SCREENING FOR RESISTANCE TO CUCURBIT YELLOW STUNTING DISORDER VIRUS, GUMMY STEM BLIGHT, AND MONOSPORASCUS ROOT ROT AND DETECTION OF RAPD MARKERS ASSOCIATED WITH QTL FOR SOLUBLE SOLIDS, SUGARS, AND VITAMIN C IN MELON (*CUCUMIS MELO* L.)

A Dissertation

by

JONATHAN WALKER SINCLAIR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2003

Major Subject: Plant Breeding

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Approved as to style and content by:

Kevin M. Crosby (Co-Chair of Committee)	Leonard M. Pike (Co-Chair of Committee)
J. Creighton Miller Jr. (Member)	Tom Isakeit (Member)
Tim D. Davis (Head of Department)	

December 2003

Major Subject: Plant Breeding

ABSTRACT

Screening for Resistance to Cucurbit Yellow Stunting Disorder Virus, Gummy Stem Blight, and Monosporascus Root Rot and Detection of RAPD Markers Associated with QTL for Soluble Solids, Sugars, and Vitamin C in Melon (*Cucumis melo* L.).

(December 2003)

Jonathan Walker Sinclair, B.S., Sam Houston State University;

M.S., Texas A&M University

Co-Chairs of Advisory Committee: Dr. K. Crosby
Dr. L. Pike

Cucurbit yellow stunting disorder virus (CYSDV) is a relatively new virus affecting cantaloupe production in South Texas and worldwide. No resistant commercial cultivars are available. A cross of ‘Dulce’ (susceptible) x ‘TGR1551’ (resistant) was made and populations were developed for screening. Although no complete resistance was recovered, ‘TGR1551’ showed some tolerance and may be useful in breeding efforts.

Sugar components such as sucrose, fructose, glucose, and total soluble solids are major factors in determining mature melon fruit sweetness, and Vitamin C is important for human health. A F_2 population was developed from the melon cross ‘Dulce’ (high values) x ‘TGR1551’ (low values) and bulked segregant analysis was used to detect random amplified polymorphic DNA (RAPD) markers associated with quantitative trait loci (QTL) for each trait. Out of 500 primers, fifteen RAPD markers were found to be significantly associated with fruit quality QTL. These markers could be useful in a

marker assisted selection program to transfer these genes into a low quality cultivar or breeding line to enhance fruit quality.

Gummy stem blight (*Didymella brioniae*) affects melon production in South Texas as well as other melon production areas in the U.S. A cross between 'TMS' (susceptible) and PI 140471 (resistant) was made and a F_2 population was screened with a strain of the disease from South Texas. F_2 plants exhibited symptoms ranging from resistant to susceptible. PI 140471 may be useful in developing commercial varieties of melon resistant to the disease in Texas.

Monosporascus root rot and vine decline (*Monosporascus cannonballus*) affects melon production in South Texas as well as other melon production areas in the US. A cross was made between 'TGR1551' (moderately resistant) and 'Deltex' (resistant) to develop a F_2 population. Both parents and the F_2 were planted in infested soil. Once symptoms appeared, plant roots were removed from the soil and rated. 'TGR1551' showed greater resistance than 'Deltex' and should be utilized in breeding to develop improved resistant cultivars.

DEDICATION

To my family:
My parents who have always stood behind me and supported my efforts
and my brother, Aaron, for his support and for showing me the joy in life.
I love you.

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CHAPTER I

INTRODUCTION

Melon (*Cucumis melo* L.) includes a horticulturally valuable and economically important group of crops grown throughout the world. The fruit flesh is a significant source of carbohydrates and water and the seeds are rich in oil and protein (Martyn and Miller, 1996a). Melons are also a source of ascorbic acid, folic acid, and potassium (Richter, 2000). The most popular melons in North America are orange-fleshed and heavily netted, commonly referred to as cantaloupes (McCreight et al., 1993). In the United States, melon (*Cucumis melo* L.) (honeydew and cantaloupe) fruit follows only banana (*Musa* x *paradisica* L.) in overall fruit consumption (bananas = 13.3 kg/capita vs. melons = 12.4 kg/capita) (Lucier, 2001).

Texas has a significant share of production of cucurbits in the United States. Cantaloupes compose about 5,000 ha. of the approximately 30,000 ha of cucurbits that are produced annually in Texas. Cantaloupe production is primarily in the Lower Rio Grande Valley of South Texas, along with some production in the Trans-Pecos of West Texas. The production value of watermelon and cantaloupe in Texas averages over $300 million annually (Martyn and Miller, 1996a).

This thesis has been prepared according to the style of the Journal of the American Society for Horticultural Science.

Three of the major disease problems having a significant impact on melon production in South Texas are cucurbit yellow stunting disorder virus (CYSDV), gummy stem blight (*Didymella bryoniae*), and monosporascus root rot and vine decline (*Monosporascus cannonballus*). Screening for and developing resistance to these diseases would be a major benefit to melon growers throughout the South Texas Valley melon producing area.

CYSDV is a relatively "new" virus now affecting melon production in North America (Kao et al., 2000). First detection of CYSDV was in the United Arab Emirates in 1982 (Hassan and Duffus, 1991); it has since spread throughout the Mediterranean region (Celix et al., 1996) and North America in the Rio Grande Valley of southern Texas and northern Mexico (Kao et al., 2000). The three other major cucurbit species under cultivation worldwide: *Cucumis sativus* (cucumber), *Citrullus lanatus* (watermelon), and *Cucurbita pepo* (squash) are also affected by CYSDV (Berdiales et al., 1999; Celix et al., 1996; Hassan et al., 1991; Louro et al., 2000). CYSDV is transmitted in a semi-persistent, non-circulative manner by whiteflies (Duffus, 1995). Resistance to CYSDV, which is controlled by a dominant allele at one locus (Lopez-Sese and Gomez-Guillamon, 2000; Sese et al., 1999), was found in a *C. melo* genotype from Zimbabwe (TGR1551) (Gomez-Guillamon et al., 1995).

Gummy stem blight is a serious disease causing considerable damage to cucurbit production (cantaloupe, watermelon, cucumber, and gourd) under greenhouse protection as well as in the field around the world particularly in tropical and subtropical areas (Blancard et al., 1994). It is commonly found in the southern United States (Sitterly and Keinath, 1996). Gummy stem blight can infect healthy plants at any time during the

growing season and all parts of melon plants may become infected (Zitter, 1992). Gummy stem blight is carried on or in infected seed and on crop debris in the soil. Gummy stem blight is very serious at humidity levels of 85% and greater (Blancard et al., 1994; Van Steekelenburg, 1985b). A plant introduction native to Texas, PI 140471, (*Cucumis melo* L.) was found to be highly resistant to gummy stem blight (Sowell et al., 1966).

Monosporascus root rot is an important disease affecting melons around the world (Martyn and Miller, 1996a). Monosporascus is now a rather serious disease affecting the Lower Rio Grande Valley of Texas (Crosby, 2000; Mertely et al., 1991). Specific losses vary by year from about 10 to 25% of the crop, but it is not uncommon for individual fields to suffer complete (100%) loss (Martyn and Miller, 1996a). The use of cultivars resistant to plant diseases is one of the best control measures, but there are currently no commercially available monosporascus resistant varieties (Cohen et al., 2000). ‘Deltex’, an Ananas type melon was found to be more tolerant to monosporascus than commonly used commercial varieties of cantaloupe such as ‘Caravelle’, a western shipper. The reduced incidence of wilt may be partially due to root system size and structure; ‘Deltex’ has a more vigorous root system, giving it better adaptation to dry-land production (Crosby and Wolff, 1998).

Fresh and frozen cantaloupe (*Cucumis melo* L.) quality is determined not only by flavor but also by color and texture (Simandjuntak et al., 1996). The concentration of sugar contained in the fruit is the best determinant of fruit quality in melon (*Cucumis melo* L.) (Yamaguchi et al., 1977). Sucrose, glucose, and fructose are the three major constituents of ripe melon fruit soluble sugars (Hubbard et al., 1989; Hughes and

Yamaguchi, 1983; Lester and Dunlap, 1985; McCollum et al., 1988; Pratt, 1971; Rosa, 1928; Schaffer et al., 1987, 1996). Vitamin C, also known as ascorbic acid, contained in melon is an important nutrient for human health (Lester and Crosby, 2002).

Developing molecular markers for traits such as these would greatly facilitate breeding programs through marker-assisted selection (MAS). Such a program could significantly reduce the time normally required to develop new improved varieties. Bulked segregant analysis (BSA) has been used as an efficient method in quick identification of markers linked closely to particular genes (utilizing bulked DNA from F_2 individuals) (Michelmore et al., 1991). Molecular markers linked to single genes of interest have been successfully developed in many crops using randomly amplified polymorphic DNA (RAPD) (Haley et al., 1994; Martin et al., 1991; Michelmore et al., 1991; Miklas et al., 1993; Park et al., 1998, 1999a, 2003; Park and Crosby, 2003). RAPD markers linked to quantitative trait loci (QTL) have also been developed using BSA (Milkas et al., 1996; Quarrie et al., 1999).

The advantage of using RAPD markers is that they are simple to use, do not require much DNA, and the fact that no radioactivity is involved (Williams et al., 1990). However, the development of markers associated with fruit quality traits in melon for use in MAS has not been reported.

RESEARCH OBJECTIVES

The overall objectives of this investigation were to develop melon cultivars with improved disease (CYSDV, gummy stem blight, and monosporascus root rot) resistance compared to commercial cultivars, and to develop molecular markers to facilitate

selection (MAS) for disease (CYSDV) and fruit quality traits (soluble solids, sucrose, and vitamin C). The specific objectives of this study were as follows:

1. Combine CYSDV resistance from ‘TGR1551’ with desirable horticultural traits from commercial varieties of melon through backcrossing.
2. Develop markers tightly linked to the resistance gene allowing early selection for resistance to CYSDV.
3. Identify RAPD markers associated with QTL affecting fruit quality traits including total soluble solids, sucrose, glucose, fructose, and vitamin C using BSA in an F_2 population.
4. Calculate Pearson correlations between total soluble solids, sucrose, glucose, fructose, and vitamin C to determine relationships.
5. Determine if resistance reported in PI 140471 is effective against the Texas strain of gummy stem blight.
6. Begin backcrossing program for incorporation of gummy stem blight resistance from PI 140471 into commercial varieties of melon.
7. Determine the amount of resistance to monosporascus root rot present in ‘TGR1551’ compared to ‘Deltex’.
8. Test a F_2 population segregating for monosporascus root rot resistance for usefulness in melon breeding efforts.

CHAPTER II

LITERATURE REVIEW

PLANT DESCRIPTION

Cucumis melo L. is in the *Cucurbitaceae* family and appears to have originated in Africa (Kerje and Grum, 2000). It comprises seven different horticulturally important groups of melon (McCreight et al., 1993), each classified as annuals and vine-like in growth habit (Wang et al., 1997). Melons are a diploid species having a base chromosome number of x = 12, 2n = 24 (McCreight et al., 1993). The most popular melons in North America are orange-fleshed and heavily netted, commonly referred to as cantaloupes (McCreight et al., 1993). The *Cucurbitaceae* family provides mankind with quite a few edible products. The fruit flesh is a significant source of carbohydrates and water, and the seeds are rich in oil and protein (Martyn and Miller, 1996a). Melons are also a source of ascorbic acid, folic acid, and potassium (Richter, 2000). In the United States, melon (*Cucumis melo* L.) fruit (honeydew and cantaloupe) follow banana (*Musa* x *paradisica* L.) in overall fruit consumption (bananas = 13.3 kg/capita vs. melons = 12.4 kg/capita) (Lucier, 2001).

Cucurbits produced in Texas are mainly watermelon (*Citrullus lanatus* (Thumb.) Matsum & Nakai) and cantaloupe (*Cucumis melo* L.). Cantaloupes compose about 5,000 ha. of the approximately 30,000 ha of cucurbits that are produced annually in Texas and are grown mainly in the Lower Rio Grande Valley of South Texas, along with some production in the Trans-Pecos of West Texas. The production value of watermelon and cantaloupe in Texas averages over $300 million annually (Martyn and Miller, 1996a). Texas is a major producer of cantaloupes averaging about $56 million worth of

production over the past three years (1999-2001) ranking third in the US over the same time period (NASS, 2002).

INTRODUCTION TO CUCURBIT YELLOW STUNTING DISORDER VIRUS

Since 1980 many 'new' viruses infecting cucurbits around the world have been described; several have become widespread causing economic damage following multiplication and spread of the vectors that transmit them (Lecoq et al., 1998*)*. CYSDV is one such virus that now affects melon and other cucurbit production in North America (Kao et al., 2000). One of the primary goals of the Texas A&M melon breeding program is to develop melon (*Cucumis melo* L.) varieties with resistance to various diseases such as CYSDV.

Origin and Spread of the Virus

First detection of CYSDV was in the United Arab Emirates in 1982 (Hassan and Duffus, 1991) where it remains in epidemic proportions (Duffus, 1995). CYSDV has since spread throughout the Mediterranean region (Celix et al., 1996) including Egypt, Israel, Jordan, Spain, Turkey (Cohen and Ben-Joseph, 2000; Sese et al., 1994; Wisler, et al., 1998), Lebanon (Abou-Jawdah et al., 2000), Portugal (Louro et al., 2000), and Morroco (Desbiez et al., 2000) where it is has caused major economic damage to cucurbit crops (Abou-Jawdah et al., 2000; Celix et al., 1996; Livieratos et al., 1999; Louro et al., 2000; Rubio et al., 1999). CYSDV also has been introduced into North America, specifically the Rio Grande Valley of southern Texas and northern Mexico (Kao et al., 2000).

Virus Symptoms

CYSDV produces initial symptoms of severe interveinal chlorosis and green spots on oldest leaves. Spots appear between 14 and 22 days post inoculation; definite symptoms are visible after 30 days (Sese et al., 1994; Celix et al., 1996). Leaves also may develop prominent yellow sectors. Severe symptoms include complete leaf lamina yellowing (except for the veins) and leaf rolling and brittleness (Celix et al., 1996). Fruit quality is severely affected; yield, fruit size, and sugar content are reduced, making fruit unacceptable for commercial market sale, resulting in economic losses for melon growers.

Since *Criniviruses* produce symptoms mainly in older leaves, CYSDV symptoms may be easily confused with physiological disorders, nutritional deficiencies, inadequate water, insect damage, natural senescence, or pesticide damage (Wisler et al., 1998). Growers, diagnosticians, and researchers may have a hard time visually diagnosing such virus infections (Lecoq et al., 1998; Wisler et al., 1998). Further complicating correct identification is the fact that CYSDV symptoms are indistinguishable from those caused by beet pseudo-yellows virus (BPYV) (Wisler et al., 1998). CYSDV symptoms also are quite similar to those caused by lettuce infectious yellows virus (LIYV) (Sese et al., 1994).

Virus Description

CYSDV not only affects *C. melo* (cantaloupe and honeydew) production, but also the three other major cucurbit species under cultivation worldwide: *Cucumis sativus* (cucumber), *Citrullus lanatus* (watermelon), and *Cucurbita pepo* (squash) (Hassan et al., 1991; Celix et al., 1996; Berdiales et al., 1999; Louro et al., 2000). Cucurbits and lettuce

are the only known CYSDV hosts to date (Duffus, 1995).

CYSDV is a member of the newly assigned *Crinivirus* genus, in the *Closteroviridae* family along with several other viruses that have primarily been discovered within the past 12 yrs (Lecoq et al., 1998) such as: abutilon yellows virus (AYV), lettuce chlorosis virus (LCV), lettuce infectious yellows virus (LIYV), sweet potato chlorotic stunt virus (SPCSV), tomato chlorosis virus (ToCV), and tomato infectious chlorosis virus (TICV) (Martelli et al., 2000). The CYSDV coat protein (CP) shares the highest level of similarity with those of SPCSV (36%) and LIYV (26%) (Livieratos et al., 1999). Criniviruses are long, flexible particles transmitted naturally by whiteflies (Duffus, 1995; Lecoq et al., 1998; Liu et al., 2000; Livieratos et al., 1998, 1999; Wisler et al., 1998). Closteroviruses use polyprotein processing, translational frameshifting, and subgenomic RNA's to express their genomes (Lecoq et al., 1998).

CYSDV is a phloem-limited virus which makes diagnosis, isolation, and purification difficult (Wisler et al., 1998). However, it has been purified with differential centrifugation and determined to have particle lengths ranging from 825 to 900 nm (Celix et al., 1996). The virus has a bipartite genome consisting of two single strand, plus sense RNA segments estimated at ~9 kb (RNA1) and ~8 kb (RNA2) encapsulated separately (Celix et al., 1996). More recently, leaf dip preparations have suggested somewhat shorter particle lengths from 750 to 800 nm (Liu et al., 2000). CYSDV contains a heat shock protein (HSP70) coding region that is unique to closteroviruses (Celix et al., 1996; Tian et al., 1996). Sequence information has been estimated for four complete CYSDV genes (first three oriented 5' to 3') and one incomplete gene. The first open reading frame (ORF) corresponds to HSP70 (1659 nt long, encoding for a protein estimated at 62 kDa);

the second ORF corresponds to p58 (1524 nt long, encoding for a protein estimated at 58 kDa); the third ORF corresponds to p9 (240 nt long, encoding for a protein estimated at 9 kDa); and the fourth ORF represents a putative CP gene (756 nt long encoding for a 28.5 kDa protein) (Livieratos et al., 1999).

Virus Transmission

CYSDV is transmitted in a semi-persistent, non-circulative manner by whiteflies (Duffus, 1995). Virus particles are transmitted efficiently world wide by *Bemisia tabaci* biotype B (also known as *B. argentifolii*), commonly known as the silverleaf whitefly (Soria et al., 1995; Celix et al., 1996), and biotype Q in Spain (Berdiales et al., 1999). It is also transmitted by *Bemisia tabaci* biotype A, but inefficiently. However, CYSDV is not transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*) which transmits BPYV and recently has been displaced over most of its former range by *B. tabaci* (Celix et al., 1996; Berdiales et al., 1999). CYSDV can persist in the vector for 9 days and has a half life of 72.2 hr which is the longest documented retention time of any known whitefly transmitted closterovirus (Wisler et al., 1998). It cannot be transmitted mechanically (Sese et al., 1994; Celix et al., 1996). Whitefly population required for virus transmission has been studied and, although one individual is able to transmit the virus, 60 individuals per plant are required for a 100 % transmission rate. As little as 2 hr of feeding time on infected plants is sufficient for whitefly acquisition of CYSDV resulting in a 50 % transmission rate, and in as little as 24 hr of feeding time individual whiteflies have the ability to transmit the virus near a 100 % infection rate (Sese et al., 1994).

Virus Detection and Differentiation

Although it produces symptoms similar to other members of the *Closteroviridae* family, such as BPYV and LIYV, CYSDV can be distinguished by host range, insect transmission characteristics, and serology (Duffus, 1995). Random cDNA cloning of viral dsRNA has been performed, and a virus-specific cDNA clone (p410) of 557 nucleotides that hybridized with the smaller of the two viral dsRNA species has been identified. Heat shock protein 70 (HSP70) homologous gene amplified with primers 410U (5'-AGAGACGGTAAGTAT-3') and 410L (5'-TTGGGCATGTGACAT-3') has allowed reverse transcription polymerase chain reaction (RT-PCR) detection of CYSDV in plants (Celix et al., 1996). *Closterovirus* degenerate primers also have been used in addition to RT-PCR to generate, clone, and characterize cDNA's from CYSDV for use in detecting plant infections (Tian et al., 1996). Oligonucloetide primers have been designed based on the CYSDV clone p410, which allow the use of RT-PCR and hybridization assays for detection of CYSDV and differentiation from BPYV in melon plants (Livieratos et al., 1998). The complete CYSDV CP gene has been cloned and purified and used to develop antiserum. As a result, reliable immunoblot and indirect enzyme-linked immunosorbent assay (ELISA) like tests have been developed for detecting CYSDV in infected plant extracts which can be used in extensive epidemiological studies (Livieratos et al., 1999). A new method of using digoxigen-labelled probes for estimating the amount of CYSDV in *B. tabaci* recently has been developed, which may allow better virus monitoring as well as the ability to develop action thresholds for managing the spread of CYSDV in the future (Ruiz et al., 2002).

Genetic Diversity of the Virus

Work on characterizing the genetic variability in CYSDV isolates from different countries has been done through single-strand conformation polymorphism (SSCP) and nucleotide sequence analysis of the CP gene (Rubio et al., 1999; Rubio et al., 2001). Based on these results, isolates were divided into two genetic groups: a 'Western' group containing samples from Spain, Jordan, Turkey, Lebanon, and North America (Rio Grande Valley of Texas and Mexico), and an 'Eastern' group containing samples from Saudi Arabia (Rubio et al., 1999; Rubio et al., 2001). The surprisingly low genetic diversity found in the geographically broad 'Western' group (nucleotide identity > 99%) may be due to the rapid expansion of CYSDV along with its vector (*B. tabaci*), negative selection related to constraints of virus-encoded proteins, or constraints due to secondary structure (Rubio et al., 2001). Also, CYSDV is only transmitted by one vector. Since host plants are annuals, infections are usually less than 60 days old (Rubio et al., 2001) disallowing greater mutation times.

Virus Control Strategies

Virus control strategies in cucurbits have been based on the use of cultural practices intent on preventing or delaying virus spread through vectors (Lecoq et al., 1998). Time of planting and other epidemiological factors may be important in determining virus severity (Berdiales et al., 1999). Since chemical control has proven ineffective at containing the spread of CYSDV, genetic resistance is the most likely method for controlling the virus.

