GENETIC MAPPING OF PHENOTYPIC AND QUANTITATIVE TRAIT LOCI UNDERLYING HORTICULTURALLY IMPORTANT TRAITS IN WATERMELON

by

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(Under the Direction of Cecilia McGregor)

ABSTRACT

QTL analysis was performed for multiple traits in an elite x egusi and an elite x citron F_2 population of watermelon (*Citrullus lanatus* var. *lanatus* Thunb.). Two linkage maps were constructed for QTL analysis using single nucleotide polymorphism (SNP) markers. A total of 16 quantitative trait loci (QTL) were identified across 10 traits in the elite x egusi population on a linkage map containing 14 linkage groups spanning a genetic distance of 1,514 cM. Eighteen QTL were identified for 12 traits in the elite x citron population on a linkage map containing 16 linkage groups covering a genetic distance of 1,144 cM. The QTL identified in this study can be useful for marker assisted selection (MAS) in watermelon breeding programs and broaden genetic diversity in commercially important elite watermelon germplasm by introducing favorable alleles from exotic germplasm sources.

INDEX WORDS: Watermelon, *Citrullus lanatus*, egusi, citron, QTL, mapping, SNP, F_2, fruit weight, fruits size, seed oil, seed size, Brix

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

Watermelon (*Citrullus lanatus* var. *lanatus* Thunb.) is a member of the *Cucurbitaceae* family. Other important members of the *Cucurbitaceae* family include cucumber (*Cucumis sativas* L.), squash (*Cucurbita maxima* L.), and melon (*Cucumis melo* L.) (Dane and Liu, 2007). Watermelon is believed to have originated in the Kalahari Desert region of Africa (Mohr, 1986). *Citrullus colocynthis* L., a wild ancestor of watermelon, grows wild in India and parts of China. Therefore, India and China are considered probable secondary centers of diversity for *Citrullus* (Wehner et al., 2001). Watermelon was first cultivated in Africa and the Middle East over 4,000 years ago as a source of food, water and animal feed. Watermelon was introduced into China during the first century AD and then introduced into the Americas in the 1500's (Guner and Wehner, 2004). Cultivated watermelon was first reported to be grown in the United States in 1629 (Wehner et al., 2001).

Watermelon is an important fruit crop globally. In 2009, the fresh market value of the watermelon crop was $460 million (United States Department of Agriculture, Nation Agricultural Statistics Service, 2010). 1.81 million metric tons of watermelons were produced in the United States on 51,110 hectares in 2009 while worldwide production of watermelon totaled 100.68 million metric tons on 3.81 million hectares. China is the leading producer of watermelon worldwide with production totaling 68.2 million metric tons on 2.21 million hectares (Food and Agricultural Organization-FAO, 2009). Major areas of watermelon production in the United States include Florida, Georgia, Texas, Arizona, and California (Wehner et al., 2001).

Watermelons are commercially produced from seed produced by diploid and triploid single cross hybrids and from open pollinated cultivars. In general, commercial production of watermelon is largely dominated by hybrid varieties. Diploid open pollinated varieties are still grown for commercial production, but at lower quantities than hybrid cultivars. Hybrid cultivars are the preferred choice in many commercially produced plant species due to their ability to take advantage of heterosis (Fehr, 1987). However, in watermelon, little inbreeding depression is observed with inbreds and heterosis is minimal in hybrids. In spite of this, hybrids are still widely produced to allow for the production of seedless watermelons and so that proprietary breeding lines are preserved (Wehner et al. 2001). It was reported in 2007 that 80 percent of watermelons produced in the United States were seedless varieties (United States Department of Agriculture, National Agricultural Statistics Service, 2009).

Traditional breeding methods are still relied upon heavily by breeders when developing watermelon varieties for commercial production because the infrastructure for routinely applying marker assisted selection (MAS) in watermelon breeding programs is not available (Wehner et al. 2001). The objective of this research is to genetically map phenotypic traits and QTL underlying several fruit, seed and morphological characteristics in the Strain II (PI 279461) x Egusi (PI560023) F_2 population and the ZWRM50 (PI 593359) x CTR-Delagoa (PI 244019) F_2 population.

CHAPTER 2

LITERATURE REVIEW

DNA Marker Resources in Watermelon

The application of marker assisted selection (MAS) in hybrid watermelon breeding programs has been limited by a lack of mapped high-throughput DNA markers and by a lack of genetic mapping information (Levi et al. 2002; Levi et al. 2006). Genetic mapping has previously been done in watermelon (2n = 2x = 22) using random-amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and inter-simple sequence repeat (ISSR) markers in wide hybrids (Levi et al., 2001; Hawkins et al., 2001; Levi et al., 2002; Hashizume et al., 2003; Zhang et al., 2004). Hashizume et al. (2003) mapped 53 RFLP, 477 RAPD, 23 I-SSR, and one isozyme marker in an elite x wild *C. lanantus* population (H-7 x SA-1). The wild parent (SA-1) was described as a wild African *C. lanatus*, is not found in public seed banks, and is of uncertain origin. The RFLP probes and probe sequences were not publicly released and consequently have not supported genetic mapping in other laboratories. Levi et al. (2002) mapped 141 RAPD and 27 I-SSR markers in a three-way hybrid: [(*C. lanatus* var. *citroides* x *C. lanatus* var. *lanatus*) x *C. colocynthis*]. While several hundred RAPD and I-SSR markers have been mapped in watermelon, both marker technologies are antiquated and inadequate for comparative and translational genomics and molecular breeding research in watermelon. Levi et al. (2006) developed an extended linkage map using sequence related amplified polymorphism (SRAP), amplified fragment length polymorphism (AFLP), SSR and I-SSR markers. A testcross population [(GRIF14113 x New Hampshire Midget) x PI 386015] was developed in this study in an attempt to extend the linkage maps developed by Hawkins et al.

(2001) and Levi et al. (2004). The extended linkage map contains 360 DNA markers on 19 linkage groups. While MAS resources in watermelon are lagging behing the resources available in other crops, efforts are being made to improve the resources available to breeders. These improved resources will help breeders to isolate genes responsible for fruit quality and pests resistance (Levi et al., 2006; 2010).

Fruit Quality and Morphology

Breeding efforts in watermelon have largely concentrated on fruit quality and morphological characteristics (Hashizume et al., 2003). These characteristics include, but are not limited to, flesh color, rind pattern, and sugar value. Sugar content, rind pattern, flesh color, and many other traits can vary greatly among watermelons that are commercially available (Levi et al., 2006). As early as the 1930's, researchers began investigating the inheritance of the genes controlling fruit quality and morphological traits (Porter, 1933; 1937). Since then, many efforts have been made to better understand these characteristics in watermelon but limited genetic mapping of these traits has been conducted.

Genes have been identified that control scarlet red, red, yellow, canary yellow, salmon yellow, orange, and white flesh in watermelon (Guner and Wehner, 2003; Gusmini and Wehner, 2006). Canary yellow (*C*) is dominant to red flesh (*c*) (Poole, 1944). Red flesh (*Y*) is dominant to salmon yellow (*y*) (Poole, 1944; Porter, 1937). Orange flesh color (y^o) is part of a multi-allelic system in which red flesh (*Y*) is dominant to orange flesh (y^o) and salmon flesh color (*y*) while orange flesh (y^o) is dominant to salmon flesh color (*y*) (Henderson, 1989). The white flesh (*Wf*) locus is believed to have an epistatic interaction over the *B* locus when the dominant allele of white flesh (*Wf*) is present, white flesh is formed (*Wf_B_* or *Wf_bb*) regardless of the genotype at the *B* locus. The recessive form of this allele (*wf*) allows for color to develop in the flesh. The

epistatic interaction of *wf* with *B* produces yellow flesh color (*wfwfB_*) while the interaction of *wf* with *b* produces red flesh color (*wfwfbb*) (Shimotsuma, 1963; Bang et al., 2007). Henderson et al. (1998) also found evidence of a third gene that interacts with the canary yellow locus. This gene was assigned the name inhibitor of canary yellow (*I*). The homozygous recessive form (*ii*) inhibits the canary yellow gene and allows for red flesh to be expressed. This finding by Henderson et al. (1989) is disputed by Bang et al. (2007) which found evidence to suggest that there is no epistatic interaction with the *I* locus. Bang argues that Henderson et al (1989) based their conclusions on probability test and that the segregation frequencies observed may be skewed. Another explanation of Bang's finding could be that each parent in the population Bang used was fixed for the homozygous dominant form (*II*) of the inhibitor gene. Another possibility exist where the *I* locus was fixed as homozygous dominant (*II*) in both parents, thus epistasis was not observed in Bang's population (Bang et al., 2007). In 2006, Gusmini and Wehner identified a new gene for scarlet red flesh. This flesh color was described as being deeper red than normal red fleshed cultivars of watermelon. They reported this trait to be controlled by a single dominant gene (*Scr*).

The soluble solid content of watermelons is closely related to the sugar content and is measured in degrees Brix (MacGillivray, 1947; Maynard, 2001; Hashizume et al., 2003). Watermelons that are marketed to the public can have a Brix value as high as 14^{o}, while a Brix value of 10^{o} is considered the minimal marketable value (Wehner et al., 2001). Wild forms of watermelon typically have low Brix values. Hashizume et al. (2003) mapped quantitative trait loci (QTL) for Brix on a genetic map with 11 linkage groups that was constructed using 477 RAPDs, 53 RFLPs, 23 ISSRs, and 1 isozyme marker in a population developed with the parents H-7 and SA-1. H-7 is a cultivated inbred line that has a high Brix value of 12^{o} while SA-1 is a

wild type from Africa that has a low Brix value of 4°. A QTL that accounted for 19% of the variation was detected on linkage group 8. A RAPD marker (RB1002A) is located near the region that harbors this QTL for Brix and was suggested for use by the author as a means of selection for sugar content. Hashizume et al. (2003) tested this marker in a BC_2 population (BC_1 x H-7). No data was given, but it was stated that most of the BC_2 progeny that contained the RAPD marker RB1002A had Brix values of 7 or higher.

A watermelon's shape can be classified as round, oval, blocky, or elongate (Wehner et al. 2001). The only identified gene to control fruit shape is the elongate fruit gene (*O*). This gene is incompletely dominant and when the homozygous dominant genotype (*OO*) is present, elongate fruit is observed. When the heterozygous genotype (*Oo*) is present, the fruit will be oval shaped and the homozygous recessive genotype (*oo*) will give fruit a round shape. The blocky phenotype is an intermediate phenotype and is observed in F_1 fruit (Weetman 1937; Poole and Grimball, 1945; Wehner, 2001).

Kumar (2009) crossed the cultivars Mountain Hoosier and Calsweet to determine the inheritance of fruit shape. Mountain Hoosier produces a round fruit and Calsweet produces an elongate fruit. A 1:2:1 (elongate:oval:round) segregation ratio was expected in the F_2 progeny. The F_1 were backcrossed to Mountain Hoosier (BC_1Pa) and Calsweet (BC_1Pb) and a 1:1 (oval:round) segregation ratio was expected in the BC_1Pa and a 1:1 (oval:elongate) segregation ration was expected in the BC_1Pb. In the F_2 population, 22 elongate, 116 oval, and 133 round fruit were observed. This was distorted from the expected 1:2:1 ratio with $\chi^2 = 96.54$ ($P = 0.0001$).For the BC_1Pa population, 22 oval and 55 round fruit was observed which does not agree with a 1:1 ratio $\chi^2 = 11.25$ ($P = 0.0008$). No chi square statistics were reported for BC_1Pb

because round fruit were observed and this was not expected. Kumar (2009) concluded that the results of this population do not support the theory of a single gene controlling fruit shape.

Seed Characteristics

Watermelon seed is defined as being large, medium, small, tomato, or tiny. Large, medium, and small seed have approximate seed length of 10 mm, 7 mm, and 5 mm, respectively. Tomato seed size are approximately the same size as tomato seed while tiny seed size is slightly smaller than small seed size (Wehner et al., 2001; Gusmini, 2005). Large and small seed sizes are also referred to as long and short, respectively. Large, medium, and small seed sizes are conditioned by the interaction of the large seed (*l*) and (*s*) genes. When *l* is homozygous recessive and *s* is homozygous dominant (*llSS*), large seed are observed. When *l* and *s* are both homozygous dominant (*LLSS*), medium seed are observed. Short seed are observed when *l* is either homozygous dominant or recessive and *s* is homozygous recessive, *LLss* or *llss* (Poole et al., 1941). Zhang (1996) found that the tomato seed size is controlled by a single recessive gene (*ts*). Tanaka et al. (1995) reported that the tiny seed size in the cultivar Sweet Princess is controlled by a single gene (*Ti*).

Egusi melons (*Citrullus lanatus* var. *lanatus*) posses a unique seed trait in which the seed is enclosed in a fleshy pericarp. The egusi melon is also commonly referred to as wild watermelon or ibara in regions where it is grown. It is native to temperate and arid regions of Africa and Asia (Gusmini et al., 2004) and its worldwide production is limited. A majority of the crop is produced in regions of Africa, particularly in Nigeria (Anuebunwa, 2000; Ezeike and Otten, 1989, 1991; Jolaoso et al., 1996). In regions where the egusi melon is produced, the seed are consumed and serve as a rich source of protein and oil. Seed are large and flat and the fleshy covering on these seed remnant of nucellar tissues. The egusi seed trait is controlled by a single

gene (*eg*) and the egusi trait (*eg eg*) is recessive to the normal seed trait (*Eg* _) (Gusmini et al., 2004).

Floral Development

Most plants species have perfect flowers. Perfect flowers contain both staminate (male) and pistillate (female) parts (Noguera et al., 2005). Some plant species have a spatial separation of the male and female reproductive parts. The two most prevalent types of reproductive structures with spatial separation are monoecy and dioecy. Monoecious plants have spatial separation of the male and female flowers on the same plant. Dioecious plants have separate male and female flowers on separate male and female plants (Whitaker, 1931; Perl-Treves, 1999). However, there are several variations of the monoecious and dioecious flowering structures. Andromonoecious plants produce male flowers and also produce hermaphroditic flowers. Gynomonoecious plants produce female flowers and also produce hermaphroditic flowers. Gynoecious plants produce all female flowers and androecious plants produce all male flowers. Trimonoecious plants contain male, female, and hermaphroditic flowering structures (Whitaker, 1931).

Most commercially produced watermelon varieties are monoecious. Andromonoecious varieties are rarely observed. The typical floral ratio expressed by varieties is 7 male: 1 female flower, although this can vary and a ratio of 4 male: 1 female flower can be observed in some varieties. There is no apparent advantage of having varieties that express the andromonoecious flowering type. The chance of having successful pollination or self-pollination in the absence of bees is no greater in andromonoecious plants than monoecious plants (Wehner et al., 2001). There are beneficial aspects to having breeding lines that express the monoecious flowering trait. Pollen control is simplified because hand emasculation is not necessary. Monoecious plants

produce fruit with smaller bottom scars (Noguera et al., 2005). A smaller bottom scar reduces the risk of fruit being infected with a pathogen and also helps improve fruit quality (Perin et al., 2002).

Other members of the *Cucurbitaceae* family such as cucumber (C. *sativus* L.) and melon (C. *melo* L.) can serve as models when studying sex determination in watermelon. Cucumber and melon have heritable patterns of sex determination which is common to *Cucurbitaceae* family members (Perl-Treves, 1999; Roy and Saran, 1990). Three genes interact in cucumber and melon to determine sexual expression. The three genes in melon that control sex determination are andromonoecious (*a*), gynomonoecious (*g*), and maleness (*M*). The dominant and recessive allele of the andromonoecious gene in conjunction with the dominant allele of the gynomonoecious gene gives rise to monoecious (*A-GG*) and andromonoecious (*aaGG*) plants. Hermaphroditic sexual expression is observed when the andromonoecious and gynomonoecious genes are homozygous recessive (*aagg*). In gynoecious (*AAgg*) plants, the phenotype of all female flowers is reported to be stabilized by the homozygous recessive state of the maleness gene (*mm*) (Poole and Grimball, 1939; Keningsbuch and Cohen, 1990; Roy and Saran, 1990). Plants expressing either andromonoecious or monoecious flowering patterns are preferred by breeders in developing breeding lines (Noguera et al. 2005). The three genes that control sexual expression in cucumber are female (*F*), male (*M*), and andromonoecious (*A*). Hybrid cucumber breeding gained popularity with breeders when gynoecious inbred lines were developed. The gynoecious trait is controlled by the female (*F*) gene. The use of gynoecious plants in a cucumber breeding program allowed for plants that were more uniform, earlier, and higher yielding when compared to monoecious lines (Peterson, 1975; Lower and Nienhuis, 1990).

The andromonoecious locus has been genetically mapped in melon. Perin et al. (2002) mapped the *a* locus to a region on linkage group II that covers 25.2 cM, but the region is poorly saturated with markers. Danin Poleg et al. (2002) developed a RAPD marker that is located on linkage group 4 and is located 16.2 cM from the *a* locus. Silberstein et al. (2003) developed a RFLP marker that is located 7 cM from the *a* locus. The genetic distance of these markers from the *a* locus makes it difficult to use them in a breeding program. Noguera et al. (2005) developed a SCAR marker that is located 3.3 cM from the *a* locus, which will be more practical for breeding purposes.

