Abstract

GUSMINI, GABRIELE. Inheritance of fruit characteristics and disease resistance in watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] (Under the direction of Todd C. Wehner, Ph.D.)

The watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] is a major vegetable crop in the United States. The genetics of this crop have been widely studied and several genes reported. Nevertheless, further investigation was needed for genes determining the appearance of the fruit (rind and flesh colors), the weight of single fruit, and resistance to gummy stem blight, a severe disease of watermelon caused by *Didymella bryoniae* (Auersw.) Rehm. In this work, the inheritance of novel rind phenotypes was measured and the genetics of flesh color verified. Three new genes were identified: *Scr* for the scarlet red flesh color of 'Dixielee' and 'Red-N-Sweet', *Yb* for the yellow belly color of 'Black Diamond Yellow Belly', and *ins* for the intermittent stripes of 'Navajo Sweet'. The spotted phenotype from 'Moon and Stars' was transferred to light green and gray cultivars for the development of novel varieties with distinctive rind patterns. Yield of 80 diverse cultivars was evaluated in replicated experiments. Some of the new, elite hybrid cultivars were in the top yielding group. However old, inbred cultivars appeared in the top group as well. Consistent and significant differences among the 80 cultivars tested indicates that there is large genetic variation for yield. Since most watermelon consumers are interested in smaller fruit, six adapted cultivars bearing the largest and smallest fruit were crossed in a half-diallel, producing F_1, F_2, and backcross generations. Genotypic variances, heritability, and gain from selection were estimated. High environmental variance and low narrow- and broad-sense heritability were recorded. Finally, the inheritance of resistance to gummy stem blight, previously attributed to the *db* gene, was verified. A genetic system more complex than a single gene seems to regulate the transmission of resistance from resistant to susceptible germplasm. Due to the complexity of phenotypic testing for this disease in watermelon, a new project for the development of molecular markers linked to resistance was initiated. Nevertheless, the use of F_3 phenotypic data and F_2 genotypic markers and the apparent complexity of the trait did not allow the identification of a tightly linked marker.

INHERITANCE OF FRUIT CHARACTERISTICS AND DISEASE RESISTANCE

IN WATERMELON [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]

by

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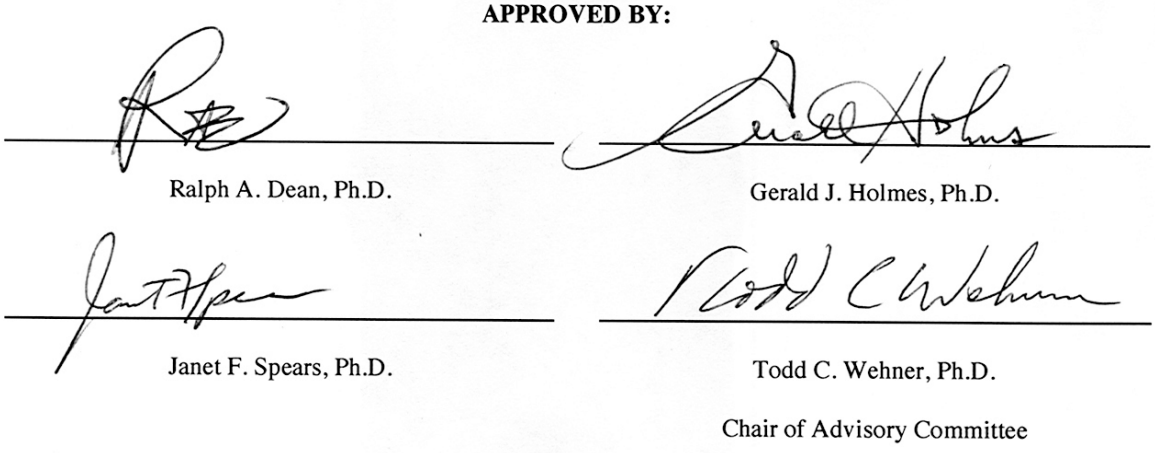

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Biography

Gabriele Gusmini was born in 1973 close to Milano (Milan), in Northern Italy. He spent his childhood in a little town called Cusano Milanino, in the province of Milano, with his mother and father. He grew up close to his other relatives, and spent vacations in the Alps during his summer and winter. As a child, Gabriele traveled with his parents to South Africa, Cyprus, Brazil, Spain, and Greece. As a teenager, he traveled to Switzerland, Germany, England, and France.

At the age of thirteen, Gabriele wanted an education both classical and scientific. Therefore, he pursued a classical curriculum during high school and a scientific education at the university. During high school he studied classical Greek, Latin, and Italian languages and literature, philosophy, history, and art, along with the basics of physics, chemistry, mathematics, and biology. He had a strong interest in politics and public administration and was active in the student senate of his school. By his graduation, Gabriele lost interest in what he called "abstract disciplines and pointless mental exercises".

The following year, Gabriele enrolled in the undergraduate program in Agricultural Sciences at the University of Perugia and became very interested in learning about agriculture, in theory at school and in practice by helping friends and neighbors on their farms. Eventually, he became familiar with the issues involved in managing small farms and making them productive by integrating new crops and techniques with traditional production. Two years into his B.S. degree, Gabriele decided to move back to his home town to be closer to his family and he transferred to the University of Milano. After one semester at his new school, he was assigned by his Professor of Arboriculture a research thesis on the propagation *in vitro* of European and Chinese chestnuts, a project that involved intense laboratory work over the next three years.

While working towards his B.S. degree, Gabriele began his professional career as a consultant and as an entrepreneur, which he expanded and continued after graduation. Gabriele's activity was quite varied but intensely focused towards creating new job opportunities for his peers and himself. He joined a group of consultants sponsored by Bayer that worked in extension management of ornamentals for homeowners in the metropolitan areas. He was certified as a public Agronomist and developed technical and financial plans for the improvement of small farms in Italy. He also directed start-up operations for environmental restoration through proper production and use of composts, an activity that culminated in the acquisition by Gabriele and his father

of a large abandoned quarry to be restored to its original condition. At the same time, Gabriele founded a consulting firm together with a lawyer and a marketing specialist to assist small and medium enterprises. In addition, he joined his father and uncle in their activity of internet marketing of databases and services for lawyers and cost-accountants. Eventually, Gabriele and his partners merged the two latter firms and initiated a new company that combined internet-based databases and consulting expertise for the benefit of their clientele. Gabriele also cooperated with the international consulting firm Ernst & Young for the development of an internet-based consulting network for the Italian corporate clients of the firm.

During those years of intense professional activity, Gabriele advanced in his horse-riding skills, with major focus in *dressage*, under the direction of famous trainers such as Giorgio Caponetti, Paolo Giani Margi, and Fabrizio Tognacci. Meanwhile, his friendship with men and women of art and culture in Milano grew stronger and his interests in art and literature resurfaced from high school times. At this point of his life, Gabriele became involved in the promotion of the preservation of the historical, artistic, and natural treasures of his country. Gabriele also developed skills and a profound passion for photography and traveled often to central Italy and Venezia (Venice) to find subjects for his pictures. Gabriele ventured several times in learning to play piano and guitar, but eventually "forcedly gave-up", or, truthfully, failed, and satisfied his passion for classical, lyrical, and jazz music by becoming an *abitueé* at the Teatro alla Scala, the Conservatorio Giuseppe Verdi, and the jazz-club Il Capolinea in Milano. Eventually, Gabriele became also a passionate scuba-diver and enjoyed free-diving and spear-fishing in the Mediterranean Sea.

In December of 2000, while shopping for a *papillon* tie for New Year's Eve, Gabriele felt the need for a sudden change in his life: a few weeks later, he left his country and his multiple activities, and moved to the United States to build a career in agricultural research. In May 2001, he enrolled in the graduate program of the department of horticultural science at North Carolina State University, Raleigh, North Carolina, to pursue M.S. and Ph.D. degrees in plant breeding. Since then, he spent most of his time as a graduate research assistant and student, but also continued his business activities in Italy and satisfied his never-ending desire for gardening. He built a small garden behind his house, where there is no longer any room left for additional plants. His garden lives and thrives in complete disrespect of all the conventions of proportion, size, and color-matching of plants set by landscape designers, yet it astonishes and fascinates his visitors.

Currently, Gabriele is planning his future as a professional in plant breeding in the United States, along with his next house, garden, orchid greenhouse, and his revived interest and passion for "the line of beauty" in nature, art, literature, music, and photography.

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GENERAL INTRODUCTION

REVIEW OF WATERMELON GENETICS FOR PLANT BREEDING

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Brief History of Watermelon Breeding and Genetics

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] is a major vegetable crop in the United States. Total production in 1999 to 2002 was 1.67 million Mg per year of marketable fruit, and the consumption *per capita* was 6.6 kg of fresh fruit (USDA-ARS, 2001; USDA-ARS, 2002; USDA-ARS, 2003; USDA-ARS, 2004b). In the same period, the major center of production was Florida (ca. 380,000 Mg per year), even though the state with the largest cultivated area was Texas (ca. 18,000 ha per year) (USDA-ARS, 2004a).