Virus Resistance

A *C. melo* genotype from Zimbabwe (‘TGR1551’) was found to be resistant to CYSDV (Gomez-Guillamon et al., 1995). Research indicates that the resistance in ‘TGR1551’ is controlled by a dominant allele at one locus (Sese et al., 1999; Lopez-Sese and Gomez-Guillamon, 2000). The locus has been designated with the symbol *Cys* for cucurbit yellow stunting. *Cys* is the first resistance gene related to a whitefly transmitted virus infecting melon to be described (Lopez-Sese and Gomez-Guillamon, 2000). The resistance is thought to be related to the existence of mechanisms that inhibit vascular transport of the pathogen, changes in cellular membranes that impede the diffusion or transport of virus particles from cell to cell, or an inhibition of virus particle replication in tissue of resistant hosts (Lopez-Sese and Gomez-Guillamon, 2000).

Breeding for Virus Resistance

Researchers in Spain have made reciprocal crosses utilizing ‘TGR1551’ and two commercial Spanish cultivars (‘Piel de Sapo’ and Bola de Oro’) (Sese et al., 1999; Lopez-Sese and Gomez-Guillamon, 2000), but currently there are no commercial varieties of *C. melo* available exhibiting resistance to CYSDV. Cucurbit viruses are one of the most complex pathosystems in the world (Lecoq et al., 1998), making breeding for virus resistance a challenge. Breeding virus resistant varieties is generally slow and inefficient due to several factors. First, environmental conditions may have a large effect on the expression of virus symptoms. Second, many viruses have multiple strains, some able to overcome resistance genes. Locating molecular markers linked to virus resistance is expected to make breeding for virus resistance more efficient and will lead to faster development of resistant cultivars (Danin-Poleg et al., 2000b). Since resistance to

CYSDV in ‘TGR1551’ is conditioned by a single dominant allele at one locus (Sese et al., 1999), effective breeding and marker utilization should be considerably easier than otherwise. ‘TGR1551’ has several undesirable characteristics including elongated fruit shape, orange color, poor fruit weight and size, white flesh color, and soluble solids content as low as 4% (Gomez-Guillamon et al., 1995). These characteristics make it a poor hybrid parent for commercial seed production; however, due to its CYSDV resistance it may be very useful in developing resistant commercial varieties over a longer time period (Gomez-Guillamon et al., 1995).

INTRODUCTION TO MARKER DEVELOPMENT FOR FRUIT TRAITS

Melon Sugars

Fresh and frozen cantaloupe (*C. melo* L.) quality is determined not only by flavor, but also color and texture (Simandjuntak et al., 1996). The concentration of sugar contained in the fruit is the best fruit quality determinant in melon (*C. melo* L.) (Yamaguchi et al., 1977). Sugar concentration in ripe fruit is a result of complex source-sink relationships including metabolism of assimilates and assimilate partitioning (particularly fruit photoassimilate metabolism) (Schaffer et al., 1996).

Sucrose, glucose, and fructose are the three major constituents of ripe melon fruit soluble sugars. Increases in sugar levels during fruit ripening are a result of sucrose accumulation; glucose and fructose levels vary minimally (Hubbard et al., 1989; Hughes and Yamaguchi, 1983; Lester and Dunlap, 1985; McCollum et al., 1988; Pratt, 1971; Rosa, 1928; Schaffer et al., 1987, 1996). In melon fruit development, the first period does not involve sucrose accumulation; sucrose accumulation begins later in fruit development and continues until the fruit abscises or is harvested. Since melon fruit does not contain

starch reserves (Rosa, 1928), sugar content cannot increase upon harvest. This makes sucrose accumulation during fruit growth to maturity highly important in ascertaining fruit quality (Burger et al., 2002).

Environmental and genetic factors may influence sucrose content in melon fruit. There is much environmental variability in the overall sugar composition of commercial hybrid varieties. This results mainly from differences in sucrose levels and not from differences in hexose (glucose and fructose) levels (Burger et al., 2000). High genetic variability in total sugar concentration observed in melon is also accounted for mainly by differences in sucrose level (Burger et al., 2000; Hubbard et al., 1989; Stepansky et al., 1999). *C. melo* contains a broad group of melon horticultural types that range from low sugar content and no sucrose accumulation to high sugar content with high sucrose content (Stepansky et al., 1999).

Vitamin C

Vitamin C, otherwise known as ascorbic acid, contained in melon is an important nutrient for human health (Lester and Crosby, 2002). It was first isolated from plants in 1928. It functions as a water soluble antioxidant in the human body (Lavine, 1986). Ascorbic acid also plays a crucial role in keeping the immune system healthy by lowering cold severity, stopping secondary viral or bacterial infections, protecting the body from free radical damage (Larson, 1997), and preventing disease of the cardiovascular system (Eichholzer et al., 2001).

Marker Development

Early genetic linkage maps in the 20th century were developed with loci related to phenotypic traits. Creation of highly saturated maps was complicated because of

environmental effects on quantitatively inherited traits. Since the development of new molecular marker technologies, plant geneticists and breeders have the ability to develop extensive plant genome maps (Bradeen et al., 2001). Genetic markers have many uses in melon breeding such as discrimination between cultivars, determining heterozygosity, testing seed purity, biosystematic studies, and taxonomic studies (Masojc, 2002; Staub et al., 1992; Staub and Meglic, 1993). However, genetic markers are most useful because of their potential for use in marker assisted selection (MAS) in plant breeding programs (Masojc, 2002).

Melon Genetics and Genetic Map

Melon genetic polymorphism ranges from about 10% to 15% (Staub et al., 1997) The genomic length of melon is 2276 to 3250 cM (Staub and Meglic, 1993). Melon gene lists, including genes for various characters such as disease resistance, pest resistance, leaf, stem, flower, fruit and seed traits, have been developed. The most recent list includes 162 loci (Pitrat, 2002). Many melon genes have been cloned (either mRNA or complete gene) but most are involved in fruit maturation (Pitrat, 2002). Melon genetic maps composed of various types of molecular markers have been developed (Baudracco-Arnas and M. Pitrat. 1996, Brotman et al., 2000, Danin-Poleg et al., 2002, Danin-Poleg et al., 2000a, Oliver et al., 2001, Perin et al., 2002a, Wang et al., 1997). Various linkages also have been reported between isozymes (Staub et al., 1998) and phenotypic mutants (Pitrat, 1991). Since the various melon maps were constructed utilizing different parental genotypes, some markers do not transfer readily from one map to another (Pitrat, 2002). Currently there is no saturated melon reference map and mapping has been accomplished on only a few phenotypic traits. Also, the number of described genes is likely inflated due

to the absence of allelism tests (Pitrat, 2002). Effectiveness and efficiency of markers will improve with the development of a saturated genetic map for melon (Staub et al., 1996).

There are several strategies for identification of molecular markers with tight linkage to genes of interest without creating genetic linkage maps. Using near-isogenic lines (NILs) differing at one or several loci is very useful in identifying molecular markers with tight linkage to genes of interest (Martin et al., 1991), but producing NILs requires time and is expensive because of the required six backcrosses (Lefebvre and Chevre, 1995; Michelmore et al., 1991). Bulk segregant analysis (BSA) with segregating F_2 or BC families is another method. It takes advantage of the linkage disequilibrium created from crossing parents of differing genetic backgrounds and the short time and low cost involved in developing such populations (Mackay and Caligari, 2000).

Types of Molecular Markers

There are several types of molecular markers commonly used. Restriction fragment length polymorphisms (RFLP) (Botstein et al., 1980) have been popularly used as molecular markers in locating genes. An advantage of RFLP markers is that they tend to be codominant, making them useful in the development of genetic linkage maps and in locating genes linked to specific traits in segregating F_2 populations. However, RFLP have disadvantages as well, including the time required, expenses involved, the necessary extensive process, and the necessity of radioactive probes (Kelley, 1995). These limitations make RFLP markers inconvenient for plant breeding work.

Randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990) has been used as an alternative to the limitations of RFLP. Molecular markers linked to genes of interest have been developed successfully in many crops using RAPD (Haley et al., 1994;

Martin et al., 1991; Michelmore et al., 1991; Miklas et al., 1993). The advantages of using RAPD markers instead of RFLP markers are the simplicity of use, the small DNA requirement, and the fact that no radioactivity is involved (Williams et al., 1990). RAPD markers are inherited in a Mendelian fashion.

Simple sequence repeat (SSR) can be used as reference points for map merging and comparative mapping in melon (Dian-Poleg et al., 1998).

Bulked Segregant Analysis

Bulked segregant analysis (BSA) has been used as an efficient method in quick identification of markers linked closely to particular genes of interest (Michelmore et al., 1991). Association between a certain allele at a certain marker locus and a gene of interest occurs only when linkage disequilibrium is present (Masojc, 2002). The BSA procedure is mainly used in populations that are segregating such as F_2 and backcross (Masojc, 2002), but doubled haploid lines, and recombinant inbred lines have also been used (Lefebvre and Chevre, 1995). F_2 populations are more useful than BC populations because there is a lower probability of false positives and there are twice as many dominant markers cutting the recombination frequency in half (Mackay and Caligari, 2000). This method involves the collection of DNA from two groups of segregating plants representing both high and low ends of the variation range. Generally differences between bulks indicate polymorphisms in regions of the genome linked tightly to traits (genes) of interest, and unlinked genetic variants are evenly distributed (Masojc, 2002). By using BSA, RAPD markers are an effective method for locating genes of interest (Lefebvre and Chevre, 1995; Michelmore et al., 1991). RAPD markers linked in repulsion phase with traits of interest are very effective when used with BSA (Staub and

Horejsi, 1998). BSA was initially developed for detection of traits controlled by a single gene (Michelmore et al., 1991), but due to its simplicity and relatively low cost it is now commonly used in studying more complex traits (Mackay and Caligari, 2000).

Quantitative Trait Loci

Most fruit quality traits show continuous variation indicative of the effect of multiple genes as well as environmental influences. Genes which condition such traits are referred to as QTL. These can be mapped in any given genome with DNA markers developed from that genome. QTL have also been developed for soluble solids content in peach [*Prunus persica* (L.) Batsch] (Etienne et al., 2002), tomato (*Lycopersicon esculentum*) (Causse et al., 2002), and watermelon (*Citrullus lanatus*) (Hashizume et al., 2003). QTL have been developed for sucrose content in corn (*Zea mays*) (Tadmor et al., 1995), peach [*Prunus persica* (L.) (Dirlewanger et al., 1999), potato (*Solanum* spp.) (Simko et al., 1999), sugarcane (*Saccharum* spp.) (Ming et al., 2001), and tomato (*Lycopersicon* spp.) (Fridman et al., 2000). However, at this time there are no known QTL related to ascorbic acid content.

QTL have been developed in melon for cucumber mosaic virus resistance (Dogimont et al., 2000; Karchi et al., 1975), ethylene production (Perin et al., 2002b), fruit length, fruit shape, fruit width, ovary length, ovary shape, ovary width (Perin et al., 2002c), and cytoplasmic yellow tip (Ray et al., 1996). However, to date none have been developed for fruit quality traits such as soluble solids content, percentage of individual sugars, or ascorbic acid content (Pitrat, 2002). In the past it was necessary to genotype each plant in a segregating population with molecular markers before QTL could be developed (Quarrie et al., 1999) making the process time intensive and costly.

Fortunately, BSA eliminates the need to screen each plant individually reducing the time and expense involved.

Marker-Assisted Selection

Using molecular markers with tight linkage to genes of interest is important to plant breeders for several reasons. Due to epistasis and gene recombination, using traditional breeding methods to pyramid single genes is complicated. Developing a pyramid of major genes through traditional breeding generally requires much time and expense (Melchinger, 1990). The various traits combined in current melon cultivars, especially F_1 hybrids, necessitate techniques that allow for multiple trait selection concurrently (Staub et al., 1996). Molecular markers make it possible to take genes from several different sources and combine them into a single cultivar (Kelley, 1995; Lefebvre and Chevre, 1995). Genetic markers may be utilized for MAS when linkage with traits of interest is close (<5 cM) (Staub et al., 1996). In this way molecular markers are used to select for one or more genes at a time (Melchinger, 1990). This is a great advantage for plant breeders in breeding programs. MAS improves efficiency of breeding efforts both through allowing earlier selection and reducing population size necessary for selection. Population size necessary for improving breeding effort efficiency through MAS is dependant upon genetic factors (heritability and additive genetic variance) and selection method (Staub and Horejsi, 1998). Genetic marker loci with loose linkage also could be useful for MAS as flanking markers (Staub and Horejsi, 1998).

Genetic pooling strategies such as BSA are more useful in detecting QTL with large or moderate effect than QTL with minor effect (Wang and Paterson, 1994). This

system is very useful for MAS because plant breeders are most interested in the detection and utilization of QTL with moderate to large effect (Miklas et al., 1996).

INTRODUCTION TO GUMMY STEM BLIGHT

Gummy stem blight is a serious disease causing considerable damage to cucurbit production worldwide (cantaloupe, watermelon, cucumber, and gourd) under protection as well as in the field, particularly in tropical and subtropical areas (Blancard et al., 1994). Gummy stem blight has a fairly broad host range affecting the *Cucurbitaceae* (St. Amand and Wehner, 1995). It is commonly found in the southern United States (Sitterly and Keinath, 1996). Gummy stem blight's causal agent is a fungus, *Didymella bryoniae,* which is the sexual stage (perithecia giving rise to ascospores) and *Phoma cucurbitacearum* which is the asexual stage (pycnidia producing conidia). Principal synonyms are: *Mycosphaerella citrullina* (C.O.Sm.) Grossenb, *Mycosphaerella melonis* (Pass. Chiu and Walker, and *Ascochyta citrullina* (Cester) C.O.Smith (Blancard et al., 1994). It is a fairly common disease of the major cucurbit crops grown and is present in most growing areas. Although both the asexual and sexual stage of the pathogen may occur on the same tissue during the growing season, they vary in their importance as sources of inoculum. (Zitter, 1992). Gummy stem blight was first reported in France, Italy, and the United States in 1891 and is now known to occur the world over on cucurbit crops (Sherf and Macnab, 1986; Sitterly and Keinath, 1996). It affects above ground plant parts such as leaves, stems, and fruit. The name, gummy stem blight, describes the leaf and stem-infecting phase of the disease, while black rot describes the fruit rot phase. Postharvest losses may be severe depending upon weather conditions.

Disease Symptoms

Gummy stem blight can infect healthy plants at any time during the growing season and all melon plant parts may become infected. Diagnosis may be difficult due to the broad range of foliar symptoms that may occur on melons (Zitter, 1992). Symptoms may include seed rot, seedling death, leaf spots, stem, petiole, and fruit-stalk cankers, stem decay, leaf and overall plant wilting, and fruit rot (Sherf and Macnab, 1986). Under weather conditions favorable to the disease many different symptom types may occur at the same time (Zitter, 1992).

Gummy stem blight often kills seedlings as they germinate and penetrate the soil surface. The fungus often develops on the plant stem near the growing point or stem axis of more mature plants (Prasad and Norton, 1967). Infected stems first show elongated, water-soaked lesions and later appear tan. Older stems, particularly of muskmelon and cucumber, show pycnidia within the affected tissue. Stem lesions often cause gummy, reddish-brown or black beads to exude, a symptom that can be confused with Fusarium wilts and injury caused by insect feeding. In the latter cases, however, pycnidia are not present. Perithecial fruiting bodies, which appear similar, also may be embedded in the same lesion (Sherf and Macnab, 1986). Symptoms of the fungal infection on fruit, called black rot, may be visible in the field, or they may later develop while fruit is in storage (Zitter, 1992).

There are differences in virulence among different strains of the fungus. Various isolates have shown various disease ratings when tested on the exact same genotypes (St. Amand and Wehner, 1995).

Pathogen Description

Gummy stem blight is carried on or in infected seed and in soil. The fungus can overwinter for over a year and a half on dry, undecomposed plant matter even when no live host plants are present. The fungus is quite resistant to dry conditions, and can survive on the structure of greenhouses (Blancard et al., 1994). Gummy stem blight survives as dormant mycelium (chlamydospores - thick-walled modified mycelium). Conidia are released through a pore (ostiole) in the pycnidia. Under moist conditions, they are readily dispersed by splashing water (Zitter, 1992). Penetration by conidia occurs directly, not requiring stomates or wounds (Zitter, 1992). Ascospores, produced any time, may be released within 3 hours after infected plant parts are wetted. The availability of visible light appears to be insignificant for disease dispersal. Spores can be dispersed through air movement (Blancard et al., 1994). Rotted fruit contains seeds that are contaminated inside and outside ensuring survival and spread of the disease (Blancard et al., 1994).

Conditions Conducive to Disease Development

Temperature and moisture conditions are crucial for the germination, sporulation, and penetration of conidia, along with subsequent symptom development. Gummy stem blight can grow and fruit at temperatures ranging from 5 to 35ºC. The optimum on cucumbers is about 23 to 25ºC. The optimum temperature on watermelons is about 24 to 25ºC. Melons have a lower optimum temperature at 19 to 20ºC and become less susceptible with temperature increase (Blancard et al., 1994; Sitterly and Keinath, 1996). However, moisture appears to be more important than temperature in disease development. Ascospore dispersal peaks subsequent to periods of rain and dew

formation. Moisture on the leaf surface for one hour is necessary for the initial infection formation, but continued wetness is necessary for expansion of lesions (up to 10 hours) (Arny and Rowe, 1991; Sitterly and Keinath, 1996). Gummy stem blight is very serious at humidity levels of 85% and greater (Blancard et al., 1994; Van Steekelenburg, 1985b). More lesions were found on leaf, petiole, main stem, and fruit tissue of plants grown for lengthy periods in humid conditions or where standing water was present) (Arny and Rowe, 1991; Van Steekelenburg, 1985a). Wounding was essential for infection of older leaves (Van Steekelenburg, 1985b).

Contributing Factors

Other factors such as mechanical damage, wounding caused by striped cucumber beetles (*Diabrotica undecimpunctata howardi* Barber and *Acalymma vittatum* Fabricus) and melon aphids, and infection by powdery mildew [*Erysiphe cichoracearum* DC. Or *Sphaerotheca fuliginea* (Schlect.) Poll.] infection make plants more susceptible to subsequent infection by gummy stem blight (Bergstrom et al., 1982; Zitter, 1992). When plants with beetle injury were inoculated they developed gummy stem blight symptoms within 3 days; lesions formed at the site of injury. Striped cucumber beetles may transmit gummy stem blight from infected cucumber plants to healthy plants (Bergstrom et al., 1982). Wounding, insect injury and powdery mildew infection may improve disease incidence by releasing nutrients (volatiles present in cucurbit tissue) for use by the fungus (Bergstrom et al., 1982; Pharis et al., 1982; Zitter, 1992).

Pathogen Control Strategies

Various control measures have been developed for control of gummy stem blight. Among them are using disease-free seed, crop residue removal, crop rotation, application

of chemicals, avoiding wounding, controlled irrigation, and proper postharvest handling of fruit (Blancard et al., 1994; Sherf and Macnab, 1986).

Only seed that is fungus free and produced in arid western climates (Sherf and Macnab, 1986) or been treated with an effective fungicide should be used for cucurbit plantings (Sitterly and Keinath, 1996; Zitter, 1992). Seed should only be obtained from dependable sources. Seed saved from open-pollinated genotypes should only be collected from plants that are disease free and harvested at locations not contaminated by airborne conidia. The use of chemicals for seed disinfection cannot guarantee completely disease-free seed. However, such disinfectants work more effectively when used in liquid form than as dry treatments (Zitter, 1992).

A minimum of two year crop rotation cycle is necessary for effective cucurbit production; to ensure adequate decomposition of plant remaining matter, refuse should be deeply plowed under immediately following harvest (Sitterly and Keinath, 1996). Crop rotations of 3 to 4 years have been recommended for adequate control (Sherf and Macnab, 1986).

In field crop production, adequate drainage is necessary to ensure that water is not allowed to remain on the ground or plants themselves for extended periods of time. Proper irrigation techniques also should be practiced; less water used at any given time but applied more frequently seems to be the best method (Blancard et al., 1994).

Various chemicals are available for control of gummy stem blight. Chemical control of both gummy stem blight and powdery mildew is necessary in reducing plant and fruit infections since powdery mildew damage allows secondary infection with gummy stem blight. Sprays with chemical combinations that can control powdery mildew

and gummy stem blight concurrently are recommended. The combination of chemicals necessary is determined by diseases present. Fungicides specifically for downy mildew control should be used if the quickly spreading disease is discovered as it can also lead to secondary gummy stem blight infections (Zitter, 1992).

Using resistant plants is the best way to reduce disease incidence. The advantage of genetic resistance in plant material is that it is not affected by environmental conditions as much as chemicals used for control and it helps in reducing pesticide use (Wehner and St. Amand, 1993). Control of the fungus with fungicides is inefficient due to the necessity of frequent applications, and ineffective control of fruit infections. Also, chemicals are not effective during some environmental conditions, such as long periods of rainy weather (Wehner and St. Amand, 1993). Plant breeders have located resistance genes in several different cucurbits, but currently there are not any commercially available GSB resistant cultivars of watermelon, melon, or cucumber (Sitterly and Keinath, 1996). In order to reduce infection by gummy stem blight, powdery mildew resistant cucumber and cantaloupe genotypes should be planted and cucumber beetles and melon aphids should be controlled (Zitter, 1992).