Hybrid Fertility

Levi et al. (2000) used 662 RAPD markers to determine the genetic similarity among 34 plant introductions from the *Citrullus* genus and 5 elite watermelon cultivars. The average genetic similarity among the 5 watermelon cultivars was calculated at 93.1%. The similarity coefficient (SC) between commercially available cultivars has been shown to be as high as 0.99 (Che et al., 2003). The narrow genetic background reported here raises concerns among breeders. The narrow genetic diversity can lead to widespread epidemics of pests and disease such as fusarium wilt or gummy stem blight (Levi et al., 2002; Harris et al., 2009). Introducing new sources of variation into breeding lines will help breeders to overcome the problem of narrow genetic diversity. These sources typically include wild or unadapted ancestors of the species a breeder is working to improve (Atlagic et al., 1993; Quillet et al., 1995). An interspecific cross of elite material to wild species is commonly used to introduce these sources of variation, but using this method can present problems. These crosses can be difficult to make, and the F_1's produced from these crosses will often show reduced fertility or complete sterility. In addition to this, undesirable traits can be introduced into breeding lines (Heiser et al., 1964;

Whelan 1978). These barriers can make introgression of alleles from wild species difficult. Embryo rescue is shown to be an effective method to recover interspecific hybrids. Kräuter et al. (1991) reported a recovery rate of 41% for interspecific hybrids using an embryo rescue method. This method is successful, but a better understanding of the factors that influence reduced hybrid fertility is needed to overcome these barriers.

Shimotsuma (1960) developed one intraspecific hybrid and two interspecific hybrids to investigate the cytology of *Citrullus* species. The parents of these hybrids consisted of two accessions of *Citrullus colocynthis* (C No.1 and C No.3) and one accession of *Citrullus lanatus*, or the cultivated watermelon (V No.1). C No.3 was crossed to C No.1 to make the intraspecific hybrid while V No.1 was crossed to C No.1 and C No.3 for the interspecific hybrids. Reduced pollen viability was observed in the the F_1 hybrids. Observation of the chromosomes at metaphase I in pollen mother cells (PMC) showed that about 50 percent of the cells showed 11 bivalents. Multivalents, trivalents and univalents were observed in the remaining cells.

Singh (1978) and Yadav (1982) used the same species for cytological study as Shimotsuma (1960) and reported observing mostly quadrivalent configurations. Sain et al. (2002) also used accessions of *Citrullus colocynthis* and *Citrullus lanatus* to develop F_1 hybrids. Two *Citrullus colocynthis* accessions and three *Citrullus lanatus* accessions were used to obtain F_1 hybrid seed from multiple interspecific crosses. Analysis of the PMC's at metaphase I showed a high number of bivalent associations. Reduced pollen fertility was observed in all of the crosses and pollen fertility was measured as low as 21.5 percent in some combinations. Sain et al. (2002) concluded that several factors could have led to the reduced pollen fertility. These factors include structural differences between chromosomes that results in gene imbalance and reduced pollen fertility. Chromosomal association in the F_1 hybrids allows for better assessment

of the genomic relatedness between species than pollen fertility. Studies such as these can improve our understanding of interspecific hybridization, which will allow breeding programs to introduce wild alleles into their breeding lines.

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CHAPTER 3

QTL ANALYSIS OF WATERMELON FRUIT AND SEED TRAITS IN AN ELITE x EGUSI F_2 POPULATION[1]

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Abstract

Watermelon (*Citrullus lanatus* var. *lanatus* Thunb.) production in the United States was valued at $460 million in 2009. Watermelon is also an important fruit crop worldwide with global production of 100.68 million metric tons in 2009. The application of marker assisted selection (MAS) in watermelon has been limited due to the lack of genetic mapping information available to breeders. An F_2 population derived from a cross between the cultivar Strain II (PI 279461) and the wild Egusi-Oyo (PI 560023) was developed with the intent to genetically map fruit and seed traits. Strain II is an elite cultivar originating from Japan that contains many of the favorable fruit morphology and quality traits common in elite cultivars. Egusi-Oyo is a wild accession that was collected in Nigeria. The egusi melon is cultivated in some regions of Africa and used for its nutrient rich seed. A linkage map was constructed that contains 357 SNP markers on 14 linkage groups. Fruit quality and morphological traits such as degrees Brix, rind thickness, and size measurements were collected. In addition to these, the egusi seed trait was phenotyped and the seed oil percentage was recorded. In total, phenotypic data was collected for 10 traits and 16 QTL were identified for these traits. The variance explained by these QTL for the traits ranged from a minimum of 8.41% to a maximum of 78.96%. The QTL analysis performed here may be a useful tool for incorporating marker assisted selection into watermelon breeding programs.

Introduction

Watermelons (*Citrullus lanatus* var. *lanatus* Thunb.) are an important vegetable crop globally. Fresh market value of the 2009 crop in the United States totaled $460 million (United States Department of Agriculture, National Agricultural Statistics Service, 2009). Traditionally, watermelon breeders focus on fruit quality traits. These traits include sugar content, flesh color, fruit size, and rind patterns (Hashizume et al., 2003). Investigations of the inheritance of fruit morphology and quality traits date back as far at the 1930's (Porter 1933; 1937). Since then, many efforts have been made to better understand traits associated with watermelon fruit quality and morphology.

Many genes have been described that control internal fruit quality and morphology in watermelon (Guner and Wehner, 2004). An internal fruit characteristic that has received attention is the Brix value. Degrees Brix is a measure of the total soluble solids in watermelon and is highly correlated with the percent sugar in watermelon (MacGillivray, 1947; Maynard, 2001; Hashizume et al. 2003). Hashizume et al. (2003) developed a mapping population with the parents H-7, an elite inbred line, and SA-1, a wild accession of watermelon from Africa. A linkage map was developed and a QTL that accounts for 19% of the variation in Brix was mapped on linkage group 8.

Fruit shape and weight are important external characteristics that breeders must consider when developing watermelon cultivars. Watermelon shape can be classified as being either round, oval, blocky or elongate (Wehner et al., 2001). The only gene described that controls fruit shape is the elongate fruit gene (*O*). Fruit weight has recently become an important consideration for breeders due to increased consumer preference for smaller sized watermelons (Gusmini and Wehner, 2007). Watermelons have traditionally been classified into five

categories based on fruit weight. These are icebox (less than 5.5 kg), small (5.5-8.0 kg), medium (8.1-11.0 kg), large (11.1-14.5 kg), giant (greater than 14.5 kg), and recently mini fruit size (1.5-4.0 kg) was added (Maynard, 2001; Gusmini and Wehner, 2007). No genes have been identified that are responsible for fruit weight in watermelon (Gusmini and Wehner, 2007).

Fruit quality and morphological traits such as the ones described above gain the most attention from watermelon breeders. There are many other important traits in watermelon that receive little to no attention. The egusi seed trait is one of these traits. Watermelons that posses the egusi trait are commonly known as egusi melons (*Citrullus lanatus* var. *lanatus*). They are also referred to as wild watermelons or ibara in Nigeria, where they are primarily cultivated. These melons are unique because they contain a large, flat seed that is enclosed in a fleshy pericarp (Figure 3.1). The flesh of the egusi melon is not edible but the seed serve as a rich source of protein, carbohydrates, and vitamins (Gusmini et al., 2004; Ntui et al., 2010). Egusi seed can also contain up to 50 percent oil (Achu et al., 2005). The oil produced from egusi seed can serve as a valuable source of energy in regions where it is grown and it has been reported that egusi oil is comparable to safflower, soybean, and sunflower oil as a feedstock for biodiesel production (Giwa et al., 2010). Gusmini et al. (2004) reported that the egusi seed trait is inherited as a single gene (*eg*).

Before recently being re-classified as *C. lanatus* var. *lanatus*, the egusi melon was classified as *Citrullus lanatus* subsp. *Mucosospermus* (Dan and Liu, 2007). In the literature the taxonomy of egusi type melons is confusing and it has commonly been referred to as *Citrullus colocynthis* (Gusmini et al., 2004). Studies have shown that the egusi type melon is closely related to the cultivated watermelon (Che et al., 2003; Dane and Liu, 2007) and that the cultivated, citron, and egusi melon have evolved from a common ancestral parent (Dane and Liu,

2007). The egusi melon is now taxonomically classified as *Citrullus lanatus* var. *lanatus* (Che et al., 2003; Dane and Liu, 2007)

Marker assisted selection (MAS) resources in watermelon lag behind the resources available in other cucurbits, such as melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.) (Levi et al., 2006; 2010). This research focuses on mapping quantitative trait loci (QTL) associated with important fruit and seed traits such as Brix, rind thickness and the egusi seed trait in a F_2 elite x egusi population. The long-term goal of this project is to improve the infrastructure of MAS in watermelon breeding programs and to broaden the genetic diversity of commercially important watermelon germplasm by introducing favorable alleles from wild germplasm sources.

Materials and Methods

Development of plant material

Seed of Strain II (PI 279461) and Egusi (PI 560023) were obtained from the Germplasm Resource Information Networks (GRIN) Southern Regional PI Station in Griffin, GA. Strain II is an elite cultivar of watermelon that was developed in Japan and donated to GRIN by the Japanese Seed Growers Cooperatives (Figure 3.2). Egusi is a wild form of watermelon that was collected in Oyo, Nigeria (Figure 3.2) (United States Department of Agriculture, Agriculture Research Service, 2010). The F_2 population that was used for mapping was developed by cross pollinating Strain II with Egusi. An F_1 fruit was harvested and the seed was collected. A single F_1 seed was planted and a self-pollinated fruit was obtained from this plant providing F_2 seed. Two hundred and fourteen individual F_2 plants were planted at the University of Georgia's Plant Science Farm in Watkinsville, GA in the summer of 2007. The soil type that this trial was planted into is Appling Coarse Sandy Loam.

Trait Evaluation

One mature fruit from 142 individuals was collected and phenotyped for multiple traits. Degrees Brix was measured using a refractometer (Atago Co., Ltd., Tokyo, Japan) from a sample of juice collected from the center of each watermelon. Rind thickness was measured with a digital caliper (Balkamp Manufacturing Corp., Indianapolis, Indiana) in the middle of the fruit, half way between the apex and the pedicel. Fruit width was measured in centimeters at the widest part of the fruit as the distance between each edge of the fruit. Fruit length was measured in centimeters as the distance between the fruit apex and the point at which the pedicel attached to the fruit. Fruit weight was measured in kilograms and was recorded at maturity. Seed was collected from each fruit and allowed to dry for oil analysis. Near magnetic resonance was used to analyze the seed for oil content (Leon et al., 1995). A Pearson Correlation Matrix was developed for all traits using the PROC CORR procedure (SAS Institute, 2003).

Linkage and QTL Analysis

Approximately four weeks after planting, fresh leaf tissue from each plant was collected and frozen. SNP genotyping was performed using the GoldenGate assay method as described by Hyten et al. (2009). The SNP panel was constructed by Monsanto using proprietary methods. Monsanto developed the linkage map used for QTL mapping that consist of 357 SNP markers on 14 linkage groups spanning a distance of 1,514.258 cM with an average distance of 4.24 cM between markers. Composite interval QTL mapping (CIM) was performed with WinQTLCart version 2.5 mapping software (Wang et al., 2010). The CIM model number 6 with backward regression method was used. Significance thresholds were determined by using 1000 permutations with a significance level of $P = 0.05$. Traits showing a segregation ratio of 3:1 were tested by chi square analysis.

Results and Discussion

An F_2 population of Strain II x Egusi was grown and horticulturally important traits were scored in this population. Nine out of the 10 traits that were measured had continuous variance (Figure 3.3). This indicates that these traits are controlled by more than one gene. The traits that showed continuous variance also exhibited transgressive segregation with phenotypic values exceeding those of the parents. Distributions of QTL across linkage groups are shown in Figure 3.4. Across the 10 traits, 16 QTL were detected (Figure 3.5). Individual R^2 values for the traits ranged from a minimum value of 8.41% for fruit weight to a maximum value of 78.96% for seed oil.

One QTL was detected for Brix on LG5 that accounts for 21.5% of the variation in this trait. Hashizume et al. (2003) previously identified a QTL for Brix that accounted for 19% of the phenotypic variation in this trait. While we did detect a single QTL for Brix, this trait can be a difficult to map because it is highly affected by environmental conditions (Hashizume et al., 2003). Advanced generations may be required to map QTL for this trait. Only one replication of the F_2 population in this study was grown and phenotyped. Since this trait is largely affected by environmental conditions, multiple replicates would be needed to account for the genotype x environment interactions. Recombinant inbred line (RIL) populations are "true-breeding", or homozygous, and a RIL population can be replicated and tested across multiple environments without any genetic change since the alleles are fixed (Collard et al., 2005). The disadvantage of using a RIL population for mapping is that only additive gene action can be estimated due to the homozygosity of the population (Vinod, 2006). Another factor that could make this trait difficult to measure is the wide ranging maturities of the F_2 fruit. Egusi requires more time to mature than Strain II and this makes determining the optimum time to measure Brix very difficult.

A single QTL was identified for rind thickness. This QTL is located on LG2 and explains 17.51% of the variation of this trait and has a maximum logarithm of odds (LOD) of 6.07. Strain II has a thinner rind than egusi. The alleles from the Strain II parent showed a positive additive effect in decreasing the thickness of the rind. However, decreasing the thickness of the rind is not always the goal when breeding watermelons. The objective when breeding for this trait should be to have a rind thickness that is a small percentage of the fruit diameter. Small fruited watermelon will have a thin rind while large fruited watermelon will have a thicker rind. The thicker rind of the large fruited watermelon will provide extra protection of the fruit during postharvest handling and shipping (Wehner et al., 2001).

Fruit length and width are highly correlated (Table 3.3). Three QTL were identified for fruit length and two QTL were identified for fruit width. Two QTL for fruit length and width were identified on in the same region on LG5. The QTL identified on LG5 account for 8.7% and 12.42% of the variation in fruit length and for 14.08% and 14.55% of the variation in fruit width. Another QTL for fruit length was identified on LG3 that explains 9.94% of the variation. Fruit length and width were combined to obtain a fruit shape ratio (length:width). No QTL were identified for fruit shape ratio (data not shown). This is not unexpected because the parents both have round fruit shape. While 2 QTL were detected for fruit length and one QTL was detected for fruit width, it is not known if either if these are associated with the elongate fruit gene. Kumar (2009) produced a population segregating for fruit shape and found that his data did not fit the single gene theory for fruit shape inheritance. Our data support Kumar's (2009) suggestion that fruit shape is inherited as more than one gene.

One QTL was identified on LG3 and two QTL were identified on LG5 for fruit weight. The QTL on LG3 explains 8.41% of the variation in this trait. The QTL on LG5 explain 15.69%

and 11.82% of the variation in this trait. One of the QTL identified for fruit weight mapped to the same position on LG5 as a QTL for fruit length. The second QTL identified on LG5 mapped to the same region on LG5 as QTL identified for fruit length and width. Fruit weight has recently become an important consideration for breeders because consumer preference is shifting away from the traditionally large fruited watermelons to smaller sized watermelons. Watermelons varieties that produce fruit that fit into the weight category most preferred by consumers must be available for growers (Gusmini and Wehner, 2007). The QTL identified here should be useful for fruit weight selection in a breeding program.

The egusi seed trait has been shown to be inherited as a single gene (Gusmini et al., 2004). Forty-six fruit expressed the egusi seed phenotype while 118 expressed the normal seed phenotype. A segregation ratio of 3 normal seeded fruit: 1 egusi seed type fruit was expected. A chi square goodness to fit test confirmed that this trait fit the expected 3:1 ratio with $\chi^2 = 0.9718$ ($\alpha < 0.05$). Two QTL were identified for this trait on LG2. The phenotypic variances explained by these QTL are 30.96% and 27.39% with maximum LOD scores of 7.88 and 7.80, respectively. One of the regions containing the QTL identified here could be the location of the egusi seed trait gene (*eg*). A significant QTL was identified for seed oil percentage. This QTL is located on LG2 and accounts for 78.96% of the variation for this trait with a maximum LOD of 44.11. This QTL falls between the locations of the two QTL identified for the egusi seed trait on LG2. All seed that expressed the egusi seed trait had oil content of 28% or higher while all normal seed had oil content of 27% or lower and this segregated in a 3:1 ratio. Seed oil for egusi and normal seed types were mapped separately and we indentified a QTL on LG2 and another QTL on LG5 and identified as “low seed oil” in figure 3.5. No QTL were identified for the oil data for seed expressing the egusi seed type. We expected to find more than one QTL affecting

the seed oil in the two classes (normal seed and egusi seed). We did for the low oil but we did not for the high oil. It is unclear how all of this is related and further investigation is needed to understand the genetics of this trait.

The genetic diversity in cultivated watermelon has been show to be very low (Levi et al., 2000, 2002; Harris et al., 2009). Wild watermelon germplasm harbors many favorable traits such as disease and pest resistance. At the same time, these germplasm lines contain many undesirable traits that can affect fruit quality and morphology. The QTL identified in this study can be used to speed up the breeding process through the use of MAS when introducing wild alleles into breeding lines.

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Table 3.1 Phenotypic values of the parents and F_2 progeny of the Strain II x Egusi population. Traits were measured on 142 fruit in the F_2 population. One replication of the population was grown.