Watermelon breeding has been going on for thousands of years, but 'official' programs in the United States began in the late 1800s. By 1900, 'Angeleno', 'Chilean', 'Florida Favorite', 'Georgia Rattlesnake', 'Cole Early', 'Kleckley Sweet', and other open-pollinated cultivars were on the market (Whitaker and Jagger, 1937). Initially, many different types were available to growers. However, as the market became more established, the cultivars converged on a few types. In addition, the limits to production imposed by new diseases favored the few resistant cultivars. In 1954, C.F. Andrus released 'Charleston Gray', with elongate fruit shape, gray rind, and red flesh (Fig. 1). It was resistant to Fusarium wilt, anthracnose, and sunburn. 'Charleston Gray' became the leading cultivar in the commercial market, although niche markets and home gardeners continued to use a diverse array of cultivars and types. In 1970, C.V. Hall released 'Allsweet' with resistance similar to 'Charleston Gray', but higher fruit quality. 'Allsweet' had elongate fruit shape and rind with wide, dark green stripes (Fig. 2). The Allsweet type was retained also in later releases of successful cultivars, even though alternative types have been introduced to the consumers during the last thirty years (i.e., 'Dixielee' and 'Crimson Sweet', both bearing fruit with narrow, dark green stripes on the light green background, or 'Sugar Baby', with round fruit and solid dark green rind).

A major change in watermelon breeding in the United States occurred in 1962 with the release of the first seedless watermelon cultivar, 'Tri-X-313', by O.J. Eigsti. However, it was not until the 1990s that seedless watermelons became commercially important, due to the slow improvement in fertility of the tetraploid parents used as the female parent in the production of triploid (seedless) hybrids. In addition to Andrus, Eigsti, and Hall, a few other major contributors have improved American watermelons in the last fifty years. In the 1950s and 1960s, J.M Crall (University of Florida, Leesburg) released 'Dixielee', a successful alternative to 'Allsweet'

for its different fruit-type and superior quality, and 'Minilee' and 'Mickylee', the first icebox (<5.5 kg/fruit) cultivars adapted to the southeastern United States. Since the 1980s, W.S. Barham improved the techniques for breeding and producing seedless cultivars. In the 1980s and 1990s, T.V. Williams developed the hybrids 'Fiesta', 'Mardi Gras', and 'Sangria', which dominated the market during that time.

In watermelon breeding, most of the useful traits subject to improvement and/or study have been either fruit characteristics or resistance to diseases of abiotic and biotic origin, often controlled by single genes. The genetics of watermelon have been studied extensively, and many genes have been characterized during the last sixty to seventy years (Cucurbit Gene List Committee, 1979; Cucurbit Gene List Committee, 1982; Henderson, 1991; Henderson, 1992; Rhodes and Dane, 1999).

Genetics of Plant Architecture and Sex Expression

Plant architecture traits have been minor objectives for watermelon breeders, often limited to the development of cultivars with short vines (dwarf type) to be used by home gardeners. The genetics of dwarfism have been studied and the inheritance of genes conferring the dwarf phenotype to normal cultivars has been described (Huang et al., 1998; Liu and Loy, 1972; Mohr and Sandhu, 1975). The dwarf type can be obtained by the introgression of one of the three genes: *dw–1*, *dw–2*, and *dw–3*. Dwarfism in watermelon is caused by shortened internodes (*dw–1*) or reduced internode number (*dw–2*). The mechanism of action of the *dw-3* allele has not been reported.

Genes regulating sex expression in watermelon have not been described, with the exception of the *a* gene for andromonoecious plants, the wild-type being monoecious (*A*) (Rosa, 1928). No gynoecious or parthenocarpic germplasm has been identified in watermelon, as opposed to cucumber (*Cucumis sativus* L.), where seedless fruit can be obtained from a gynoecious, parthenocarpic cultivar grown in isolation from other sources of cucumber pollen. Thus, seedless watermelons are produced only on sterile triploid plants, by inducing fruit formation with diploid pollen.

Male-sterility has been reported in two mutant lines and two genes have been described to confer this trait. The *ms* gene causes the development of small anthers and pollen abortion (Zhang et al., 1994; Zhang and Wang, 1990). The *gms* gene, instead, induces male sterility due to chromosome desynapsis, accompanied by

absence of trichomes on the leaves (glabrous male sterile) (Watts, 1962; Watts, 1967). Technological application of these two genes has been pursued for the production of hybrid seed, even though hand-pollination and daily removal of male flowers are still the most common techniques, probably due to the low cost of labor in the countries of seed production (Asia and Central America) (Maynard, 2001).

Genetics of Fruit Characteristics

The watermelon fruit consists of the exocarp, mesocarp, and endocarp. The endocarp is the seed-containing part that is consumed as food, and the mesocarp (white crisp inner layer) and exocarp (thin green outer layer) are usually referred to as the rind. Fruit traits of interest in watermelon can be grouped into six categories: 1) yield, 2) shape, 3) weight, 4) rind (or skin), 5) flesh, and 6) seeds. Each of these categories may include different important characteristics. Yield may be determined by the number of fruit per unit of production and may be separated into marketable and non-marketable yield (culls), based on the overall external appearance of the fruit. The rind may differ in color, pattern, thickness, toughness, and flexibility. The flesh may differ in color, texture, sweetness (sugar or total soluble solids content), and resistance to hollowheart (internal voids). The seeds may differ for color, size, and resistance to formation of hard seed coats in triploid (seedless) watermelons.

The inheritance of yield components in watermelon was studied extensively in the 1960s and 1970s. Heterosis and general (GCA) and specific (SCA) combining ability were measured by Brar and Sidhu (1977), Brar and Sukhija (1977), Nandpuri et al. (1974, 1975), Sidhu and Brar, (1977, 1985), Sidhu et al. (1977a,b). More recent studies of the effects of reciprocal crosses on yield components in watermelon have been contradictory (Gill and Kumar, 1988; Rajendran and Thamburaj, 1993; Sachan and Nath, 1976). Heterosis was inconsistent over experiments, and the studies involved diallel or top crosses of elite inbreds, not a random set of lines from a population. Furthermore, the experiments included only a small number (N_{max}=10) of non-randomly chosen elite cultivars as parents, so the results are valid only for those specific crosses and are not generally applicable.

Watermelon fruit can be round, oval, blocky, or elongate in shape (Maynard, 2001). The genetics of fruit shape has not been widely studied, but the round, oval, and elongate phenotypes were determined by the

incomplete dominance of the *O* gene. The homozygous dominant plants had elongated fruit, the homozygous recessive fruit were round (spherical), and the heterozygous fruit were oval (Poole and Grimball, 1945; Weetman, 1937). In addition, the shape of the fruit can be predicted by the shape of the ovary at anthesis, thus making ovary shape a useful marker for things such as hybrid seed production (Warid and Abd el Hafez, 1976).

The fruit of the cultivated watermelon may vary in weight from 1 to 100 kg. In the United States, commercial fruit are usually classified into four categories: icebox (<5.5 kg), small or pee-wee (5.5-8.0 kg), medium (8.1-11.0 kg), large (11.1-14.5 kg), and giant (>14.5 kg) (Maynard, 2001). Fruit weight in watermelon production is a yield component, which contributes to total yield per unit of land. However, since fruit of different sizes are marketed in different categories, fruit weight should be regarded as a descriptor of fruit type. Nevertheless, fruit weight. In two preliminary studies of inheritance of fruit weight, significant additive, dominant, and epistatic effects were reported, dominance and dominance-by-dominance being the largest gene effects (Brar and Nandpuri, 1974; Sharma and Choudhury, 1988). Nevertheless, single genes or quantitative trait loci have not been identified for the weight of watermelon fruit.

Both mechanical characteristics and color of the watermelon rind are of great importance to the development of cultivars with good shipping ability, long shelf-life, and attractive appearance. The only gene reported to influence the mechanical characteristics of the rind (toughness and flexibility) was the *t* gene for the thin, tender rind, bursting when cut, from 'California Klondike' (Porter, 1937), renamed *e* for explosive rind by Poole (1944). So far, no study has described the inheritance of rind toughness among watermelon families with non-explosive rind.

The inheritance of rind color and pattern has been studied since the 1930s. The most common rind colors are solid green (dark, medium, and light), striped (narrow, medium, and wide dark green stripes on a light green background), and gray (medium green lines on a light green background). The genes described for the different skin colors and patterns are part of a three-allele series at the *g* locus: *G* for dark green, g^s for striped, and *g* for light green (Weetman, 1937). However, this model does not seem to apply in all watermelon cultivars. Furthermore, the inheritance of some rind colors such as gray or medium green have not been published, even though these have been two common colors during the last century of watermelon breeding.

The genetics of the flesh color in watermelon have been studied and genes for the white, red, orange, salmon yellow, and canary yellow colors have been reported. A triple-allelic series was identified at the *y* locus to regulate red, orange, and salmon yellow flesh colors (*Y*, y^o, and *y*, respectively) (Henderson et al., 1989; Henderson et al., 1998; Poole, 1944; Porter, 1937). The canary yellow color (*C* gene) was dominant to pink (*c*) and epistatic to red (*Y*) (Henderson et al., 1998; Poole, 1944). In addition, the red color was also recorded in individuals homozygous for *C*, where the production of the yellow pigment was inhibited by the *i-C* gene (inhibitor of canary yellow) (Henderson et al., 1998; Rhodes and Dane, 1999). A third gene for the yellow flesh (*B*) has also been described in a breeding line. The white flesh gene (*Wf*) was epistatic to *B* (Henderson, 1992; Shimotsuma, 1963). Nevertheless, breeding for specific flesh colors has often been challenging, due to frequent distortion of the inheritance of some of these genes, thus suggesting that a more complex model may determine flesh color in segregating populations of watermelon.

