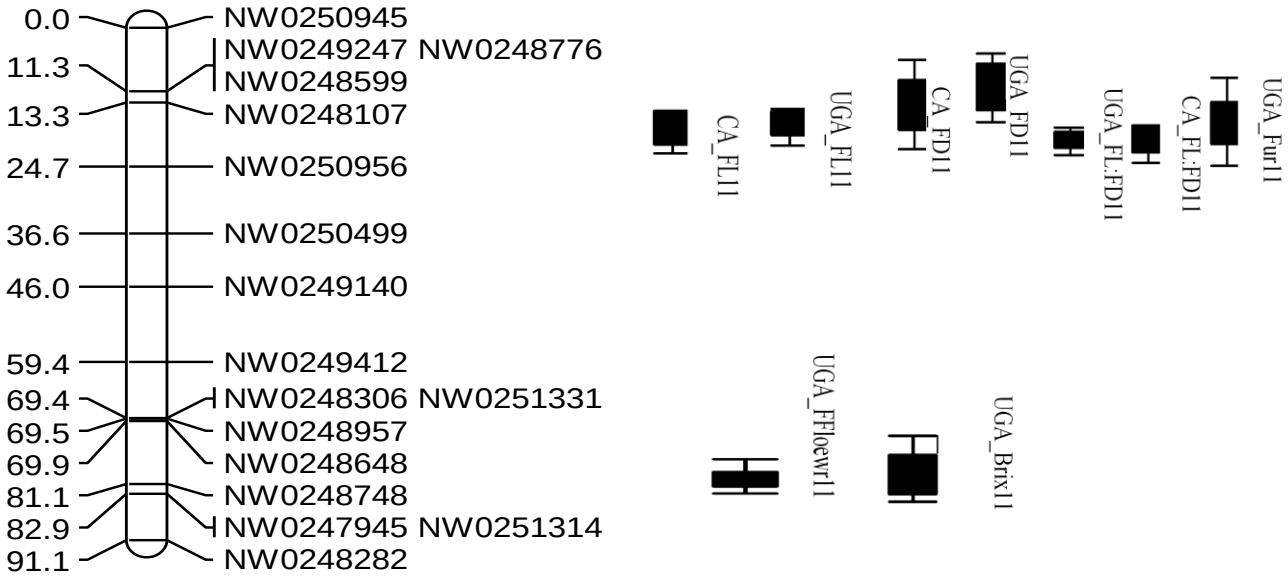


Figure 3.3 continued

LG11



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