Trait	Parents		F_2			
	Strain II	Egusi	Min	Max	Mean	SD
Brix (degrees)	8.70	2.20	3.50	9.00	5.48	1.074
Rind Thickness (cm)	0.95	2.22	0.95	2.54	1.53	0.391
Fruit Length (cm)	18.42	26.67	13.97	34.29	23.17	3.065
Fruit Width (cm)	18.42	23.50	13.97	28.58	21.15	2.789
Fruit Length/Width	1.00	1.14	0.78	1.12	0.91	0.055
Fruit Weight (kg)	2.72	8.85	1.59	10.89	5.32	1.878
Seed Oil (%)	25.20	40.60	17.80	37.69	26.23	5.051

Table 3.2 Genomic regions significantly associated with QTL for the traits phenotyped in the Strain II x Egusi F_2 population

Trait	Linkage Group	Map QTL Position	Flanking Markers	Map Position	Max LOD	R^2	Additive[1]	Dominant
Brix	5	0.01	NW0250803	0	8.51	21.5	0.6431	0.0574
			NW0248892	4.244				
Rind Thickness	2	45.58	NW0250854	29.58	6.07	17.51	0.0974	-0.0110
			NW0250242	49.22				
Fruit Length	3	187.86	NW0250460	181.85	3.81	9.94	0.5988	-0.2429
			NW0250483	198.04				
	5	1.01	NW0250803	0.00	3.90	8.7	0.3983	0.2321
			NW0248892	4.24				
	5	99.08	NW0248254	97.07	5.08	12.42	-0.5299	-0.1740
			NW0248077	104.07				
Fruit Width	5	0.01	NW0250803	0.00	7.39	14.08	0.4565	0.2488
			NW0248892	4.24				
	5	92.21	NW0249185	83.20	7.27	14.55	-0.4538	-0.2035
			NW0248254	97.07				
Fruit Weight	3	186.86	NW0250460	181.85	4.93	8.41	1.9685	-1.2468
			NW0250483	198.04				
	5	1.01	NW0250803	0.00	8.53	15.69	2.0894	0.5939
			NW0248892	4.24				
	5	99.08	NW0248254	97.07	6.83	11.82	-1.9764	-0.2353
			NW0248077	104.07				
Egusi Seed Trait	2	31.58	NW0250854	29.58	7.88	30.96	-0.2357	-0.1285
			NW0250242	49.22				
	2	78.73	NW0248325	52.72	7.80	27.39	0.2402	-0.1235
			NW0250248	80.02				

Trait	Linkage Group	Map QTL Position	Flanking Markers	Map Position	Max LOD	R^2	Additive[1]	Dominant
Seed Oil Percentage	2	61.73	NW0251010	54.45	44.11	78.96	-2.4191	-9.6871
			NW0250480	70.07				
Low Seed Oil	2	40.58	NW0250854	29.58	7.07	29.08	1.8591	-0.7277
			NW0250242	49.22				
	5	131.59	NW0252521	127.58	5.20	16.19	-0.3589	-1.1803
			NW0248192	133.39				
Fruit Shape (Length:Width)[2]								

[1]Positive values of the additive effect indicate that alleles from Strain II are contributing a positive effect on the phenotypic value of the trait
[2]No QTL detected

Table 3.3 Pearson correlations for traits measured in the Strain II x Egusi F_2 population. Shaded boxes indicate significant ($P<0.05$) correlations.

TRAIT	BRIX	Rind Thickness	Fruit Length	Fruit Width	Fruit L/W	Fruit Weight
Rind Thickness	0.1399					
Fruit Length	0.3713	0.5050				
Fruit Width	0.3920	0.4795	0.8946			
Fruit Length/Width	-0.0419	0.0592	0.2407	-0.2145		
Fruit Weight	0.3793	0.4398	0.8912	0.9207	-0.0419	
Seed Oil Percentage	-0.3019	-0.2158	-0.0439	-0.1236	0.1624	-0.0723

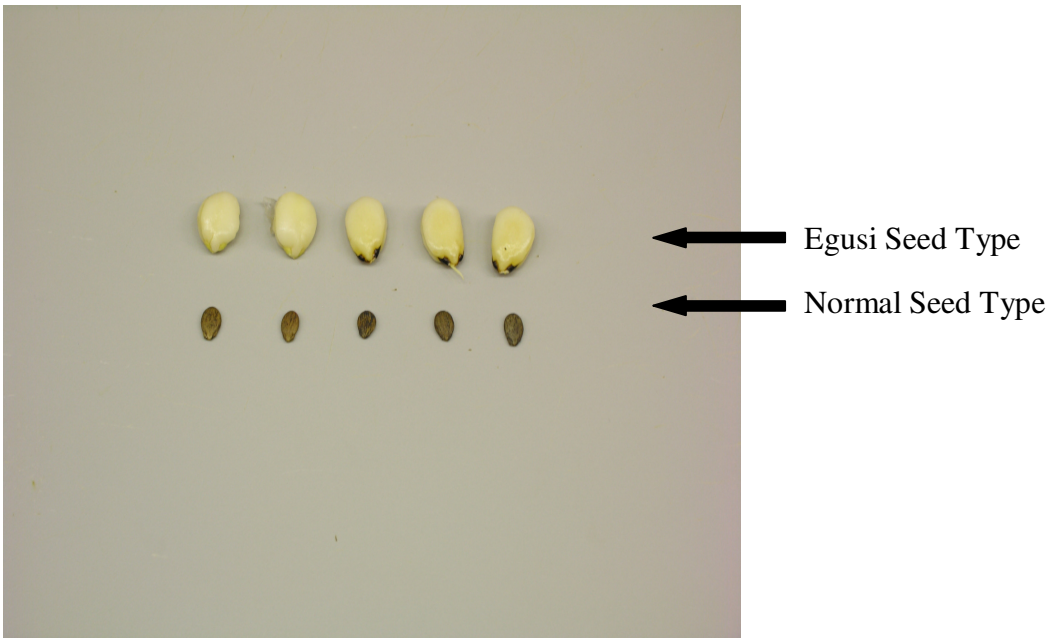


Figure 3.1 Egusi seed trait compared to normal seed trait

Strain II (PI 279461)

Egusi (PI 560023)

Figure 3.2 Cross section through mature fruit of parents of the F_2 mapping population

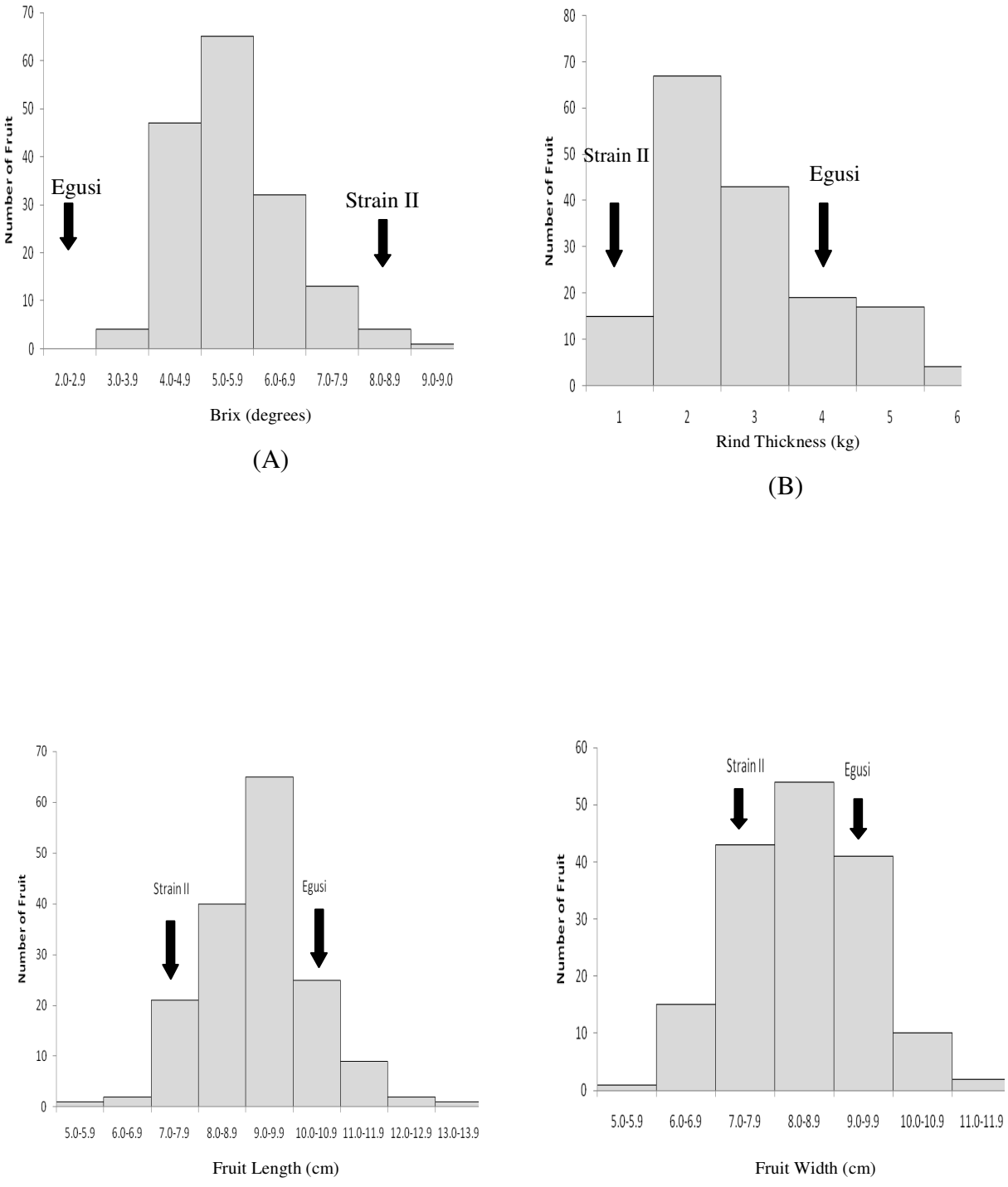
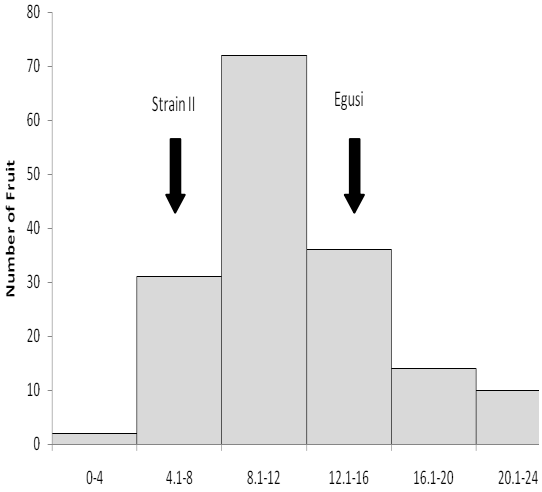
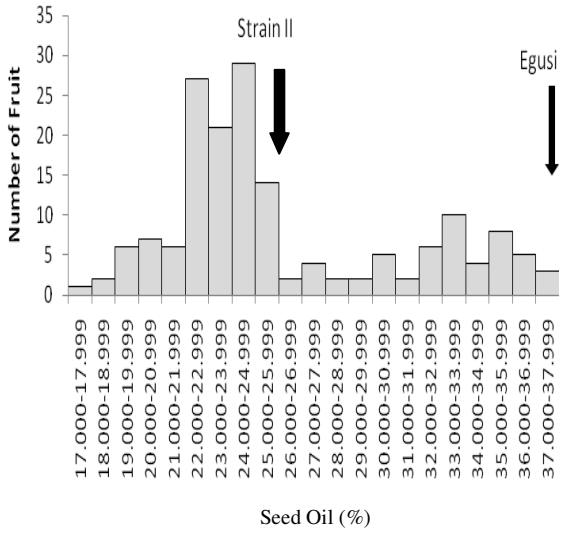


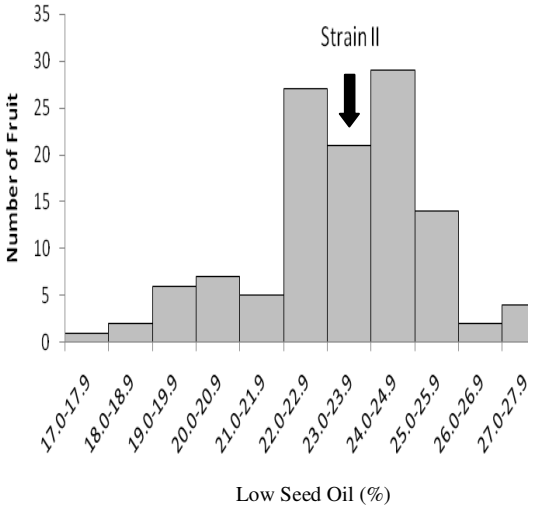
Figure 3.3 Frequency distribution for horticultural traits in the F_2 progeny. Arrows represent phenotypic values of the parents. (A) Brix, (B) Rind Thickness, (C) Fruit Length, (D) Fruit Width, (E) Fruit Weight, (F) Seed Oil, (G) Low Seed Oil, (H) High Seed Oil


Number of Fruit
Strain II
Egusi
0-4
4.1-8
8.1-12
12.1-16
16.1-20
20.1-24
Fruit Weight (kg)

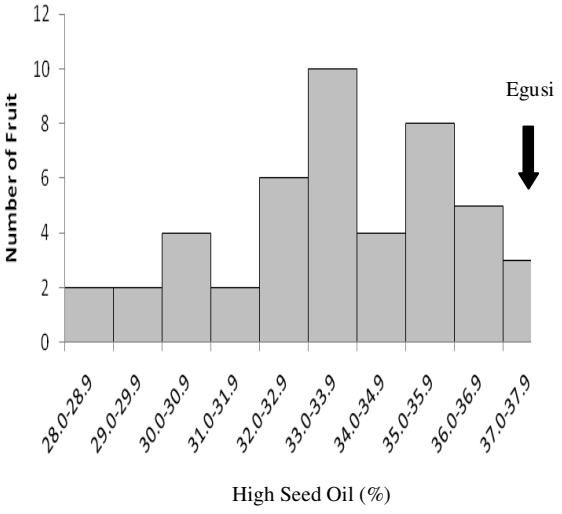
(E)


Number of Fruit
Strain II
Egusi
Seed Oil (%)

(F)


Number of Fruit
Strain II
Low Seed Oil (%)

(G)


Number of Fruit
Egusi
High Seed Oil (%)

(H)

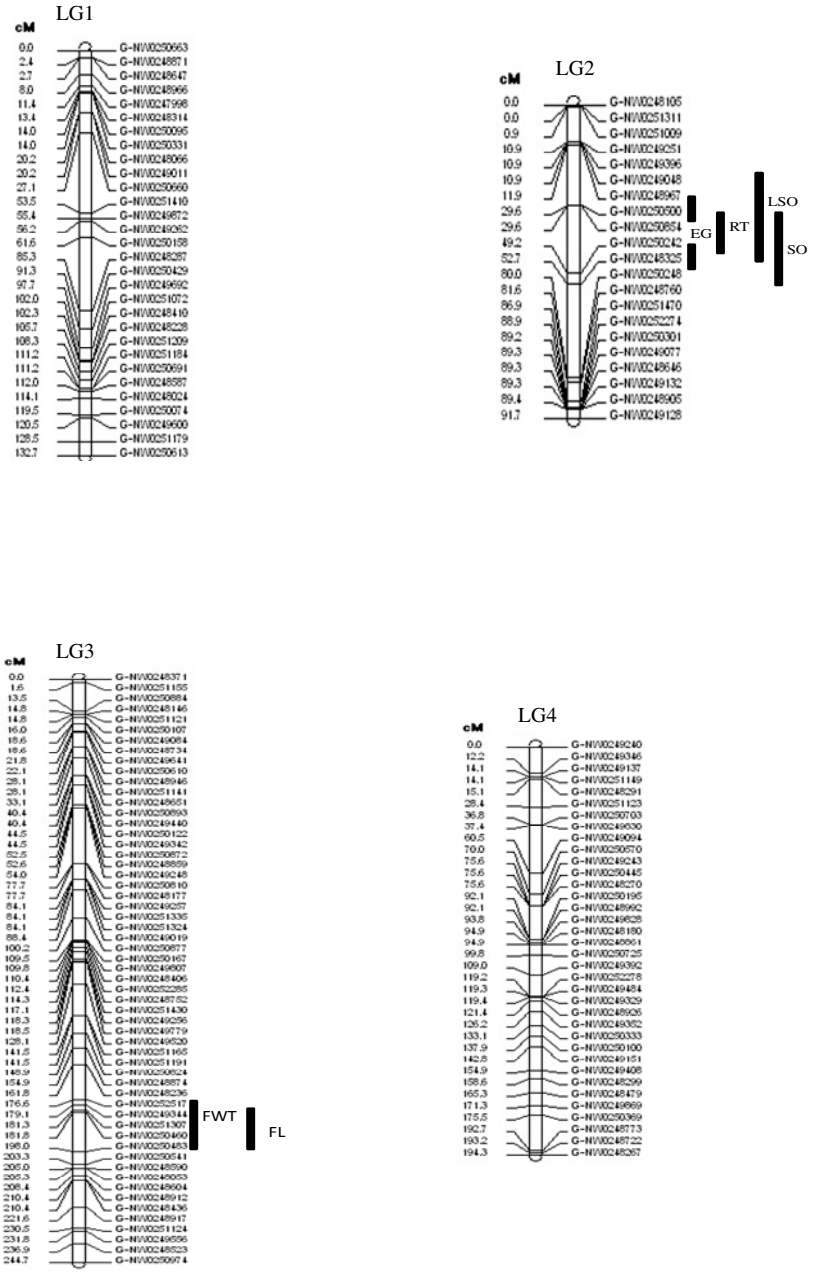
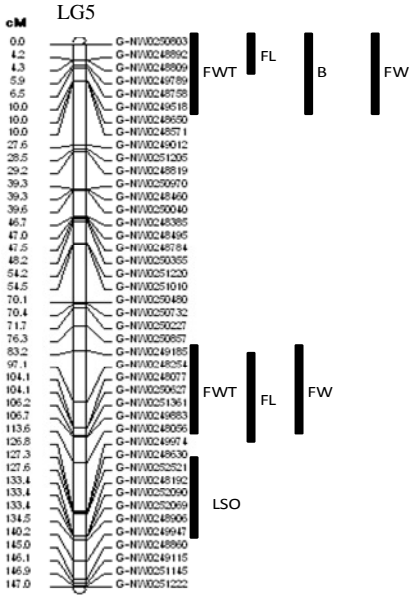
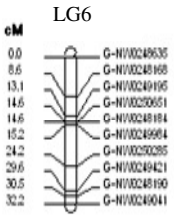
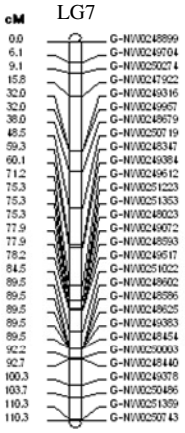
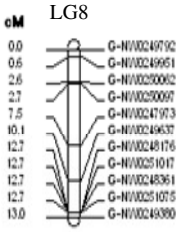