Sugar content of the flesh in watermelon is measured as total soluble solids (degree Brix). It is the major component of taste, and may vary from 1 to 16 °Brix (Maynard, 2001). So far, no study of the genetics of total soluble solids based on a set of cultivars representative of the watermelon crop has been reported. Furthermore, preliminary studies included parents that differed by 2 to 3 °Brix in sugar content (Abd el Hafez et al., 1985; Brar and Nandpuri, 1977). A study of crosses between cultivars at the extremes of the range of watermelon sweetness would be useful to the improvement of the organoleptic properties of the flesh of watermelon.

Genetics of Seed Characteristics

Watermelon seeds usually are classified according to their color and size, both traits of great interest to breeders. Seed color may affect the appearance of cut fruit greatly (seed color that contrasts with flesh color are usually preferred), while their size can limit the edibility of the fruit itself. Large seeds usually are removed from the flesh, when consumed, while tiny seeds might be ingested.

The main colors of watermelon seeds are white, tan, brown, black, red, green, and mottled (Maynard, 2001). In addition, seeds may have lighter or darker margins (ring), or may be covered by an additional layer of

fleshy pericarp in Egusi cultivars, as induced by the *eg* gene (Fig. 3) (Gusmini et al., 2004; Gusmini and Wehner, 2003).

The genes *r*, *t*, and *w* determine seed color. Black is given by triple dominant; mottled is homozygous recessive only for *w*; tan is homozygous recessive only for *t*; white with tan tip is homozygous dominant only for *R*; red is homozygous dominant only for *W*. Finally, triple recessive homozygosis generates white seeds with pink tip (Poole et al., 1941). The mottled (renamed from dotted) seed type is determined by the *d* gene, acting as a modifier of black: *RR TT WW DD* gives solid black and *RR TT WW dd* gives dotted black seed-coat (Kanda, 1951; Poole, 1944; Poole et al., 1941).

Commercially, watermelon seeds are categorized by size: large (or long), medium, small (or short) (approximately 10, 7, and 5 mm), and tomato (watermelon seeds of the size of tomato seeds). According to Poole et al. (1941), the *l* and *s* genes interact to determine seed size for the large, medium, and small classes (*ll SS* for long, *LL SS* for medium, and *LL ss* or *ll ss* for short). The tomato seeds were studied in a cross between a 'Sugar Baby' mutant with tomato seeds and 'GN-1', with short seeds. The tomato seed trait was inherited as a single recessive gene (*ts*) (Zhang, 1996). The interactions of *ts* with *l* and *s* have not been described, so far. In addition, a gene (*Ti*), dominant over medium seeds, has been described for the so-called "tiny" seed-size in 'Sweet Princess' (Tanaka et al., 1995). Tiny seeds have similar size as small seeds (Fig. 4).

Seeds of certain cultivars show vertical cracks (parallel to the longest axis of the seed) of the seed-coat, usually less than one mm wide and long about 50-75% of the seed length. The development of these cracks has been determined to be under the genetic control of *cr* (Abd el Hafez et al., 1981). It is not known if this phenotype has any advantage in germination and whether growers would perceive seeds with cracks as defective, compared to normal seeds.

Genetics of Resistance to Pathogens

Very little information is available on the genetics of resistance to abiotic stresses in watermelon, even though traits such as drought and heat tolerance would be useful traits. A single dominant gene (*Ctr*) was identified for tolerance of cold temperatures (<20ºC at night) at the seedling stage (Provvidenti, 1992; Provvidenti, 2003).

The primary nematode species attacking watermelon are the peanut [*Meloidogyne arenaria* (Neal) Chitw.], southern [*M. incognita*, (Kofoid and White) Chitw.], and Javanese [*M. javanica* (Treub) Chitw.] root-knot nematodes. Even though losses due to these parasites may be as much as 50% (Maynard, 2001), the extensive use of fumigants has delayed the search for nematode resistant germplasm and the study of the genetics of resistance. Currently, the phasing-out of methyl bromide is renewing interest in the development of nematode resistant cultivars.

Genetic resistance to insect pests does not have a very important role in the protection of the watermelon crops. So far, insect control is mostly achieved chemically. Nevertheless genes for resistance to the red pumpkin beetle (*Aulacophora foveicollis* Lucas) and to the fruit fly (*Dacus cucurbitae* Coquillett) have been identified. Both *Af* and *Fwr* were reported as dominant to susceptibility (Khandelwal and Nath, 1978; Vashistha and Choudhury, 1972). Most insect pests in the United States are typical of specific regions of watermelon production, thus making it economically less advantageous to develop insect resistant cultivars. Nevertheless, some pests are common to different areas, i.e. the melon aphid (*Aphis gossypii* Glover) and the cucumber beetles (*Acalymma vittatum* Fabricius and *Diabrotica undecimpunctata howardi* Barber) (Maynard, 2001), and genetic resistance may be a valid alternative to chemical control.

Fungi, viruses, and bacteria are the causal agents of some of the most destructive diseases of watermelon. In the United States, fungal diseases are a major limit to the watermelon industry in the southeastern region, while viruses are more damaging in western production areas. The most important fungal diseases and their causal agents are: anthracnose (caused by *Colletotrichum lagenarium* (Pass.) Ellis & Halst), downy mildew (caused by *Pseudoperonospora cubensis* Berk. & M.A. Curtis), Fusarium wilt (caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans), gummy stem blight (caused by *Didymella bryoniae* (Auersw.) Rehm), Monosporascus root rot and vine decline (caused by *Monosporascus cannonballus* Pollack & Uecker), Phytophthora blight (caused by *Phytophthora capsici* Leonian), Pythium damping-off (caused by *Pythium* spp.), and powdery mildew (caused by *Podosphaera xanthii* (Castagne) U. Braun & N. Shishkoff). The main viral diseases and causing viruses are: cucumber mosaic (CMV), papaya ring spot (PRSV; also known as PRSV type W and previously known as WMV), watermelon mosaic (WMV; previously known as WMV-2), squash mosaic (SqMV), and zucchini yellow

mosaic (ZYMV). A threatening bacterial disease is bacterial fruit blotch, caused by *Acidovorax avenae* subsp. *citrulli* Schaad et al.. Despite the high number of biotic pathogens listed, the genetics of resistance have been described only for the control of Fusarium wilt, gummy stem blight, anthracnose, watermelon mosaic, and zucchini yellow mosaic (Guner and Wehner, 2003; Xu et al., 2004).

Seven races of anthracnose have been reported, but races 1, 2, and 3 appear to be the most important in watermelon (Maynard, 2001). Most cultivars are resistant to races 1 and 3, and resistance sources to race 2 have been found, PI 512385 being the most resistant (Boyhan et al., 1994). Resistance to all three races was inherited as a dominant gene in multiple crosses: *Ar-1* conferred resistance to races 1 and 3 (Layton, 1937), and *Ar-2*^{1} to race 2 (Winstead et al., 1959).

Resistance to three races (0, 1, and 2) of Fusarium wilt has been found and watermelon differentials have been determined. 'Black Diamond' and 'Sugar Baby' are susceptible to all races, 'Quetzali' and 'Mickylee' are resistant to race 0, 'Calhoun Gray' is resistant to races 0 and 1, and PI 296341 and PI 271769 are resistant to all races (Table 1) (Maynard, 2001). In addition, the inheritance of resistance to race 1 has been described. Resistance was inherited as a single dominant gene (*Fo-1*) in crosses of the resistant 'Calhoun Gray' or 'Summit' with the susceptible 'NH Midget' (Henderson et al., 1970).

Resistance to gummy stem blight was found in PI 189225 and PI 271778 and deployed as a single recessive gene (*db*) to develop the resistant cultivars 'AU-Producer', 'AU-Golden Producer', 'AU-Jubilant', and 'AU-Sweet Scarlet' (Norton, 1979; Norton et al., 1993; Norton et al., 1995; Norton et al., 1986). Nevertheless, these cultivars were less resistant than their resistant parents, thus suggesting a more complex genetics for this trait.

Watermelon resistance to viruses has been identified for PRSV, WMV, and ZYMV (Maynard, 2001). So far, identification of two genes for resistance to zucchini yellow mosaic has been reported. Recessive homozygosis at the *zym*-FL locus was reported to induce resistance to a strain of ZYMV from Florida (Provvidenti, 1991). More recently, resistance to a Chinese strain of the same virus was inherited as the single recessive gene *zym-CH* (Xu et al., 2004). In addition, Xu et al. (2004) estimated that tolerance to watermelon mosaic in two crosses was controlled by a minimum of two genes with large broad-sense heritability.

Finally, one recessive gene (*pm*) for the susceptibility to powdery mildew was reported (Robinson et al.,

1975), but no gene has been found for resistance to powdery mildew. The genetics of resistance to this disease is becoming an important information, because powdery mildew is an emerging disease in watermelon.

Other genes of minor interest to breeders have been studied and described, as reported in the current watermelon gene list (Guner and Wehner, 2003), such as genes for green flower color (*gf*), golden leaf color (*go*), along with several genes coding for specific proteins. On the contrary, watermelon breeders are selecting for many different traits, such as flesh color, rind pattern, plant architecture, leaf shape, and many others and their genetics have not been studied so far. Current efforts are underway to collect mutants by the Cucurbit Genetics Cooperative watermelon gene curators (T.C. Wehner and S.R. King).

Objectives

The objective of this dissertation was to enhance the knowledge of the genetics of the cultivated watermelon, to the benefit of those interested in watermelon improvement. Specifically, we were interested in 1) studying the genetics of rind color and pattern; 2) studying the inheritance of novel skin phenotypes and verifying the genetics of the white, red, salmon yellow and canary yellow flesh colors; 3) establishing the genetic foundations for yield improvement; 4) estimating the heritability and predicted gain from selection for the improvement of fruit-weight; 5) verifying the inheritance of resistance to gummy stem blight; and 6) identifying molecular markers linked to gummy stem blight resistance.