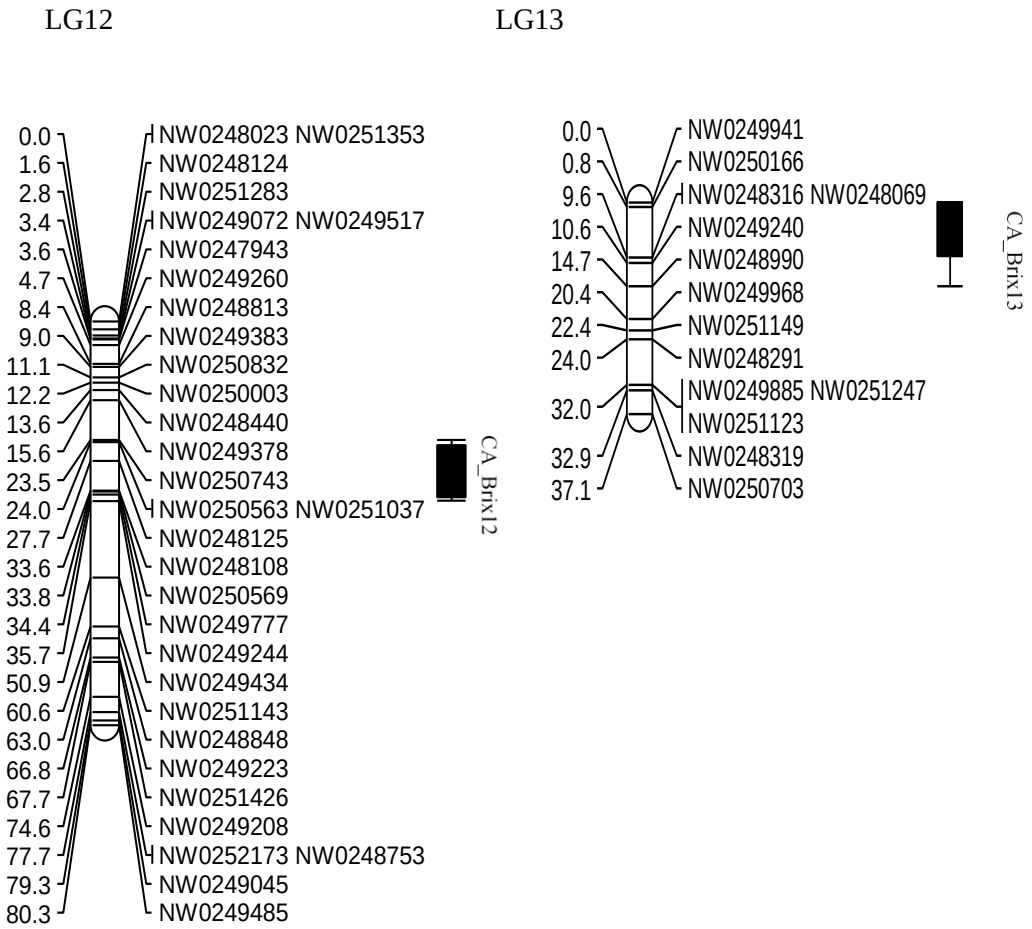
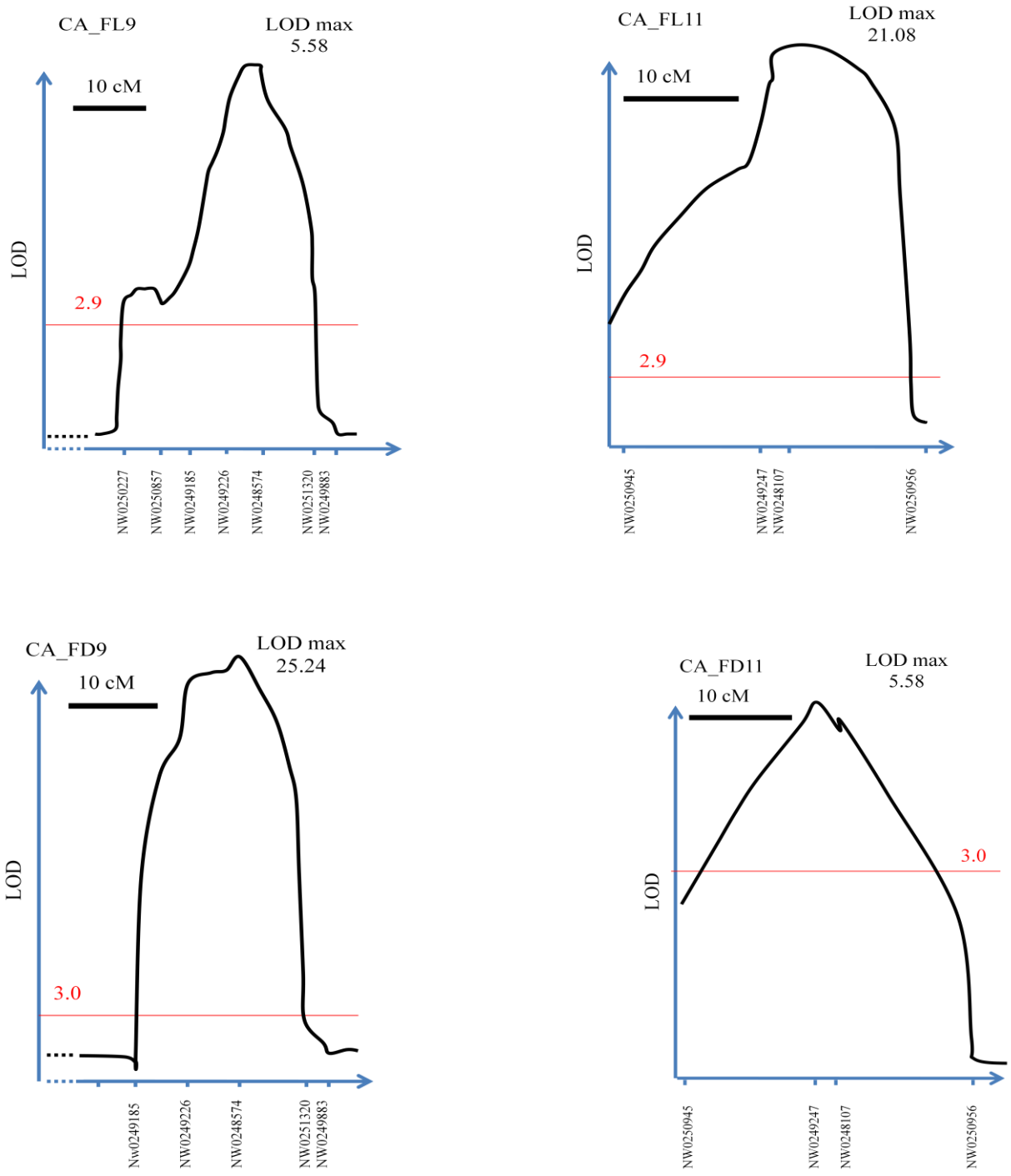