Figure 3.4 Map positions of each significant quantitative trait loci on each linkage group. *B* Brix, *RT* Rind Thickness, *FL* Fruit Length, *FW* Fruit Width, *FWT* Fruit Weight, *EG* Egusi Seed Trait, *SO* Seed Oil, *LSO* Low Seed Oil

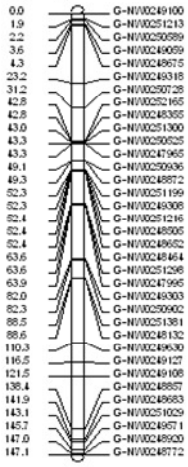

LG5
cM
FWT
FL
B
FW
FWT
FL
FW
LSO


LG6
cM

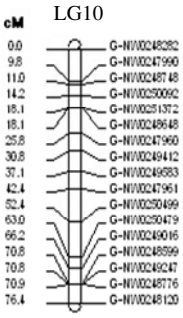

LG7
cM


LG8
cM

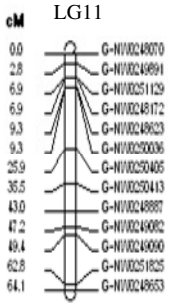
LG9



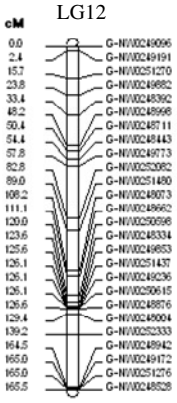
LG10



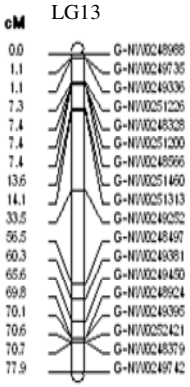
LG11



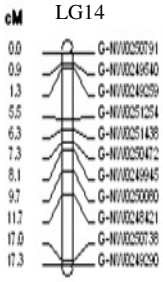
LG12



LG13


cM
0.0
1.1
1.1
7.3
7.4
7.4
7.4
13.6
14.1
33.5
56.5
60.3
65.6
69.8
70.1
70.6
70.7
77.9
G-NW002489681
G-NW002497135
G-NW002493361
G-NW002512261
G-NW002483281
G-NW002512001
G-NW002489661
G-NW002514601
G-NW002513131
G-NW002492521
G-NW002484971
G-NW002493811
G-NW002494501
G-NW002489241
G-NW002493951
G-NW002524211
G-NW002483791
G-NW002497421

LG14


cM
0.0
0.9
1.3
5.5
6.3
7.3
8.1
9.7
11.7
17.0
17.3
G-NW002507911
G-NW002496401
G-NW002492591
G-NW002512541
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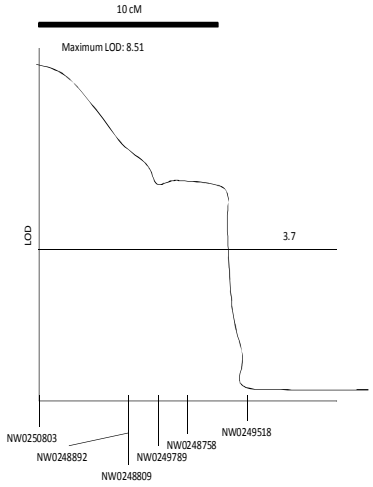
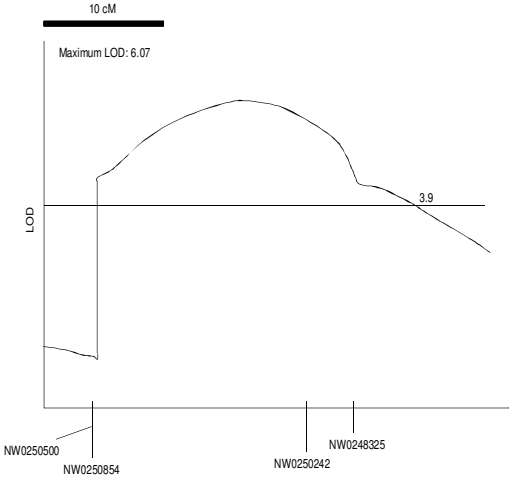
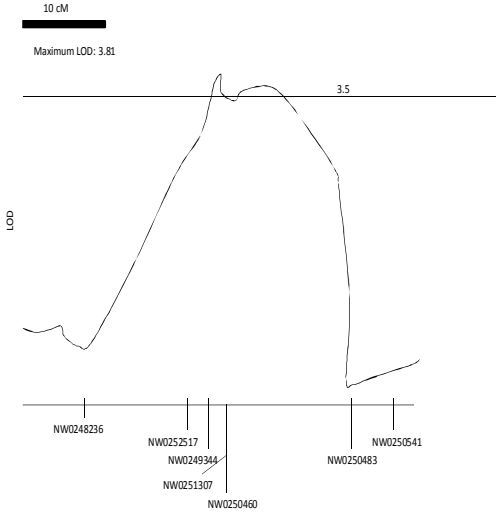
G-NW002484211
G-NW002507381
G-NW002492901



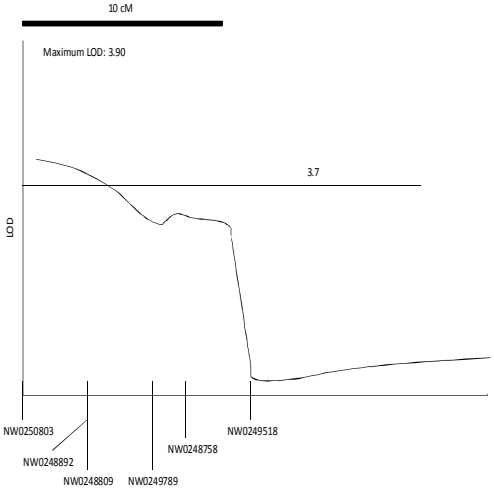
Brix LG5
(A)



Rind Thickness LG2
(B)

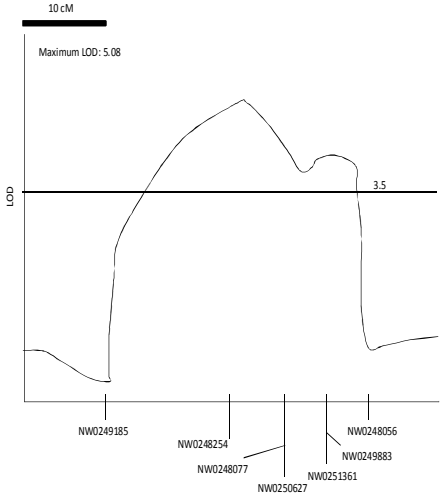


Fruit Length LG3
(C)

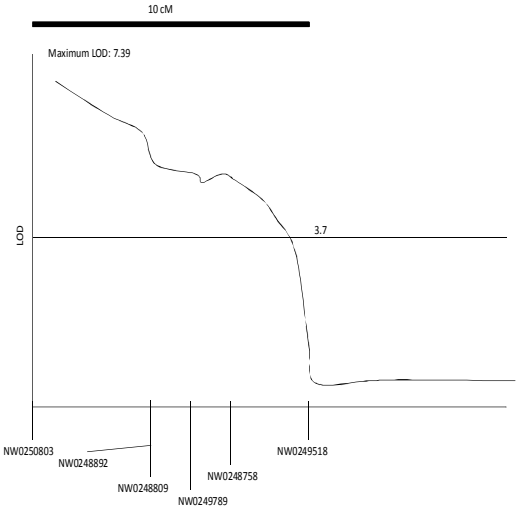


Fruit Length LG5
(D)

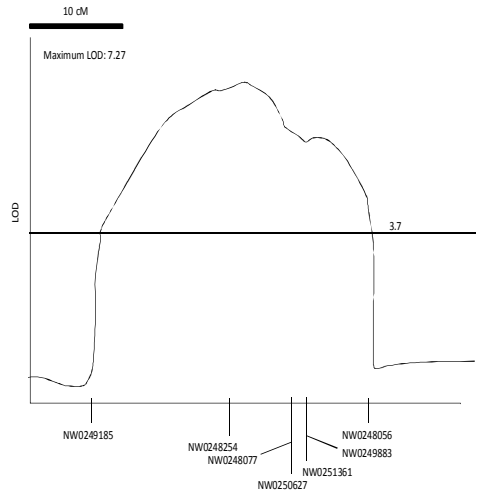
Figure 3.5 Maximum likelihood plots identifying genomic regions of quantitative trait loci associated with horticultural traits in the F_2 progeny of the Strain II x Egusi population. (A) Brix LG5, (B) Rind Thickness LG2, (C) Fruit Length LG3, (D) Fruit Length LG5, (E) Fruit Length LG5, (F) Fruit Width LG5, (G) Fruit Width LG5, (H) Fruit Weight LG3, (I) Fruit Weight LG5, (J) Fruit Weight LG5, (K) Egusi Seed Trait LG2, (L) Egusi Seed Trait LG2, (M) Seed Oil Percentage LG2, (N) Low Seed Oil LG2, (O) Low Seed Oil LG5.



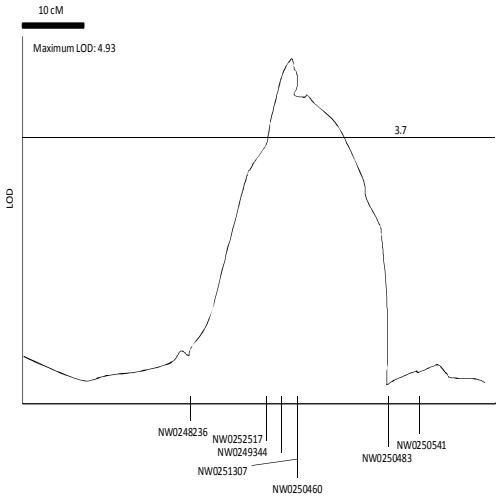
Fruit Length LG5
(E)



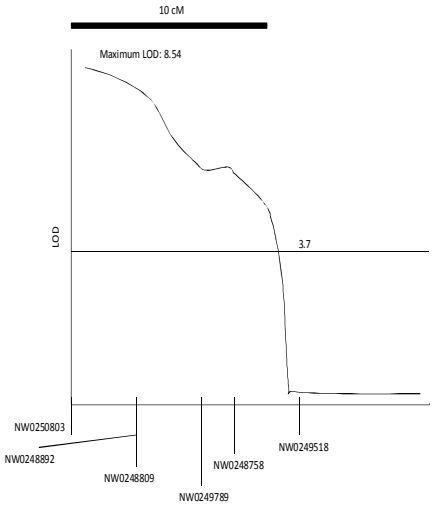
Fruit Width LG5
(F)



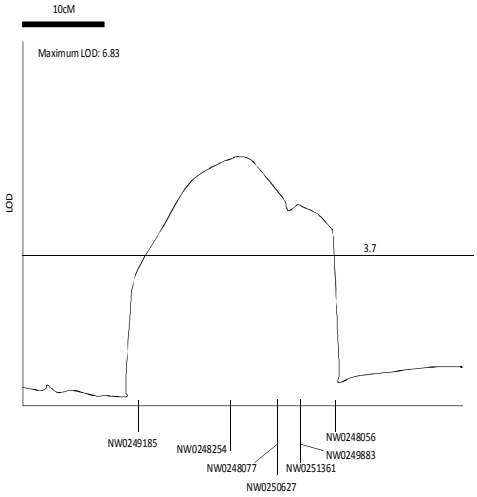
Fruit Width LG5
(G)



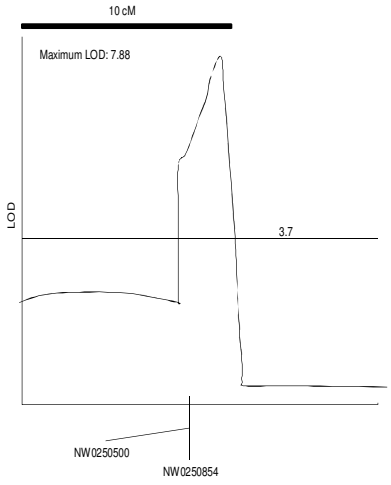
Fruit Weight LG3
(H)



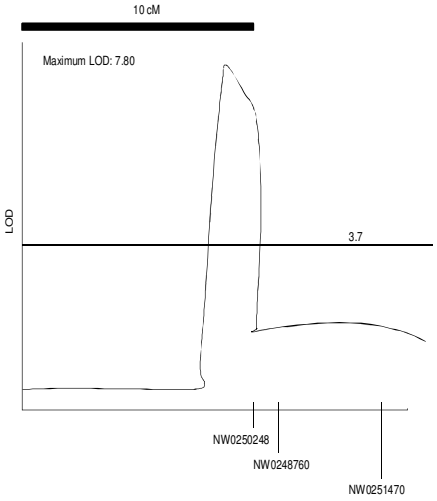
Fruit Weight LG5
(I)



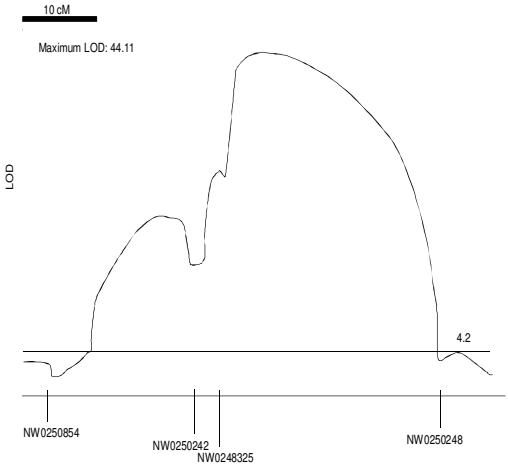
Fruit Weight LG5
(J)



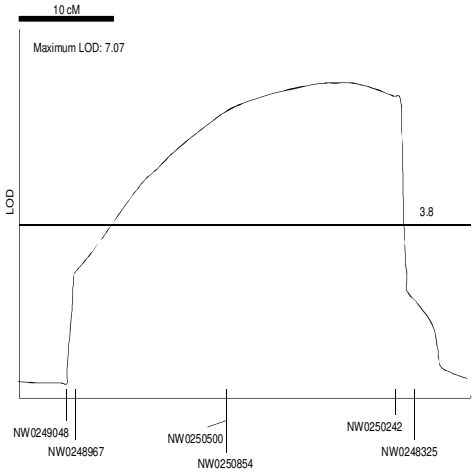
Egusi Seed Trait LG2
(K)



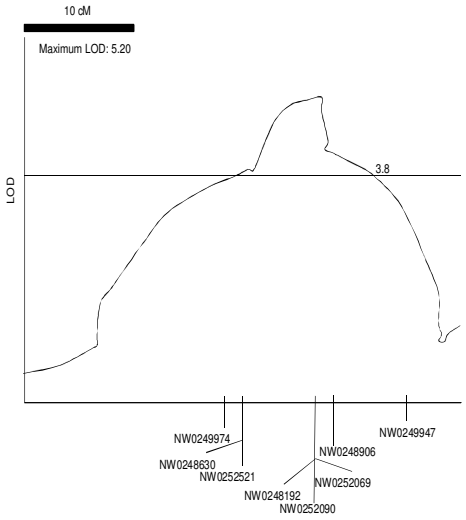
Egusi Seed Trait LG2
(L)



Seed Oil Percentage LG2
(M)



Low Seed Oil LG2
(N)



Low Seed Oil LG5
(O)

CHAPTER 4

QTL ANALYSIS OF WATERMELON FRUIT, SEED AND REPRODUCTIVE TRAITS IN AN ELITE x CITRON F_2 POPULATION[1]

[1]Prothro, J., A. Heesacker, N. Khalilian, E. Bachlava, V. White, W. Xiang, E. Chan, S.J. Knapp, and C. McGregor. To be submitted to *Journal of the American Society for Horticultural Science*.