In order to prevent post harvest fruit rot associated with gummy stem blight, wounding of fruit during harvest should be avoided and fruit should be stored at 7.2 to 10ºC (Sherf and Macnab, 1986).

Resistance to the Pathogen

Many different genotypes (both PI’s and commercial varieties) have been screened for resistance to gummy stem blight. Work in Wisconsin using field screening showed ‘Homegrown #2’ and PI 200818 melons to be resistant to gummy stem blight

strains present there (Wyszogrodzka et al., 1986). A plant introduction native to Texas, PI 140471, was found to be highly resistant to gummy stem blight also (Sowell et al., 1966). Greenhouse screening in the Netherlands was used to identify gummy stem blight resistance in PI 200818 as well (Van Der Meer et al., 1978).

About 1200 melon plant introductions were screened for resistance to gummy stem blight in 1966 and PI 140471 was found to be the most resistant both in terms of mean diameter of lesions and disease ratings. In field tests PI 140471 showed complete resistance to gummy stem blight being completely free of disease damage from the pathogen. All F_1 plants resulting from PI 140471 also exhibited resistance (Sowell et al., 1966). Approximately 600 additional muskmelon PI's were screened and compared to PI 140471. Although several additional sources of resistance, PI266934 and PI266935, were identified they were both inferior to PI 140471 in field testing (Sowell, 1981). PI 266934 was found to be highly resistant to gummy stem blight in another test (McGrath et al., 1993), but was not compared to PI140471 in disease screening.

Breeding for Resistance

Several cultivars have been developed using 140471 since 1966 in Alabama. 'Gulfcoast' in 1971 (Norton, 1971), Chilton' in 1972 (Norton, 1972), and 'Cosper' in 1985 (Norton et al., 1985). Resistance to gummy stem blight was tested in the greenhouse on a segregating F_2 population and found to be controlled by a single dominant gene (segregation ratio of 3 resistant to 1 susceptible). This also was confirmed in field trials. The gene involved in the resistance was named Mc (Prasad and Norton, 1967). Unfortunately the resistance was overcome during severe disease outbreaks (Sowell,

1981). It has since been suggested that this resistance is more complex than single dominant gene control (Kyle, 1995).

INTRODUCTION TO MONOSPORASCUS ROOT ROT

Diseases causing vine decline in mature melon plants have been classified into three groups. The first group contains the vascular wilts which are represented by *Fusarium oxysporum* and *Verticillium dahliae*. The second group contains the crown-rot fungi represented by *Myrothecium roridum* and *Macrophomina phaseolina*. The third group contains the root-rot fungi that incite melon declines represented by *Monosporascus cannonballus* and *Acremonium cucurbitacearum* (Bruton et al. 1998).

Monosporascus root rot and vine decline (*M. cannonballus*) is an important disease affecting melons around the world and it is on the increase (Martyn and Miller, 1996a). It also has been referred to as sudden wilt, sudden death, melon collapse, Monosporascus wilt, and black pepper root rot. Monosporascus is adapted to hot, arid climates such as those found in the Rio Grande Valley of Texas (Martyn and Miller, 1996b). Monosporascus root rot is now a fairly serious disease affecting the Lower Rio Grande Valley of Texas (Crosby, 2000; Mertely et al., 1991). This is partially due to continuous cultivation with melons which is a common practice providing the fungus ideal growth conditions (Mertely et al., 1993a).

Origin and Spread of the Fungus

The original discovery of Monosporascus occurred in Arizona in 1970 (Pollack and Uecker, 1974), and it has since been confirmed in Texas in the mid 1980's (Champaco et al., 1988) and the Imperial Valley of southern California (Bruton et al., 1995) in the United States. It has also been reported in thirteen other countries including

Israel (Reuveni et al., 1983), Japan (Uematsu et al., 1985), Spain (Ruano, 1990), Mexico (Martyn et al., 1996), Tunisia (Martyn et al., 1994), Honduras (Bruton and Miller, 1997a), and Guatemala (Bruton and Miller, 1997b). It most severely affects the southwestern United States and southern Spain (Martyn and Miller, 1996a).

Affected Plants

Although *M. cannonballus* was originally reported as a pathogen of cantaloupe, watermelon is highly susceptible, cucumber and summer squash are somewhat susceptible, and pumpkin, several winter squashes, bottle gourd (*Lagenaria siceraria*) and sponge gourd (*Luffa aegyptiaca*) have been shown through greenhouse tests to be susceptible as well (Mertely et al., 1993b).

Economic Impact

Significant economic losses related to *M. cannonballus* in the Lower Rio Grande Valley of Texas were first noted in 1986. However, it is most likely that the disease was a problem earlier and was undetected or symptoms were blamed on other causes. Its ability to persist is a major limiting factor for melon production in many areas. Specific losses vary by year from about 10 to 25% of the crop, but it is not uncommon for individual fields to suffer complete (100%) loss (Martyn and Miller, 1996a). In Israel, melon crops have been totally destroyed by *M. cannonballus* in the fall growing season; disease severity and occurrence is not as severe in spring crops (Cohen et al., 1996).

Possible Causes of Disease Appearance as a Problem

It may be difficult to determine exactly what factors changed dramatically enough to cause the Monosporascus problem. Possible explanations could be related to the drastic changes in melon culture during the 1980's including the use of black plastic

mulches, drip irrigation, and hybrids. Soil temperature greatly increases with use of plastic mulch and tunnels. Also, hybrid melon varieties commonly produce fruit that is earlier and bigger than that of previously used, open-pollinated varieties causing more physiological stress. New hybrids have higher shoot to root ratios than older open-pollinated varieties not allowing them to tolerate root damage as well (Martyn and Miller, 1996b).

Disease Symptoms

First symptoms observed in melon fields are often the stunting of young plants. When the field infection is uniform, such symptoms may not be noticed. Usually, the older crown leaves start to turn yellow and senesce within 2 to 3 weeks of harvest (Martyn and Miller, 1996b). First appearance of wilt-like symptoms in infected melons is often associated with fruit set and maturity (Pivonia et al., 2002). Between 10 to 14 days pre-harvest, the whole canopy typically collapses, exposing the fruit to the sun’s solar radiation (Martyn and Miller, 1996b). Subsequently chlorosis and death of leaves moves rapidly along the vines, and the entire canopy may be dead in as many as 3-10 days of first foliar symptom appearance depending on the climate and fruit maturity (Cohen et al., 1996; Martyn and Miller, 1996b). Stem lesions are not present. However, aboveground symptoms may be otherwise confused easily with other vine declines such as charcoal rot and gummy stem blight. The fruit of affected plants is usually smaller with less sugar. It may be sunburned and easily cracked due to lack of cover from the canopy. Foliar symptoms are the result of below ground damage to the root system, occurring quite a bit earlier (Pivonia et al., 2002).

Belowground symptoms may include root lesions, root rot, loss of secondary and tertiary feeder roots, and under extreme, wet conditions, secondary root rot and tap root death. Visible perithecia may form in the dead and necrotic roots. Monosporascus is caused by a soil-borne ascomycete uniquely adapted to climates that are hot and arid. The infection of young roots occurs fairly early from either mycelium or ascospores. However, tissue colonization occurs later being favored by the higher soil temperature that occur. The roots closest to the soil surface are affected first (Martyn and Miller, 1996b).

Reason for Vine Collapse

The *M. cannonballus* mediated collapse of melon plants is a result of plant-pathogen interaction, sink-source effects along with fruit maturity, and environmental conditions (Pivonia et al., 2002). Monosporascus causes production of tyloses in xylem vessels (Alcantara et al., 1995). Tyloses cause a reduction of hydraulic conductance in secondary roots as well as sap flow throughout the plant. There is an incremental increase of tylose formation during fruit maturation. High water demand by plants during later stages of plant development results in an imbalance in water uptake from roots vs water flow to leaves for transpiration, which eventually leads to collapse by the plant. The uptake and water flow through the roots is constrained, and thereby causes wilting. For this reason, monosporascus collapse does not inevitably include total root destruction. The disease may do the most damage to thin roots, as although they make up a small percentage of the root mass, they contribute substantially to plant water uptake. Infected plants showed reduced root growth even after fruit removal compared to healthy plants,

indicating that vine collapse is due in part to the negative effects on growth and root system size (Pivonia et al., 2002).

Low temperatures can stave off wilting by reducing water demand and allow some fruit to be harvested before plants collapse. Monosporascus subjects plants to increased water loss until death of the plant; this process is facilitated by higher temperatures. The removal of fruit increases resistance of leaf stomates and root growth giving plants better ability to survive even though disease damaged (Pivonia et al., 2002).

Pathogen Detection

Detection and identification of plant pathogens residing in the soil is often quite difficult. Several characteristics of monosporascus make it even more difficult to positively identify. Ascospores are the only spore stage and they rarely germinate; isolation and identification of vegetative mycelium in the lab is also difficult (Martyn and Miller, 1996b). *M. cannonballus* is fairly similar to other fungal diseases; analysis of fungal sequence showed a 74 to 90% similarity of the 18S, 5,8S, and 28S gene sequences in *M. cannonballus* to the same genes in other fungi.

Five sequences (Primers A to E) from the ITS region were developed and tested by amplification of *M. cannonballus* DNA as well as DNA from other fungi (Lovic et al., 1995a). The primers were shown to be specific for monosporascus; they amplified fragments from each isolate of monosporascus but not from any related fungi. Such genus-specific primers along with DNA probes obtained by digoxigenin-labeling along with PCR have been used to develop a protocol for diagnosing the pathogen in both plant tissue and from the soil (Lovic et al., 1995b). Utilizing the protocol, DNA from small (5- to 10-mg) root tissue sample is extracted and probed with the primers. Ascospores may

be extracted from soil through sieving, centrifugation, crushing, and processing the DNA for testing (Lee and Taylor, 1990). This method can be used to extract DNA from a single ascospore for identification, allowing positive fungal identification from either cultures or plant tissues (Martyn et al., 1994). The development of this protocol has improved testing accuracy and improved the rapidness with which disease confirmation can be accomplished; instead of 2 to 3 weeks, testing is now possible in 2 days (Martyn and Miller, 1996a).

Disease Control

Traditionally, methyl bromide has been effective at controlling fungal infections such as monosporascus. In fields infested with *M. cannonballus*, the fumigation of melon beds before planting effectively reduces stunting of the plants and increases melon yield (Martyn and Miller, 1996b). However, its impending phase out for use in developed countries presents a challenge to the established agriculture scientific community to develop effective alternatives that are environmentally acceptable (Cohen et al., 2000). Such alternatives may include manipulating irrigation, grafting onto more resistant genotypes, good drainage, breeding for resistance, improved soil solarization, fungicides, crop rotation, and biological control. Since no single method is currently available to replace methyl bromide, a combined management approach will likely be most effective. Continuous monitoring of fields for early detection of pathogen escape will be necessary (Cohen et al., 2000).

In comparison with fumigants, soil fungicides are usually more cost effective. Another advantage is that fungicide makeup and application often allow for targeting of specific organisms with less likelihood of having detrimental effects on microorganisms

in the soil. Fluazinum and kresoxim-methyl are two of the most effective fungicides tested; both inhibit *M. cannonballus* growth at concentrations of 10 ug a.i./ml (Cohen et al., 2000).

It may be possible to manipulate the root system size through modifications in an irrigation scheme effectively reducing disease incidence. However, irrigation cannot be used effectively as the only management practice. (Cohen et al., 2000). In order to effectively control monosporascus, the use of long-term crop rotation of melons in conjunction with crops that are not susceptible may be necessary. Any field that has a known infestation with *M. cannonballus* should not be used for melon or other cucurbit production (Martyn and Miller, 1996b).

Soil solarization has not proven effective at controlling the pathogen, due to its ability to survive at high temperatures (Martyn and Miller, 1996b).

In field trials performed, monosporascus incidence on plants grafted to *Cucurbita* and bottle gourd rootstock was significantly less severe than on plants that were not grafted even though such plants are hosts as well (Mertely et al., 1993b; Uematsu et al., 1992). Reduced disease development, along with their significantly more extensive root system, enable plants that were grafted to better compete during the production season. Apart from their disease response, performance of grafted plants is also determined by rootstock compatibility, growing season, and cultivation methods employed on the specific crop. The effective ability of certain rootstock's vigor in absorbing water and nutrients may explain why they outperform non-grafted plants; they may also supply endogenous plant hormones further contributing to improved performance. Melon plants

grafted onto cucurbit rootstock perform better when grown along the ground in the field than when trellised in the greenhouse (Cohen et al., 2000).

Several methods of biological control have shown promise. Inoculation with a gliotoxin producing strain of *Trichoderma virens* has produced a significant reduction in Monosporascus disease on muskmelon roots (Zhang et al., 1999) and hypovirulent isolates of *M. cannonballus* show potential for reducing infection by monosporascus on cantaloupe (Batten et al., 2000). However, neither has been used on a commercial basis.

Developing Resistance

The use of cultivars resistant to plant diseases is one of the best control measures, but there currently are no commercially available monosporascus resistant varieties (Cohen et al., 2000). Through extensive screening of germplasm utilizing fields heavily infested with *M. cannonballus*, several possible sources of resistance have been identified. In the United States, Ananas and Honeydew melons showed more tolerance than US cantaloupes (Mertely et al., 1993a; Wolff and Miller, 1998). Vine decline ratings showed ‘Deltex’ had the best tolerance to MRR/VD over two seasons of testing, but yield response was not determined (Wolff and Miller, 1998). ‘Deltex’, an Ananas type melon, was found to be more tolerant to monosporascus than commonly used commercial varieties of cantaloupe such as ‘Caravelle’, a western shipper. The reduced wilt incidence may be partially due to root system size and structure; ‘Deltex’ has a more vigorous root system giving it better adaptation to dry-land production (Crosby and Wolff, 1998). In another study performed in Israel, ‘Deltex’ was found to be susceptible , but the results may be due to variance in inoculum level or pathogen virulence (Cohen et al., 2000).

‘TGR1551’ has been found to be moderately resistant to monosporascus (Dr. Crosby, personal communication)

Although the number of genes that control tolerance is unknown, results indicate gene action is additive in nature (Cohen et al., 1996) and tolerance inheritance is likely complex (Crosby, 2000). However, high heritability of several root traits suggests the possibility of making efficient selections for improved tolerance. Although the tolerance mechanisms are possibly both morphological and biochemical, the mechanisms are not extremely important as long as improvement is possible in breeding programs (Crosby, 2000). Correlation analysis indicated that by selecting for shorter vine length and more substantial root systems disease tolerance could be improved (Martyn and Miller, 1996b).

Since certain non-genetic factors such as fruit maturity and environmental stresses may contribute to disease progression (Pivonia et al., 1998; Pivonia et al., 1999; Wolff, 1995), they need to be studied further and taken into account in breeding programs when selecting for tolerant plants (Cohen et al., 2000).

CHAPTER III

INTROGRESSION OF RESISTANCE TO CUCURBIT YELLOW STUNTING DISORDER VIRUS (CYSDV) INTO CANTALOUPE (*CUCUMIS MELO* L.)

MATERIALS AND METHODS

2001-2002 Experiments

Plant materials – Source and Selection

Seed from various sources was used in 2001 experiments (Table 1). Plants used in crossing were grown in a greenhouse to ensure pollination control (eliminating uncontrollable contamination due to whiteflies, houseflies, honeybees, etc.).

Table 1. Melon cultivars used in crossing program with origin and useful attributes.

Cultivar:	Origin	Useful attributes:
Caravelle	Asgrow	Western Shipper cantaloupe with powdery mildew resistance
Cruiser	Harris Moran	Western Shipper cantaloupe
Deltex	Nunhems	Cantaloupe type with high sugars, Fom 0,2 and *Monosporascus* resistance
Dulce	TAES	Cantaloupe type with powdery mildew and Fom 0,2 resistance
Morning Ice	Harris Moran	Honeydew type with good fruit quality
Primo	Rogers NK	Western Shipper cantaloupe with powdery mildew resistance
TDI	TAES	Honeydew type with good fruit quality, powdery and downy mildew and Fom 0,2 resistance
1409	TAES	Western shipper with excellent fruit quality and powdery mildew resistance
1405	TAES	Western shipper with vine decline and Fom 0,2 resistance
TGR1551	Zimbabwe USDA	Resistance to Cucurbit Yellow Stunting Disorder Virus

An initial cross was made using ‘Dulce’ and ‘TGR1551’ seeds obtained from the USDA. Resulting F_1 plants were self pollinated to produce a F_2 generation and crossed to

commercial varieties listed in Table 2 (at least 2 plants each). Fruit from crosses was collected and seeds were removed, processed, cataloged, and stored for further use.

Plant materials – Treatment and Care

Seeds from crosses, F_2 and both original parents were then planted in 32 cell seedling flats with Redi-earth plug and seedling mix (Scotts-Sierra Horticultural Products Company, Marysville, OH). Seedlings were fertilized once per week with Peters 20-20-20 professional water soluble fertilizer (Smurfit-Stone, Wellsburg, WV). After seedlings had grown for approximately two weeks, 18 plants from each cross, as well as 200 F_2 plants from the original cross, and 5 plants of both original parents were mass inoculated with CYSDV. Silverleaf whiteflies previously fed on cantaloupe were collected and placed in 3’x5’x3’ enclosed mesh cages with two plants positively identified with CYSDV for 72 hours, for adequate virus acquisition. Seedlings in trays were then placed inside cages with viruliferous whiteflies and inoculated for 72 hours to ensure adequate virus inoculation occurrence (Sese et al., 1994). After inoculation, seedlings were removed from cages. All plants were then planted into three gallon plastic pots in soil-less media (Sunshine Mix #4) (Sun Gro Horticulture Inc., Bellevue, WA) and fertilized with Osmocote 14-14-14 controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH).

2002-2003 Experiments

Plant materials – Source and Selection

An initial cross was made using ‘Dulce’ and ‘TGR1551’ seed obtained from Spain. Resulting F_1 plants were self pollinated to produce a F_2 generation and crossed back to ‘Dulce’ to produce a BC_1 generation. Plants used in crossing were grown in a

greenhouse to ensure pollination control (eliminating uncontrollable contamination due to whiteflies, houseflies, honeybees, etc.). Fruit from crosses was collected and seeds were removed, processed, cataloged, and stored for further use.

Plant materials – Treatment and Care

Seeds from 'Dulce', 'TGR1551', F_1, F_2 and BC_1 were then planted in 96-cell seedling flats with Redi-earth plug and seedling mix (Scotts-Sierra Horticultural Products Company, Marysville, OH) Seeds were replanted as needed for adequate seed stand. Seedlings were fertilized once per week with Peters 20-20-20 professional water soluble fertilizer (Smurfit-Stone, Wellsburg, WV). After seedlings had grown for approximately two weeks, 16 'Dulce' (Parent #1), 16 'TGR1551' (Parent #2), 16 F_1 ('Dulce' x 'TGR1551'), 16 BC_1 [('Dulce' x 'TGR1551') x 'Dulce'], and 144 F_2 (Selfed F_1) plants were mass inoculated with CYSDV. Silverleaf whiteflies previously fed on cantaloupe were collected and placed in 3'x5'x3' enclosed mesh cages with two plants positively identified with CYSDV for 72 hours, for adequate virus acquisition. Seedlings in trays were then placed inside cages with virus carrying whiteflies and inoculated for 72 hours to ensure adequate virus inoculation occurrence (Sese et al., 1994). After inoculation, seedlings were removed from cages. A selected number of plants (five each of both parents, F_1, and BC_1, and 100 F_2) were then planted into three gallon plastic pots in soil-less media (Sunshine Mix #4) (Sun Gro Horticulture Inc., Bellevue, WA) and fertilized with Osmocote 14-14-14 controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). Observations on virus incidence were made at 30 day intervals, with the final observation at 90 days. A field trial was also planted using the same number of plants from the inoculated population. Spraying with appropriate

chemicals was done to control powdery mildew, spider mites, mealy bugs, and aphids, as this was not intended to screen for resistance to those pests / diseases. Temperature data is shown in Appendix A.

Measurement and Statistical Analysis

Data analysis for this paper was generated using SAS software, Version 8 of the SAS System for Windows (SAS Institute Inc., Cary, NC.). The analysis of variance (ANOVA) was generated using the General Linear Model command. Mean separations were performed using Duncans Multiple Range Test. (SAS Institute, 2003).

RESULTS

In the 2001 experiments, no complete resistance as reported previously (Gomez-Guillamon, 2000) was detected at 90 days (3 months) after inoculation in any of the F_2 plants. All plants were destroyed to prevent the spread of CYSDV to other non-inoculated plants in production in the vicinity.

In the 2002 experiments, no complete resistance as reported previously (Gomez-Guillamon, 2000) was detected at 90 days (3 months) after inoculation in either the parents, F_1, F_2, or BC_1 plants. However, some plants appeared to be somewhat tolerant (virus spread was slow) as symptoms had not yet appeared at 21 days post inoculation and date of first symptom appearance varied within the population. 'Dulce' virus symptoms are shown in Appendix B. *Begomoviruses*, which are also transmitted by whiteflies were tested for using PCR with DNA specific primers and were not detected. Plants were also tested to make sure there was no contamination with BPYV which looks very similar to CYSDV. No infection with BPYV was detected in any of the tested plants (Park et al., 2002). RT-PCR was performed to test for CYSDV particles in "resistant"

TGR1551 plants, and TGR1551 plants were found to be CYSDV positive (Park et al., 2002) (Fig 1).