Figure 3.4 Linkage groups and QTL for the elite x elite F_7 RIL population. The number and red line represent the confidence intervel of 0.05% caluclated for the trait by 1,000 permutations. Marker positions are shown along the x-axis of the graph.

A.



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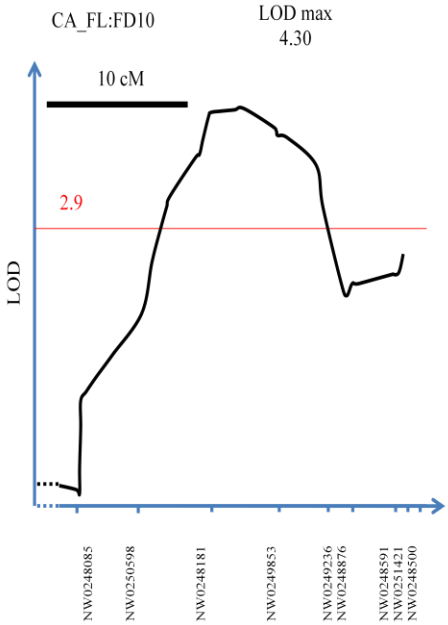

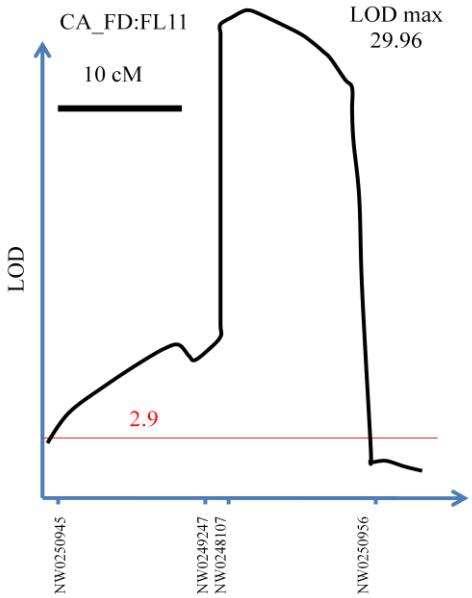