Abstract

Watermelons (*Citrullus lanatus* var. *lanatus* Thunb.) are an important vegetable crop worldwide. The fresh market value of watermelon in the United States in 2009 was $460 million. Watermelons are believed to have originated in the Kalahari Desert region of Africa and were first cultivated over 4,000 years ago. Watermelon was first introduced into the America's in the 1500's. Since then, many improvements have been made to the watermelon and an estimated 80 percent of the crop produced in the United States is seedless varieties. Breeders focus on improving a wide variety of characteristics ranging from disease resistance to fruit quality. Traits such as Brix have high value in watermelon, but low heritability can make improvement a long and arduous task. Implementation of marker assisted selection (MAS) in watermelon has lagged behind MAS application in other crop species because limited genetic mapping information is available to breeders. In this study, an F_2 population was developed between the cultivar ZWRM50 (PI 593359) and the wild accession Delagoa (PI 244019) with the intent to genetically map multiple fruit quality, morphological, and seed traits. ZWRM50 is an elite watermelon cultivar originating from China and Delagoa is a citron type melon originating from South Africa. A linkage map was constructed that contains 338 single nucleotide polymorphism (SNP) markers on 16 linkage groups. Twelve fruit quality, seed, and morphology traits such as Brix and sexual determination were scored in this population and 18 QTL were identified for these traits. Individual R^2 values for identified QTL ranged from a minimum of 6.57% for flesh firmness to 69.82% for seed length.

Introduction

Watermelon (*Citrullus lanatus* var. *lanatus* Thunb.) is a member of the *Cucurbitaceae* family (Dane and Liu, 2007) and is believed to have originated in the Kalahari Desert region of Africa (Mohr, 1986). Watermelon was first cultivated 4,000 years ago in Africa and the Middle East as a source of food, water, and animal feed and was first grown in the Americas in the 1500's (Guhner and Wehner, 2004). Today, watermelon is an important vegetable crop with global production worldwide in 2009 totaling 100.68 million metric tons (Food and Agricultural Organization-FAO, 2009). The value of the fresh market crop in the United States in 2009 was estimated at $460 million (United States Department of Agriculture, National Agricultural Statistics Service, 2010). Commercial watermelons are produced from seed of diploid or triploid single cross hybrids and from open pollinated cultivars. Hybrid varieties dominate the commercial market and in 2007, 80 percent of the watermelons produced were reported to be seedless watermelons (United States Department of Agriculture, National Agricultural Statistics Service, 2009).

Several fruit quality and morphology traits need to be considered when breeding watermelons. Brix is a high value trait in watermelon and is measured as degrees Brix. Brix is directly correlated with the total soluble solid (TSS) content of watermelon (MacGillivray, 1947; Maynard, 2001; Hashizume et al., 2003). Commercial cultivars of watermelon have high Brix which gives them a sweet flavor while wild types of watermelon have low Brix (Wehner et al., 2001). Hashizume et al. (2003) mapped a quantitative trait locus (QTL) for Brix in a BC_1 population derived from the parents H-7 (*C. lanatus*) and SA-1 (*C. lanatus*). H-7 is an elite inbred and SA-1 is a wild form of watermelon originating from South Africa. The locus mapped for Brix accounted for 19% of the variation in the trait. The linkage map developed in their

study contained 11 linkage groups with 477 random amplified polymorphic DNA (RAPD), 53 restriction fragment length polymorphism (RFLP), 23 inter-simple sequence repeat (ISSR), and one isozyme markers. Hashizume et al. (2003) suggest that Brix has a very low heritability and identifying QTL with genetic markers linked to them can help to make the selection process for Brix quicker and more efficient.

While Brix is one of the most valued traits in watermelon, there are other traits that watermelon breeders must consider. The genetics of these traits have not been extensively studied. Watermelon shape is shown to be controlled by the incompletely dominant elongate fruit gene (*O*). Watermelon fruit can either be elongate (*OO*), oval (*Oo*), round (*oo*), or blocky shape. The blocky shape phenotype is described as being an intermediate shape and only observed in F_1 fruit (Wehner et al., 2001). Rind thickness of watermelons must be maintained as a small percentage of the diameter of a fruit. Small fruited watermelons must have a very thin rind while larger fruited watermelon will have a thicker rind. The thicker rind of the large fruited watermelons will help protect the melon during post harvest handling and shipping (Wehner et al., 2001). Fruit weight is used to classify watermelons based on size and is also a yield component. Watermelon fruit is classified as being giant (more than 14.5 kg), large (11.1-14.5 kg), medium (8.1-11.0 kg), small (5.5-8.0 kg), or icebox (less than 5.5 kg). Recently, mini fruit size (1.5-4.0 kg) was added to fruit size classification (Gusmini and Wehner, 2007). Growers expect to be able to harvest 50.5 metric tons ha^{-1} of marketable watermelons (Wehner, 2008). No genes have been identified for watermelon weight and this trait is shown to have low heritability (Gusmini and Wehner, 2007).

Consumers prefer seedless watermelons or watermelons with a small seed size. The small seed size allows for easier consumption of watermelon fruit. Watermelon seed can be

large (10 mm), medium (7 mm), small (5 mm), tomato, or tiny seed size. Tomato seed size is approximately the same size as tomato seed while tiny seed size is slightly smaller than small seed size (Wehner et al., 2001; Gusmini, 2005). An interaction between the large seed (*l*) and small seed (*s*) genes determines whether seed is large (*llSS*), medium (*LLSS*), or small (*LLss* or *llss*). Tomato seed size is controlled by the *ts* gene (Zhang, 1996) while tiny seed size is controlled by the *Ti* gene (Takayuki et al., 1995).

There are numerous fruit quality and morphological traits that must be considered when breeding watermelons, but other phenotypic traits such as sex determination must also be considered. Most plant species contain perfect flowers that have both staminate and pistillate flowering structures (Noguera et al., 2005). However, some plants species have spatial separation of male and female flowering structures. Monoecious plants have separate male and female flowers that are contained on the same plant. Dioecious plants have separate male and female flowers that are contained on separate plants. (Whitaker, 1931; Perl-Treves, 1999). Several variations of these flowering types exist. Andromonoecious plants have male and hermaphroditic flowers while gynoecious plants have female and hermaphroditic flowers. Gynoecious plants have all female flowers while androecious plants have all male flowers. Trimonoecious plants contain male, female, and hermaphroditic flowers on the same plant (Whitaker, 1931). Commercial cultivars of watermelon express monoecious flowering type with a typical flower ratio of 7 male: 1 female flower (Wehner, 2008). There are several advantages to having the monoecious flowering type in watermelon breeding lines. Hand emasculation becomes unnecessary because the male and female flowering structures are separated. Monoecious plants produce fruit with smaller bottom scars (Noguera et al., 2005). Smaller

bottom scars help protect the fruit from pathogen invasion and help improve fruit quality (Perin et al., 2002).

The andromonoecious (*a*) gene has been described in watermelon and is recessive (*aa*) to monoecious flowering type (Wehner, 2008). While few studies have investigated sex determination in watermelon, members of closely related species such as cucumber (*Cucumis sativus* L.) and melon (*Cucumis melo* L.) can serve as models when investigating sex determination in watermelon (Perl-Treves, 1999; Roy and Saran, 1990). Three genes have been described in cucumber and melon that control sex expression. The female (*F*), male (*M*), and andromonoecious (*A*) gene are responsible for sex expression in cucumber (Peterson, 1975; Lower and Nienhuis, 1990). The andromonoecious (*a*), gynomonoecious (*g*) and maleness (*M*) gene are responsible for sex expression in melon (Poole and Grimball, 1939; Keningsbuch and Cohen, 1990; Roy and Saran, 1990). The a locus has been mapped several times in melon (Danin Poleg et al., 2002; Perin et al., 2002; Silberstein et al., 2003), most recently by Noguera et al. (2005) who developed a sequence characterized amplified region (SCAR) marker that is located 3.3 cM from the locus.

Improving fruit quality and morphological traits such as the ones discussed here are essential for a successful watermelon breeding. The genetic base of commercial watermelon cultivars has been reported to be very narrow (Navot and Zamir, 1987; Zhang et al., 1994; Lee et al., 1996; Levi et al., 2001, 2004). Similarity coefficients (SC) between commercially available cultivars have been shown to be as high as 0.99 (Che et al. 2003). The narrow genetic diversity raises concerns because commercial cultivars can be more susceptible to widespread outbreaks of pathogens and disease due to the limited genetic diversity (Levi et al., 2002; Harris et al., 2009). The narrow genetic base can be alleviated by the introduction of alleles from wild

germplasm (Atlagic et al., 1993; Quillet et al., 1995). Interspecific crosses between elite and wild material are a common method used to introduce variation into breeding lines but problems with reduced fertility can make this process difficult (Heiser et al., 1964; Whelan 1978).

Shimotsuma (1960) developed an intraspecific hybrid and two interspecific hybrids with accessions of Citrullus colocynthis and Citrullus lanatus for cytological and morphological observation. Reduced pollen viability was observed in the F_1 hybrids and about 50 percent of the pollen mother cells (PMC) showed 11 bivalents. Multivalents, trivalents and univalents were observed in the remaining cells. Singh (1978) and Yadav (1982) used the same species for cytological study and reported observing mostly quadrivalent configurations. Sain et al. (2002) also used accessions of Citrullus colocynthis and Citrullus lanatus to develop F_1 hybrids. A high number of bivalent associations were observed in PMC's during metaphase I but reduced fertility was also observed. Sain et al. (2002) concluded that several factors could be responsible for reduced pollen fertility such as structural differences between chromosomes that result in gene imbalance and reduced fertility. Studies such as these can help to improve our understanding of interspecific hybridization and allow for breeders to better plan how to introduce wild alleles into breeding lines.

In addition to understanding the genetic factors that affect the introduction of wild alleles into breeding lines, breeders must evaluate germplasm diversity to effectively make use of these genetic resources (Che et al., 2003). Levi and Thomas (2005) used RAPD markers to classify watermelons into three major phenetic groups. *Citrullus lanatus* var. *citroides* is comprised of citron type melons, *Citrullus lanatus* var. *lanatus* is made up of wild watermelon accessions and cultivated watermelons. *Citrullus colocynthis*, also known as the bitter apple, is native to north and western regions of Africa and have been proposed as being an ancestor of watermelon

(Wehner, 2008). *C. lanatus* var. *citroides* is believed to be the wild progenitor of *C. lanatus* var. *lanatus* (Navot and Zamir, 1987). Nimmakayala et al. (2009) used amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers to determine the relationship between *Citrullus* spp. This study indicated that extensive introgression has occurred with *citroides* and *colocynthis*. They further explain that because of this extensive introgression, *citroides* is more closely related to *colocynthis* than it is related to *lanatus*. However, the results of this study do not agree with the results of similar studies that reported *C. lanatus* var. *citroides* was more closely related to *C. lanatus* var. *lanatus* than to *C. colocynthis* (Levi et al., 2001; Dane and Liu, 2004; Levi and Thomas, 2005).

Marker assisted selection (MAS) is a valuable tool that enable breeders to efficiently introduce favorable alleles for fruit quality and morphological traits such as disease resistance from wild germplasm sources into breeding lines. This study focuses on mapping QTL for important fruit, seed, and morphological traits in a F_2 elite x citron population. The long-term goals of this project are to enhance the infrastructure for applying MAS in watermelon breeding programs and broaden genetic diversity in commercially important elite watermelon germplasm by introducing favorable alleles from exotic germplasm sources.

Materials and Methods

Development of Plant Material

Seed of ZWRM50 (PI 593359) and Delagoa (PI 244019) were obtained from the Germplasm Resource Information Networks (GRIN) Southern Regional PI Station in Griffin, GA. ZWRM50 is an elite cultivar of watermelon originating from Shaanxi, China and Delagoa is a citron type melon that originates from Transvaal, South Africa (Figure 4.1) (United States

Department of Agriculture, Agriculture Research Service, 2010). The F_2 population that was used for mapping in this study was developed by cross pollinating ZWRM50 with Delagoa. F_1 seed was collected from the fruit that resulted from the pollination and a single F_1 seed was grown. The F_1 was self-pollinated and F_2 seed was collected from the self-pollinated fruit. Individual F_2 plants were planted in containers using Fafard 3B Potting Mix (Conrad Fafard, Inc., Agawam, Massachusetts). The plants were trained vertically on a trellis system.

Trait Evaluation

Two hundred individual F_2 seeds were planted in a greenhouse at the University of Georgia's campus Athens, GA in the summer of 2007. One mature self-pollinated fruit from 139 individuals was harvested and phenotyped. Degrees Brix was measured using a refractometer (Atago Co., Tokyo, Japan) from a sample of juice collected from the center of each watermelon. A digital caliper (Balkamp Manufacturing Corp., Indianapolis, Indiana) was used to measure the rind thickness in the middle of the fruit, half way between the apex and the pedicel of the fruit. Fruit width was measured in centimeters at the widest part of the fruit as the distance between each edge of the fruit. Fruit length was measured in centimeters as the distance between the fruit apex and the point at which the pedicel attached to the fruit. Fruit weight was measured in kilograms and was recorded at maturity. Seed length and width were measured in millimeters with a digital caliper. Seed width was measured at the widest part of the seed while seed length was measured at the longest part of the seed. Flowering pattern for the sex determination study were determined by phenotyping the first 20 flowering nodes on each plant as male, female, or hermaphrodite. For hybrid fertility, a single flower was collected from each plant before anthesis and placed on ice. The pollen staining procedure described by Alexander (1969) was used to

differentiate between aborted and non-aborted pollen cells. A Pearson Correlation Matrix was developed for all traits using the PROC CORR procedure (SAS Institute, 2003).

Linkage and QTL Analysis

Approximately four weeks after planting, fresh leaf tissue from each plant was collected and frozen. SNP genotyping was performed using the GoldenGate assay method as described by Hyten et al. (2009). The SNP panel was constructed by Monsanto using proprietary methods. Monsanto developed the linkage map used for QTL mapping that consist of 338 SNP markers on 16 linkage groups spanning a distance of 1,144.057 cM with an average distance of 3.384 cM between markers. Composite interval QTL mapping (CIM) was performed with WinQTLCart version 2.5 mapping software (Wang et al., 2010). The CIM model number 6 with backward regression method was used. Significance thresholds were determined by using 1000 permutations with a significance level of $P = 0.05$. Traits showing a segregation ration of 3:1 were tested by chi square analysis.

Results and Discussion

Twelve traits were scored for QTL mapping in an F_2 population of ZWRM50 x Delagoa. Eleven of these traits showed continuous variation (Figure 4.2) which is suggested that these traits are controlled by more than one gene. The traits also showed transgressive segregation with the values of the F_2 population exceeding those of the parents (Figure 4.3). Individual loci R^2 values for these traits ranged from a minimum 6.57% for flesh firmness to a maximum value of 69.82% for seed length (Table 4.1) and individual QTL profiles are shown in figure 4.4.

Brix is a high value trait in watermelon with a low heritability. Improvement of this trait in watermelon requires a long breeding process using traditional breeding methods (Hashizume et al., 2003). Marker assisted selection (MAS) for QTL affecting Brix can speed up

the breeding process. One QTL for Brix was identified in this study. The QTL is located on LG4 and accounts for 18.28% of the variation. All individuals evaluated in this population had low Brix values. The citron parent had a low Brix (1.4) while the elite parent had a high degrees Brix (10.3). The F_2 population mean was 2.42 degrees Brix while values ranged from 1 to 4.2 (Table 4.2). The parents used in this study were similar (elite x citron) to the ones used by Hashizume et al. (2003) used to develop a BC_1 population for QTL mapping. They identified a QTL for Brix and reported that the F_1 individual had a low Brix, suggesting partial dominance of the loci associated with low degrees Brix. We observe a similar trend in our population.

Consumers demand a wide variety of fruit sizes (Wehner et al., 2001). Fruit length and width were measured in this population and mapped separately. These two traits were also combined to determine a fruit shape ratio (length:width). Two QTL were detected for fruit length and for fruit width. For fruit length, QTL were identified on LG11 and LG12 that account for 11.41% and 40.09% of the variation, respectively. Two QTL identified for fruit width are located on LG11 and LG15 and explain 16.38% and 9.24% of the variation, respectively. The QTL identified on LG11 for both fruit length and fruit width mapped to the same position on that linkage group. One QTL was identified for fruit shape. This QTL mapped to LG12 and explains 32.08% of the variation in this trait. The QTL identified for fruit shape mapped to the same region as the QTL identified on LG12 for fruit length. No genes have been described specifically for fruit length or width, but the elongate fruit gene (*o*) has been reported as being responsible for fruit shape (Wehner et al., 2001). The genomic region described here on LG12 that harbors QTLs for fruit length and fruit shape ratio could be a potential location of the elongate fruit gene. However, recent research has shown that fruit shape may be controlled by

more than a single gene (Kumar, 2009) and it is possible that more than one gene is interacting to control fruit shape in this population.

One QTL for fruit weight was identified on LG11. This QTL explains 15.94% of the variation in this trait. Fruit weight is an important characteristic for both growers and consumers. Consumers demand a wide variety of fruit weights and growers need to maximize their yield while producing a marketable watermelon (Wehner, 2008). As with Brix, watermelon weight has a very low heritability and improvement of this trait takes time and effort (Gusmini and Wehner, 2007). As expected, fruit weight is significantly correlated with fruit length and width.

Two QTL were identified for seed length. These QTL are located on LG9 and LG11 and explain 69.82% and 19.49% of the variation in this trait. Two QTL were also identified for seed width. One QTL is located on LG9 and explains 68.40% of the variation. The other QTL is located on LG11 and accounts for 25.76% of the variation. The QTL identified on LG9 for seed length and seed width map to the same region of that linkage group and account for a large amount of the variation seen in those traits. The QTL identified on LG11 for each trait also map to the same region.