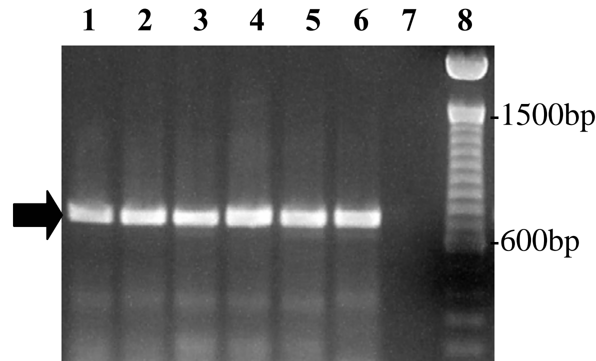

Fig. 1. RT-PCR showing a 753 pb product for the CYSDV CP gene generated by CYSCPf/CYSCPr in 'TGR1551' plants. 1-6= CYSDV infected 'TGR1551' plants, 7= CYSDV free 'TGR1551' plant (control), 8= molecular size marker. Arrow indicates band of interest. Photo courtesy Soon Park, TAES, Weslaco.

The ANOVA showed highly significant effects for genotype in the population used for analysis (Table 2).

Table 2. Significance of genotype in population developed for CYSDV resistance in 2002 with source, degrees of freedom, mean squares, and f values.

Source	**Degrees of freedom**	**Type I SS**	**Mean Square**	**F value**	**Significance**
Genotype	4	6.11	1.53	5.12	***$_z$

$_z$***. Significance level $\leq$ 0.001.

TGR1551 had significantly better disease ratings than did Dulce, F_2, or BC_1 populations and was as good as or better than the F_1 population (Table 3).

Table 3. Mean comparison of genotypes developed for CYSDV screening.

	Genotype Dulce	**TGR1551**	**F1**	**F2**	**BC1**	**Overall mean**
Mean[y]	2.40 c[z]	1.30 a	1.70 ab	2.28 bc	2.20 bc	2.22

[y]CYSDV disease rating scale: No symptoms = 0, Slight symptoms = 1, Moderate symptoms = 2, Severe symptoms = 3
[z]Different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

All five 'TGR1551' plants, as well as selected F_2 plants exhibiting some degree of tolerance, were saved for making crosses. Plants exhibiting some degree of tolerance were pruned back and fertilized with Osmocote 20-20-20 (Scotts-Sierra Horticultural Products Company, Marysville, OH) to induce more vigorous growth when moved to the greenhouse from the seedling house. First inspection of resumed growth appeared to be virus free. Virus tolerant plants used for making the crosses were R1 2-1, R1 4-8, R2 2-1, R2 4-8, R2 4-10, R3 2-1, R3 4-6, R4 2-1, R4 4-5, R4 4-15, R5 2-1, R5 4-19. The following controlled backcrosses were made in the greenhouse: R1#4-8 x 'Caravelle', R2#4-8 x 1409, R5#2-1 x R5#3-10 (gummy stem blight resistant), R5#4-19 x 146-19.

Disease incidence in the F_2 population derived from the cross of 'Dulce' x 'TGR1551' is shown in Fig. 2. The F_2 population rating was as follows: #1 = 5, #1.5 = 12, #2 = 28, #2.5 = 32, #3 = 30.

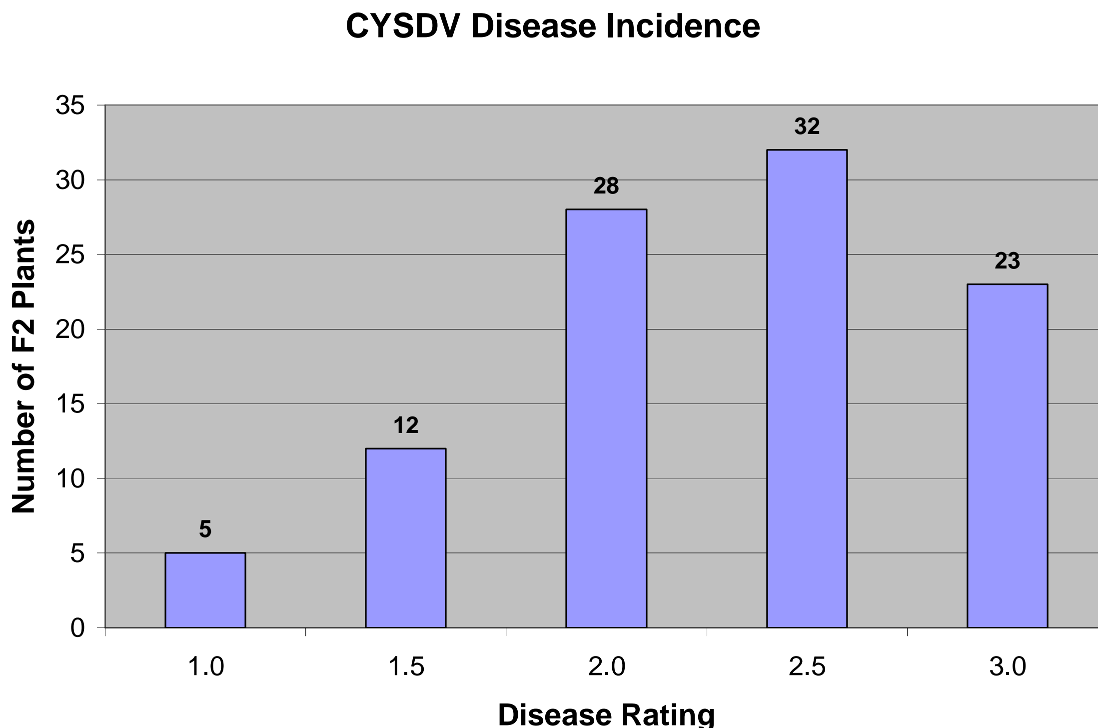

Fig. 2. CYSDV incidence on F_2 population developed from the cross 'Dulce' x TGR1551. 1 = slight virus symptoms, 2 = moderate virus symptoms, and 3 = severe virus symptoms. Columns labeled with the number of plants contained in each category.

Fruit type, for the most part, resembled the undesirable oblong shape of 'TGR1551' even in the F_1. However, BC_1 plants had fruit that was much closer to 'Dulce' and adequate in appearance. Fruit size was generally poor throughout the population compared to field grown melons due to greenhouse growing conditions. Powdery mildew became a severe problem on most plants in the seedling house even with spraying, but only at about 9 weeks after virus inoculation. Plant infestation with aphids and spider mites was sporadic and reoccurred several times during the study, but was adequately controlled.

DISCUSSION

In the 2001-2002 experiments, it was assumed that the wrong seed for ‘TGR1551’ had been used in the original cross due to no resistance detection in any of the F_2 plants. The seed in question was acquired from the USDA and presumably there could have been a mix-up in seed lots. As a result, seed of ‘TGR1551’ was acquired directly from Spain, where original resistance was reported (Gomez-Guillamon et al., 1995), and experiments were repeated the following year (2002-2003). In 2002, no complete resistance was found again in any of the F_2 plants. RT-PCR was performed to test for CYSDV particles in “resistant” ‘TGR1551’ plants, and plants were found to contain CYSDV (Park et al., 2002). It may be definitively stated that ‘TGR1551’ is not completely resistant to the strain of CYSDV present in the Lower Rio Grande Valley of South Texas. Although preliminary screening showed promise and previous reports championed it’s CYSDV resistance, ‘TGR1551’ should have been fully screened before going through all the work to develop the various populations screened in this study.

Although no single gene dominant resistance, as previously reported from research done in Spain (Sese et al., 1999; Lopez-Sese and Gomez-Guillamon, 2000) was found, there may be partial tolerance. Results from graft transmissibility experiments indicate that ‘TGR1551’ may possess either resistance or tolerance to CYSDV depending upon the mode of infection (Marco et al., 2003). Therefore, the resistance/tolerance mechanism may function by slowing or inhibiting movement of the virus (possibly through the vascular system through membrane permeability mechanisms) from leaves infected initially to newer, younger leaves. The heritability of tolerance appears to be low. Recovery of any kind of tolerance approaching resistance in BC_1 or F_2 plants will

likely require screening much larger populations. Either at least 2 genes must be involved instead of one dominant gene as previously reported, or the virus may have mutated and overcome the dominant resistance gene. This may be due to differences in virus strain from South Texas compared to Spain, as well as the intensity of various pest, disease, and environmental pressures in the Rio Grande Valley of South Texas. Many other viruses have multiple strains allowing some to overcome resistance genes (Danin-Poleg et al., 2000). While CYSDV particles showed high similarity in the coat protein area of the genome (Rubio et al., 2001), differences in virulence and symptom severity may be due to the effects of differences in other segments of the virus genome. A recent study indicated that supposed resistance to CYSDV in 'TGR1551' may simply be a form of tolerance which inhibits virus particle spread and or multiplication (Marco, et al., 2003).

'TGR1551' may still be a useful source of resistance to CYSDV, but due to its lack of complete resistance from a single dominant gene and many poor quality traits previously mentioned, other more suitable sources of resistance are needed for adequate breeding for resistance. More screening of PI's and other wild-type melons should be carried out with accurate records kept not only on final disease ratings, but also on the number of days until first symptom appearance and effect on fruit load. In this way, plants can be rated both on possible resistance as well as tolerance to CYSDV.

CHAPTER IV

DETECTION OF RAPD MARKERS ASSOCIATED WITH QTL FOR TOTAL SOLUBLE SOLIDS, SUGARS, AND VITAMIN C IN MELON (*CUCUMIS MELO* L.)

MATERIALS AND METHODS

Plant Material – Source and Selection

Seeds from two sources were used in marker selection experiments in 2002; the parents of the original cross were 'Dulce' (TAES) and PI 482420 ('TGR1551' - USDA). 'Dulce' is a cantaloupe type with good fruit quality. 'TGR1551' is a wild type with poor fruit quality. Some of the important fruit and quality traits of the two parents are listed in Table 4.

Table 4. A summary of ten selected fruit and quality characteristics of the two melon parents 'Dulce' and TGR1551 used in marker development experiments.

Fruit and quality trait	Dulce	TGR1551
Total Soluble Solids	High	Low
Sucrose	High	Low
Glucose	Moderate	High
Fructose	Moderate	High
Ascorbic Acid	High	Low
Fruit weight	Moderate	Low
Fruit length	Moderate	Moderate
Fruit diameter	Moderate	Low
Fruit flesh weight	Moderate	Low
Fruit dry weight	Moderate	Low

The F_1 seed of this cross was grown in the greenhouse and self pollinated and backcrossed to 'Dulce' to produce F_2 and BC_1 seed populations, respectively. Plants used

in the crossing program were grown in a greenhouse to ensure pollination control (eliminating uncontrollable contamination due to whiteflies, houseflies, honeybees, etc.). One fruit per plant was grown to ensure maximum fruit quality. When mature, fruit from crosses was collected and seeds were removed, processed, cataloged, and stored for further use.

Plant Material – Treatment and Care

Seeds from parents, F_1, BC_1, and the F_2 were grown in the greenhouse in the fall of 2002, Weslaco, TX. One plant was planted per pot into three-gallon plastic pots in soil-less media (Sunshine Mix #4) (Sun Gro Horticulture Inc., Bellevue, WA) and fertilized with Osmocote 14-14-14 controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). Total number of plants grown was 5 ‘Dulce’, 5 TGR1551, 5 F_1, 110 F_2, and 5 BC_1.One fruit per plant was grown to ensure maximum fruit quality. When mature, fruit from crosses was collected (between 0800h and 0900h) and seeds were removed, processed, cataloged, and stored for further use.

A nutrient solution of Peters 20-20-20 professional water soluble fertilizer (Smurfit-Stone, Wellsburg, WV) was applied one time per week during the plant growth period utilizing drip fertigation. Pesticides were applied as needed during plant growth to control pests and diseases (Table 5).

Table 5. Chemicals used to control pests and diseases on melon plants at the Texas A&M Experiment Station, Weslaco 2002.

Pest / Disease	**Chemical used for control**	**Comments**
Aphids	Aphid Star®	Systemic
	Contact®	Used in rotation
Looper Worms	Ambush®	
Mealybugs	Admire®	
Mites	Agrimek®	Used in rotation
	Kelthane®	
White Fly	Thiodan®	Systemic
	Admire®	Used in rotation
Downy Mildew	Ridomil Gold®	Used in rotation
	Bravo®	
Powdery Mildew	Nova®	Used in rotation
	Quadris®	
	Procure 50 W®	

Soluble Solids Testing

A tissue sample was removed from the middle of the fruit with a common 5 cm x 1.5 cm fruit core. After removing the portion in contact with rind and seed cavity, juice from the mesocarp was extracted using a hand held garlic press. Juice was collected in extra small weigh boats. A small amount of juice (1 to 2 drops) was placed on a temperature corrected refractometer with digital readout (Reichert Scientific Instruments, Buffalo, NY). The machine was adjusted with each sample and readings were taken to 2 decimal places.

Dry Weight Determination

The percentage dry weight of melon fruit was determined by first weighing a 50-mL plastic centrifuge tube to 2 decimal places (PM 4000; Mettler, Columbus, OH). Then 5.0 g of diced middle mesocarp (1 cm cubes) was placed into the centrifuge tube. The tubes were covered with KimWipes, secured with rubber bands, and stored overnight at – 80° C. Samples were subsequently dried down in a freeze dryer for approximately 2 days.

Weight of dried sample and tube was recorded. Samples were placed in sealed freezer bags and stored at –20° C in a desiccator until carbohydrate and ascorbic acid analyses were performed.

Carbohydrate Analysis

Carbohydrate analysis was performed using a modified version of the procedure developed by Lester and Dunlap (1985). First, tissue previously freeze dried was pulverized to a fine powder. Ethanol (80%) was heated to 80-85 °C using a heat bath (Reacti-Therm heating/stirring module; Peirce Chemical Company, Rockford, IL) and 5 mL was added to the pulverized tissue with a dispensette (Brinkmann Instruments Inc., Westbury, N.Y.). The resulting mixture was placed on a shaker (Orbit shaker; Lab-Line, Dubuque, IA) @ 1800 rpm for 1-1/2 minutes at room temperature. The mixture was then filtered through filter paper (No. 1; Whatman, Maidstone, United Kingdom) to remove the solid material and collect the liquid. Tubes were rinsed with an additional 5 mL ethanol (80% heated to 80-85 °C.) and filtered as well. At least 5 mL of filtrate was collected from each sample. Samples were then stored at 4°C. A nitrogen (N_2) dry down (Model 18780 ReactiVap Evaporating Unit; Pierce Chemical Company, Rockford, IL) set on low heat was used to remove ethylene from the samples to a final volume of 0.2 mL to 0.3 mL. Samples were filtered through cartridge filters (C18 SepPac; Waters, Milford, MA) previously rinsed with 2 mL of Milli-Q water. The filtrate was stored at 4° C if analysis on HPLC was completed on same or next day. Samples not analyzed within one day of extraction were stored at -20° C. The filtrate was diluted for HPLC analysis(1.0 mL sample + 4.0 mL Milli-Q water). A 250 µL sample of each standard (fructose, glucose, and sucrose) was injected into the 20 µL loop in triplicate and averages were

used to develop a standard curve. Standards were injected at the beginning of the day, at noon, and at the end of the day to ensure continued proper HPLC calibration. 250 μL of each sample was injected into the 20 μL loop. HPLC consisted of a metering pump (ConstaMetric III; Milton Roy, Ivyland, PA), carbohydrate analysis column (Aminex HPX-87C 300 x 7.8 mm; Bio-Rad Laboratories, Hercules, CA), Detector (HP 1047A RI; Hewlett Packard, Houston, TX), and Integrator (HP 3396 II; Hewlett Packard, Houston, TX).

Ascorbic Acid Analysis

Ascorbic acid analysis was performed using the procedure developed by Hodges et al. (2001). A 5.0 g. sample of frozen (-20º C) melon tissue was homogenized with 10 mL 5% meta-phosphoric acid using a homogenizer Brinkman Instruments Inc., Westbury, NY). The homogenizer then was washed with another 5 mL of 5% meta-phosphoric acid to collect as much of the sample as possible. The homogenizer was washed with water and dried between samples to reduce cross contamination of samples. Samples then were centrifuged at 7.000 x g for 15 minutes at 4º C. (Sorvall RC-5 Superspeed Refrigerated Centrifuge; Dupont Instruments, Wilmington, DE) The supernatant was saved and residue was set aside until testing was finished in the event of problem occurrence. Tubes were labeled "+" Blank, "-" Blank, sample #1 "+", sample #1 "-", sample #2 "+", sample #2 "-", etc. Then 100 uL 5% meta-phosphoric acid and 500 uL KH_2PO_4 were added to "+" and "-" blanks. Then 100 uL of each sample was added to each "+" and "-" tube along with 500 uL KH_2PO_4. Then 100 uL DTT was added to each "+" tube and 100 uL Milli Q water was added to each "-" tube. Vortexing was done after each addition to ensure a proper reaction. The tubes were kept at room temperature (22.5

- 23º C.) for 60 minutes. Then 100 uL Ethylmaleimide was added to “+” tubes and 100 uL Milli Q water was added to “-“ tubes. Then 400 uL TCA, 400 uL ortho-phosphoric acid, 400 uL dipyridyl, and 200 uL Ferric chloride was added to each tube. Vortexing was again done after each addition to ensure a proper reaction. The tubes were incubated at 40º C for 60 minutes; marbles were placed on tube tops to prevent desiccation. Tubes were remove from heat, stored at room temperature (22.5 - 23º C.) for 5 minutes, and vortexed. Absorbance was recorded at 525 nm using a spectrometer (20 Genesys; Spectronic, Rochester, NY). The “+” blank was set as the standard followed by reading of the “+” samples. Then the “-“ blank was set as the standard and the “-“ samples were read.

DNA Extraction

DNA extraction was done according to a slightly modified previously developed method (Skroch and Nienhuis, 1995). Fully mature leaves of ‘Dulce’, TGR1551, F_1, BC_1, and F_2 plants were collected at approximately one month after planting. Total genomic DNA was extracted from lypholized leaf tissue as follows.

Several normal sized mature leaves (0.5 to 0.75 grams fresh tissue) were harvested from each plant to be analyzed. Leaf tissue was placed in cold storage (–80 °C) within 10 minutes of harvesting. Tissue from approximately 1 leaf per sample was removed from the freezer and immediately ground to complete homogenization using a mortar and pestle. Then 500 µl of extraction buffer containing 2% CTAB, 100mM TRIS (pH = 8.0), 20mM EDTA (pH = 8.0), 1.4M NaCl, and 1% PVP (polyvinylpyrolidone) was added to a 1.5 ml micro centrifuge tube marked for each ground sample using a fume hood (due to the unpleasant odor). After grinding of tissue was completed, as much

ground leaf tissue as possible from each sample was placed into its correspondingly labeled micro centrifuge tube containing extraction buffer and mixed by vortexing briefly. Tissue/extraction buffer mixture was incubated at 65 °C for at least 60 minutes. Vortexing was done every 10 minutes for the first 30 minutes to facilitate the extraction process. Then the mixture was spun at 11,000 RPM in a micro centrifuge for 10 minutes. The supernatant was transferred to a correspondingly labeled clean 1.5 ml micro centrifuge tube. Nucleic acids were precipitated by filling the tubes with a 6:1 mixture of Ethanol and 7.5M Ammonium Acetate. Tubes were mixed by inverting at least 10 times and allowed to precipitate for 30 minutes at room temperature before storing in the freezer at -20 °C. over night. Tubes were removed from the freezer (12 at a time) and shaken manually to break up precipitate. Precipitated nucleic acids were pelleted by spinning the samples in a micro centrifuge for 5 minutes at 5000 RPM. The supernatant was then poured off under the hood (due to unpleasant odor). Each tube was dried before being closed by inverting and blotting remaining moisture on paper towels. Then 300 µl TE buffer (1 mM Tris, pH 7.5 and 0.1 mM EDTA, pH 8.0) was added to each tube. Also 10 µl RNAase A (10mg/ml solution) was added and mixed by vortexing. The mixture then was incubated at 37 °C for 1 hour vortexing slightly every 10 minutes for the first 30 minutes. The tubes were spun in a micro centrifuge (Spectrafuge 16M; National Labnet Co., Edison, NJ) at 14,000 RPM for 10 seconds to pellet any remaining plant debris (time not including spin up and spin down to and from desired RPM). The supernatant then was transferred to a correspondingly labeled clean 1.5 ml micro centrifuge tube. DNA was precipitated by filling each tube with a 20:1 mixture of ethanol and 3M Sodium Acetate. Tubes were mixed by inverting and allowed to precipitate for 30 minutes at room

temperature before storing in the freezer at 20 °C overnight. Tubes were removed from the freezer and agitated manually to break up the precipitate. Samples were spun for 5 minutes at 5000 RPM to pellet the DNA. Ethanol was poured off and tubes were dried by inverting and blotting on paper towels to remove moisture before closing. Pellets were washed by filling tubes with 1 ml of 70% ethanol and vortexing slightly to clean the insides of the tubes (not to resuspend the pellet). The pellet was collected by spinning for 15 seconds at 14,000 RPM (time not including spin up and spin down to and from desired RPM). The alcohol was poured off and tubes were dried by blotting on paper towels to remove moisture and setting them inverted on paper towels for 30 minutes. Pellets were rehydrated by adding 300 µl TE and allowing them to sit for 1 hour at ambient temperature (22.5 - 23° C.). Tubes were vortexed slightly to resuspend the pellet. After 10 minutes tubes were vortexed again and the process was repeated as necessary for complete resuspension of the pellet. Suspended DNA then was placed in boxes labeled with population name, date, and “Original DNA” and stored at 20 °C.