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Table 1. Watermelon cultivars with vertical resistance to Fusarium wilt, caused by *Fusarium oxysporum* Schlechtend.:Fr. f. sp. *niveum* (E.F. Sm.) W.C. Snyder & H.N. Hans.

Cultivar	Race 0	Race 1	Race 2
'Black Diamond', 'Sugar Baby'	-	-	-
'Quetzali', 'Mickylee'	+	-	-
'Calhoun Gray'	+	+	-
PI 296341, PI 271769	+	+	+

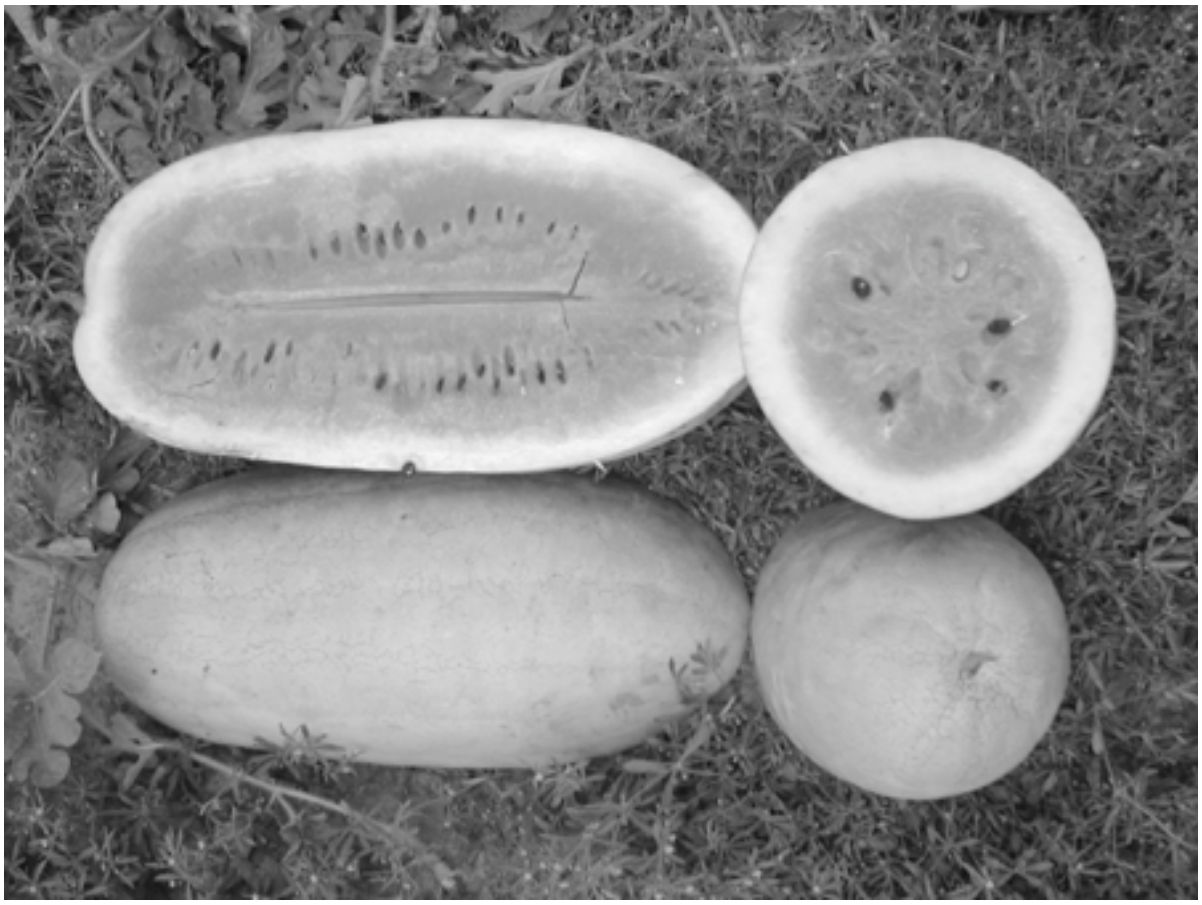

Figure 1. Cultivar Charleston Gray, released in 1954 by C.F. Andrus. Resistant to Fusarium wilt, anthracnose, and sunburn.

Figure 2. Cultivar Allsweet, released in 1970 by C.V. Hall. Resistance similar to 'Charleston Gray', but higher fruit quality.

Figure 3. Watermelon seeds covered by an additional layer of fleshy pericarp in Egusi cultivars, as induced by the *eg* gene.

Figure 4. Watermelon seeds size: 1 = tomato (watermelon seeds of the size of tomato seeds), *LL ss tsts* or *ll ss tsts*; 2 = small (or short) (~5 mm), *LL ss* or *ll ss*; 3 = medium (~7 mm), *LL SS*; and 4 = large (or long) (~10 mm), *ll SS*.

CHAPTER ONE

A REVIEW OF INHERITANCE OF RIND PATTERN IN WATERMELON

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Introduction

The inheritance of rind pattern and color in watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) has been studied since the 1930s. The most common rind (or skin) colors in watermelon are currently described as solid green (dark, medium, and light), striped (narrow, medium, and wide dark green stripes on a light green background), and gray (a light green background with a medium or dark green network).

The genes described for the different rind colors and patterns do not seem to behave consistently in all watermelon cultivars. Furthermore, the inheritance of some rind types such as gray or medium green have not been published, even though these have been two common rind colors throughout the last century of watermelon breeding.

This review presents the available information and highlights questions still open on the inheritance of the main rind colors and patterns in watermelon.

The Triple-Allelic Series at the *g* Locus and the Linkage Hypothesis by L.M. Weetman

According to the model reported in all the gene lists for watermelon (Cucurbit Gene List Committee, 1979; Cucurbit Gene List Committee, 1982; Cucurbit Gene List Committee, 1987; Guner and Wehner, 2003; Henderson, 1991; Henderson, 1992; Rhodes and Dane, 1999; Rhodes and Zhang, 1995; Robinson et al., 1976) and proposed by Weetman in 1937, three alleles at a single locus would determine the inheritance of striped and solid green (dark and light) rind. The *D* allele for dark green was dominant to the *d* allele for light green rind, and the d_s allele produced stripes, being dominant to *d* and recessive to *D* (Weetman, 1937). The allelic series was renamed to *G,* g_s, and *g* by Poole in 1944.

Other rind colors in watermelon may derive from combinations of stripes on different shades of green background (from light to dark), as is the case of the dark green stripes on a dark green background in 'Sugar Baby' or 'Moon and Stars'. These cases pose doubts about the behavior of the g_s allele and point to the second hypothesis originally suggested by Weetman in 1937: two genes (*S* dominant for striping and *D* dominant for dark green rind) could be present and their reciprocal distance could determine the phenotype of the background and the stripes on the foreground. The two genes would have been tightly linked in his crosses. Weetman showed that both hypotheses are equally plausible and should be further studied. Nevertheless, the linkage

hypothesis was disregarded by Poole in 1944 and the three allele series has been the accepted hypothesis ever since.

Data suggesting the dominance of dark green over light green were published almost one year earlier than the article by Weetman (Porter, 1937). In this study, Porter reported that dark green was completely dominant to light green (yellowish white, in his description) in two crosses involving two different dark green cultivars ('Angeleno' and 'California Klondike'). He reported incomplete dominance of dark green, instead, in the cross 'California Klondike' × 'Thurmond Gray', the latter cultivar being described as yellowish green.

In conclusion, there is no strong evidence for either of the two hypotheses proposed by Weetman for the inheritance of different shades of solid green rind and striped rind in watermelon. Nevertheless, dark green (*D*, renamed *G*) is completely dominant to light green (*d*, renamed *g*) in crosses with a light green parent. On the other hand, in crosses of dark green cultivars with gray cultivars (light green background), genes for rind color behave as incomplete dominant and produces the medium green type that is also commonly observed in watermelon. Possibly, the multi-allelic series at the *g* locus needs to include an allele for the background of the gray watermelons that is different from the *g* allele for light green rind.

Gray, Pencilled, Netted, and Mottled Rind

The gray rind in modern watermelon cultivars may be described as a grayish light green background with a medium green netting foreground. This network or netting may be evenly present over the entire watermelon fruit or may be more evident in the furrows (longitudinal depressions). The inheritance of the gray rind color has not been studied directly and a gene for this trait has never been published.

Porter in 1937 made a cross between the gray cultivar Thurmond Gray and 'California Klondike' (solid dark green) (Fig. 1), but he was interested only in the inheritance of the background color and he disregarded the netted trait from 'Thurmond Gray'. In fact, he described this cultivar as "yellowish green" as opposed to the "yellowish white" 'Snowball' and the "dark green" cultivars 'Angeleno' and 'California Klondike'.

Shimotsuma studied the inheritance of striped rind by crossing a striped cultivar with what we would now call a gray cultivar (Shimotsuma, 1963). Like Porter, he defined the gray cultivar as "whole-colored with a fine reticulation" and then did not rate the progenies for the reticulation, but only for the presence/absence of

stripes. In his study, Shimotsuma confirmed the dominance of g_s over g from the triple-allelic series of Weetman. These results may have suggested to watermelon breeders a link of the gray type to the g allele. Nevertheless, Shimotsuma did not measure the gray phenotype, but the light green supposedly determined by the g allele.