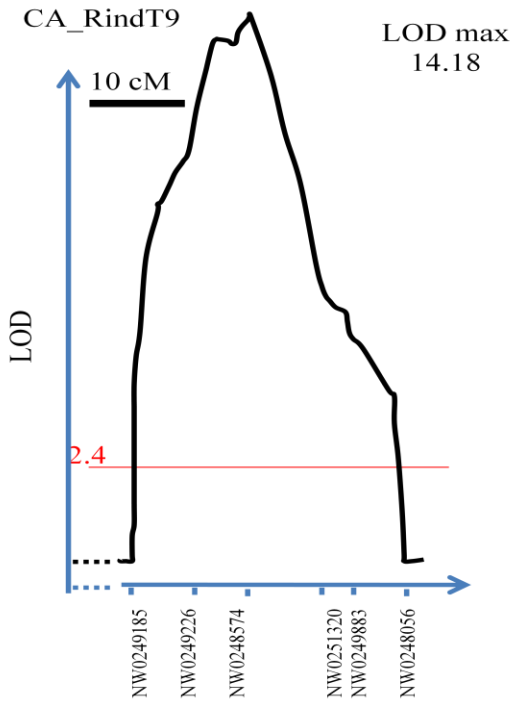

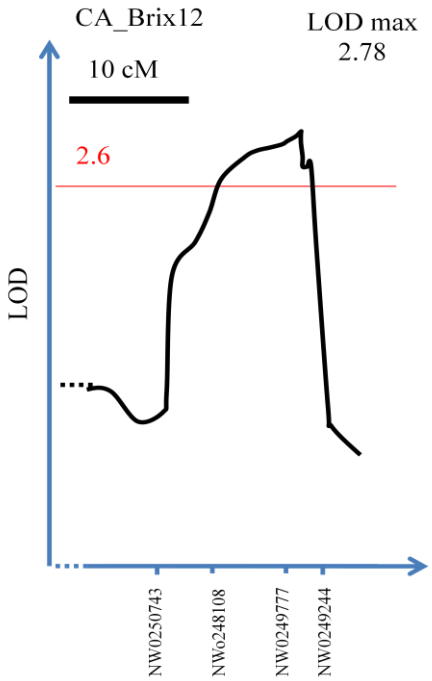

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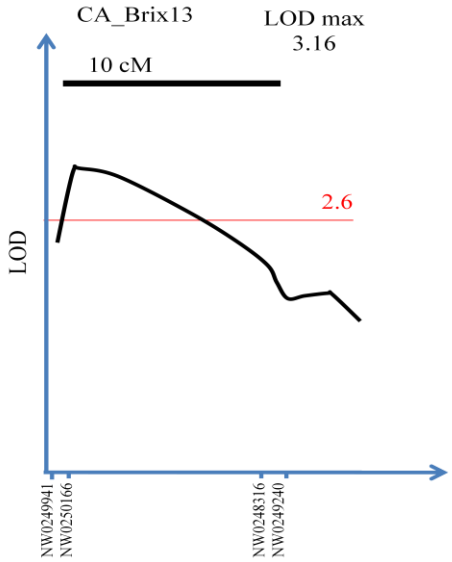
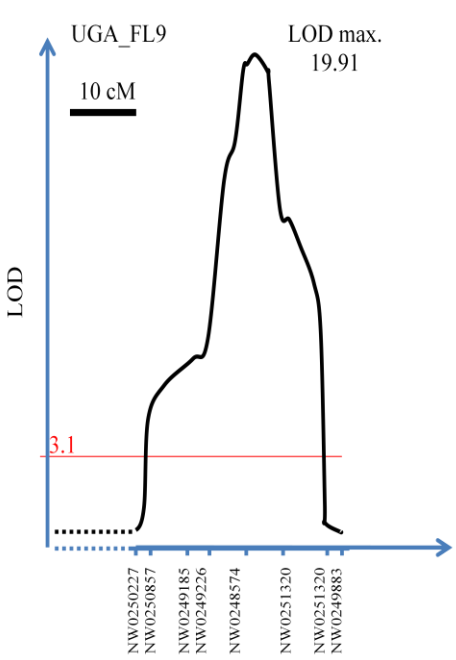
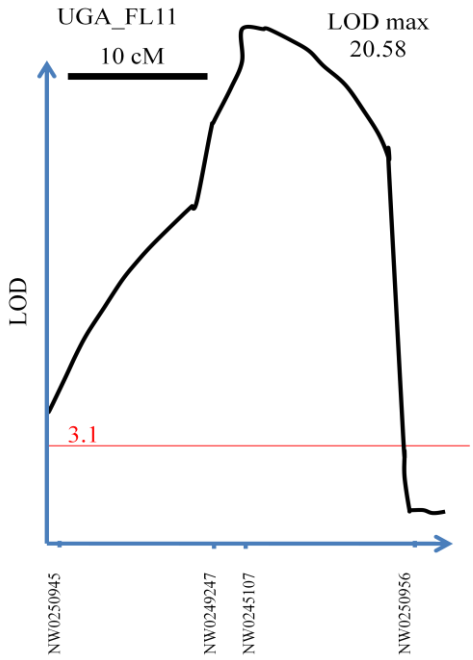


B.





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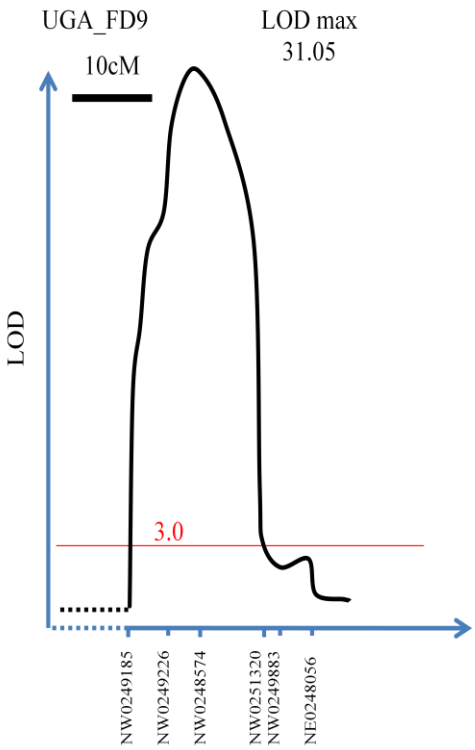