Development of DNA bulks for Fruit Quality Traits

Before use, DNA concentration was measured and adjusted appropriately (10 ng/ul) with a spectrometer (DU 530 Lifescience; Beckman, Fullerton, CA). Three low and high DNA bulk pairs were prepared from equal volumes of standardized DNA (10 ng/ul) from selected F_2 plants with the highest and lowest values for total soluble solids, sucrose, and ascorbic acid for a total of 6 bulks. Eight F_2 plants per bulk were used for screening the first 240 primers and six F_2 plants per bulk were used for screening the final 260 primers. Specific plants used in DNA bulks are listed in Appendices C, D, and E.

Screening RAPD Markers Associated with Fruit Quality Traits

Random 10-mer primers (Operon Technologies, Almedia, CA) were used to simultaneously screen between high and low DNA bulks and between the two parents for each of the three traits of interest. A total of 500 10-mer primers were screened simultaneously on all three traits using high and low value bulks.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed in a PTC-100 thermacycler machine (MJ Research, Waltham, MA) in PCR plates (96 well polypropylene microplates MJ Research, Waltham, MA). PCR plates for testing 10 primers at a time were prepared utilizing various components in a mixture. Water (30.6 ul), 5x buffer (17.6 u;), each primer (17.6 ul), DNTP Mg (3.5 ul), and Taq polymerase (1.1 ul). Buffer, primers, and DNTP were vortexed before pipeting to ensure adequate mixing (Scroch and Neinhuis, 1995).

After the addition of all components a pipet was used to mix each cell individually. Then 8 uL of the mixture was placed into the first row (first 8 wells) of PCR grid. This was continued consecutively until 10 rows were finished. After vortexing, DNA (2.1 uL) from each parent and bulks was placed in each row. Rows were as follows: 1 = 'Dulce' (parent #1), 2 = 'TGR1551' (parent #2), 3 = high soluble solids bulk, 4 = low soluble solids bulk, 5 = high percent sucrose bulk, 6 = low percent sucrose bulk, 7 = high ascorbic acid bulk, and 8 = low ascorbic acid bulk.

The final 10 ul volume of reactants in each cell of the PCR plate included 2 ng/ul template DNA and 8uL of the mixture. A microseal "A" Film (MJ Research, Waltham, MA) was placed over the top of each PCR plate and gently rubbed with a pipet handle

until all wells were sealed. The plate then was placed in a PCR machine (PTC-100 Programmable Thermal Controller; MJ Research, Waltham, MA), and the cover was closed securely. The PCR machine then was set and run for approximately 2 hours. The first two cycles had a thermal profile of 60 seconds at 92˚ C, 7 seconds at 42˚ C, and 70 seconds at 72˚ C. Subsequently, 38 additional cycles were carried out for denaturation (1 second at 92˚ C), annealing (7 seconds at 42˚ C), and elongation (70 seconds at 72˚ C). The product was held for 4 minutes at 72˚ C before cooling to 4° C (Scroch and Neinhuis, 1995). Once PCR was finished each plate was removed from the PCR machine and stored at 4° C.

Gel Electrophoresis

Agarose gel for electrophoresis (1.5 % W/V) was prepared by mixing 4.5 g Agarose (Invitrogen Corporation, Carlsbad, CA) and 300 ml 1x TBE buffer solution. The solution was heated in a microwave for 3 minutes, stirred, and heated for another 1.3 minutes. The solution then was cooled under agitation to a temperature of 50-60° C. and poured onto an electrophoresis gel tray. Four 30 well combs, for a total of 120 wells, were inserted equally spaced from the top. The gel was allowed to set for 40 minutes. Combs then were removed from the gel and gel board was placed into the electrophoresis box. After ensuring adequate buffer coverage the gel was filled by pipeting from PCR grids (8 at a time) from left to right and top to bottom. Three PCR grids at 80 wells each fill 2 gels at 120 wells each for a total of 240 wells. Once the gel was loaded the cover was placed on top of the electrophoresis box (Submarine/Horizontal Gel Unit; C.B.S. Scientific Co., Del Mar, CA) making sure polarity lined up properly, the voltage was set to 180 or 200 and the current was started. The current was turned off after 1.5 to 2.0

hours when the dye had moved about ¾ inch across the gel. A staining solution was prepared using 30 uL Ethidium Bromide and 600 ml water. The gel was cut in half along the third row of wells using a razor blade. The gel was placed in staining solution for 1 hour. Then ½ gel at a time was removed and placed on a light box to determine if adequate staining of DNA had occurred. Upon adequate staining, the gel was placed in a destaining solution of water for about 20 minutes. After adequate destaining, ½ of the gel at a time was placed onto an ultraviolet illuminator (T1202; Sigma, St. Louis, MO.) with light box and digital camera setup attached (EDAS 290; Eastman Kodak Company, Rochester, NY) and photographed. Images were transferred to PC using specialized software for that purpose (1DLE Version 3.5; Eastman Kodak Company, Rochester, NY). Gels were disposed of in appropriate container when no longer needed.

Names of RAPD Marker Fragment

Size markers from a 100 base pair (bp) ladder (Life Technologies, Grand Island, NY) were used to measure the location of RAPD marker bands on the agarose gel. Marker size was determined to the nearest 50 base pairs (bp) based on their migration distance compared to size markers. The RAPD marker names were developed from the Operon kit identification number, the Operon number, and the approximate length (bp) of the marker (Park and Crosby, 2003; Park et al., 2003).

Linkage Analysis

Band presence was designated D and band absence was designated B for analysis of each marker in the F_2 population. To detect segregation distortion of markers the F_2 population marker data was tested for goodness-of-fit to a 3:1 ratio. Due to the dominant nature of RAPD markers, the linkage analysis of markers obtained from ‘Dulce’ and

TGR1551 was performed separately on the data for F_2 plants from the cross 'Dulce' x TGR1551. MAPMAKER version 3.0 (Lander et al., 1987) was used for linkage analysis. On the basis of a logarithm of odds (LOD) score of 3.0 and a linkage threshold of 0.4, linkage groups were displayed using the Group command. Recombination fractions and the Kosambi mapping function (Kosambi, 1944) were used to calculate map distances (centiMorgan, cM) between ordered marker loci.

Detection of QTL

All statistical analyses were conducted using the Statistical Analysis System (SAS, 2003). The analysis of variance was generated using the GLM command. Correlations of fruit quality traits were determined using the proc CORR command. Mean separations were performed using Duncan's Multiple Range Test.

Single-factor analysis of variance (ANOVA) for each pairwise combination of quantitative trait and marker locus was used to analyze the data for detection of QTL affecting total soluble solids, sucrose, fructose, glucose, and ascorbic acid. Significant differences in trait associations were based on F-tests ($P<0.05$) (Edwards et al., 1987). Loci with the lowest P value per QTL were chosen and then added in a stepwise regression to select the best set of markers ($P< 0.05$) for prediction of the total trait phenotypic variation explained by the detected QTL (Paterson et al., 1991).

RESULTS

Differences in the F_2 Population

Fruits were collected from 105 of 110 F_2 plants. The remaining 5 plants were omitted from analysis due to lack of phenotypic data. Fruit weight, fruit length, fruit diameter, flesh weight, dry weight, total soluble solids, percent sucrose, percent glucose, percent fructose, and ascorbic acid content were measured (data not shown for first five). The ANOVA showed significant differences in the population (‘Dulce’, ‘TGR1551’, F_1, F_2, and BC_1 plants developed from the melon cross ‘Dulce’ x ‘TGR1551’) for soluble solids ($P \leq .001$), percent sucrose ($P \leq .001$), percent glucose ($P \leq .001$), percent fructose ($P \leq .001$), and ascorbic acid ($P \leq .01$). Differences by genotype for various characteristics were tested (Duncan’s multiple range test). ‘Dulce’ showed the highest soluble solids values followed by F_1, F_2, and BC_1 plants; ‘TGR1551’ showed the lowest values. ‘Dulce’ and BC_1 plants showed higher percent sucrose values than ‘TGR1551’, F_1, and F_2 plants. ‘TGR1551’, F_1, and F_2 plants had higher percent glucose values than ‘Dulce’ and BC_1 plants. ‘TGR1551’, F_1, and F_2 plants had higher percent fructose values than ‘Dulce’ and BC_1 plants. ‘Dulce’ plants showed higher ascorbic acid values than ‘TGR1551’, F_1, F_2, and BC_1 plants.

Correlations of Fruit Quality Traits

A significant positive correlation was noted in the F_2 population between total soluble solids and sucrose (r=0.34), whereas a significant negative correlation was detected between soluble solids and fructose (r= -0.38) (Table 6). A significant negative correlation was observed between sucrose and glucose (r= -85) or fructose (r= -0.58). A positive correlation between ascorbic acid and sucrose (r=0.24) and a negative correlation

between ascorbic acid and glucose (r= -0.40) were observed in the population, respectively.

Table 6. Pearson correlations of total soluble solids, sugars (sucrose, glucose, and fructose), and ascorbic acid in the F_2 population derived from the cross 'Dulce' x 'TGR1551'.

Fruit quality trait	**Soluble solids**	**Sucrose**	**Glucose**	**Fructose**	**Ascorbic acid**
Ascorbic acid	.064NS	.236*	-.398****	.166NS	1
Fructose	-.380****	-.584****	.070NS	1	
Glucose	-.168NS	-.850****	1		
Sucrose	.337***	1			
Soluble solids	1				

[z]*, **, ***, ****, NS. Significance levels ≤ 0.05, 0.01, 0.001, 0.0001, and non-significant respectively.

Frequency Distributions for Fruit Quality Traits

Frequency distributions for total soluble solids, sucrose, and ascorbic acid were skewed towards low values, while a frequency distribution for glucose was skewed towards high values (Fig. 3). Continuous frequency distributions for total soluble solids, sucrose, glucose, fructose, and ascorbic acid were observed in the F_2 population in the greenhouse experiment, indicating that the five fruit quality traits were quantitatively inherited.

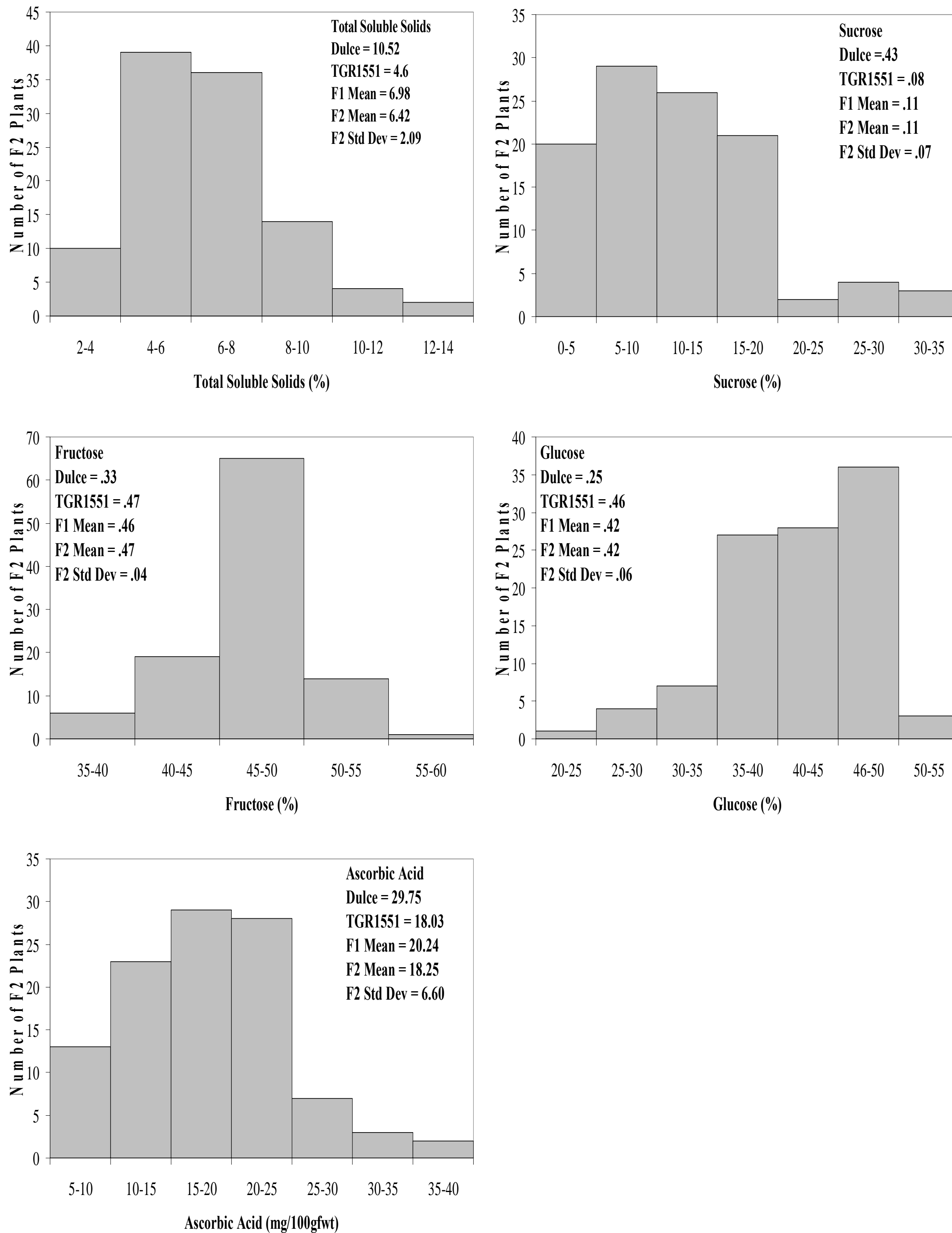

Fig. 3. Frequency distributions for total soluble solids, sucrose, glucose, fructose, and ascorbic acid of F_2 plants derived from the melon cross 'Dulce' (high quality) x 'TGR1551' (low quality). Means for 'Dulce', TGR1551, F_1, and F_2 populations are shown along with standard deviation of the F_2 population.

Screening RAPD Markers for Fruit Quality Traits Using Bulked Segregant Analysis

A total of 500 random 10-mer primers were used for the RAPD analysis of three different bulk pairs developed from low and high value F_2 plants for fruit quality traits along with their parents 'Dulce' and 'TGR1551'. Forty-five RAPD markers were polymorphic for the low and high bulk pairs of total soluble solids, sucrose, and ascorbic acid. Thirty displayed an amplified DNA fragment in the low DNA bulk that was absent in the high DNA bulk (Figs. 4, 5, and 6). Fifteen showed an amplified DNA fragment in the high bulk that was absent in the low bulk (Fig. 4).

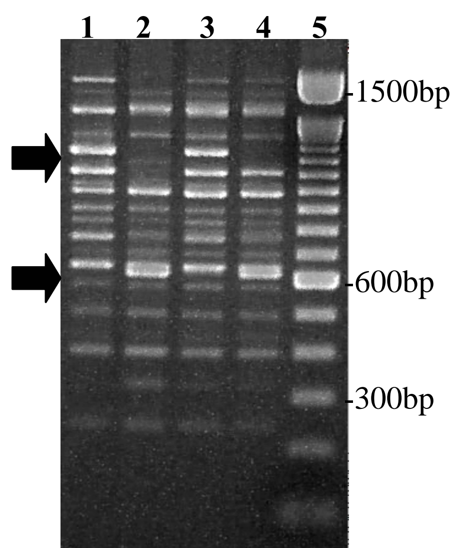

Fig. 4. RAPD markers OAW06.1250 (upper) and OAW06.600 (lower) expressing polymorphism between two DNA bulks from high and low soluble solid F_2 plants, and between the high soluble solids parent 'Dulce' and the low soluble solids parent TGR1551. 1='Dulce', 2='TGR1551', 3=DNA bulk from high soluble s F_2 plants, 4=DNA bulk from low soluble solids F_2 plants, and 5=molecular size marker.

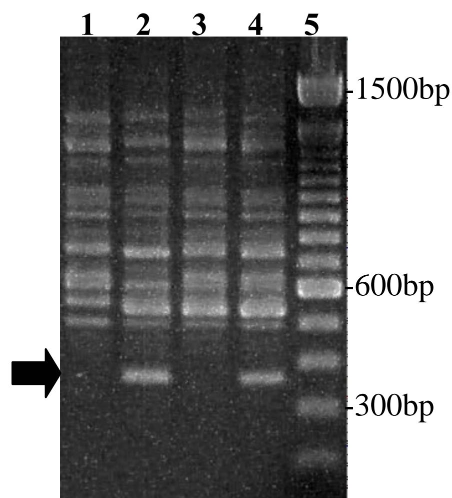

Fig. 5. RAPD marker OAA09.350 from 'TGR1551' expressing polymorphism between two DNA bulks from high and low sucrose F_2 plants, and between the high sucrose parent 'Dulce' and the low sucrose parent 'TGR1551'. 1='Dulce', 2='TGR1551', 3=DNA bulk from high sucrose F_2 plants, 4=DNA bulk from low sucrose F_2 plants, and 5=molecular size marker. Arrow indicates band of interest.

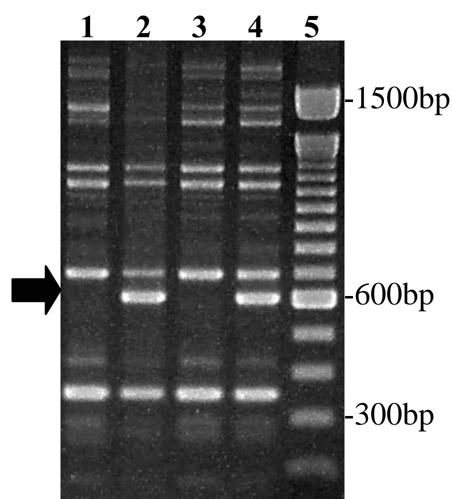

Fig. 6. RAPD marker OAU02.600 from 'TGR1551' expressing polymorphism between two DNA bulks from high and low ascorbic acid F_2 plants, and between the high ascorbic acid parent 'Dulce' and the low ascorbic acid parent 'TGR1551'. 1='Dulce', 2='TGR1551', 3=DNA bulk from high ascorbic acid F_2 plants, 4=DNA bulk from low ascorbic acid F_2 plants, and 5=molecular size marker. Arrow indicates band of interest.

The 45 marker fragments segregated in the F_2 population of the cross 'Dulce' x 'TGR1551'. An example of RAPD marker OAA09.350 obtained from 'TGR1551' is shown in Fig. 7.

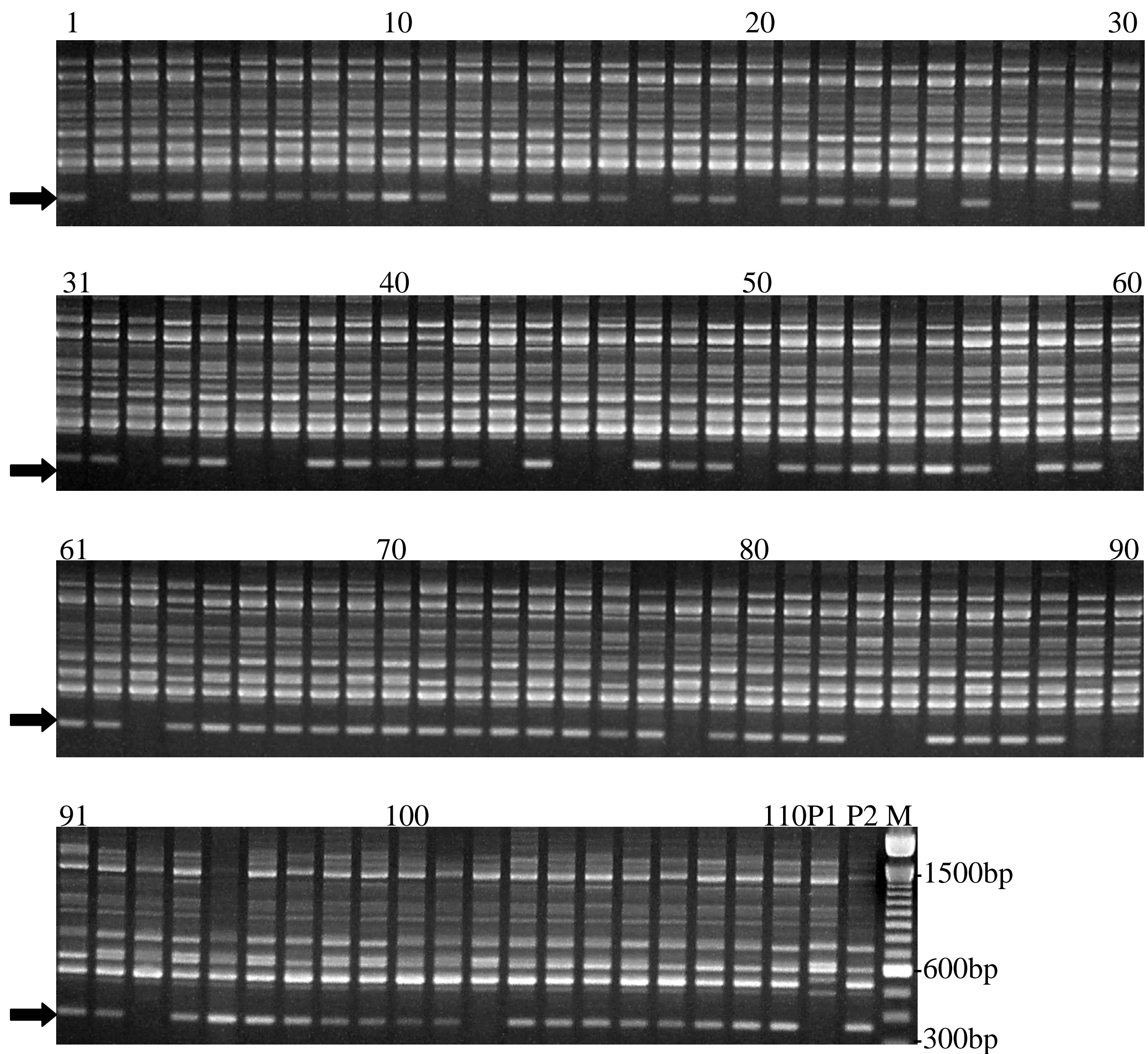

Fig. 7. Segregation of RAPD marker OAA09.350 from 'TGR1551' in a F_2 population derived from the cross 'Dulce' x 'TGR1551'. First image=F_2 plants from #1 to #30, second image=F_2 plants from #31 to #60, third image=F_2 plants from #61 to # 90, fourth image=F_2 plants from #91 to #110, P1 (Parent 1) ='Dulce', P2 (Parent 2) =TGR1551, and M=molecular size marker. Arrows indicate band of interest.