The watermelon gene list includes two genes (*p* and *m*) that determine the pencilled, netted, and mottled rind types. Since the type lines for the genes are not available and the articles describing them are difficult to interpret, it would be useful to determine whether *p* or *m* were the genes responsible for gray rind.

The watermelon gene *p* for pencilled rind pattern has been reported in the gene lists since 1976 (Robinson et al., 1976). The name "penciled" first appeared in 1944 to describe inconspicuous lines on self-colored rind of 'Japan 6' (Poole, 1944), but the spelling was changed later to "pencilled" in the gene lists. The cross 'Japan 6' × 'China 23' was used by Weetman to study the inheritance of solid light green vs. striped rind and lined (later renamed pencilled) vs. netted rind (Weetman, 1937). 'Japan 6' had solid light green rind with inconspicuous stripes, usually associated with the furrow (Fig. 2). 'China 23' had dark green stripes on a light green background and a network running through the dark stripes (netted type) (Fig. 2). Weetman confirmed his hypothesis of two independent genes regulating the presence of stripes and the lined vs. netted pattern, recovering four phenotypic classes in a 9:3:3:1 ratio (striped, netted : striped, lined : non-striped, netted : non-striped, lined) in the F_2 generation and in a 1:1:1:1 ratio in the backcross to the double recessive non-striped, lined 'Japan 6'. However, Weetman did not name the two genes.

In 1944 Poole, based on the experiment of Weetman, named the single recessive gene *p* for the lined type. The inheritance of the *p* gene was measured by Weetman against the netted type in 'China 23' and not a self-colored type as reported by Poole. Previously, Porter reported that studies of rind striping were underway and specifically cited a pencilled pattern in the F_1 of the cross 'California Klondike' × 'Golden Honey' (Porter, 1937). The results of these studies have never been published, but they may have been used by Poole to describe the *P* allele in 1944 as 'self-colored skin' vs. the *p* allele for the lined rind. Apparently, with his studies between 1937 and 1944, Poole dismissed Porter's hypothesis of incomplete dominance for the pencilled type, in favor of the single recessive gene *p*. This explanation seems possible: a geneticist interested in verifying the inheritance of the *p* allele would have crossed a pencilled inbred with a solid light green (self-colored) inbred,

as apparently Porter did. We propose that the *P* allele produces the netted type, as originally described by Weetman.

Seeds of the two type lines used by Weetman are not currently available, nor are Porter's data and germplasm, thus making it difficult to confirm the inheritance of the *p* gene or to identify current inbreds allelic to pencilled and netted rind patterns.

The *m* gene for mottled rind was first described by Weetman in 'Long Iowa Belle' and 'Round Iowa Belle' (Weetman, 1937). Weetman described the rind as "medium-dark green with a distinctive greenish-white mottling", the 'Iowa Belle' (IB) type (Fig. 3). In the cross 'Iowa Belle' × 'China 23', Weetman observed that the IB type was inherited as a single recessive gene. However, in the cross 'Iowa Belle' × 'Japan 6', he recovered the two parental types (IB and non-IB, respectively) along with an intermediate type (sub-IB), described as inconspicuous mottling. In the backcross to 'Iowa Belle' (the recessive parent for the mottled rind), though, the traits segregated with a perfect fit to the expected 1:1 ratio. He explained the presence of the intermediate type as determined by interfering genes from 'Japan 6'. There was no other mention of the IB-type until Poole (1944) attributed its inheritance to the *m* gene from 'Iowa Belle', based on the article by Weetman. 'Iowa Belle' is not currently available and the IB mottling has not been identified in other mutants since the 1937 study by Weetman.

Based on the description of the mottled, or IB, rind type by Weetman, we can exclude any association of the *m* gene with the gray rind trait. The inheritance of the gray rind may involve, instead, the *P* allele as described by Weetman in 1937 (netted type) expressed on a light green background (maybe the *g* or a fourth new allele from Weetman's three allele series at the *g* locus). Nevertheless, since this hypothesis has not been tested, the *P* gene should not be considered responsible for the gray rind. Unfortunately, there are no published studies of crosses of inbreds having the gray netted type (possibly due to the *P* gene) on a solid light green background with inbreds having the solid light green color without netting.

Gene Combinations for Rind Colors

The homozygous genotypes produced by the genes known to regulate rind color and pattern in watermelon should have the following phenotypes: *GG MM* or *GG PP* or *GG pp* = solid dark green ('Black

Diamond'), *GG mm* = mottled dark green ('?'), *gg MM* = solid light green ('King and Queen'), and *gg mm* = mottled white green ('Charleston Gray'). GG PP = ???? g_sg_s PP = medium-stripe netted ('Crimson Sweet'), gg PP = gray ('Charleston Gray'), and *gg pp* or g_sg_s *pp* = pencilled ('?').

Future Directions for Watermelon Breeders and Geneticists

Based on the description of the pencilled and netted types reported by Weetman and Poole, and on the images of 'Japan 6' and 'China 23' in the article by Weetman (Fig. 2), we suggest that two inbred cultivars should be identified for the *p* gene among those currently available as substitute type lines for 'Japan 6' and 'China 23'. The netted type is present on fruit of 'Crimson Sweet', a currently available inbred cultivar (Fig. 4). We suggest that this cultivar be crossed with a non-netted striped cultivar, to confirm the single-gene segregation for the netted trait. If so, the *P* allele should be attributed to this new type line and further verified against *p*, should a replacement for 'Japan 6' be found.

A new type line for the mottled rind type could be identified by tracing the pedigree of 'Iowa Belle'. The cultivar was released in 1932 by Porter, having 'Conqueror' and another unknown line as parents (Wehner, 2002). The unknown parent is suspected to be 'Kleckley Sweet', but this cultivar does not show the mottled rind type. 'Conqueror' is not presently available, but its pedigree included citron (*C. lanatus* var. *citroides*) as the source of Fusarium wilt resistance. It is possible that the mottled type was already present in 'Conqueror' from the citron parent. Evaluation of the citrons available in the USDA-ARS watermelon germplasm collection may allow the identification of a new type line carrying a gene for the mottled rind type. Finally, several heirloom and modern cultivars had 'Iowa Belle' in their pedigree, including 'Charleston Gray', 'Congo', 'Fairfax' and 'Garrisonian', but none had mottled rind.

Further research is needed on the inheritance of the gray rind color in watermelon, and the effect of the *G* allele on the light green background of gray cultivars. The inheritance of fruit netting and background color in gray watermelons should be studied and a linkage hypothesis of two genes for these traits should be tested.

The study of the inheritance of striped rind should expand the original model by Weetman to include different width of the stripes and different shades of the background. Narrow-, medium-, and wide-striped, and dark, medium, and light green cultivars should be crossed in a half-diallel, and F_1, F_2 and BC_1 generations

should be developed. Should a model be established, then it should be verified against the inheritance of gray color to develop a model for rind color in watermelon.

To avoid the loss of type lines for new genes, in the future seeds of the mutants used in inheritance studies should be delivered to the watermelon gene curators for the Cucurbit Genetics Cooperative (T.C. Wehner and S.R. King).

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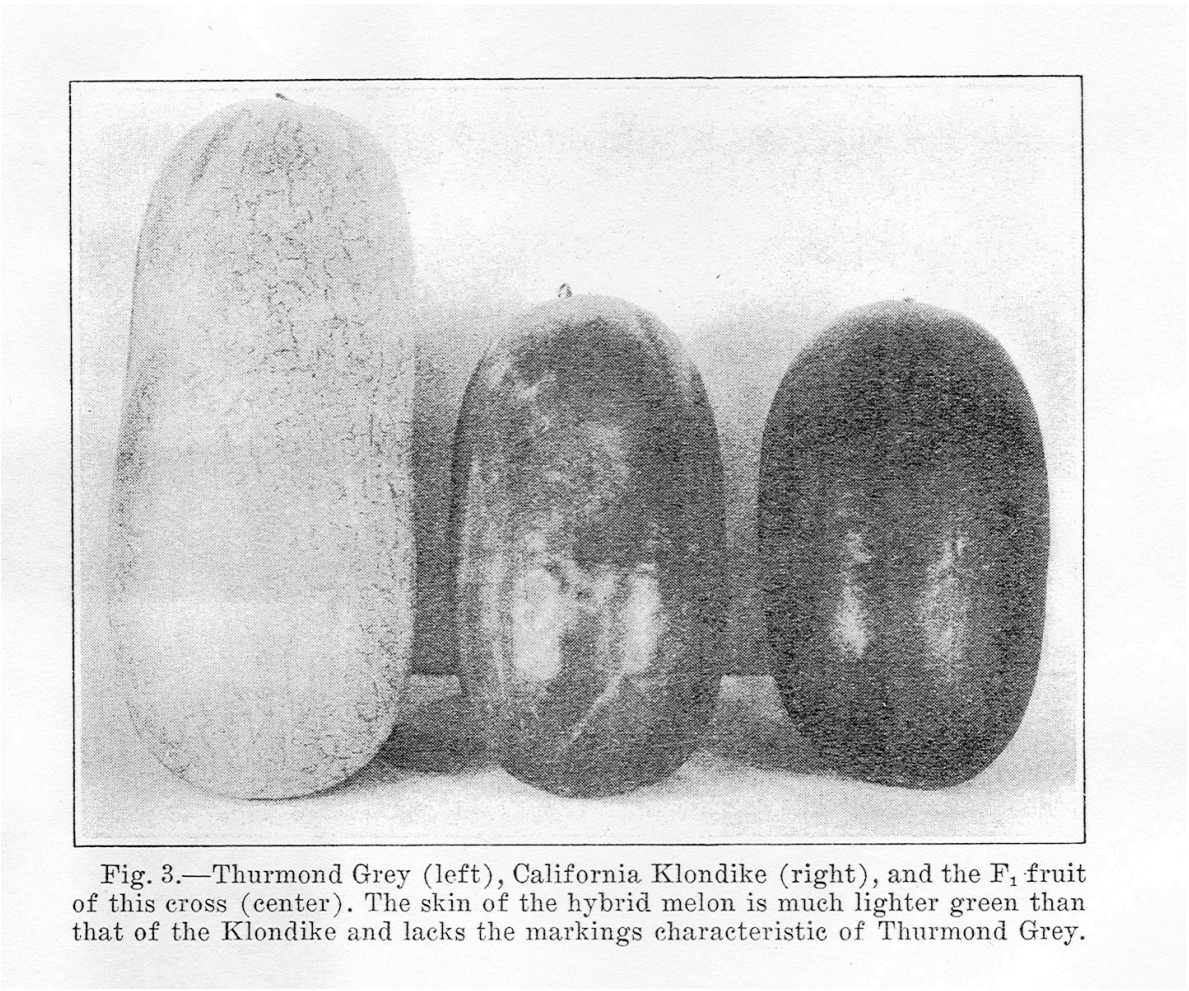