One hundred seed weight was also recorded in this population. QTL for this trait were identified on LG9 and LG11. The QTL on LG9 mapped to the same region as the QTL on LG9 for both seed width and seed length and accounts for 65.42% of the variation. The QTL on LG11 for 100 seed weight mapped to the same region as the QTL on LG11 for seed length and width. Two genes (*l*, *s*) have been described as controlling seed size in watermelon (Poole et al., 1941). The QTL located on LG9 for seed length, width and 100 seed weight map to the same region and have a very large effect. The QTL identified on LG11 for seed length, seed width and

100 seed weight could also be another location of one of these genes. Genes for seed size have not been genetically mapped, but this could be a likely location of one or both of these genes. Seed size is an important trait that consumers consider when purchasing watermelons. If seedless melons are not available, small seeded watermelons are preferred. Significant advances in seed size should be expected if one selects for the QTL located on LG9 and LG11.

Two QTL were identified for the trait "percent aborted pollen". One QTL is located on LG7 and explains 9.46% of the variation while the other identified QTL is located on LG12 and explains 9.40% of the variation in this trait. Watermelon (Citrullus lanatus var. lanatus) and citron (Citrullus lanatus var. lanatus) are classified as being botanical varieties (Wehner et al., 2001) but hybrid fertility is apparently reduced in watermelon x citron hybrids (Dr. Steve Knapp, personal communication). It is suspected that these botanical varieties carry chromosomal rearrangements that can produce meiotic abnormalities and reduce pollen viability. The QTL identified here may indicate the breakpoints that have led to chromosomal rearrangements between watermelon and citron.

Flowering pattern was recorded in the F_2 population with the intent of identifying QTL that are responsible for sexual expression in watermelon. The frequency of flower type (male, female and hermaphroditic) for each individual plant was calculated and used for QTL analysis. Three QTL were identified for female flowering frequency. These QTL are located on LG8, LG9 and LG12. The QTL on LG8 and LG9 explained 7.68% and 7.69% of the variation in this trait respectively. The QTL located on LG12 explained 28.6% of the variation in this trait. Two QTL were identified for hermaphroditic flowering frequency and are located on LG12 and LG16 and these QTL account for 38.77% and 6.82% of the variation in this trait respectively. No QTL were identified for male flowering frequency. The QTL identified for female flowering

frequency and hermaphroditic flowering frequency on LG12 both map to the same position on the linkage group and explain a large amount of the phenotypic variation in this trait. The andromonoecious gene (*a*) is the only gene in watermelon that has been described as controlling sexual expression (Guner and Wehner, 2004). These significant QTL identified on LG12 could be the possible location of the andromonoecious gene. As described here, several other QTL were identified for these traits that have smaller effects. The andromonoecious gene has been identified in cucumber and melon along with several other genes that are responsible for modifying sexual expression (Perl-Treves, 1999; Roy and Saran, 1990). The QTL that were mapped in this study could be possible locations of genes that have a modifying effect on the flowering trait in watermelon.

No significant QTL were identified for rind thickness. One explanation for finding no QTL in rind thickness is there was difficulty encountered when measuring this trait in this particular population. Citron produces small fruit with tough flesh that is green to white in color. These two factors make it difficult to determine where the rind of the fruit ends and where the flesh begins. A different approach may be necessary when measuring this trait in this population. Mapping in a different population in which the progeny have a more defined rind may be necessary.

The genetic base of cultivated watermelon is very narrow (Levi et al., 2000, 2002; Harris et al., 2009) and the infrastructure for applying MAS in watermelon does not exist. The QTL identified in this study can help to broaden the genetic base of commercially important watermelon cultivars by introducing favorable alleles from wild germplasm accessions.

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Table 4.1 Genomic regions significantly associated with QTL for the traits phenotyped in the ZWRM50 x Delagoa F_2 population

Trait	Linkage Group	Map QTL Position	Flanking Markers	Map Position	Maximum LOD	R^2	Additive[1]	Dominance
BRIX	4	91.20	NW0250697	75.19	3.86	18.28	0.479	0.022
			NW0249088	108.24				
Fruit Length	11	29.97	NW0248254	24.96	5.47	11.41	-0.003	1.335
			NW0249313	34.59				
	12	17.34	NW0248107	13.33	18.06	40.09	1.271	1.164
			NW0249365	20.37				
Fruit Width	11	29.97	NW0248254	24.96	6.64	16.38	0.389	0.538
			NW0249313	34.59				
	15	19.88	NW0249313	18.87	4.03	9.24	-0.565	0.187
			NW0248714	22.41				
Fruit Shape (length:width)	12	18.34	NW0248107	13.33	13.76	32.08	0.206	0.327
			NW0249365	20.37				
Fruit Weight	11	74.81	NW0250470	70.80	6.45	15.94	1.254	-0.252
			NW0248650	77.71				
Seed Length	9	21.13	NW0248118	20.12	40.19	69.82	0.887	-1.006
			NW0248583	28.30				
	11	26.97	NW0248254	24.96	13.52	19.49	0.547	0.980
			NW0249313	34.59				
Seed Width	9	23.13	NW0248118	20.12	40.19	68.40	-0.880	-1.071
			NW0248583	28.30				
	11	22.15	NW0248796	20.14	18.20	25.76	0.447	1.042
			NW0248254	24.96				
100 Seed Weight	9	24.13	NW0248118	20.12	28.21	65.42	-2.135	-2.191
			NW0248583	28.30				
	11	19.50	NW0249883	17.49	10.81	18.16	0.668	2.377
			NW0248796	20.14				

Trait	Linkage Group	Map QTL Position	Flanking Markers	Map Position	Maximum LOD	R^2	Additive[1]	Dominance
Percent Aborted Pollen	7	74.11	NW0248861	74.10	4.15	9.46	-2.904	-3.011
			NW0248560	77.96				
	12	50.15	NW0248648	46.14	4.11	9.40	6.317	-5.419
			NW0251028	50.85				
Female Flowering Frequency	8	5.07	NW0248268	5.06	4.27	7.68	0.035	0.009
			NW0248392	12.54				
	9	11.02	NW0251455	6.01	3.88	7.69	-0.030	-0.009
			NW0248118	20.12				
	12	23.38	NW0249365	20.37	14.00	28.6	0.041	0.027
			NW0250956	26.45				
Hermaphroditic Flowering Frequency	12	23.38	NW0249365	20.37	18.44	38.77	-0.056	-0.040
			NW0250956	26.45				
	16	6.11	NW0252173	2.10	3.95	6.82	-0.024	-0.009
			NW0249208	6.39				
Rind Thickness[2]								
Male Flowering Frequency[2]								

[1]Positive values of the additive effect indicate that alleles from Strain II are contributing a positive effect on the phenotypic value of the trait

[2]No QTL's detected

Table 4.2 Phenotypic values of the parents and F_2 progeny of the ZWRM50 x Delagoa population

Trait	Parents		F_2			
	ZWRM50	Delagoa	Min	Max	Mean	SD
Brix (degrees)	10.30	1.40	1.00	4.20	2.42	0.676
Rind Thickness (cm)	1.27	0.32	2.74	38.00	9.25	6.476
Fruit Length (cm)	20.64	9.53	4.76	32.39	18.17	5.467
Fruit Width (cm)	16.51	7.94	4.45	17.15	10.38	2.588
Fruit Length/Width	3.18	3.05	2.40	9.36	4.51	1.252
Fruit Weight (kg)	2.61	0.34	0.05	3.74	1.11	0.756
Seed Length (cm)	30.84	16.56	7.98	34.06	18.67	5.732
Seed Width (cm)	15.82	11.61	4.37	19.94	11.24	3.552
100 Seed Weight (g)	8.65	4.61	8.11	15.30	3.95	3.543
Aborted Pollen Cells (%)	3.00	2.00	0.50	61.00	13.28	11.699

Table 4.3 Pearson correlations for traits measured in the ZWRM50 x Delagoa F_2 population. Shaded boxes indicate significant (P<0.05) correlations.

Trait	BRIX	Rind Thickness	Fruit Weight	Fruit Length	Fruit Width	Fruit L/W	Seed Length	Seed Width	100 Seed Weight
Rind Thickness	0.23906								
Fruit Weight	0.26033	0.37263							
Fruit Length	0.17206	0.30886	0.64169						
Fruit Width	0.24759	0.33138	0.77462	0.57039					
Fruit L/W	-0.04985	0.03411	-0.00918	0.62132	-0.25104				
Seed Length	-0.23159	0.0623	0.12367	0.27124	0.23044	0.09994			
Seed Width	-0.235	0.03909	0.12748	0.25176	0.20252	0.09983	0.95478		
100 Seed Weight	0.33991	0.0634	0.03276	0.04537	0.01065	0.0323	0.34263	0.37268	
Percent Aborted Pollen	0.07497	0.07806	0.08517	0.05982	0.08137	-0.0336	0.04835	0.08309	-0.08794

ZWRM50 (PI 593359)

Delagoa (PI 244019)

Figure 4.1 Cross section through mature fruit of parents of the F_2 mapping population

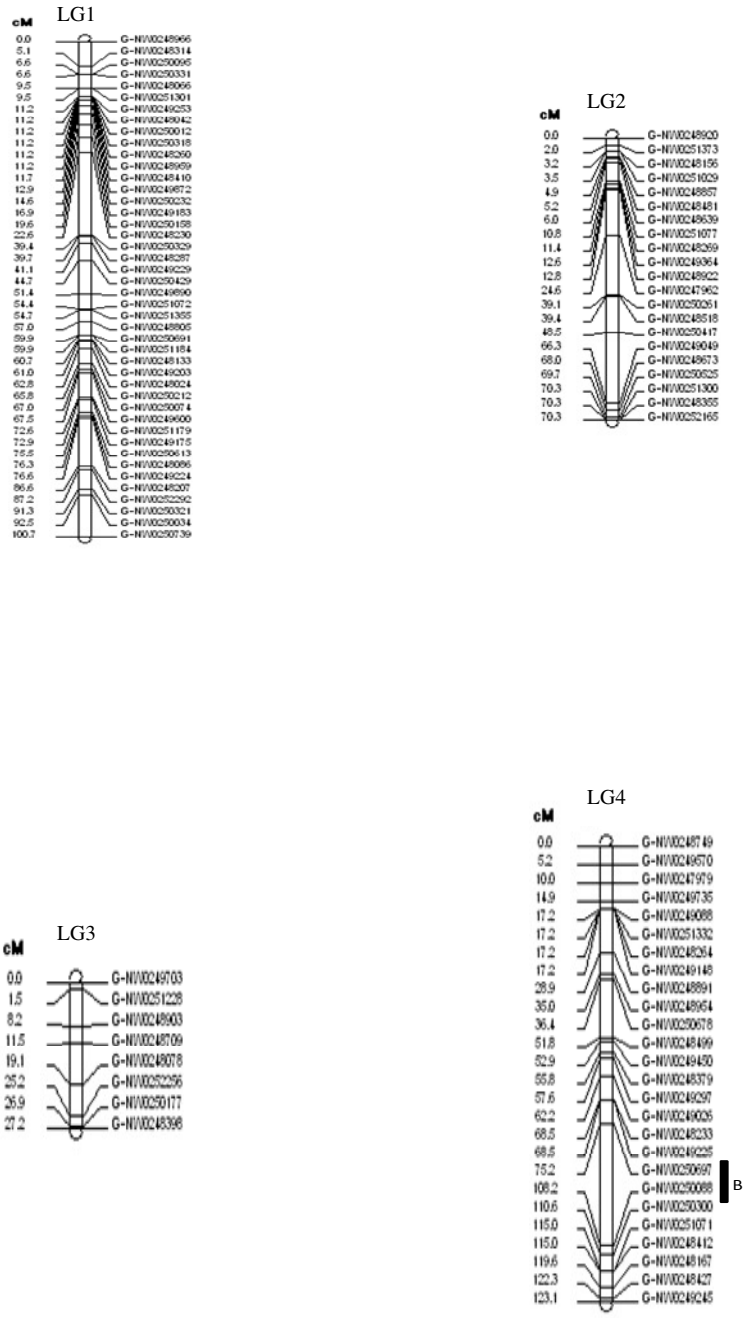
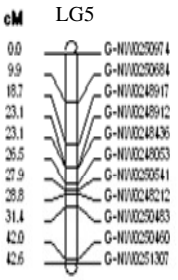
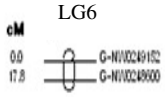
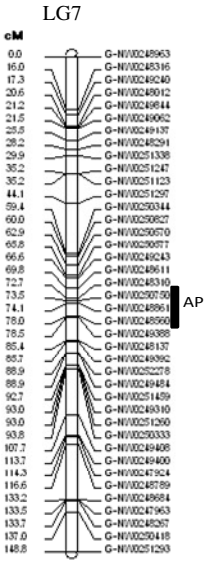
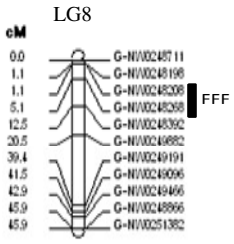


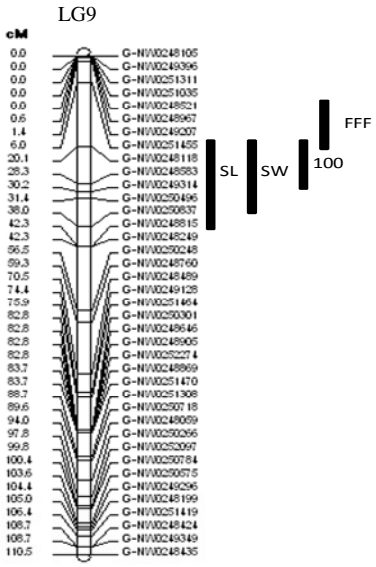
Figure 4.2 Map positions of each significant QTL on each linkage group. *B* Brix, *FL* Fruit Length, *FW* Fruit Width, *FS* Fruit Shape, *FWT* Fruit Weight, *SL* Seed Length, *SW* Seed Width, *100* 100 Seed Weight, *AP* Aborted Pollen, *FFF* Female Flower Frequency, *HFF* Hermaphroditic Flower Frequency


LG5
cM
0.0
9.9
18.7
23.1
23.1
26.5
27.9
28.8
31.4
42.0
42.6
G-NV0250974
G-NV0250684
G-NV0248917
G-NV0248912
G-NV0248436
G-NV0248053
G-NV0250541
G-NV0248212
G-NV0250483
G-NV0250460
G-NV0251307