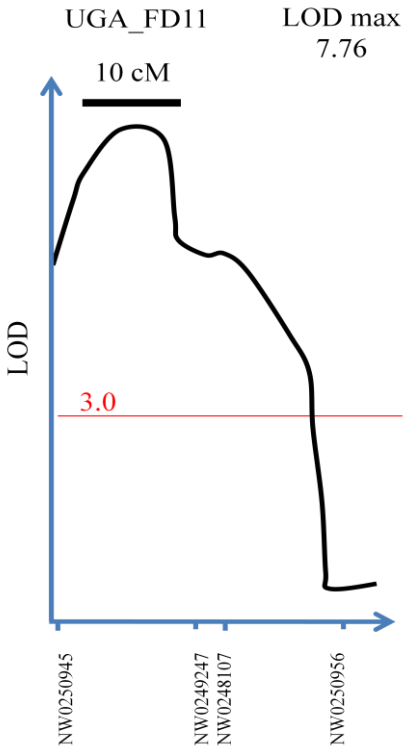

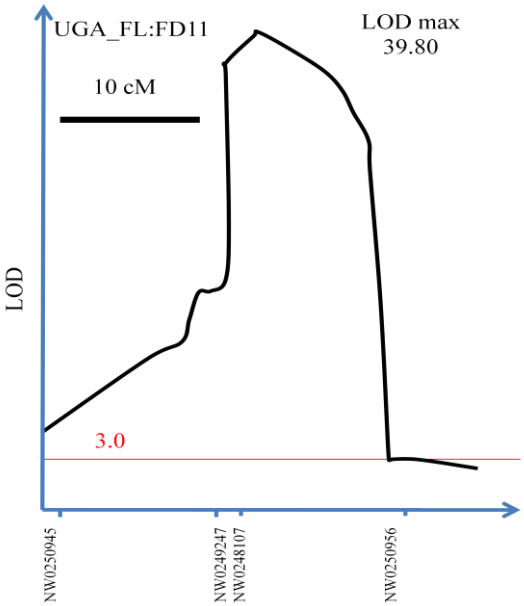

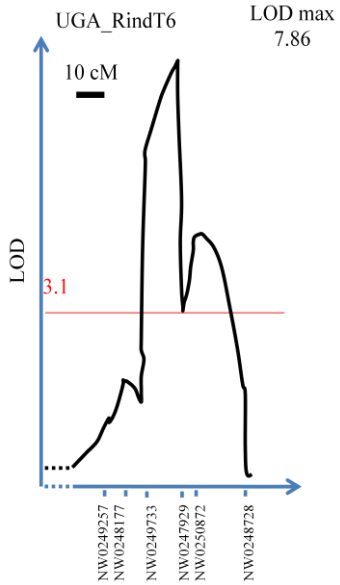

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