Fig. 3.—Thurmond Grey (left), California Klondike (right), and the F_1 fruit of this cross (center). The skin of the hybrid melon is much lighter green than that of the Klondike and lacks the markings characteristic of Thurmond Grey.

Figure 1. Cultivars Thurmond Gray and California Klondike used by Porter in 1937 in the first reported study of the inheritance of dark vs. light green rind colors in watermelon.

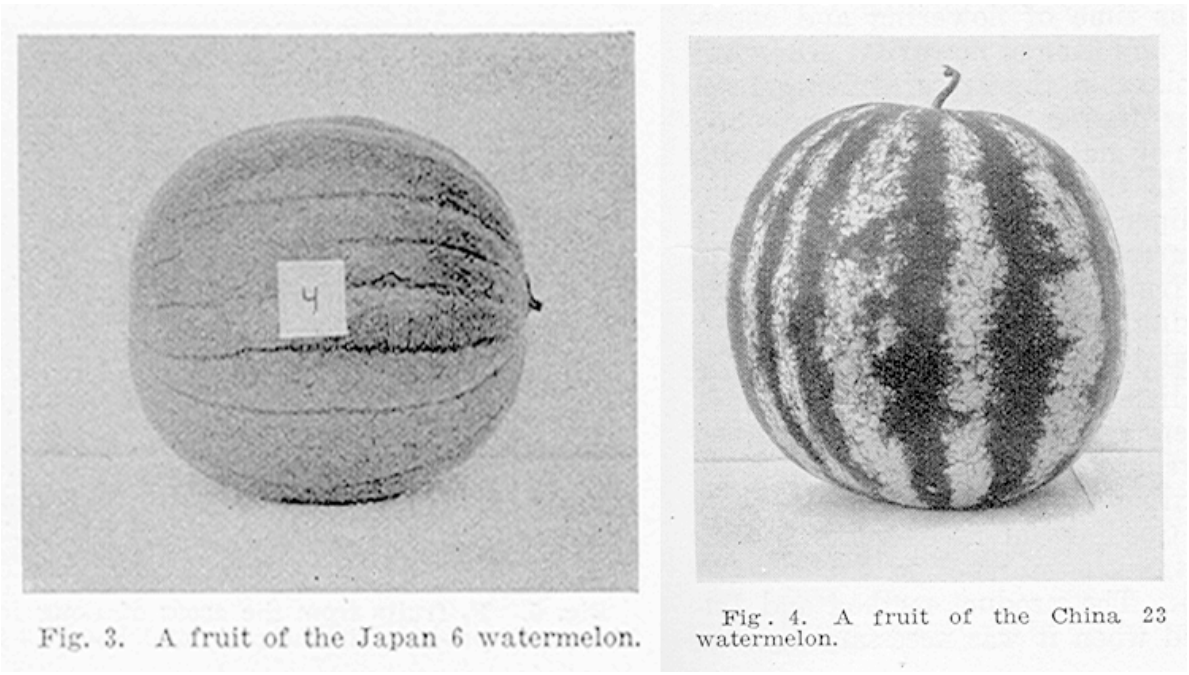

Fig. 3. A fruit of the Japan 6 watermelon.

Fig. 4. A fruit of the China 23 watermelon.

Figure 2. Cultivars Japan 6 and China 23 used by Weetman in 1937 to determine the inheritance of the lined and netted rind patterns (*p* and *P* alleles, respectively) in watermelon.

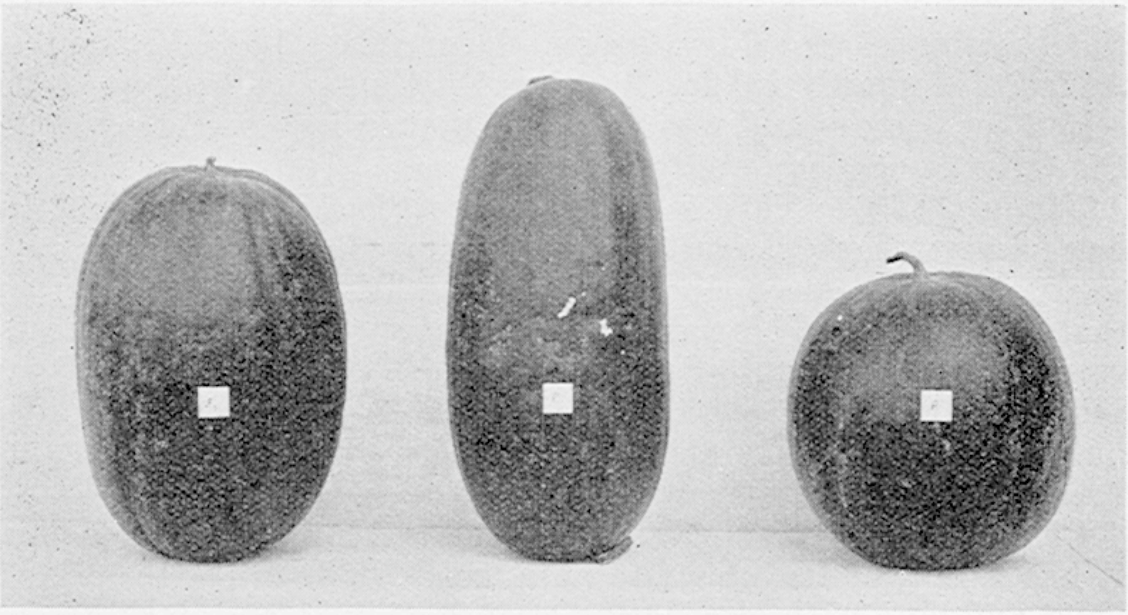

Fig. 1. Left, F_1 fruit from the cross of Long Iowa Belle × Round Iowa Belle. Center, Long Iowa Belle. Right, Round Iowa Belle.

Figure 3. Cultivars Long Iowa Belle and Round Iowa Belle used by Weetman in 1937 to determine the inheritance of the mottled rind pattern (a.k.a. Iowa Belle or IB-type) and regulated by the *m* gene in watermelon.

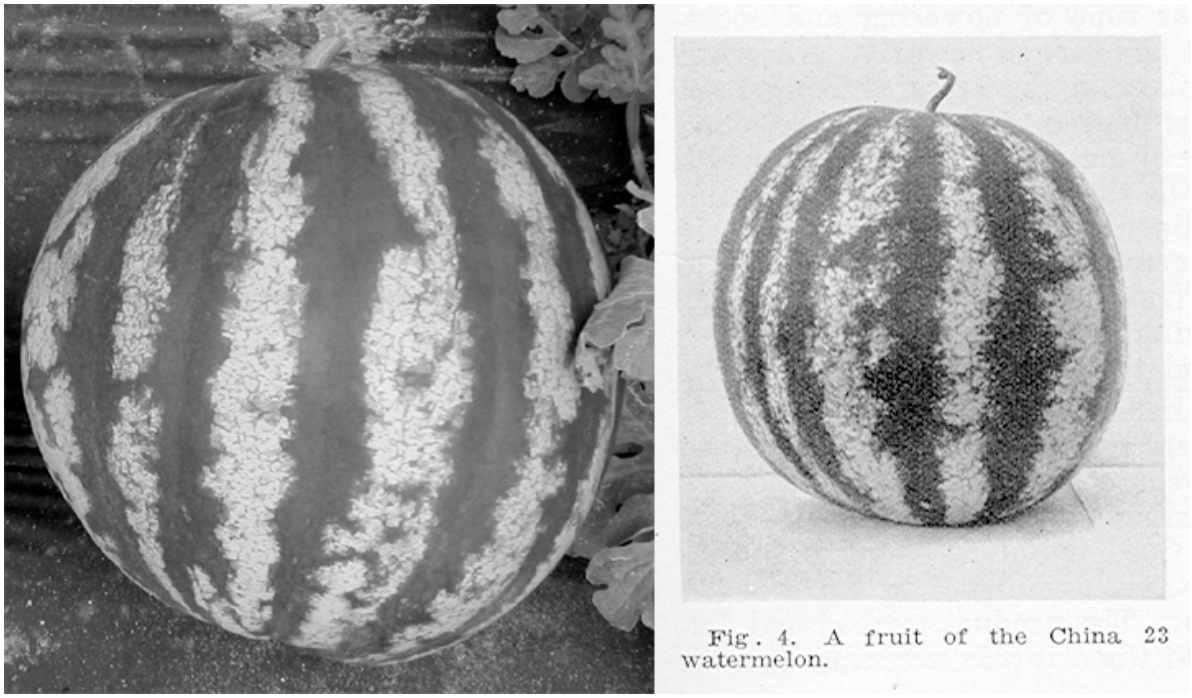

Figure 4. Cultivars Crimson Sweet (fruit on the left) and China 23 (fruit on the right) showing a similar netted phenotype. 'Crimson Sweet, pending verification of the inheritance of the netted phenotype, could be the new type line in substitution of 'China 23' for future genetic studies and breeding work.