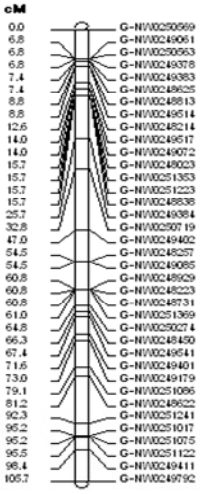

LG6
cM
0.0
17.8
G-NV0249102
G-NV0249600


LG7
cM
AP

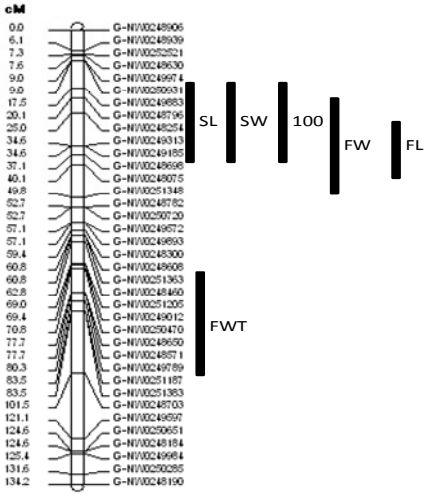

LG8
cM
FFF


LG9
cM
SL
SW
100
FFF

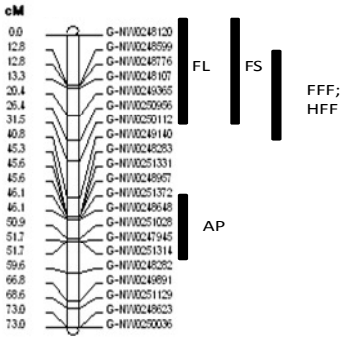
LG10



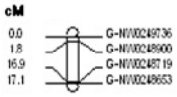
LG11



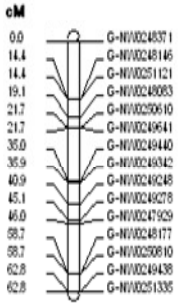
LG12



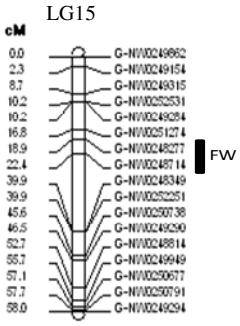
LG13



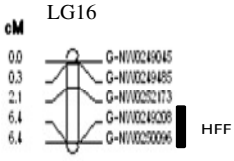
LG14



LG15



LG16



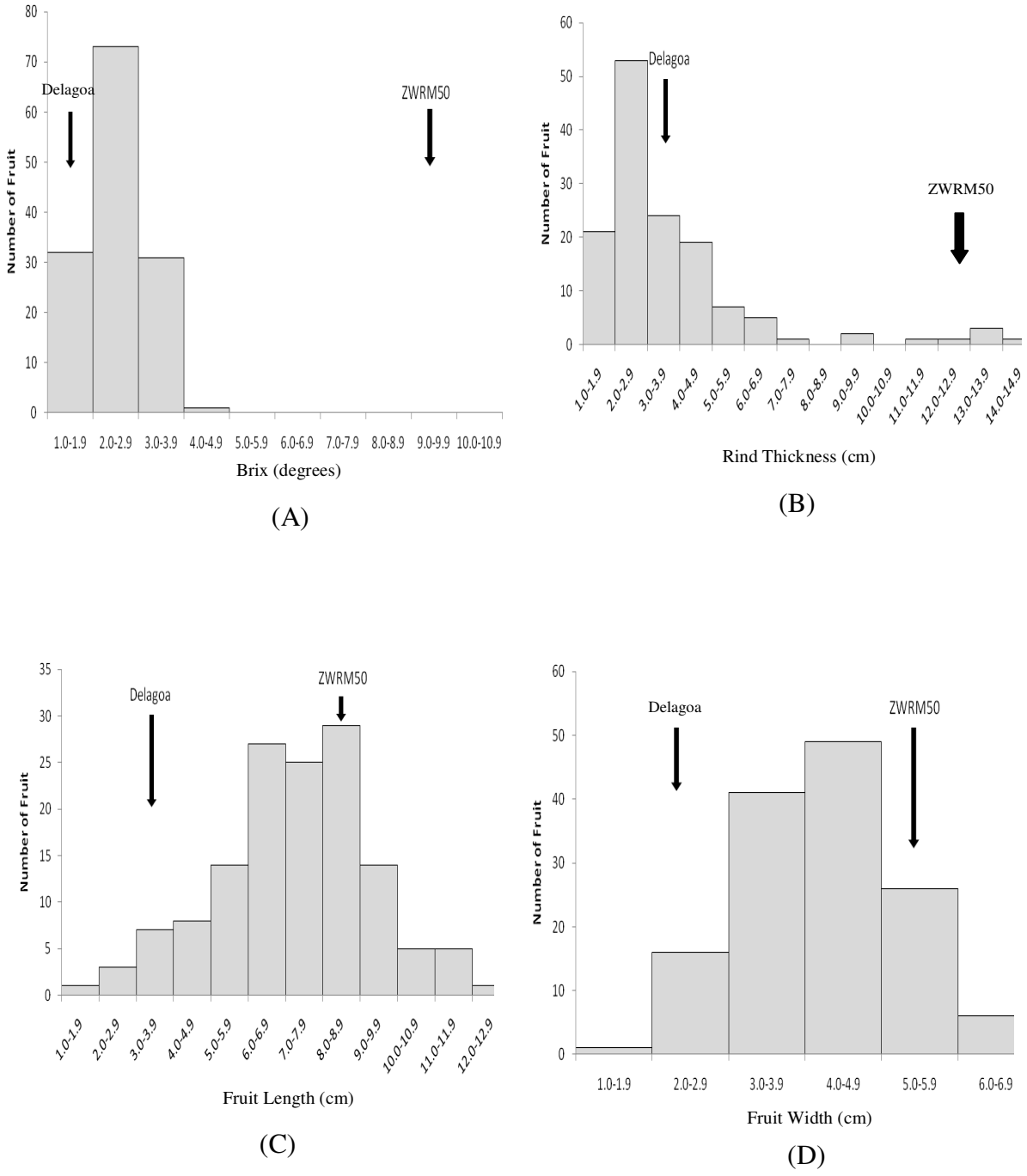
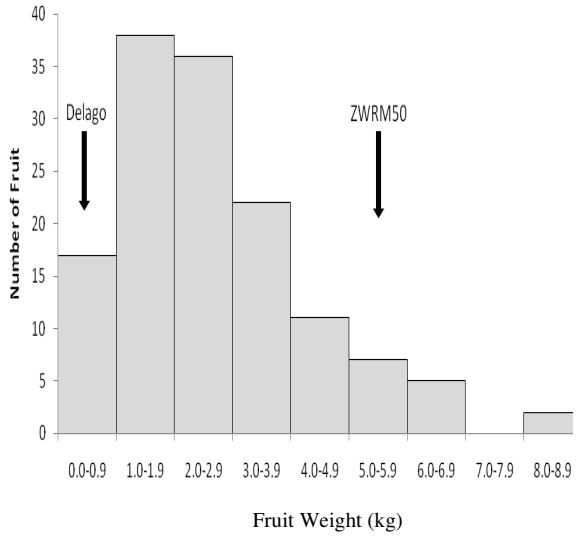
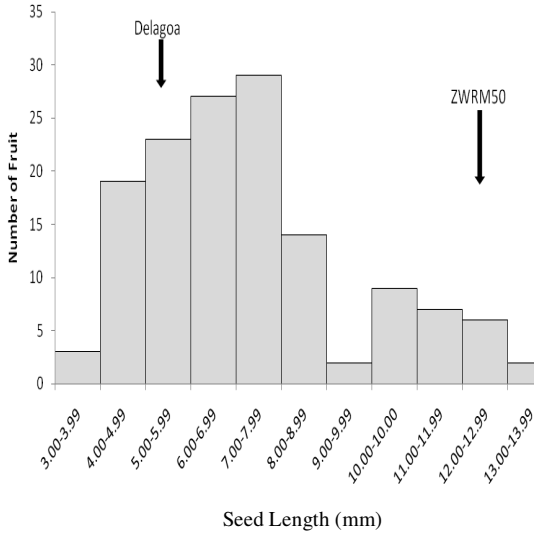


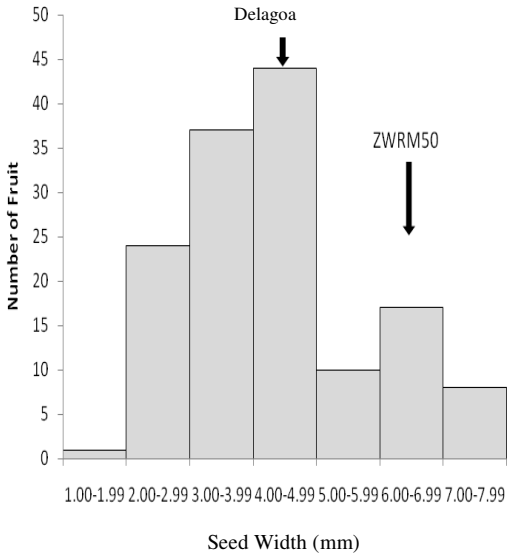
Figure 4.3 Frequency distribution for horticultural traits in the F_2 progeny. Arrows represent phenotypic values of the parents. (A) Brix, (B) Rind Thickness, (C) Fruit Length, (D) Fruit Width, (E) Fruit Weight, (F) Seed Length, (G) Seed Width, (H) 100 Seed Weight, (I) Aborted Pollen, (J) Female Flower Frequency, (K) Hermaphroditic Flower Frequency, (L) Male Flower Frequency


Delago
ZWRM50
Number of Fruit
0.0-0.9
1.0-1.9
2.0-2.9
3.0-3.9
4.0-4.9
5.0-5.9
6.0-6.9
7.0-7.9
8.0-8.9
Fruit Weight (kg)

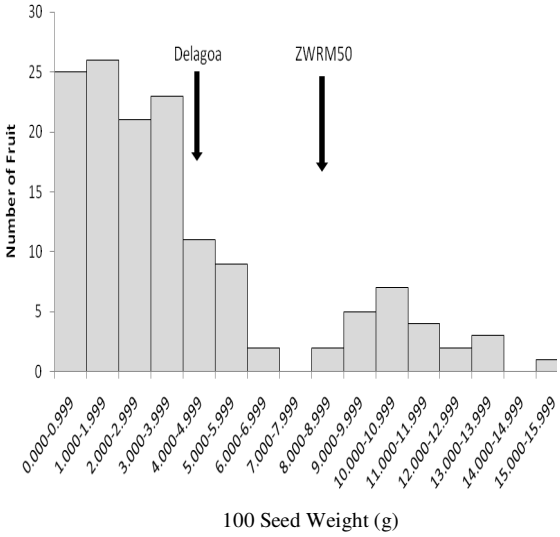
(E)


Delagoa
ZWRM50
Number of Fruit
3.00-3.99
4.00-4.99
5.00-5.99
6.00-6.99
7.00-7.99
8.00-8.99
9.00-9.99
10.00-10.00
11.00-11.99
12.00-12.99
13.00-13.99
Seed Length (mm)

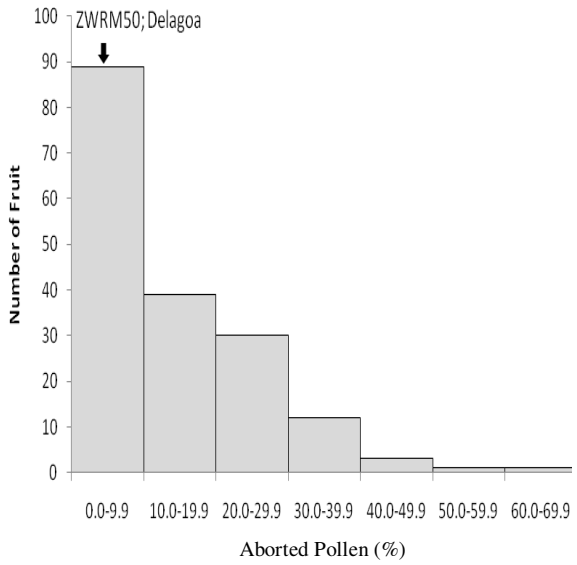
(F)


Delagoa
ZWRM50
Number of Fruit
1.00-1.99
2.00-2.99
3.00-3.99
4.00-4.99
5.00-5.99
6.00-6.99
7.00-7.99
Seed Width (mm)

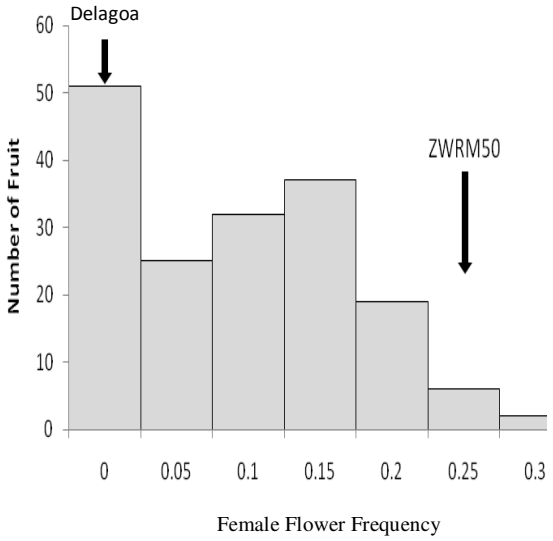
(G)


Delagoa
ZWRM50
Number of Fruit
0.000-0.999
1.000-1.999
2.000-2.999
3.000-3.999
4.000-4.999
5.000-5.999
6.000-6.999
7.000-7.999
8.000-8.999
9.000-9.999
10.000-10.999
11.000-11.999
12.000-12.999
13.000-13.999
14.000-14.999
15.000-15.999
100 Seed Weight (g)

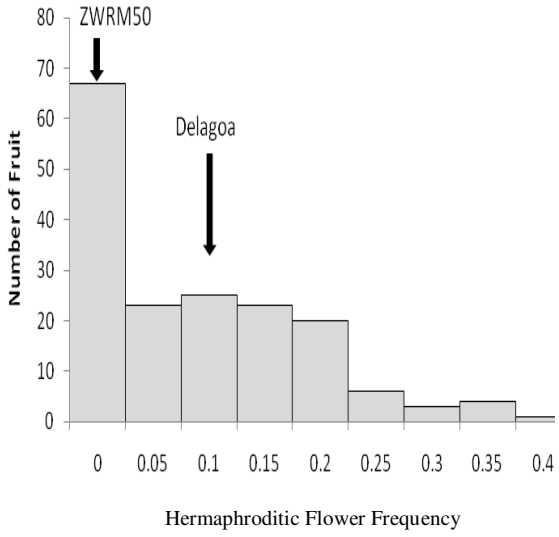
(H)


ZWRM50; Delagoa
Number of Fruit
0.0-9.9
10.0-19.9
20.0-29.9
30.0-39.9
40.0-49.9
50.0-59.9
60.0-69.9
Aborted Pollen (%)

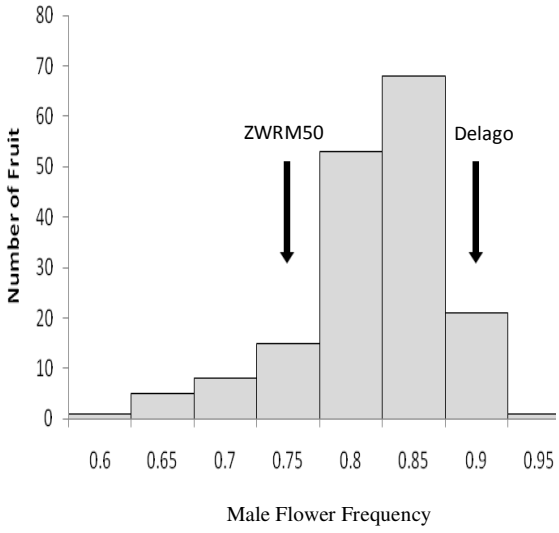
(I)


Delagoa
ZWRM50
Number of Fruit
Female Flower Frequency

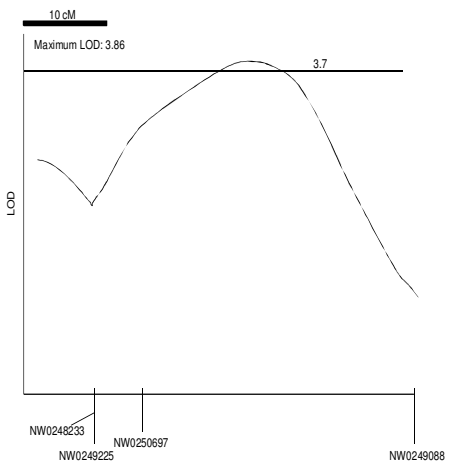
(J)


ZWRM50
Delagoa
Number of Fruit
Hermaphroditic Flower Frequency

(K)

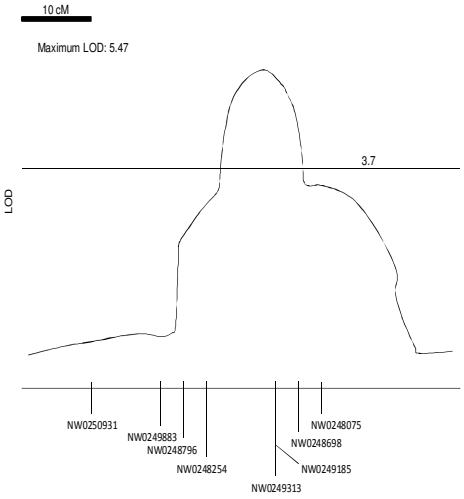

ZWRM50
Delago
Number of Fruit
Male Flower Frequency

(L)



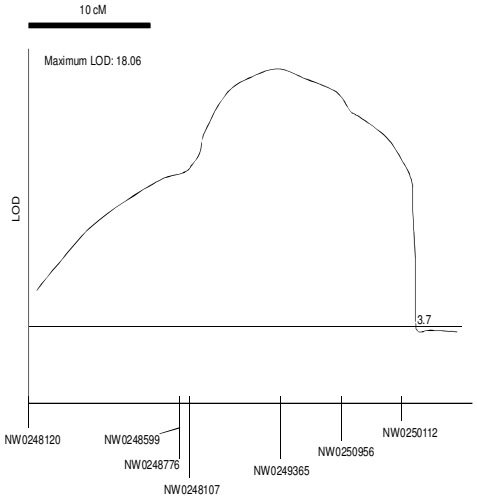
Brix LG4

(A)



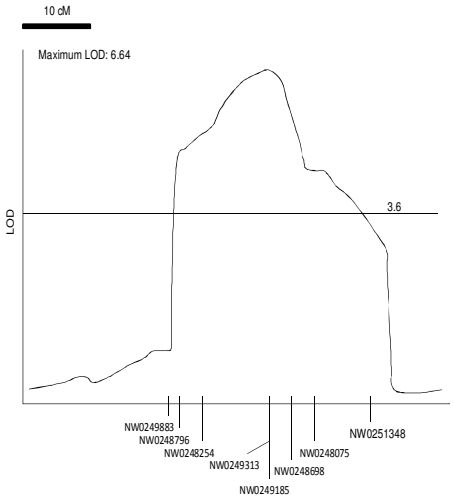
Fruit Length LG11

(B)



Fruit Length LG12

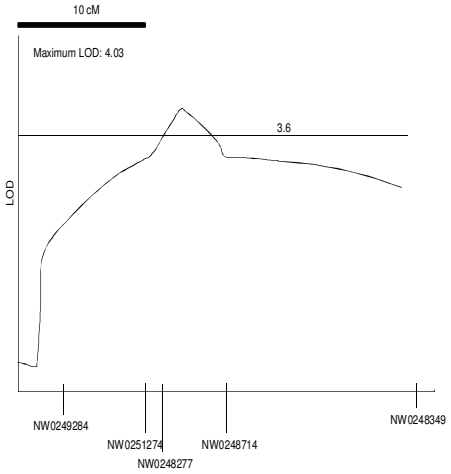
(C)