An example of three RAPD markers (OAW06.1250 from ‘Dulce’, OAW06.1100 from ‘Dulce’, and OAW06.600 from ‘TGR1551’) obtained from one primer is shown in Fig 8.

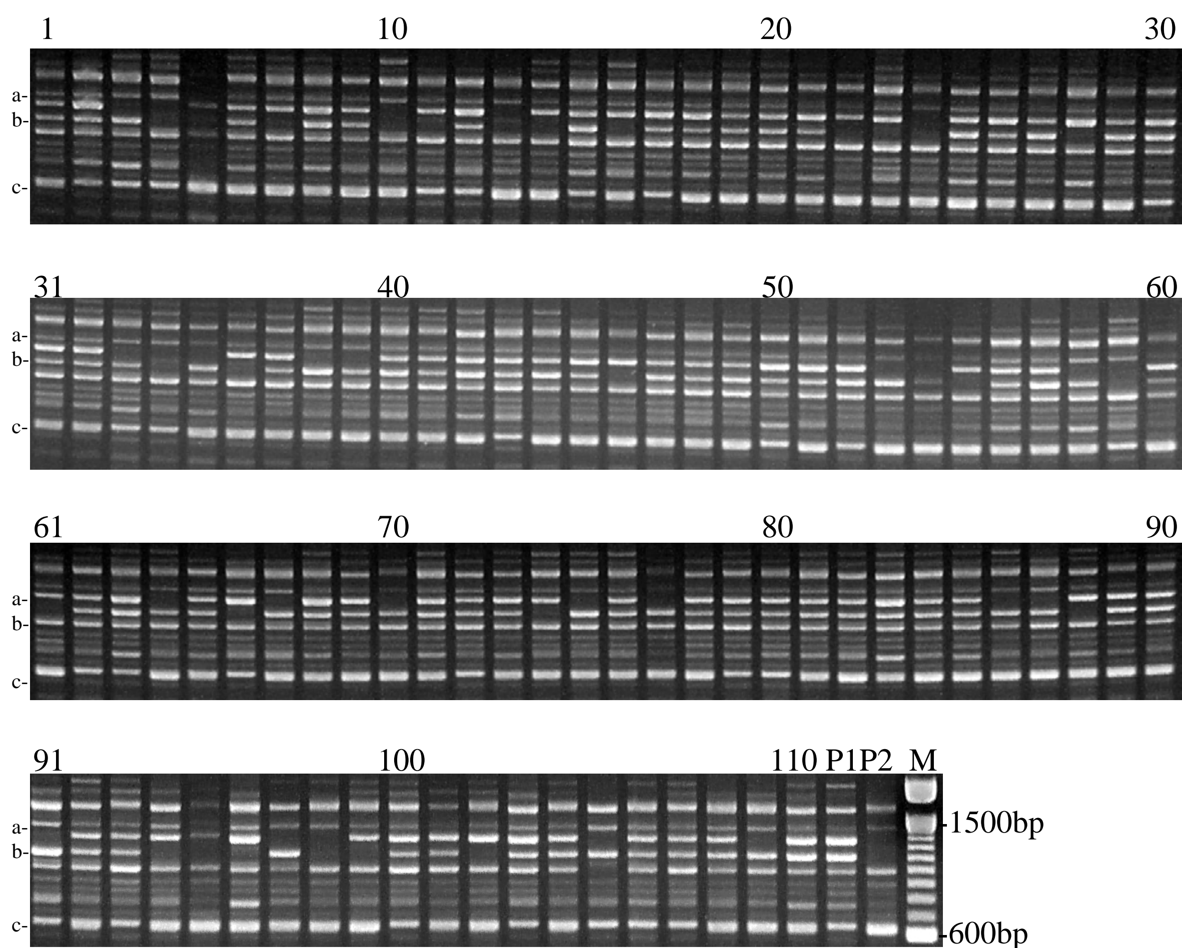

Fig 8. Segregation of RAPD markers OAW06.1250 from ‘Dulce’, OAW06.1100 from ‘Dulce’, and OAW06.600 from TGR1551 in a F_2 population derived from the cross ‘Dulce’ x ‘TGR1551’. First image= F_2 lines from #1 to #30, second image= F_2 lines from #31 to #60, third image= F_2 lines from #61 to # 90, fourth image= F_2 lines from #91 to #110, Parent 1 (P1)= ‘Dulce’, Parent 2 (P2)= ‘TGR1551’, and M= molecular marker. a= band at 1250bp, b= band at 1100bp, c= band at 600bp.

Of the 45 markers detected, 15 were identified to be associated with at least one of the fruit quality traits in the F_2 population on the basis of single-factor ANOVA (Edwards et al., 1987). The remaining 30 markers proved to be false positives. The sizes of the 15 associated markers varied from 250 to 1600 bp based on separation of RAPD amplification products on agarose gel (Table 7). A goodness-of-fit to a 3:1 ratio for band presence to band absence for each of the associated markers except OAS03.450 was observed in 105 F_2 plants (Table 7). Markers OAS14.800, OAU02.600, OAU03.700, and OAW10.400 had poor P values but were within the acceptable limit (Table 7).

Table 7. The chi-square tests for segregation of RAPD fragments for five markers from 'Dulce' and ten markers from TGR1551 associated with fruit quality traits in a F_2 population derived from the melon cross 'Dulce' (high sugars) x 'TGR1551' (low sugars).

Marker	**Marker source**	**Number of F_2 plants**		**Expected ratio**	χ^2	**P**
		Presence	**Absence**			
OAT03.1600	Dulce	79	26	3:1	0.002	0.96
OAU13.1350	Dulce	75	30	3:1	0.536	0.46
OAW06.1250	Dulce	80	25	3:1	0.028	0.87
OAW06.1100	Dulce	80	25	3:1	0.028	0.87
OAT03.250	Dulce	75	30	3:1	0.536	0.46
OAA09.350	TGR1551	82	23	3:1	0.384	0.54
OAP03.800	TGR1551	81	24	3:1	0.155	0.69
OAQ13.750	TGR1551	79	26	3:1	0.002	0.96
OAS03.450	TGR1551	65	40	3:1	8.917	0.00
OAS14.800	TGR1551	72	33	3:1	1.984	0.16
OAU02.600	TGR1551	72	33	3:1	1.984	0.16
OAU03.700	TGR1551	72	33	3:1	1.984	0.16
OAU05.600	TGR1551	77	28	3:1	0.080	0.78
OAW10.400	TGR1551	70	35	3:1	3.457	0.06
OAW06.600	TGR1551	80	25	3:1	0.028	0.87

Development of One Linkage Group for Ascorbic Acid

Four of the ten associated RAPD markers that displayed an amplified DNA fragment in the low DNA bulk for the fruit quality traits were included in one linkage group (Fig. 9). These four RAPD markers were significantly associated with only ascorbic acid in the F_2 population based on single-factor ANOVA. This linkage group included four loci spanning a length of 8.5 cM. However, on the basis of linkage analysis the remaining six RAPD markers were not linked in the F_2 population. Also, five associated RAPD markers that showed an amplified DNA fragment in the high DNA bulk were unlinked in this population, suggesting that they are differently located on a chromosome or from different chromosomes.

Detection of QTL for Total Soluble Solids

A total of five RAPD markers were found to be significantly associated with total soluble solids in the greenhouse experiment in the F_2 population based on single-factor ANOVA (Table 8). Two of the five markers that were unlinked on the basis of linkage analysis were amplified from 'Dulce' and three were amplified from 'TGR1551'. Marker OAW06.1250 from 'Dulce' and marker OAS03.450 from 'TGR 1551' explained 9% and 8% of the phenotypic variation for the total soluble solid content, respectively (Table 8) (R^2 values are rounded to the nearest %). Also, markers OAT03.250, OAP03.800, and OAW06.600 accounted for 5% to 6% of the variation for the trait respectively. The two unlinked makers from 'Dulce' and the three unlinked markers from 'TGR1551' were significant in the stepwise regression analysis (Paterson et al., 1991) explaining 14% and 19% of the variation for the trait (Table 8).

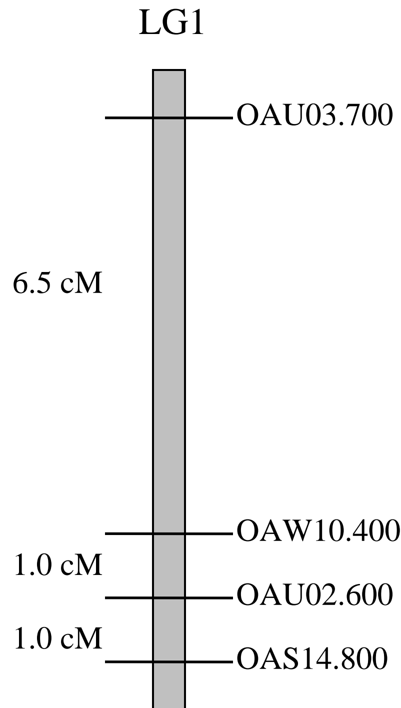

Fig. 9. Linkage group 1 including four RAPD markers from 'TGR1551' associated with ascorbic acid developed using a F_2 population of the melon cross 'Dulce' x 'TGR1551'. Marker names are given on the right and the length in centiMorgans between markers is indicated on the left of linkage group 1.

Table 8. Single-factor ANOVA and stepwise regression analyses of RAPD marker and data for detection of QTL associated with total soluble solids in a F_2 population derived from the melon cross 'Dulce' (high total soluble solids) x 'TGR1551' (low total soluble solids).

Marker	**Marker Source**	**Linkage group**	**Single factor ANOVA**		**Stepwise regression**	
			P	**R^2**	**P**	**R^2**
OAW06.1250	Dulce	unlinked	.00177	9	.0018	9
OAT03.250	Dulce	unlinked	.02951	5	.0144	5
					Cumulative R^2	14
OAS03.450	TGR1551	unlinked	.00483	8	.0048	8
OAP03.800	TGR1551	unlinked	.00580	7	.0112	6
OAW06.600	TGR1551	unlinked	.01096	6	.0116	5
					Cumulative R^2	19

'Dulce' contributed high total soluble solid alleles for five markers (Table 9).

Table 9. Average values of band absence and presence for each of the 15 RAPD markers associated with fruit quality traits including soluble solids, sucrose, glucose, fructose, and ascorbic acid in a F_2 population from the melon cross 'Dulce' (high sucrose) x 'TGR1551' (low sucrose).

Marker	Marker source	Soluble solids	Sucrose	Glucose	Fructose	Ascorbic acid
OAT03.1600	Dulce			0.437[z]/0.410[y]		15.666/19.097
OAU13.1350	Dulce		0.055/0.126	0.469/0.408		13.970/19.013
OAW06.1250	Dulce	5.204/6.819	0.091/0.124			
OAW06.1100	Dulce					20.615/17.508
OAT03.250	Dulce	5.723/6.703	0.089/0.124	0.445/0.406		15.968/19.159
OAA09.350	TGR1551		0.171/0.101	0.389/0.424	0.439/0.476	
OAP03.800	TGR1551	7.455/6.116			0.449/0.474	
OAQ13.750	TGR1551		0.154/0.104	0.387/0.426		
OAS03.450	TGR1551	6.964/6.076			0.456/0.476	
OAS14.800	TGR1551					21.020/17.111
OAU02.600	TGR1551					21.020/17.111
OAU03.700	TGR1551					21.217/17.019
OAU05.600	TGR1551		0.144/0.105	0.401/0.422	0.455/0.473	
OAW10.400	TGR1551					21.070/16.917
OAW06.600	TGR1551	7.344/6.135	0.165/0.098	0.374/0.430		20.778/17.456

[z] An average value of band absence for marker.
[y] An average value of band presence for marker.

Detection of QTL for Sucrose

Seven significant RAPD marker locus-sucrose trait associations were detected in this population by single-factor ANOVA (Table 10). Three RAPD markers were amplified from 'Dulce', while four markers were amplified from 'TGR1551'. All seven markers were not linked in the population. The high sucrose parent 'Dulce' contributed high sucrose alleles for seven markers (Table 9). Markers OAU13.1350, OAT03.250, and OAW06.1250 from 'Dulce' explained 4% to 13% of the phenotypic variation for sucrose. One (OAU13.1350) of the three unlinked markers was detected using stepwise regression. This marker explained 13% of the variation for this sucrose. Markers

OAW06.600, OAA09.350, OAU05.600, and OAQ13.750 from TGR1551 accounted for 6% to 17% of the variation for the sucrose trait. Three (OAW06.600, OAA09.350, and OAU05.600) of the four unlinked markers were significant in the multilocus model. These three markers combined explained 31% of the phenotypic variation for this trait (Table 10).

Table 10. Single-factor ANOVA and stepwise regression analyses of RAPD marker and data for detection of QTL associated with sucrose in a F_2 population derived from the melon cross 'Dulce' (high sucrose) x 'TGR1551' (low sucrose).

Marker	Marker Source	Linkage group	Single factor ANOVA		Stepwise regression	
			P	R^2	P	R^2
OAU13.1350	Dulce	unlinked	.00017	13	.0002	13
OAT03.250	Dulce	unlinked	.02756	5		
OAW06.1250	Dulce	unlinked	.05207	4		
					Cumulative R^2	13
OAW06.600	TGR1551	unlinked	.00001	17	.0001	17
OAA09.350	TGR1551	unlinked	.00020	13	.0006	9
OAU05.600	TGR1551	unlinked	.00768	7	.0080	5
OAQ13.750	TGR1551	unlinked	.01387	6		
					Cumulative R^2	31

Detection of QTL for Glucose

Seven RAPD markers were significantly associated with glucose on the basis of single-factor ANOVA (Table 11). The seven markers were unlinked based on linkage analysis. Three markers were derived from 'Dulce' and four markers were derived from 'TGR1551'. High glucose parent 'TGR1551' contributed high glucose alleles for the seven markers (Table 9). Marker OAU13.1350 from 'Dulce' explained 19% of the phenotypic variation for the glucose trait, while two markers AT03.250 and OAT03.1600 from 'Dulce' accounted for 4% and 9% of the variation. However, only marker locus OAU13.1350 explaining 19% of the variation for this glucose was detected using the

stepwise regression analysis (Table 11). Marker OAW06.600 from ‘TGR1551’ explained 18% of the variation, and three markers OAQ13.750, OAU05.600, and OAA09.350 from ‘TGR1551’ accounted for 4% to 7% of the variation. In the stepwise regression analysis three markers OAW06.600, OAQ13.750, and OAU05.600 explaining 25% of the variation for the glucose trait were significant (Table 11).

Table 11. Single-factor ANOVA and stepwise regression analyses of RAPD marker and data for detection of QTL associated with glucose in a F_2 population derived from the melon cross ‘Dulce’ (moderate glucose) x ‘TGR1551’ (high glucose).

Marker	**Marker Source**	**Linkage group**	**Single factor ANOVA**		**Stepwise regression**	
			P	**R^2**	**P**	**R^2**
OAU13.1350	Dulce	unlinked	.00000	19	.0001	19
OAT03.250	Dulce	unlinked	.00247	9		
OAT03.1600	Dulce	unlinked	.04169	4		
					Cumulative R^2	19
OAW06.600	TGR1551	unlinked	.00001	18	.0001	18
OAQ13.750	TGR1551	unlinked	.00822	7	.0263	4
OAU05.600	TGR1551	unlinked	.05340	4	.0376	3
OAA09.350	TGR1551	unlinked	.03699	4		
					Cumulative R^2	25

Detection of QTL for Fructose

Significant associations of four markers with the fructose trait were detected using single-factor ANOVA (Table 12). These four markers were amplified from ‘TGR1551’, and were not linked in this population. High fructose parent ‘TGR1551’ contributed high fructose alleles for the four markers. Marker OAA09.350 explained 13% of the phenotypic variation for the fructose trait, while three markers OAS03.450, OAP03.800, and OAU05.600 accounted for 4% to 6% of the fructose variation. In the stepwise regression analysis two markers OAA09.350 and OAS03.450 provided significant associations, and accounted for 19% of the variation for the trait (Table 12).

Table 12. Single-factor ANOVA and stepwise regression analyses of RAPD marker and data for detection of QTL associated with fructose in a F_2 population derived from the melon cross ‘Dulce’ (moderate fructose) x TGR1551 (high fructose).

Marker	**Marker Source**	**Linkage group**	**Single factor ANOVA**		**Stepwise regression**	
			P	**R^2**	**P**	**R^2**
OAA09.350	TGR1551	unlinked	.00017	13	.0002	13
OAS03.450	TGR1551	unlinked	.01121	6	.0077	6
OAP03.800	TGR1551	unlinked	.03832	4		
OAU05.600	TGR1551	unlinked	.04034	4		
					Cumulative R^2	19

Detection of QTL for Ascorbic Acid

Nine significant RAPD marker locus-ascorbic acid trait associations were found based on single-factor ANOVA (Table 13). Four of the nine markers were obtained from ‘Dulce’, while five were obtained from ‘TGR1551’. ‘Dulce’ contributed high ascorbic acid alleles for these markers except OAW06.1100. Four of the five markers from ‘TGR1551’ were linked within a distance of 8.5 cM and included into linkage group 1 (Fig. 9). These markers on linkage group 1 were associated with only the ascorbic acid trait. The four unlinked markers (OAT03.1600, OAT03.250, OAW06.1100, and OAU13.1350) from ‘Dulce’ accounted for 4% to 5% of the phenotypic variation for this trait. Using the stepwise regression analysis three markers (OAT03.1600, OAT03.250, and OAW06.1100) were identified and explained 14% of the variation for the ascorbic acid trait (Table 13). The five RAPD markers from TGR1551 accounted for 5% to 9% of the variation for the trait. Two markers (OAW10.400 and OAW06.600) were significant in the stepwise regression analysis explaining 12% of the variation for this quality trait (Table 13).

Table 13. Single-factor ANOVA and stepwise regression analyses of RAPD marker and data for detection of QTL associated with ascorbic acid in a F_2 population derived from the melon cross 'Dulce' (high ascorbic acid) x TGR1551 (low ascorbic acid).

Marker	**Marker Source**	**Linkage group**	**Single factor ANOVA**		**Stepwise regression**	
			P	**R^2**	**P**	**R^2**
OAT03.1600	Dulce	unlinked	.02069	5	.0207	5
OAT03.250	Dulce	unlinked	.02439	5	.0255	5
OAW06.1100	Dulce	unlinked	.03915	4	.0336	4
OAU13.1350	Dulce	unlinked	.02663	5		
					Cumulative R^2	14
OAW10.400	TGR1551	1	.00250	9	.0025	9
OAW06.600	TGR1551	unlinked	.02727	5	.0500	3
OAU02.600	TGR1551	1	.00773	7		
OAS14.800	TGR1551	1	.00773	7		
OAU03.700	TGR1551	1	.00389	8		
					Cumulative R^2	12

Common RAPD Markers Associated with the Fruit Quality Traits

Ten of the fifteen associated RAPD markers were found to be consistently associated with two to four fruit quality traits in this population based on single-factor ANOVA (Table 14). Marker OAU13.1350 from 'Dulce' was associated with sucrose, glucose, and ascorbic acid, and accounted for 5% and 19% of the phenotypic variation for these traits. Marker OAT03.250 from 'Dulce' was consistently associated with total soluble solids, sucrose, glucose, and ascorbic acid, and explained 5% to 9% of the variation for the four traits. Marker OAW06.600 from TGR1551 was also associated with the four traits, and explained 5% and 18 % of the variation for the traits. Marker OAA09.350 from TGR1551 was consistently associated with sugars including sucrose, glucose, and fructose, and accounted for 4% to 13% of the variation for the sugar traits (Table 14).

Table 14. Common RAPD markers associated with at least more than two fruit quality traits in a F_2 population derived from the melon cross 'Dulce' (high sucrose) x TGR1551 (low sucrose).

Marker	Marker source	Soluble solids	Sucrose	Glucose	Fructose	Ascorbic acid
OAT03.1600	Dulce	NS	NS	*	NS	*
OAU13.1350	Dulce	NS	***	****	NS	*
OAW06.1250	Dulce	***	*	NS	NS	NS
OAT03.250	Dulce	*	*	**	NS	*
OAA09.350	TGR1551	NS	***	*	***	NS
OAP03.800	TGR1551	**	NS	NS	*	NS
OAQ13.750	TGR1551	NS	**	**	NS	NS
OAS03.450	TGR1551	**	NS	NS	**	NS
OAU05.600	TGR1551	NS	**	*	*	NS
OAW06.600	TGR1551	**	****	****	NS	*

[z]*, **, ***, NS. Significance levels $\leq$ 0.05, 0.01, 0.001, 0.0001, and non-significant respectively.