CHAPTER TWO

INHERITANCE OF QUALITATIVE FRUIT TRAITS IN WATERMELON

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Abstract

The watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] is a diverse crop, with fruit of different size, shape, rind patterns, and flesh colors. This study measured the inheritance of novel rind phenotypes and verified the genetics of the white, red, salmon yellow and canary yellow flesh colors. For each of eleven crosses, six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) were produced to form eleven families. Each family was tested in 2004 in North Carolina. Phenotypic data were analyzed with the χ^2 method to test the segregation of Mendelian genes. Three new genes were identified: *Scr* for the scarlet red flesh color of 'Dixielee' and 'Red-N-Sweet', *Yb* for the yellow belly (ground-spot) of 'Black Diamond Yellow Belly', and *ins* for the intermittent stripes of 'Navajo Sweet'. The inheritance of the *C* gene for the canary yellow flesh color was verified and a new inbred type line was identified for that gene. Aberrations in the segregation of red, white, and salmon yellow flesh colors were recorded, raising questions on the inheritance of these traits. Finally the spotted phenotype from 'Moon and Stars' was combined with light green and gray rind patterns for the development of novel cultivars with distinctive rind patterns.

Introduction

The watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] has been bred to improve yield, quality, and disease resistance, to diversify fruit and plant type (i.e., seeded vs. seedless fruit, and large vs. dwarf vines), and to adapt useful cultivars to different production areas around the world.

Watermelon breeders have contributed to the development of new cultivars and to the understanding of the genetics of useful traits in this crop. In the United States, many cultivars were released in the late 1800s and early 1900s with adaptation to the western or eastern production areas: e.g., 'Angeleno', 'Chilean', and 'Kleckley Sweet' were popular in California, while 'Florida Favorite' and 'Georgia Rattlesnake' were popular in the southeastern United States (Whitaker and Jagger, 1937).

The first reported genetic studies on watermelon were from the late 1930s and early 1940s and involved the adapted inbred cultivars developed in the previous few decades of watermelon breeding. The emphasis of these investigations was on major traits, such as rind, flesh, and seed-coat colors, fruit shape and

weight, and sex expression (Poole, 1944; Poole and Grimball, 1945; Poole et al., 1941; Porter, 1933; Porter, 1937; Weetman, 1937).

The genetics of the flesh color in watermelon have been studied and genes for the white, red, orange, salmon yellow and canary yellow colors have been reported (Guner and Wehner, 2003). Three alleles were identified at the *y* locus to regulate red, orange, and salmon yellow flesh colors (*Y*, y^o, and *y*, respectively). *Y* was dominant to y^o and *y*. The gene y^o was dominant to *y* (Henderson et al., 1989; Henderson et al., 1998; Poole, 1944; Porter, 1937). The canary yellow color (*C* gene) was dominant to pink (*c*) and epistatic to red (*Y*) (Henderson et al., 1998; Poole, 1944). In addition, the red color was also recorded in individuals homozygous for *C*, due to the expression of the *i-C* gene (inhibitor of canary yellow) (Henderson et al., 1998; Rhodes and Dane, 1999). A third gene for the yellow flesh (*B*) has also been described in a breeding line as hypostatic to the white flesh gene (*Wf*) (Henderson, 1992; Shimotsuma, 1963).

Nevertheless, breeding for specific flesh colors has often been challenging, due to frequent distortion encountered in the inheritance of some of these genes. Watermelon breeders often recovered in their populations recombinant for red and salmon yellow flesh abnormal phenotype. These fruit had differential coloration among portions of the flesh, i.e., having a colored center and white margin, or white flesh with colored blotches within the carpellar remnants.

The inheritance of the *C* gene has been stably confirmed in many crosses and breeding populations. Nevertheless, it has never been verified against the *Wf* gene for the white flesh. In addition, the *C* gene was fixed only in heterozygous condition in the F_1 hybrids 'Yellow Baby' and 'Yellow Doll' and a homozygous type line was not available.

Cultivars with distinctive flesh colors included 'Dixielee' and 'Red-N-Sweet', released in 1979 and 1987. These cultivars had firm scarlet red flesh of a much darker color than usual cultivars. 'Angeleno Black Seeded' was the red (*Y* gene) type line, having a much lighter flesh than the new mutant type. Many current hybrids list in their parentage 'Dixielee' or 'Red-N-Sweet' as sources of genes for the scarlet red color and high quality flesh, but the inheritance of scarlet red has not been studied so far.

The rind (skin) colors and patterns of watermelon fruit have been a major objective of breeding. Watermelon is considered to have a green rind, ranging from light to dark, and from solid to striped (Guner and Wehner, 2003), and the inheritance of these rind types has been studied.

During the 1900s, inbred cultivars with interesting mutations of rind colors have been released by watermelon breeders in the United States and Japan, but their seeds have been lost during the years. 'Japan 6' had inconspicuous and pencilled lines on the rind (*p* allele), 'China 23' had a medium green colored network on the striped rind (*P* allele), and 'Long Iowa Belle' and 'Round Iowa Belle' had randomly-distributed and irregularly-shaped greenish-white spots on a mostly solid dark green rind (*m* gene) (Gusmini and Wehner, 2005). Other interesting mutations have been maintained until now in inbred cultivars.

'Navajo Sweet' has medium green intermittent stripes on a light green rind, becoming more incomplete towards the stem-end of the fruit. 'Black Diamond Yellow Belly' has solid dark green rind with a dark yellow to orange ground spot unlike the usual creamy white ground spot of 'Black Diamond'. The genetics of these two mutations have not been studied. The phenotypes may be useful to breeders interested in the solid dark green rind pattern and ground spot color. The intermittent stripes might be interesting to use on new specialty cultivars, such as the mini type (1.5 to 4.0 kg fruit weight), while the yellow belly could confer more uniformity of the ground spot color to cultivars with dark green rind. The normal color usually varies greatly depending on the position of the fruit on the ground and consumers may interpret this variation as different degrees of maturity of the fruit, while mutant fruit would have a consistently yellow ground spot.

'Moon and Stars' has been a popular novelty cultivar since its release in 1926 (Wehner, 2002), having large yellow areas (moons) and small yellow areas (stars) over a dark green rind. Fruit were elongate, with sweet, red flesh, thick rind, and brown seeds with black speckles in the center of the seed. The word ‘clump’ has been used to describe this seed-coat color, possibly referring to the concentration of black speckles in the center of the seed and their absence along the margins. Following the first release, many selections of 'Moon and Stars' have been released which were different from the original type. Major differences were in the shape of the fruit (round or oval vs. elongate), color of the seed-coat (tan or black or white vs. clump), and color of the flesh (yellow vs. red). In addition, in some cultivars the moons were not present. The single dominant spotted gene (*Sp*) has been identified as controlling that trait (Rhodes, 1986). However, there are no reports of research

on the behavior of the spotted type in a cross with inbreds having gray (white-green or yellow-green) fruit rind pattern.

The objectives of these experiments were to study the inheritance of the new scarlet red flesh trait from 'Dixielee' and 'Red-N-Sweet' crossed with coral red flesh of 'Angeleno Black Seeded'; the inheritance of yellow belly (yellow ground spot) from 'Black Diamond Yellow Belly'; the inheritance of intermittent striped rind pattern in 'Navajo Sweet'; the interaction of canary yellow (NC-517), coral (light) red ('Charleston Gray'), and salmon yellow ('Golden Honey') lines crossed with a white fleshed line ('Cream of Saskatchewan'); and the interaction of spotted and gray or solid light green rind traits in segregating families.

Materials and Methods

Traits and Crosses

A total of 11 families were developed from 11 crosses of watermelon inbred cultivars or lines (Table 1). We developed six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) for each family using the greenhouses at North Carolina State University in Raleigh, North Carolina.

Two scarlet red flesh color parents, 'Dixielee' and 'Red-N-Sweet', were crossed with the coral red 'Angeleno Black Seeded' to study the inheritance of scarlet red flesh color, the darkest red we know of. We also crossed 'Dixielee' and 'Red-N-Sweet' with each other to test for allelism for scarlet red.

'Black Diamond Yellow Belly' was crossed with 'Black Diamond' to study the inheritance of yellow belly, a dark yellow to orange coloration of the ground spot. 'Black Diamond Yellow Belly' had the yellow belly phenotype and 'Black Diamond' had the regular white ground spot, common to most watermelon cultivars.

'Navajo Sweet' has intermittent striped rind (Figure 1), where the usual stripes are just speckles of dark green pigment on the medium green background color. The speckles may become more continuous near the stem-end of the fruit, forming partial stripes extending from the stem-end towards the equator of the fruit. For the study of intermittent stripes, 'Navajo Sweet' was crossed with 'Crimson Sweet', having continuous stripes extending from the stem- to the blossom-end of the fruit.