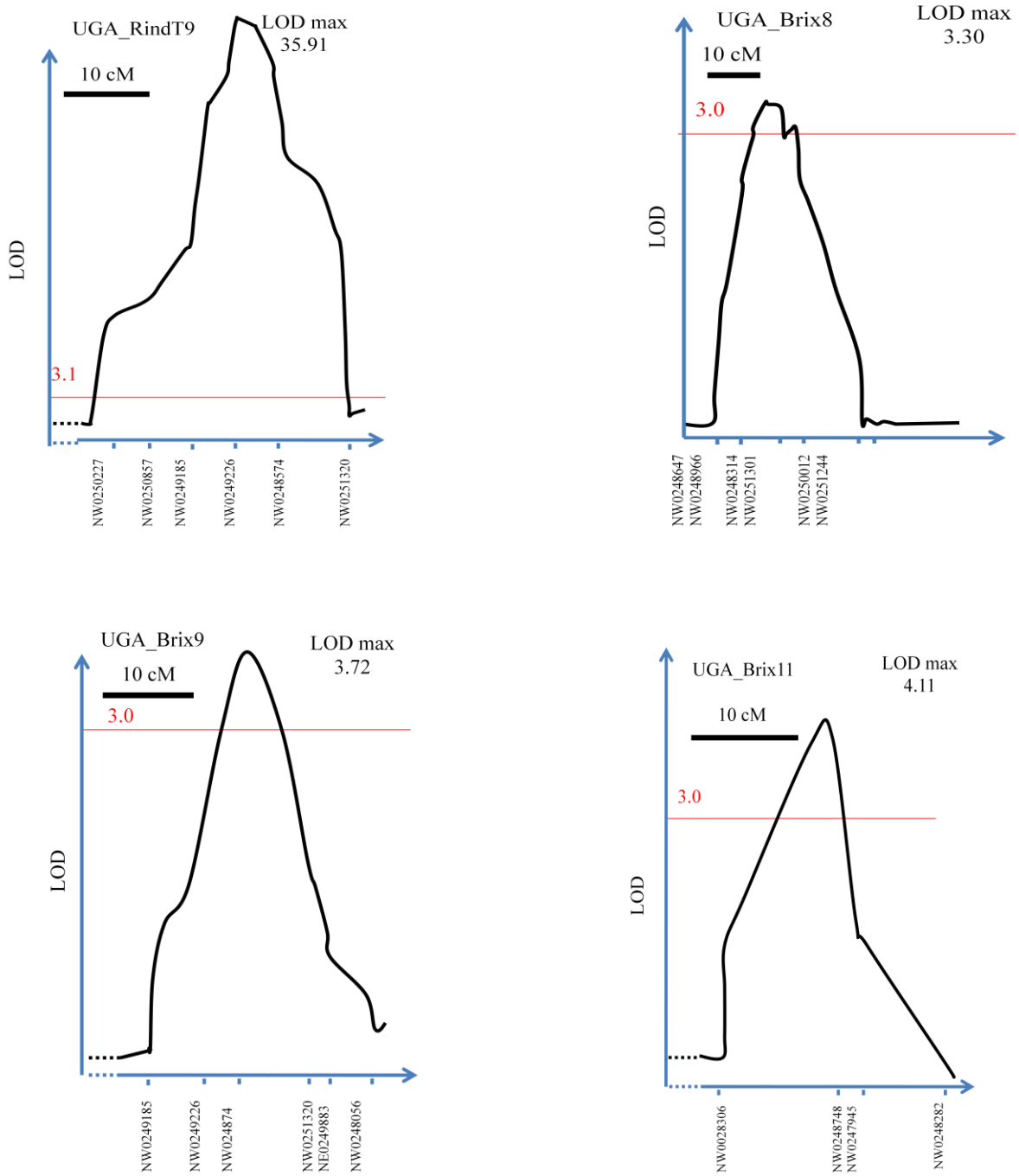

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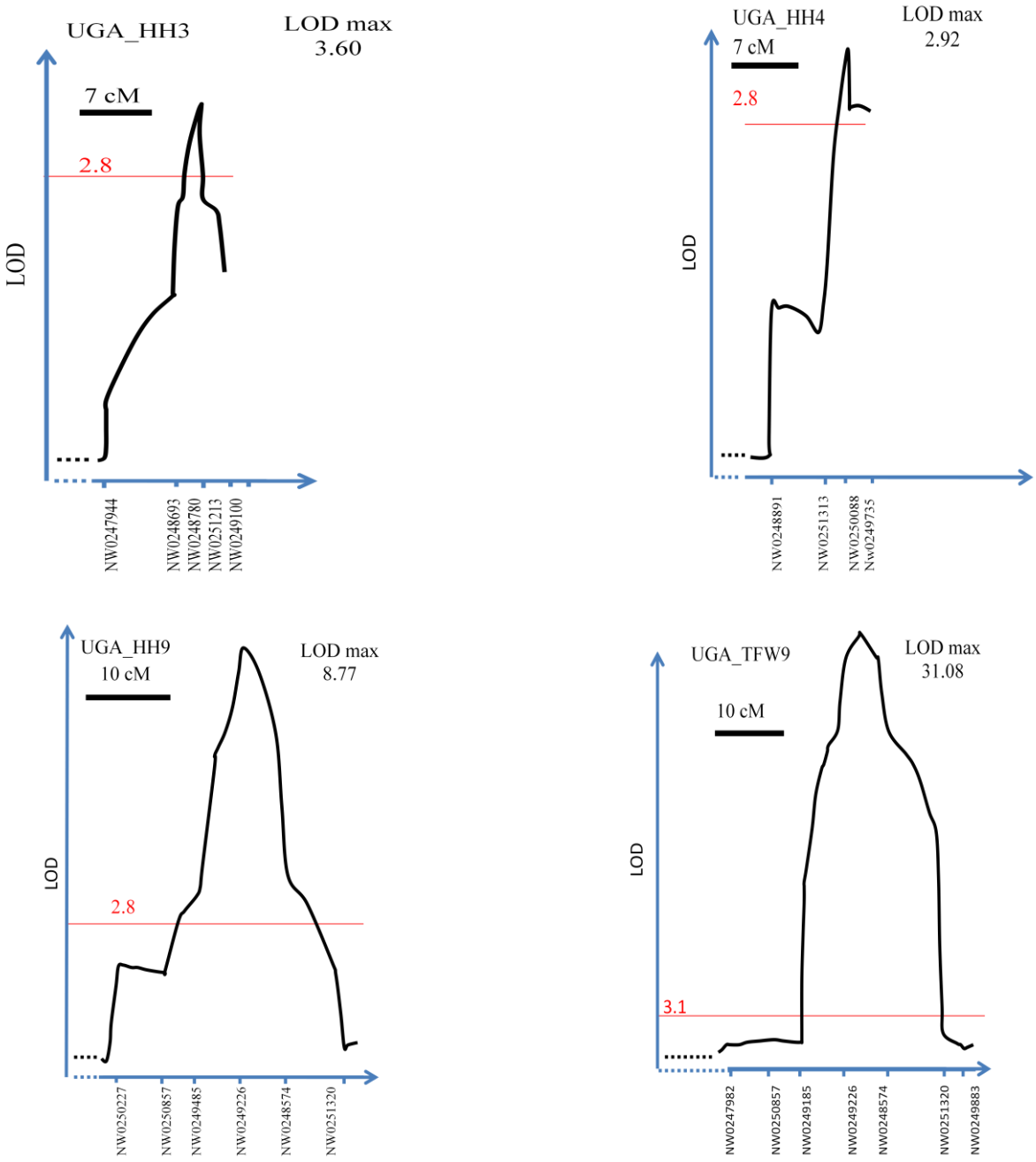