Fruit Width LG11

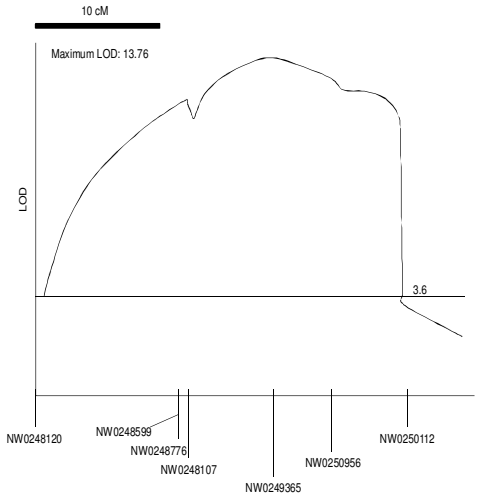
(D)

Figure 4.4 Maximum likelihood plots identifying genomic regions of quantitative trait loci associated with horticultural traits in the F_2 progeny of the ZWRM50 x Delagoa population. (A) Brix LG4, (B) Fruit Length LG11, (C) Fruit Length LG12, (D) Fruit Width LG11, (E) Fruit Width LG15, (F) Fruit Shape LG12, (G) Fruit Weight LG11, (H) Seed Length LG9, (I) Seed Length LG11, (J) Seed Width LG9, (K) Seed Width LG11, (L) 100 Seed Weight LG9, (M) 100 Seed Weight LG11, (N) Percent Aborted Pollen LG7, (O) Percent Aborted Pollen LG12, (P) Female Flower Frequency LG8, (Q) Female Flower Frequency LG9, (R) Female Flower Frequency LG12, (S) Hermaphroditic Flower Frequency LG12, (T) Hermaphroditic Flower Frequency LG16



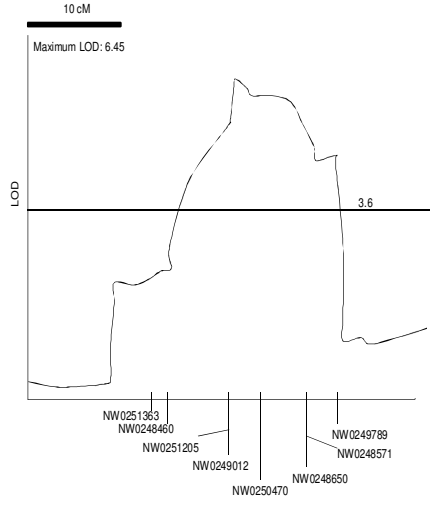
Fruit Width LG15

(E)



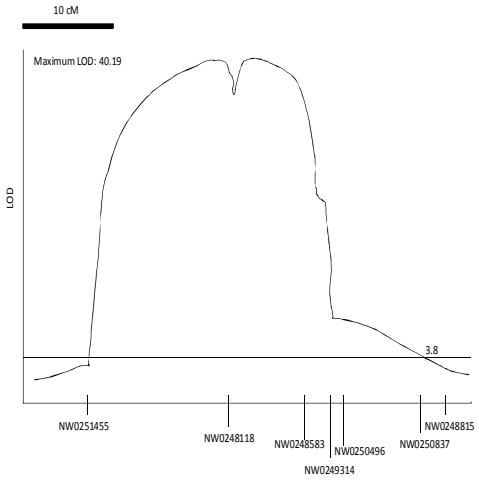
Fruit Shape LG12

(F)



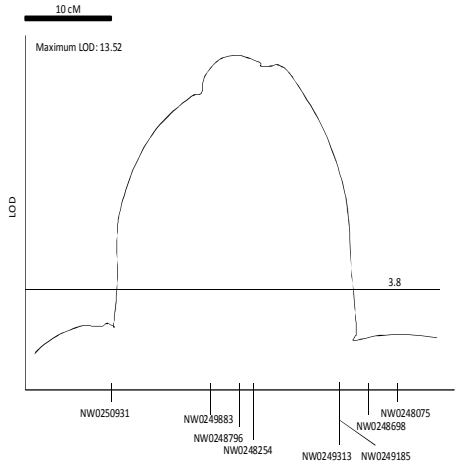
Fruit Weight LG11

(G)

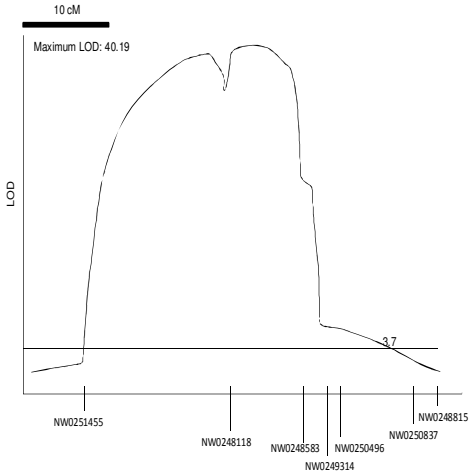


Seed Length LG9

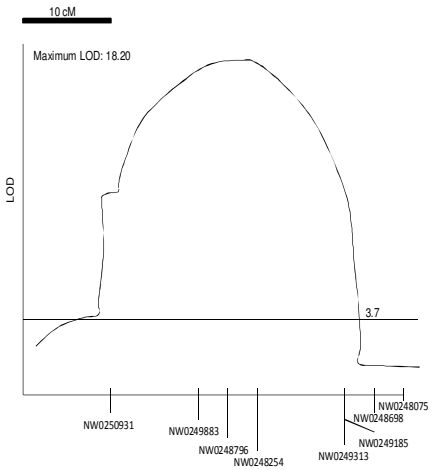
(H)



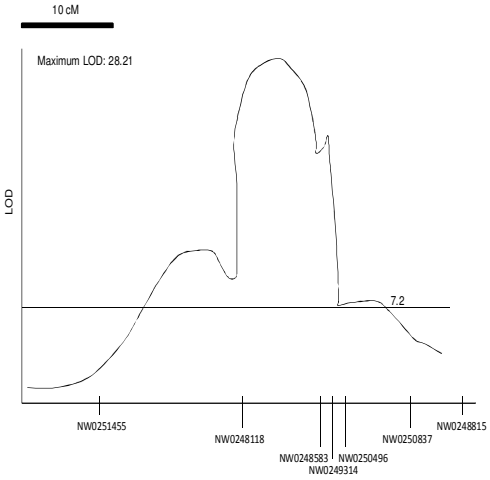
Seed Length LG11
(I)



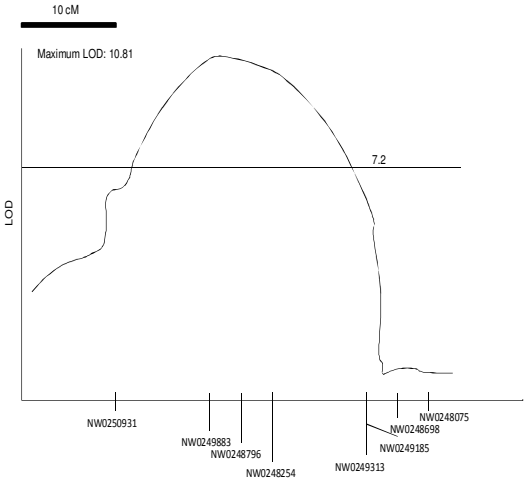
Seed Width LG9
(J)



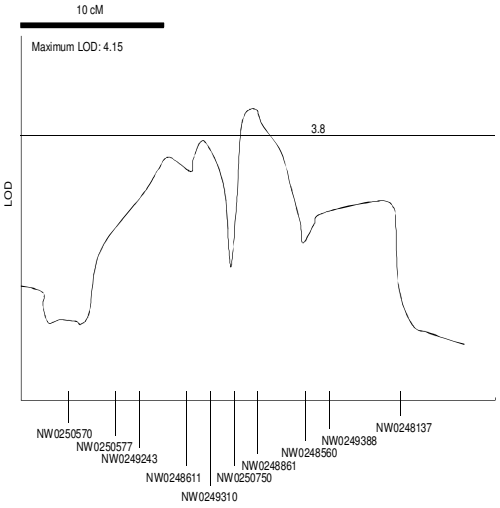
Seed Width LG11
(K)



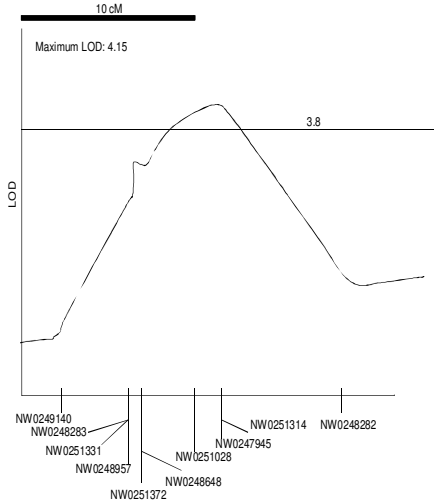
100 Seed Weight LG9
(L)



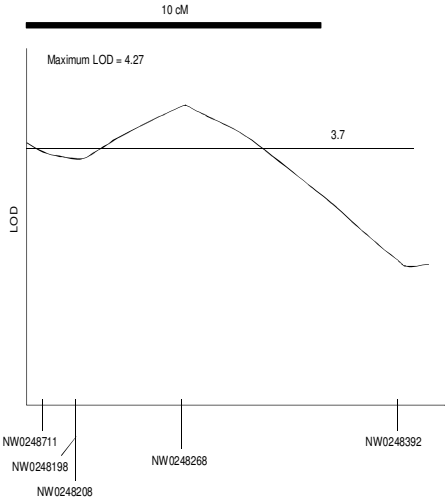
100 Seed Weight LG11
(M)



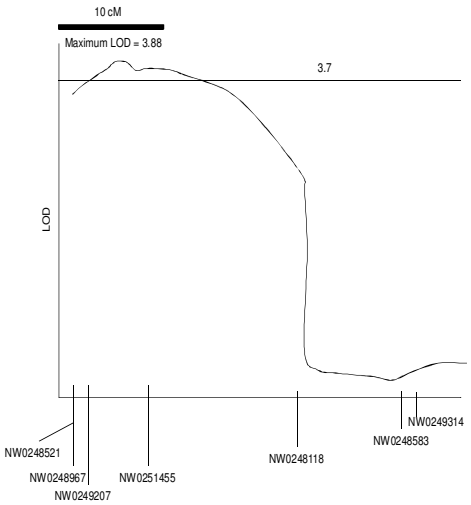
Percent Aborted Pollen LG7
(N)



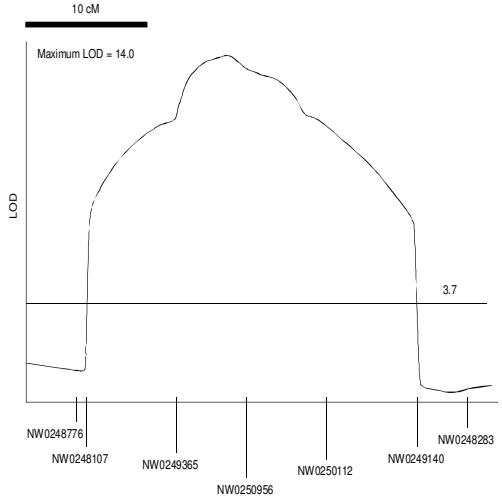
Percent Aborted Pollen LG12
(O)



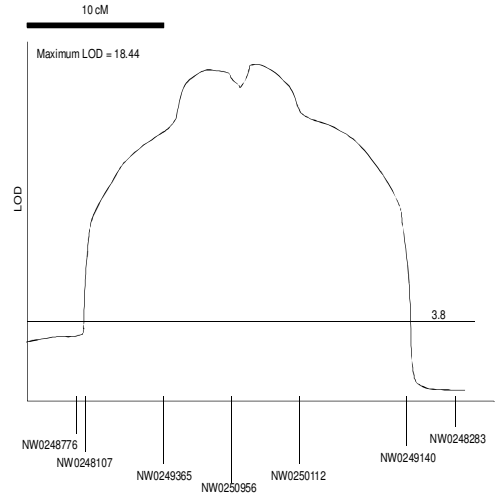
Female Flowering Frequency LG8
(P)



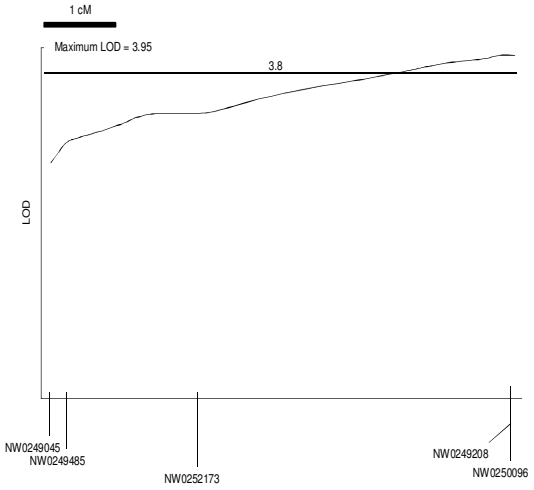
Female Flowering Frequency LG9
(Q)



Female Flowering Frequency LG12
(R)



Hermaphroditic Flowering Frequency
LG12
(S)



Hermaphroditic Flowering Frequency
LG16
(T)

CHAPTER 5

SUMMARY

Three hundred and fifty-seven single nucleotide polymorphism (SNP) markers were used to construct a linkage map for the elite x egusi watermelon (*Citrullus lanatus* var. *lanatus* Thunb.) population that contains 14 linkage groups and covers a distance of 1,514.258 cM. Fruit quality traits that were scored in this population include Brix and rind thickness. One QTL was detected for Brix but this trait may require mapping in advanced generations due to its low heritability. A single QTL for rind thickness detected on LG2 accounts for 17.51% of the variation.

Morphological traits measured in this population include fruit length, fruit width, and fruit weight. Three QTL were identified for fruit length. One QTL is located on LG3 and accounted 9.94% of the variation while the other two QTL were located on LG5. The two QTL on LG5 accounted for 8.7% and 12.42% of the variation in this trait. Two QTL for fruit width were identified on LG5 that explained 14.08% and 14.55% of the variation. Three QTL were identified for fruit weight. As with fruit length, one QTL was located on LG3 while two QTL were located on LG5. The QTL for fruit weight on LG3 mapped to the same region as a QTL for fruit length. Two regions on LG5 contained significant QTL for each of these three traits indicating that a cluster of genes in this region may be responsible for overall fruit size. Fruit length and width were combined to obtain a fruit size ratio (length:width) but a QTL was not identified for this measurement.

The egusi parent of this population contributed a unique characteristic known as the egusi seed trait. Watermelons with this trait have large, flat seed enclosed in a fleshy pericarp. These seed are a rich source of protein and oil in regions of the world where it is cultivated. The egusi seed trait is said to be controlled by a single gene. Two significant QTL on LG2 were identified for this trait. One of these QTL could be the possible location of the egusi seed trait gene. A QTL was identified on LG2 for seed oil that accounts for 78.96% of the variation in this trait. We expected to detect more QTL for seed oil and further study of the genetics controlling this trait is needed.

An elite x citron population was also developed for QTL analysis of several fruit quality, seed, and morphological traits. A linkage map was developed with 338 SNP markers that contains 16 linkage groups and spans a distance of 1,144.057 cM. As with the elite x egusi population, fruit quality traits scored in this population included degrees Brix, flesh firmness, and rind thickness. One QTL was identified for Brix in this population on LG4 and it accounted for 18.28% of the variation in this trait. Three QTL were identified for flesh firmness on LG9, LG11 and LG14 but no QTL were identified for rind thickness. This could be due to the possibility that the rind thickness in the citron parent is difficult to measure.

Fruit length, width, and weight were also recorded in this population. Two QTL for fruit length were identified on LG11 and LG12 that accounted for 11.41% and 40.09% of the variation, respectively. Two QTL were identified for fruit width. One QTL was located on LG11 at the same position as the QTL identified for fruit length on LG11. The other QTL for fruit width was located on LG15. Fruit length and width were also combined in this population to obtain a fruit shape ratio (length:width). A QTL on LG12 was identified that accounted for 32.08% of the variation in this trait.

Seed length, width, and 100 seed weight were measured in the elite x citron population. Several QTL were identified for each trait. Significant QTL were identified on LG9 for each trait and these QTL mapped to the same region on this linkage group. QTL were also identified for each of these traits on LG11 and they mapped to the same region of this linkage group. Studies have shown that watermelon seed size is controlled by one or a few genes. The QTL detected on LG9 and LG11 could be possible locations of the genes that control seed size.

The percent aborted pollen was measured from each plant and these data were used to map breakpoints that underlie chromosomal rearrangements. These rearrangements can lead to reduced fertility in hybrids between citron and watermelon. A QTL on LG7 was identified that accounts for 9.46% of the variation while another QTL on LG12 was identified that accounts for 9.40% of the variation in this trait.

Flowering pattern was scored in this population to map the factors affecting sexual expression in watermelon. Several plants in the F_2 population expressed a trimonoecious flowering pattern while other plants expressed a monoecious or andromonoecious flowering pattern. Three QTL were identified for female flower frequency that is located on LG 8, LG9 and LG12. Two QTL were identified for hermaphroditic flower frequency that is located on LG12 and LG16. The QTL located on LG12 for each trait mapped to the same position on the linkage group and could be the possible location of the andromonoecious gene.

The genetic diversity of cultivated watermelons has been shown to be very low. Wild types of watermelons such as the citron and egusi parent in these populations can serve as valuable sources of beneficial alleles such as disease and pest resistance. Many of the QTL identified in this study would be useful in selecting against undesirable traits such as low Brix when introducing alleles from a wild parent such as citron. These QTL can help to enhance the

infrastructure of marker assisted selection (MAS) in watermelon and help to broaden the genetic base of commercial watermelon germplasm.