DISCUSSION

Many correlations were detected between the five traits tested (Table 6). Many of the markers detected were associated with multiple traits (Table 14). This supports previous work indicating that correlated traits are associated with common markers (Paterson et al., 1991). Previous results indicated that Vitamin C content is correlated with the refractive index of melon juice (Wagner et al., 1940). In this study, sucrose content was significantly correlated with ascorbic acid content (Table 6). The fact that melon breeders use sugar content as one of their selection criteria may explain why commercial melons are relatively high in vitamin C, although it is not normally selected for in breeding populations.

Although a major gene may control sugar accumulation in certain crops like carrot (Freeman and Simon, 1983), the continuous distribution of total soluble solids, sucrose percentage, glucose percentage, and fructose percentage observed (Fig 3),

suggests a quantitative mode of inheritance for sugar related traits and not a single gene as recently suggested for melon (Burger et al., 2002).

The markers associated with sugar trait QTL could be useful for transferring the genes involved into low sugar cultivars and enhancing fruit quality. The fact that four markers for ascorbic acid content are located in the same linkage group (Fig. 9) indicates that a major gene for ascorbic acid accumulation may be present in melon. This will be useful to select for enhanced vitamin C content in melon breeding.

As indicated by work done with tomato and common bean, even though all QTL are not detected under all environmental conditions, some QTL are always expressed under any environmental condition (Paterson et al., 1991; Park et al., 1999b). Thus, it is necessary to test the consistency of markers with QTL associations under different environmental conditions (Park et al., 1999a). Also, few QTL are expressed consistently in all populations tested (Park et al., 1999b).

In order to maximize utilization of QTL regions, the complex epistatic interactions involved must be better understood. MAS may be useful in population and inbred line development, but such improvements will not diminish the requirement for field testing in replicated trials (Staub, et al., 1996).

Among molecular markers available, RAPDs allow the most efficient use of time and money when sample sizes are small (Darvasi and Soller, 1994) such as the 105 F_2 plants used in this study. At this point in time, RAPD markers are still not cost effective compared to phenotypic selection. However, aside from costs involved, markers developed will be useful in MAS for traits of interest.

MAS efficiency depends on the collection of accurate phenotypic data, since that determines the degree to which markers and traits of interest are linked. MAS has much potential to improve selection gain compared to traditional phenotypic selection, but utilization of molecular markers in plant improvement programs will be regulated by the cost of development and use (Edwards and Page, 1994).

As molecular marker systems improve in cost and efficiency, MAS will become more feasible for use as a component in plant breeding. In the future, MAS may improve selection for yield in agronomic and horticultural crops. However, the many complicated attributes (aesthetic, culinary, organoleptic, and other) involved in horticultural crop improvement will still require the expertise of plant breeders for genetic improvement (Staub, et al., 1996).

The time required to develop RAPD markers tightly linked to genes of interest is highly variable. Due to the random nature of RAPD primers used, a marker may be detected in the first primers tested or not until 1000 have been tested. In this study 500 primers were screened for 3 traits (1500 total) and a total of 15 significant markers were detected. Although it may be possible to develop linked markers more quickly with RAPD marker systems, time required to screen enough primers will always be difficult to predict.

As far as BSA is concerned, smaller DNA pools results in more false positives and larger DNA pools results in less markers detected. Obtaining an acceptable balance gives optimal results. In this study bulks of 6 F_2 plants gave better results than did bulks of 8 F_2 plants. Although BSA was initially developed for used with qualitatively inherited traits it has also successfully be used to quantitatively inherited traits as seen in this study.

The combination of BSA, RAPD, and QTL systems is the most efficient method for detecting QTL of moderate to large effect in melon. Development of separate linkage maps for each population in a breeding program is not feasible. By developing markers directly related to traits of interest the development of genetic linkage maps is not necessary saving both time and money making it more feasible for MAS and thus more attractive to plant breeders. Conversion of RAPDs to sequence characterized amplified regions (SCARs) (Piran and Michelmore, 1993) may be done to improve reliability when used outside of the initially screened population. MAS with quantitative traits will become more of an option for plant breeders as technology for development and use of QTL markers improves (Kelly and Miklas, 1998).

CHAPTER V

DEVELOPMENT OF RESISTANCE TO GUMMY STEM BLIGHT IN CANTALOUPE (*CUCUMIS MELO* L.) THROUGH TRADITIONAL BREEDING AND DISEASE SCREENING

MATERIALS AND METHODS

Plant Material – Source and Selection

Seed from two sources was used in 2002 gummy stem blight experiments; the parents of the original cross were 'TMS', a honeydew type from TAES with good fruit quality, and PI 140471, a wild type from the USDA-GRIN with resistance to gummy stem blight. The F_1 seed of this cross was grown in the greenhouse and self pollinated to produce a F_2 seed population. Plants used in crossing were grown in a greenhouse to ensure pollination control (eliminating uncontrollable contamination due to whiteflies, houseflies, honeybees, etc.). Fruit from crosses was collected and seeds were removed, processed, cataloged, and stored for further use.

Plant Material – Treatment and Care

Seed from both parents and the F_2 was planted out into 15 cell (~10 x 10 cm) seedling trays (~50 x 30 x 10 cm) in soil-less media (Sunshine Mix #4) (Sun Gro Horticulture Inc., Bellevue, WA) and fertilized with Osmocote 14-14-14 controlled release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). Two seeds were planted per cell to ensure adequate germination. Each parent was planted in a total of 5 cells and 105 cells were planted with F_2 seed in a completely randomized design. One week after germination cells were thinned to one plant each.

A nutrient solution of Peters 20-20-20 professional water soluble fertilizer (Smurfit-Stone, Wellsburg, WV) was applied once per week during the growing period using drip fertigation lines. Pesticides were applied as needed during plant growth to control whiteflies (Admire®), aphids (Aphid Star®), and spider mites (Agrimek®). Powdery mildew was controlled with Quadris®.

Preparation of the Inoculum

One *D. bryoniae* culture, TX 97-128 (Marvin Miller - TAES) collected from a melon field in the Rio Grande Valley of South Texas, was used throughout the experiment. It exhibited common gummy stem blight cultural characteristics including symptom development on inoculated melon plants. Cultures were increased on Petri plates containing 10 ml of ¼ Potato Dextrose Agar (PDA) (Becton, Dickinson, and Company, Sparks, MD) using mycelial plug inoculation from cultures grown previously. The inoculated plates were incubated for about 7 days about 21 °C, under fluorescent light 12 hours per day (~60 μmol m^{-2} s^{-1} photosynthetic photon flux) to promote the formation of spore containing pycnidia. The inoculum was prepared by adding ~10 ml sterile distilled water, gently scraping the surface to dislodge spores, and collecting the resulting liquid. Plates were washed with a further ~10 ml of sterile distilled water and added to the liquid to make sure all spores had been collected. The liquid spore suspension was then strained through cheesecloth to remove any remaining agar or other particles. A spore density count was taken with a hemacytometer and a final concentration of 1 x 10^5 spores / ml was prepared. The resulting spore preparation was kept at 4 °C. for ~12 hours until use the following day.

Inoculating Plants

Plants were sprayed at the 2-4 true leaf stage to runoff using a hand held spray bottle. The inoculum (strain TX 97-128) was applied on 9-20-2002 both morning and evening. Overhead humidity was applied with a hand sprayer at 2 hour intervals to ensure adequate disease development.

Backcrossing Program

Resistant F_2 plants as well as several plants of the resistant parent (PI 140471) were maintained by placing in black plastic pots (3 gallon) in the greenhouse for continued use in the breeding program. A nutrient solution of Peters 20-20-20 professional water soluble fertilizer (Smurfit-Stone, Wellsburg, WV) was applied once per week during the plant growth period using drip fertigation lines. Pesticides were applied as needed during plant growth to control whiteflies, aphids, scale, and spider mites. Powdery mildew was controlled with Quadris® (Syngenta, Greensboro, NC). The approximate greenhouse day/night temperatures were $27 \pm 2^\circ$ C/ $24 \pm 2^\circ$ C. Natural day/night lengths were approximately 13/11 hours.

Disease Rating Scale

Symptoms were measured at one week intervals based on a scale of 0 to 5. Symptom rating system: 0. No gummy stem blight symptoms, 1. Slight lesions visible, 2. Lesion cracked but < 1” long, 3. Lesion not encircling the stem but ~ 1” long, 4. Lesion encircling the stem and >1” long, 5. Dead plant (due to gummy stem blight).

Date symptom data was taken: (one time each week for 6 weeks following inoculation). Week 1: (07 days post inoculation). Week 2: (14 days post inoculation), Week 3: (21 days post inoculation), Week 4: (28 days post inoculation), Week 5: (35 days post inoculation), Week 6: (42 days post inoculation), Final disease ratings taken 42 days post inoculation.

Statistical Analysis

Data analysis for this paper was generated using SAS software, Version 8 of the SAS System for Windows (SAS Institute Inc., Cary, NC.). The analysis of variance (ANOVA) was generated using the General Linear Model command. Mean separations were performed using Duncan's Multiple Range Test. (SAS, 2003).

RESULTS

The ANOVA shows significant effects for genotype but not for repetition or genotype x repetition in the gummy stem blight trial (Table 15).

Table 15. Significance of genotype with degrees of freedom, sum of squares, mean square, and f value for population developed for gummy stem blight testing.

Source	Degrees of freedom	Sum of Squares	Mean Square	F value	Significance
Genotype	2	59.44	29.72	9.89	*** [z]

[z]***. Significance level ≤ 0.001.

PI 140471 had significantly better gummy stem blight disease ratings than did either 'TMS' or the F_2 population (Table 16, Fig 10, Appendix G).

Table 16. Mean comparison of genotypes involved in gummy stem blight screening.

	Genotype			
	TMS	**140471**	**F2**	**Overall mean**
Mean^{y}	4.80 c^{z}	0.00 a	1.95 b	1.99

^{y}Disease screening scale 0= no disease to 5= dead

^{z}Different letters indicate significant differences according to Duncan's multiple range test.

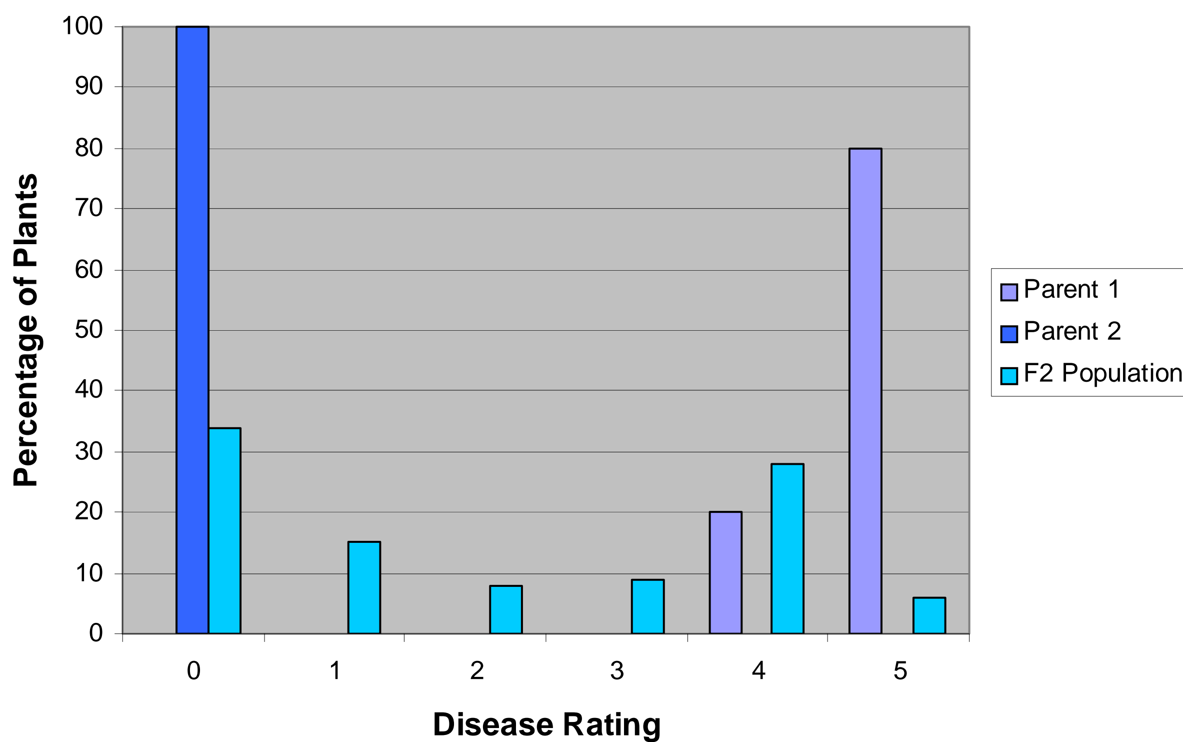

Fig 10. Incidence of gummy stem blight disease on 'TMS', '140471', and F_2 plants ('TMS' x '140471' self) six weeks after inoculation with South Texas strain TX 97-128.

Gummy stem blight severity increased from week 1 to week 6 in the F_2 population tested (Fig. 11).

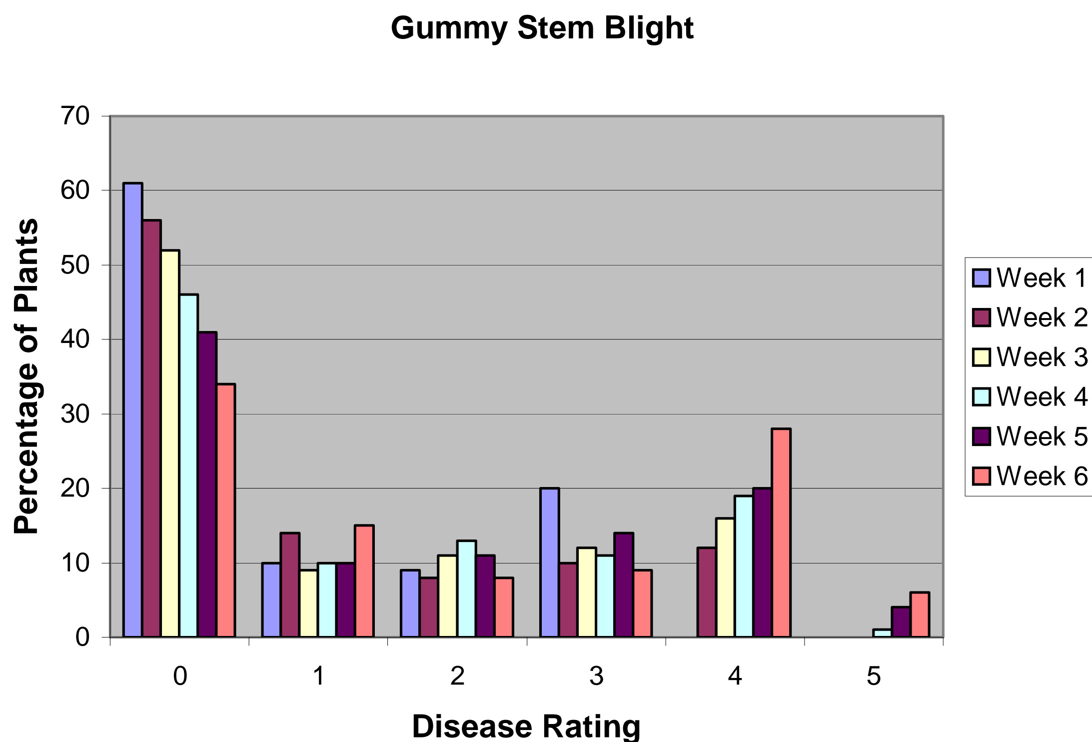

Fig 11. Incidence of gummy stem blight disease on F_2 plants from a cross of 'TMS' x '140471' one to six weeks after inoculation with South Texas strain TX 97-128.

Backcrosses

Backcrosses were made under controlled conditions in the greenhouse with several of the most highly resistant F_2 plants selected (all showing disease rating of 0 or no disease) (Table 17).

Table 17. Backcrosses made with gummy stem blight resistant F_2 plants selected in disease trial.

Commercial variety used as female parent	GSB resistant F2 plant used as male parent
'Deltex'	R3#3-2
'Deltex'	R4#3-5
'Deltex'	R5#3-10
'TMS'	R5#3-10

DISCUSSION

Results indicate that PI 140471 is a useful source for resistance to gummy stem blight in Texas (Table 16, Fig. 10) and may be useful in developing commercial varieties of disease resistant melons. Previous tests indicated that resistance to gummy stem blight is not specific to certain isolates, allowing effective resistance screening using only one strain of the disease (St. Amand and Wehner, 1995). This suggests that resistant varieties developed with PI 140471 and GSB strain TX 97-128 might also be resistant to strains of the disease present in other areas of the country. Results of this study indicate that resistance is not controlled by a single dominant gene as previously described (Prasad and Norton, 1967). Results from the present study correspond with other studies indicating that resistance is more complex (Kyle 1995). The number of disease free F_2 plants was 35 and number of diseased F_2 plants was 65 at six weeks after inoculation (Fig. 11). Single gene dominant resistance would have resulted in a 3:1 resistant to susceptible ratio. However, results were closer to a single recessive gene control ratio of 1:3. Ratings taken on disease free plants four months after inoculation indicate that many were not truly resistant and either a dominant and recessive gene control ratio of 3:13 or a double recessive gene control ratio of 1:15 may be more appropriate (K.M. Crosby, personal communication). Thus, there is a high probability that more than one gene is

involved in the resistance. However, at this point exactly how many genes are involved is not known.

Seedling screening is useful for initial resistance studies. However, for any breeding program, field screening under severe disease conditions provides a more practical and effective assessment of resistance. Therefore, progeny from the BC_1 generation should be planted out in the field for evaluation in making further resistant selections in the gummy stem blight resistance backcrossing program.

CHAPTER VI

SCREENING FOR RESISTANCE TO MONOSPORASCUS ROOT ROT IN MELON (*CUCUMIS MELO* L.) THROUGH TRADITIONAL BREEDING AND DISEASE SCREENING

MATERIALS AND METHODS

Plant Material – Source and Selection

Seed from two sources was used in monosporascus experiments; the parents of the original cross were 'TGR1551', a melon plant introduction from Zimbabwe via the USDA-GRIN with moderate resistance to monosporascus, and 'Deltex', an Ananas type melon from Nunhems with resistance to monosporascus. The F_1 seed of this cross was grown in the greenhouse and self pollinated to produce a F_2 seed population. Plants used in crossing were grown in a greenhouse to ensure proper pollination (eliminating uncontrollable contamination due to whiteflies, houseflies, honeybees, etc.). Fruit from crosses was collected and seeds were removed, processed, cataloged, and stored for further use.

Plant Material – Treatment and Care

Plants from both parents, and the F_2 population were planted on black plastic covered raised beds in a field known to be heavily infested with *M. cannonballus*. A randomized complete block design with 4 reps was used for the experimental design (Table 18).

Table 18. Genotypes used in monosporascus screening with genotype description, number of plants, and number of plants per rep.

Genotype	**Genotype Description**	**Number of plants**	**Number of plants per rep**
TGR1551	Parent 1	8	2
Deltex	Parent 2	8	2
TGR1551 x Deltex self	F2	120	30
Total		136	34

Water was applied once per week or as necessary through a drip irrigation system. Plants were sprayed with the proper chemicals (Ridomil Gold®, Nova®, Admire®) to keep pests and diseases, other than monosporascus, under control. Weeds were removed on a periodic basis as necessary.

Disease Ratings

When plants began showing above-ground symptoms of monosporascus development (wilting) roots were removed from the field, taken to the lab, washed to remove any remaining soil, and rated for monosporascus disease severity. The monosporascus root rot disease rating scale ranged from 0 = no vine decline disease, 1 = slight root damage, 2 = moderate root damage, 3 = extensive root damage, 4 = severe root damage, to 5 = dead plant.

Statistical Analysis

Data analysis for this paper was generated using SAS software, Version 8 of the SAS System for Windows (SAS Institute Inc., Cary, NC). The analysis of variance (ANOVA) was generated using the General Linear Model command. Mean separations were performed using Duncan’s Multiple Range Test. (SAS Institute, 2003).

RESULTS

The ANOVA showed significant effects for genotype and replication but not for genotype x replication in the monosporascus trial (Table 19).

Table 19. Significance of genotype, replication, and genotype x replication with degrees of freedom, sum of squares, mean squares, and f values.

Source	**Degrees of freedom**	**Sum of Squares**	**Mean Square**	**F value**	**Significance**
Genotype	2	11.21	5.61	4.82	** [z]
Replication	3	15.75	5.25	4.52	***
Genotype x Replication	6	8.66	1.44	1.24	NS

[z]**, ***, NS. Significance levels $P \leq 0.01$, $P \leq 0.001$, and non-significant, respectively.

'TGR1551' showed significantly better monosporascus root rot disease resistance than did either 'Deltex' or the F_2 population (Fig. 12, Appendix H, Appendix I).