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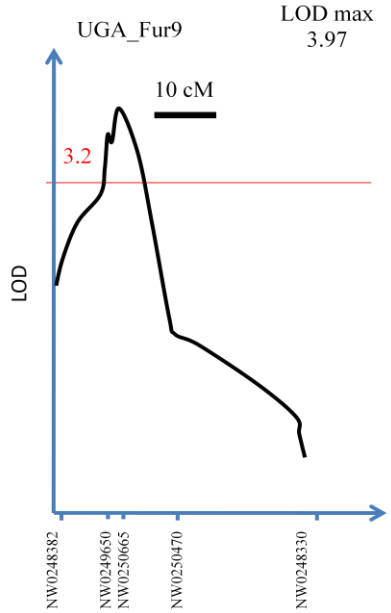

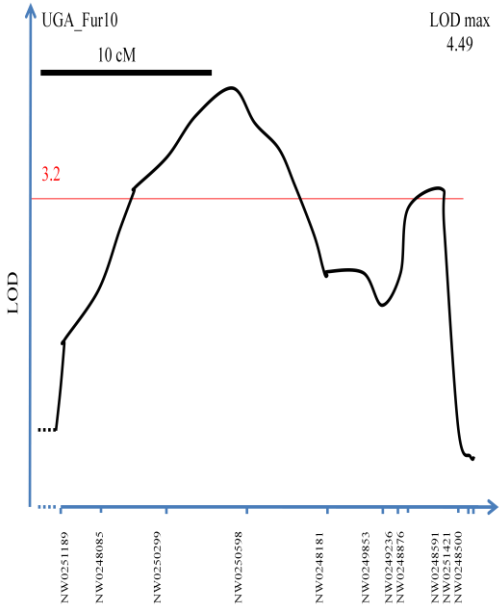

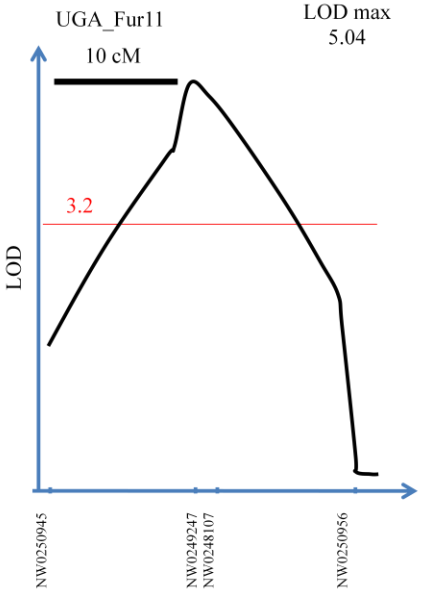

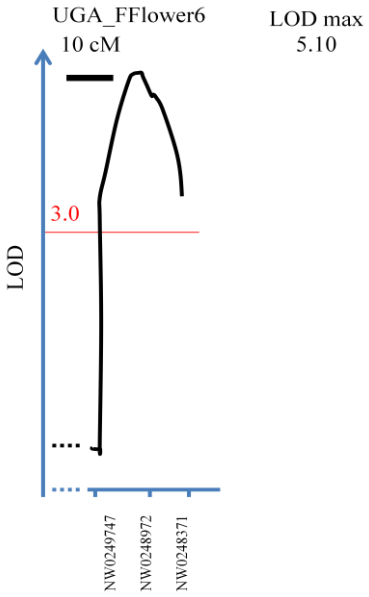

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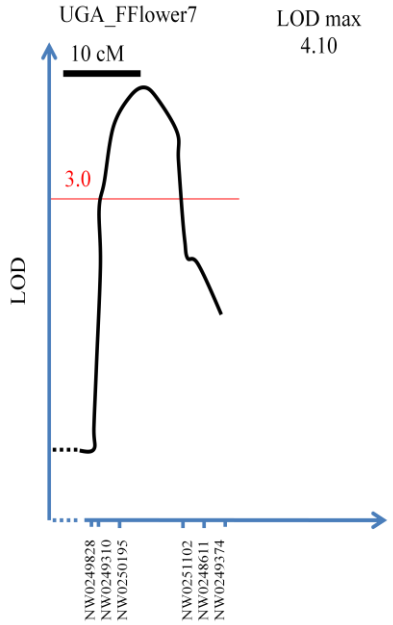

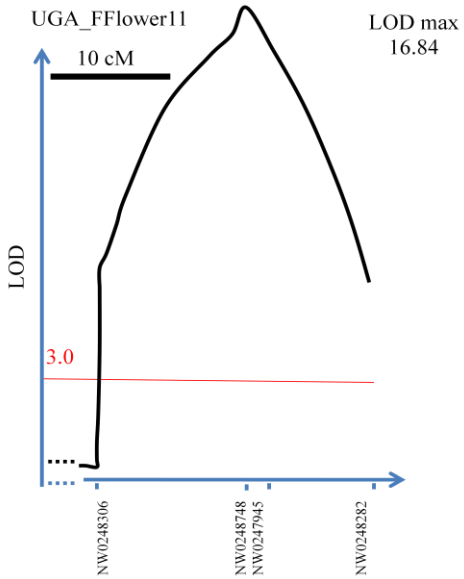

CHAPTER 4

SUMMARY

Previous attempts towards the production of a linkage map for elite *Citrullus lanatus* var. *lanatus* (Thumb.) Matsum. and Nakai watermelon cultivars were restricted by the low genotypic diversity available for marker development. This study overcame this restriction with the development of a 1,536 SNP Illumnia GoldenGate array for the species. Through the use of these markers an elite x elite, elite x egusi, and elite x citron populations were mapped. The elite x elite linkage map consisted of 379 markers with a length of 1,438.05 cM and an average marker distance of 3.79 cM, the elite x citron linkage map consisted of 357 markers with a length of 1,514.26 cM and an average distance between markers of 4.24 cM and the elite x citron population linkage map consisted of 338 markers with a length of 1,114.06 cM and an average marker distance of 3.38 cM. Previous mapping studies encountered relatively high levels of marker segregation distortion. The percentages of marker segregation distortion for the elite x elite and elite x egusi populations in this study were relatively low, at 3.7% and 2.8%, but the elite x citron population still had a higher level of segregation distortion of 12.7%. About half of the distorted markers for the elite x egusi and