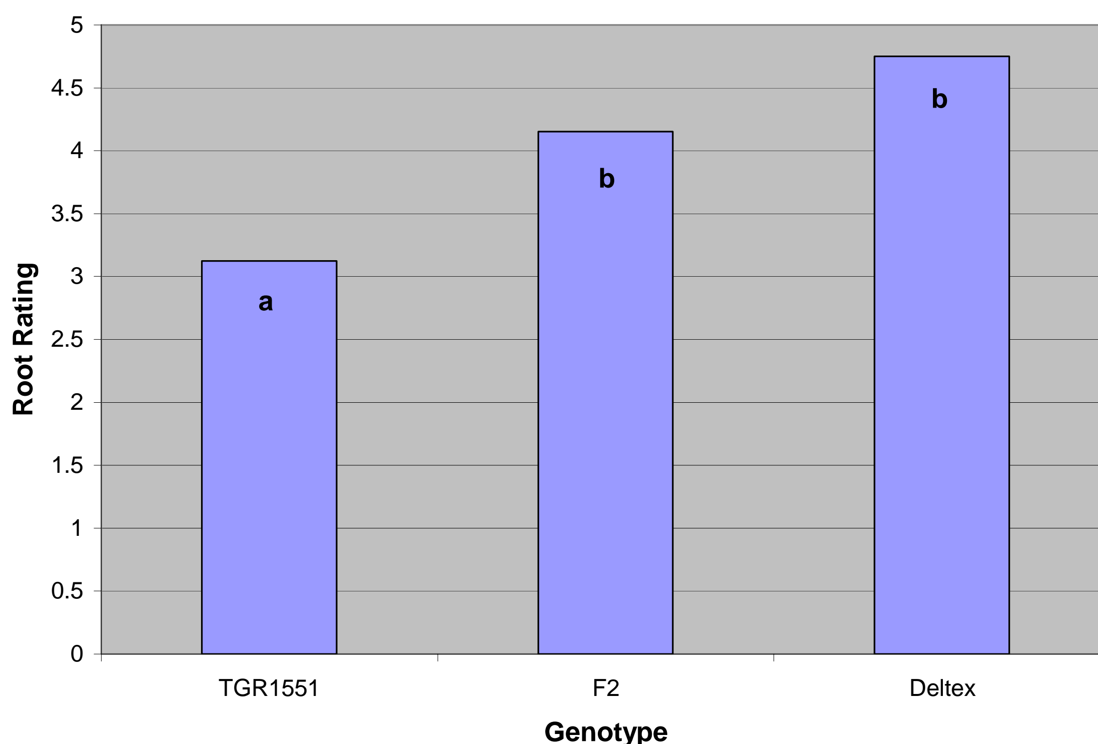

Fig. 12. Comparison of genotypes used in monosporascus screening. All roots were rated by Kevin Crosby, Marvin Miller, and Jonathan Sinclair. Disease screening scale 1=no disease to 5=dead. Different letters indicate significant differences according to Duncan's multiple range test.

Replication 1 was significantly different from repetition 2, 3, and 4 in the monosporascus trial (Table 20).

Table 20. Mean comparison of replication involved in monosporascus screening.

	Replication Number				
	1	**2**	**3**	**4**	**Overall mean**
Mean[y]	3.49 a	4.31 b	4.27 b	4.35 b	4.13

[y]Disease screening scale 0= no disease to 5= dead.
[z]Different letters indicate significant differences according to Duncan's multiple range test.

Seed of the most resistant F_2 plants was saved for further evaluation / testing. Seed was saved from plants 1-3-5 (2.33 RR), 1-3-17 (3 RR), 2-3-14 (2 RR), 2-3-17 (2.33 RR), 2-3-18 (2 RR), 3-3-4 (3 RR), 3-3-15 (3RR), 4-3-4 (3RR), 4-3-21 (2RR), and 4-3-26 (2.33 RR).

DISCUSSION

While ‘TGR1551’ did show significantly better disease ratings compared to ‘Deltex’, (Fig. 12) mean scores were still fairly high. On first inspection, it would seem that ‘TGR1551’ does not have potential for use in plant breeding efforts in relation to development of monosporascus resistance. However, since the season that plants were grown was extremely conducive to disease development and ‘Deltex’ had proven much more resistant in previous trials done at the same location (Wolff and Miller, 1998) the differences in disease expression may be due to environmental conditions. Also, the disease rating system used was slightly different than the one used in the previous study and ratings were taken at a later maturity stage. Possible reasons that ‘TGR1551’ outperformed ‘Deltex’ are that ‘TGR1551’ has a later maturity stage, has lower sugar accumulation in the fruit, and is resistant to leaf diseases resulting in less overall stress on the plant. Also, because of possible contamination with other root affecting diseases future trials should examine root samples along with taking root ratings to determine what other, if any, diseases are present.

Since ‘TGR1551’ did show significantly better disease ratings than ‘Deltex’, which has been shown to be resistant in the past, it should be considered for use in breeding efforts for monosporascus resistance. More testing is needed to determine

resistance of selected F_2 plants in relation to parents as well as other cultivars. Successful melon production in areas affected by *M. cannonballus* will likely include not only breeding for resistance, but improved cultural practices and other control measures as well.

CHAPTER VII

SUMMARY

Wild type 'TGR1551' was found susceptible to CYSDV. As a result no molecular markers were developed. However, as 'TGR1551' was less susceptible than 'Dulce', a common commercial cantaloupe-type melon, it may have tolerance to the virus. Tolerance may be related to impeded phloem transport or reduced virus replication as previously reported. Several of the most tolerant F_2 plants were saved for backcrossing.

Fifteen RAPD markers significantly linked to melon fruit quality QTL for total soluble solids, percent sucrose, percent glucose, percent fructose, and ascorbic acid were detected using BSA. The percentage of phenotypic variation explained by each marker ranged from 3.2% to 19.2%. Cumulative phenotypic variation explained for each trait ranged from 12.9% to 30.9%. These markers may be useful in a MAS melon breeding program.

Wild type PI 140471 was found to be completely resistant to gummy stem blight. Plants from a F_2 population developed using PI 140471 and commercial honeydew type melon 'TMS' showed a broad range of symptoms from resistant to susceptible. This suggests the involvement of more than one gene in the resistance mechanism as previously reported. F_2 plants which exhibited a high level of resistance to gummy stem blight were saved for backcrossing.

Wild type 'TGR1551' showed better disease tolerance to monosporascus than 'Deltex' which had shown good resistance in previous experiments. Ratings for 'Deltex' were higher than in previous experiments probably because of differences in environment

or plant maturity stage. Seed from highly resistant F_2 plants was saved for further evaluation and inclusion in the backcrossing program.

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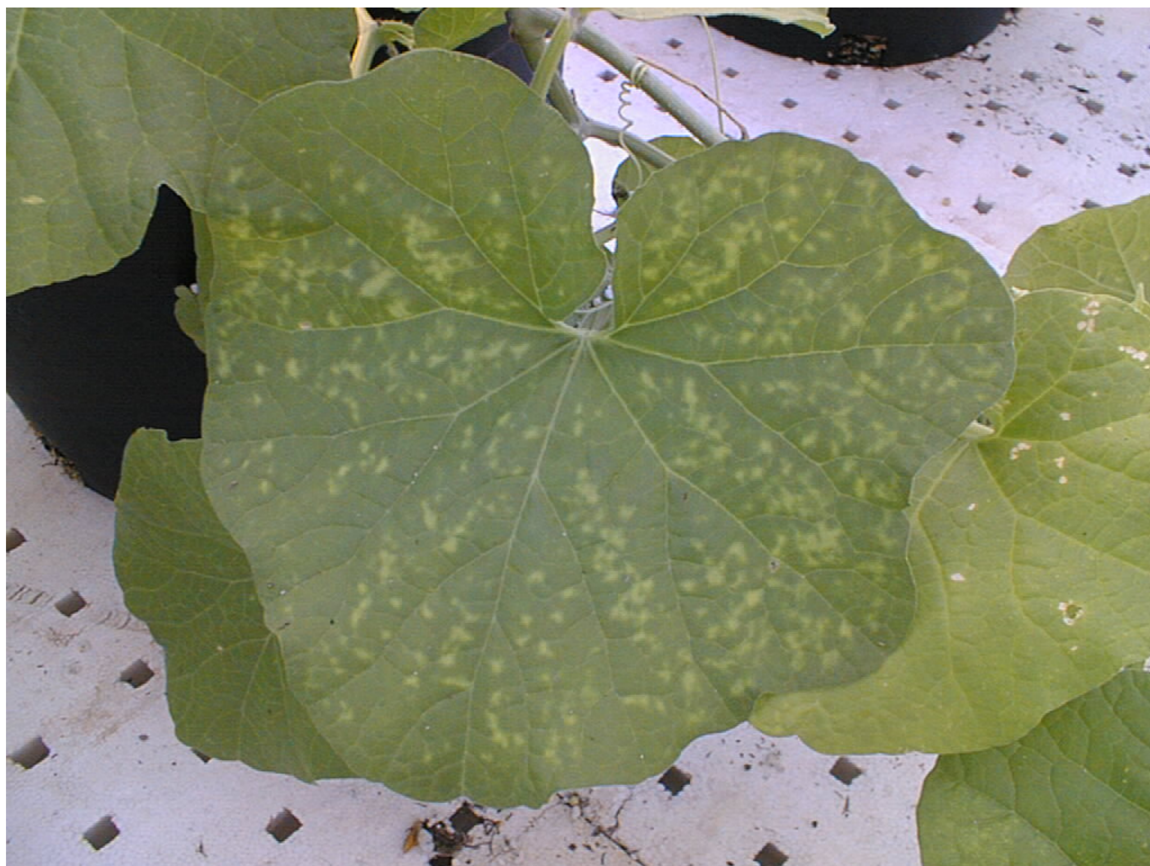
APPENDIX A

Temperature data for Weslaco, Texas 2002 in Celcius (USDA).

Month	Minimum	Maximum	Average Minimum	Average Maximum	Average
January	-2	32	11.3	23.5	17.4
February	5	32	10.0	22.5	16.2
March	3	36	16.0	26.6	21.3
April	13	36	21.7	31.5	26.6
May	17	37	22.6	33.0	27.8
June	21	37	23.8	34.4	29.1
July	23	37	24.9	34.6	29.8
August	24	38	25.3	36.3	30.8
September	18	37	23.1	32.9	28.0
October	14	34	21.5	30.1	25.8
November	6	31	13.2	23.6	18.4
December	4	29	11.7	22.6	17.2
Overall Average	12	35	19	29	24

APPENDIX B

Cucurbit yellow stunting disorder virus symptoms on 'Dulce' at three weeks post inoculation.



APPENDIX C

Total soluble solids bulks created from F_2 population developed from the melon cross 'Dulce' x 'TGR1551'.

High Soluble Solids Bulk	
DNA number	**Percent Soluble Solids**
63	12.8
15	12.0
79	11.5
41	11.3
16	10.8
49	10.8
76	9.8
52	9.8
Low Soluble Solids Bulk	
DNA number	**Percent Soluble Solids**
64	3.9
80	3.8
61	3.5
6	3.5
92	3.4
86	2.9
106	2.8
77	2.3

Eight highest and lowest valued plants were used for bulks tested on first 240 primers. Six highest and lowest valued plants were used for bulks tested on final 260 primers.

APPENDIX D

Percent sucrose bulks created from F_2 population developed from the melon cross 'Dulce' x 'TGR1551'.

High Sucrose Composition Bulk	
DNA number	**Sucrose Composition**
50	0.34
17	0.31
34	0.30
100	0.29
27	0.28
78	0.28
25	0.27
31	0.21
Low Sucrose Composition Bulk	
DNA number	**Sucrose Composition**
39	0.03
61	0.03
24	0.03
98	0.03
86	0.02
56	0.02
87	0.02
67	0.01

Eight highest and lowest valued plants were used for bulks tested on first 240 primers. Six highest and lowest valued plants were used for bulks tested on final 260 primers.

APPENDIX E

Ascorbic acid (Vitamin C) bulks created from F_2 population developed from the melon cross 'Dulce' x 'TGR1551'.

High Vitamin C Content Bulk		
DNA number	**uM/gfwt**	**Vitamin C (mg/100gfwt)**
4	2.22	39.13
47	2.09	36.78
95	1.98	34.92
12	1.76	31.04
36	1.74	30.60
104	1.67	29.48
34	1.65	29.02
22	1.60	28.10
Low Vitamin C Content Bulk		
DNA number	**uM/gfwt**	**Vitamin C (mg/100gfwt)**
60	0.49	8.59
61	0.46	8.15
6	0.46	8.06
53	0.42	7.37
78	0.40	7.11
44	0.39	6.91
109	0.37	6.54
2	0.36	6.38

Eight highest and lowest valued plants were used for bulks tested on first 240 primers.
Six highest and lowest valued plants were used for bulks tested on final 260 primers.

APPENDIX F

Molecular and phenotypic data set for the Fifteen markers in the 105 F_2 plants derived from the melon cross 'Dulce' x 'TGR1551'.

Marker	Molecular and phenotypic data
OAA09.350	DBDDDDDDDDDBDDDDBDDBDDDDBDBBDBDDDDBDDDDDBDBBDDDBDDD DDDBDDBDDBDDDDDDDDDDDDBDDDDBBDDDDBBDDBDDDDDDDBDDDDD DDD[z]
OAP03.800	DDDDDDDBDDDDDDBDDDDDDDDDDBDBDBBDDDDDDDDBDBDBDDDDBDDDB DDDBDDDDBBDDDDBDDDDBDBDBDDDDDBDDDBBBDDDDDBDDDDDDDDD DDB
OAQ13.750	DDDDDDDDDDDDDDDDBDBBDDDDDBDDDBDDBDDBDBBBDDBDDBBDDD DDDBDDDBDBDBDDDBBDDDDDBBDDDDDDDDDDDDBDDDDBDDBDDDDDBB DDD
OAS03.450	BBDDDDBBDDDDDDBBDDBBDBBDBDBDBBBDDDDDBBDDDDDDDDDBD DDDDDBBBBBDDDDDDDDBBDBBBDDBBDDDDBDDDDDDDDBDDBBBBDBDD DBB
OAS14.800	BDDBBDBDDBBBDDDDDBBDDDDDBBDDBDDBBBDDDBBDDDDBDDDDDDD DDBDDBDBBBDDDDDDDDDDDDBDDBDBDDBDDBDDDDBDBDDDBDDDBDDD DDB
OAT03.250	DDDDDBBBBDDDBBDDDBBDDBDDDDBDBDDDDDDDBDDDDBBBDDBDBDB DDDDDDBBDDBDDDBDDDDDDDDDDDBBDBBBBDBBDDDDDDDDDDDDDDDD DDD
OAT03.1600	DBDDBDDBDDDDDDDDDDDDDBDBDDDBBDDDDDDDDDDDDDBBDDDDDDDBDB BDDDDDBDDDDDDDDDBDDBDDBDBDBDDDDDDBDDDDDDDBBDBBDBDDDDDB DBD
OAU02.600	BDDBBDBDDBBBDDDDDBBDDDDDBBDDBDDBBBDDDBBDDDDBDDDDDDD DDBDDBDBBBDDDDDDDDDDDDBDDBDBDDBDDBDDDDBDBDDDBDDDBDDD DDB
OAU03.700	DDBBBDBDDBDBDDDDDDBDDDDDBBDDBDDBBBDDDBBDDDDBDDDDDDD DDBDDBDBBBDDDDDDDDDDDDDDDBDBBDBDDBDDDDBDBDDDBDDDBBBD DDB
OAU05.600	BDDDBDDDDDDDDDDDDDDDDDDDDBDDDBDDDBDBDDDBDDDBBDDDDBBD BBDDDDBDDBDDDDDDDBDBBBBBBBDBDDDDDBDDDDDDDDDDDBBBDDDDD DDD
OAU13.1350	DBDDBDDBDDDDBBDDDDBDDDDBDDDDBDDBDDDDBDDDDDDDDDBDDDB BBDDDDBBDDBDBDDDDDDDDDBDDDBBDBDBDDBBDDDDBDBDDDDDBBDD DBD
OAW06.600	DBBBDDDDDDBBDDBDBDDDDDDDDDDDDDBDDBDDDDDDDDBDDDDDDBDBD DDDDDBDDBBDDDDDDDBDDDDDDBBDDDDDDDDDDDDBDBDDDDDBDDBDBBB DDD
OAW06.1100	DDDBBDBDDBBDBBDBDDDDDBBBDDDBDDDDBDBDDDDDDDDDBDDDDDDD DBDDDBDBDDDDDDDDDDDDBDDDDDDDDDDDDDDBDDDDDDBBBDBDDBDDDDD DDD
OAW06.1250	DDBBDDDDDBDDBDDDDDDDDDDDBDDDDDDDDDBBDBBDDDDDDDDDDDDDB BDDDBBDDDDBDBDDDDDDBDBDDDDDDDDDBBDDDBDDDBDBBDDDDDDBDD DBD
OAW10.400	BDBBBDBDDBBBDDDDDBBDDDDDBBDDBDDBBBDDDBBDDDDBDDDDDDD BDBDDBDBBBDDDDDDDDDDDDBDDBDBDDBDDBDDDDBDBDDDBDDDBDDD DDB

[z]D = band present, B = band absent

APPENDIX G

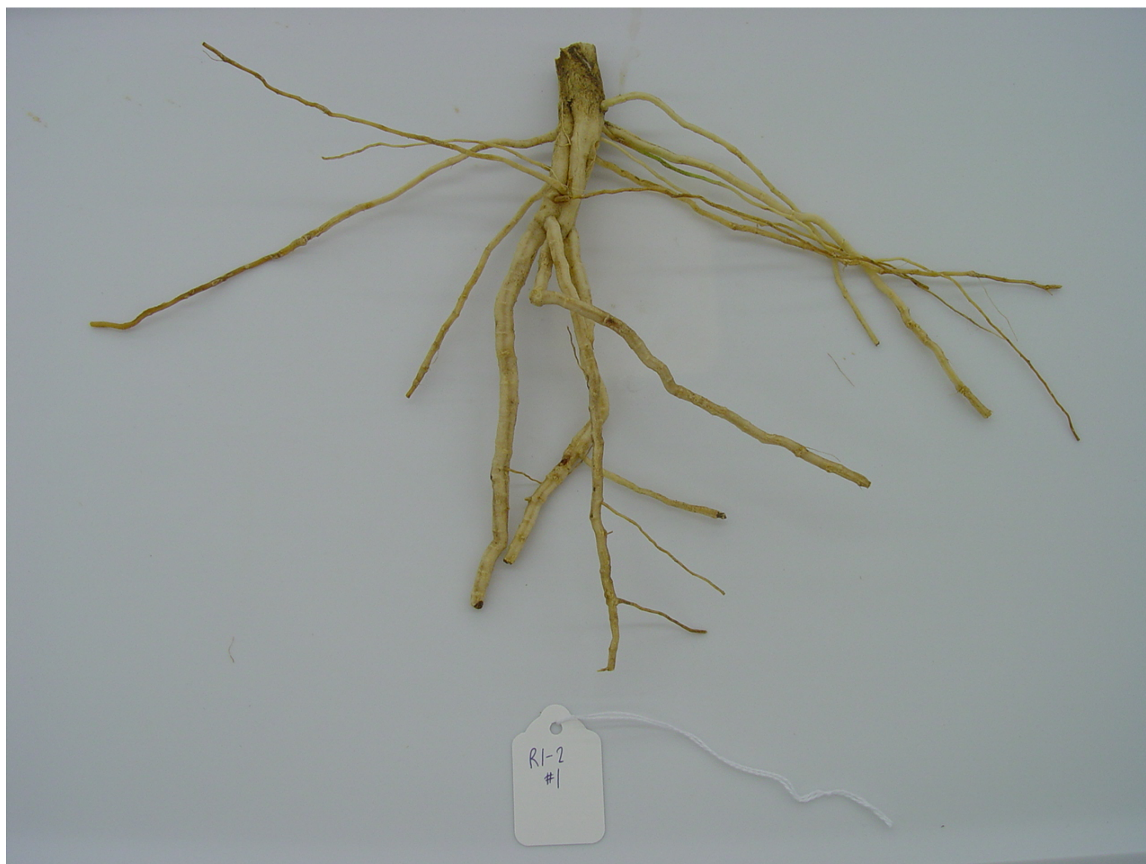
Gummy stem blight symptoms on parents used in gummy stem blight trial at six weeks after inoculation. 'TMS' on the left shows severe symptoms whereas PI 140471 on the right is disease free.

APPENDIX H

Monosporascus symptoms on female parent used in Monosporascus root rot trial. ‘TGR1551’ shows moderate disease damage.

APPENDIX I

Monosporascus symptoms on male parent used in Monosporascus root rot trial. ‘Deltex’ shows severe disease damage.



VITA

Jonathan Walker Sinclair was born on October 8, 1974, in Houston, Texas. His interest in horticulture began at an early age working in his mother's flowerbeds and steadily increased as he grew older. Upon graduation from high school he attended Wharton County Junior College in Wharton, Texas, from fall 1993 through spring 1995. He transferred to Sam Houston State University in Huntsville, Texas, fall 1995 and graduated summa cum laude with a Bachelor of Science degree in horticulture / crop science, in May 1997. He began graduate work fall 1997 under Dr. David Byrne at Texas A&M University in College Station. While there he worked for the Texas A&M stone fruit and rose breeding program. He earned a Master of Science in horticulture with emphasis on plant breeding, in December 2000. He entered a Ph.D. program spring 2001 under Dr. Kevin Crosby and Dr. Leonard Pike at Texas A&M University in College Station. While there he worked for the Texas A&M melon and pepper breeding program. He earned a Doctorate of Philosophy in plant breeding in December 2003. His knowledge of breeding various crops includes peach, rose, onion, carrot, melon, and pepper.

His permanent address is: 8130 Blase Rd., Rosenberg, Texas 77471.