elite x citron population could be found in clusters on the linkage groups. With the exclusion of the distorted markers and the few markers with order discrepancies, the markers from the three linkage maps were organized into a consensus marker order. By combining the three linkage maps, the total number of linkage groups was reduced to 11, the haploid chromosome number of the species (2n=22).

The elite x elite map was further used to analyze quantitative trait loci (QTL) for horticulturally important traits. The F_7 generation was phenotyped at the University of Georgia' plant science farm in Watkinsville, GA and Monsanto's facilities in Woodland, CA. The traits analyzed were fruit length, fruit

diameter, fruit shape, thickness of the rind, Brix, presence of hollow heart, fruit weight, degree of fruit furrowing, and the number of days from sowing to the first female flower. The data was analyzed for QTL, for each location separately, using composite interval mapping with the program WinQTLCart 2.5. Thirty-three QTL were found for the 7 traits measured in Georgia and the 5 traits measured in California. Several of the important morphological fruit traits co-localized to LGs 9 and 11.

The formation of these linkage maps represents the first genetic maps for elite x elite or elite x egusi populations. The QTL analyzed for the elite x elite population are the first to be found using an elite x elite population, and this was the first time some of the traits have been analyzed for QTL in watermelon. This study provides a significant step towards the development of molecular breeding tools for the species *Citrullus lanatus*.