The *C* gene for the canary yellow flesh phenotype was studied in the cross 'Cream of Saskatchewan' × NC-517. NC-517 was used as an inbred line developed from 'Yellow Baby' and 'Yellow Doll' F1 hybrids. The

two hybrids are the original canary yellow type lines, but we preferred to use an inbred line as the canary yellow parent. 'Cream of Saskatchewan' has white flesh. The white color in this cultivar was less bright than in citrons we have studied, making it difficult to classify white and canary yellow individuals when partially mature. Therefore, at harvest some of the fruit were discarded and only data from fully mature fruit were recorded.

Two crosses were made to study the inheritance of the *y* and *Wf* genes for salmon yellow and white flesh color in watermelon. 'Golden Honey' was homozygous recessive for the *y* gene (salmon yellow flesh). The cross 'Golden Honey' × 'Cream of Saskatchewan' segregated for salmon yellow vs. white flesh. 'Charleston Gray' was homozygous recessive for the *wf* gene (non-white flesh). The cross 'Charleston Gray' × 'Cream of Saskatchewan' segregated for non-white vs. white flesh.

The inheritance of spotted rind and leaves (*Sp* gene) in 'Moon and Stars' was verified in a cross with 'Black Diamond'. 'Moon and Stars' had yellow spots (1 to 5 mm in diameter), called stars, on the dark green rind and on the leaves. Larger spots, called moons, can be present on the fruit of spotted cultivars and their shape and size may vary greatly. The moons were not consistently present on fruit of all the plants of 'Moon and Stars' evaluated, as it is often the case with this cultivar. 'Black Diamond' had dark green rind and leaves free of spots and was considered homozygous for *sp*. The interaction of spotted rind with light green rind was studied in 'Moon and Stars' × 'King and Queen', and the interaction of spotted rind with gray rind was studied in 'Moon and Stars' × 'Charleston Gray'. The presence of spots on rind and leaves was recorded separately, because the light green or gray background colors of the fruit made it difficult to identify the presence of spots on the fruit.

Cultural Practices

Seeds of the six generations for each family were sown in 72-cell polyethylene flats in the greenhouses at North Carolina State University. An artificial soilless growing medium was used (Canadian sphagnum peat moss, perlite, vermiculite, processed pine bark). The flats were moistened to capacity after seeding and held in the greenhouse at constant temperature (25-30 ºC) until full emergence. The transplants were moved to an open cold frame at the field site for acclimation two weeks prior to transplanting. The seedlings were transplanted by hand at the two-true-leaf stage. Missing or damaged transplants were replaced a week after transplanting.

In the field, raised beds were made up with drip irrigation tubes and covered with black polyethylene mulch. The experiment was conducted using horticultural practices recommended by the North Carolina Extension Service (Sanders, 2004). Soil type was an Orangeburg loamy sand at Clinton, and a Norfolk sandy loam at Kinston.

In order to keep families, generations, and plants separate for data collection, each plant was manually trained each week into a spiral shape by turning all the vines in a clockwise circle around the crown until about 70% of the plants in the field set fruit (Fig. 2). The vine training allowed easy tracing of the fruit to the plant that produced it, giving high accuracy to the system.

Experimental Design and Data Analysis

Our field test was run in the summer of 2004 at two locations: Horticultural Crops Research Station in Clinton, North Carolina, and Cunningham Research Station in Kinston, North Carolina. Even though this was a study of Mendelian traits, and replication was not necessary over locations, dividing the families into two sets was a precautionary measure in case of adverse environmental conditions or unpredicted disease epidemics at one location. All six generations of each family were planted at each location. Transplants were placed in rows in the following order and number: P_aS_1, (10), P_bS_1 (10), BC_1P_a, (30) BC_1P_b (30), F_1, (20) F_2 (100) at Clinton and P_aS_1 (10), P_bS_1 (10), F_1 (20), BC_1P_a (30), BC_1P_b (30), F_2 (110) at Kinston. At Clinton each field was 0.4 ha with eight rows 60 m long, and each family occupied four rows. At Kinston each field was 0.4 ha with six rows 85 m long and each family occupied three rows. The fields had raised, shaped beds (rows) on 3.1 m centers with single hills 1.2 m apart.

We analyzed the data by family and then pooled data over families for the same gene after testing for homogeneity of variances using the heterogeneity χ^2 test (Ostle and Malone, 1988; Steel et al., 1997). We performed segregation analysis and goodness-of-fit tests using the SAS-STAT statistical package (SAS Institute, Cary, North Carolina) and the SASGene 1.2 program (Liu et al., 1997), based on χ^2 testing of the expected segregation ratios for a single gene. All χ^2 tests were performed with a 95% confidence level (α=0.05).

Names and symbols for new genes proposed herein are in conformance with gene nomenclature rules for the *Cucurbitaceae* family (Cucurbit Gene List Committee, 1982).

Results and Discussion

Scarlet Red Flesh

The heterogeneity χ^2 for the F_2, BC_1P_a, and BC_1P_b data were non significant (0.01, 0.27, and 0.04, respectively). Therefore, we analyzed the data pooled over families (Table 2). In the F_1 generation, all fruit in the two crosses had scarlet red flesh, demonstrating that the scarlet red flesh color was inherited as a single dominant gene. The pooled F_2 individuals segregated 169:52 (scarlet red:coral red flesh), and the χ^2 was 0.25 (P-value=0.61) showing that the data were consistent with a 3:1 ratio. The fruit in the BC_1P_a generation (P_a having coral red flesh) segregated 1:1 (scarlet:coral red flesh), as expected: the χ^2 was 0.01 (P-value=0.91). The BC_1P_b generation had two fruit with coral red flesh in one cross, but a misclassification due to the maturity of the fruit may have been possible. The pooled χ^2 was 0.04 (P-value=0.83) confirming the 1:0 expected ratio and the misclassification of the two fruit with coral red flesh.

In the test for allelism between the two parents with scarlet red flesh color ('Dixielee' × 'Red-N-Sweet'), all the fruit had scarlet red flesh, thus confirming that the two cultivars have the same allele at this locus. Our results confirmed that the scarlet red flesh phenotype in 'Dixielee' and 'Red-N-Sweet' is controlled by a single dominant gene. We propose naming this new gene *Scarlet red* flesh color, with the symbol *Scr*.

Yellow Belly

In the cross 'Black Diamond Yellow Belly' × 'Black Diamond' only the color of the ground spot segregated, since 'Black Diamond Yellow Belly' is a mutant of 'Black Diamond' differing only for that trait (Table 3). The F_1 generation had all fruit with yellow belly. Therefore, in the next generations we tested the hypothesis that the yellow color was controlled by a single dominant allele. The F_2 plants segregated 91:32 (yellow:white belly), and the χ^2 of 0.07 (P-value=0.79), consistent with our hypothesis. For the backcross to the

homozygous recessive parent the segregation was 27:26 (yellow:white belly) and the χ^2 for the 1:1 expected ratio was 0.02 (P-value=0.89).

The evaluation of the color of the ground spot is difficult if the fruit are not fully mature. At full maturity, however, the wild-type had a creamy white colored belly that could be called white, vs. the mutant type with a dark yellow to orange belly. In addition, the yellow ground spot usually had a shape more regular than the white one.

This test confirmed the hypothesis of a single gene controlling yellow belly in 'Black Diamond Yellow Belly'. We propose naming this new dominant gene *Yellow belly*, with the symbol *Yb*.

Intermittent Stripes

For the intermittent stripe rind pattern, the F_1 generation indicated control by a single recessive gene (Table 4). The F_2 plants segregated 154:53 (continuous:intermittent stripes), and the χ^2 was 0.04 (P-value=0.84). The plants in the BC_1P_a generation (P_a having intermittent stripes) segregated as expected with a 1:1 ratio and a χ^2 of 0.27 (P-value=0.60). Our hypothesis testing confirmed that the intermittent stripes in 'Navajo Sweet' were controlled by a single recessive gene. We propose naming this new gene *intermittent stripes*, with the symbol *ins*.

Canary Yellow Flesh

In the F_1 generation, all 34 fruit had canary yellow flesh (Table 5). The F_2 segregated 135:49 canary yellow:white flesh, and the χ^2 was 0.26 (P-value=0.60) showing that the data were consistent with a 3:1 expectation. The fruit in the BC_1P_a generation (P_a having white flesh) segregated as expected with a 1:1 ratio, and the χ^2 was 0.27 (P-value=0.60).

The experiment confirmed that the *C* gene for canary yellow flesh color was inherited as a single dominant gene (Henderson et al., 1998; Poole, 1944). Originally, this gene was identified in 'Yellow Baby' and 'Yellow Doll', both F_1 hybrids, since it was not available in an inbred line. We propose the type line NC-517 be used in the future, an inbred developed from 'Yellow Baby' and 'Yellow Doll', and having the *C* gene.

Salmon Yellow and Red Flesh

During the development of salmon yellow and red flesh cultivars, we recovered in the segregating populations some unexpected phenotypes. For example, some fruit had a colored center and a white margin, or patches of color in a generally white flesh, or colored flesh with white carpel walls, or other intermediate combinations. Similar phenotypes were recovered in this study as well. In both families, the parental fruit were uniform for the expected flesh color. The F_1 and the F_1-derived generations, instead, had the unexpected colors described above. We did not observe any Mendelian segregation pattern for flesh color in the progenies of the crosses 'Golden Honey' × 'Cream of Saskatchewan' and 'Charleston Gray' × 'Cream of Saskatchewan' (Figure 5).

The separate rating of the color of different parts of the flesh could be an alternative approach to gather more information on the genetics of the color of different portions of the fruit. The portions of the fruit to be rated should include: 1) the endocarp between the carpel walls and the mesocarp (white rind); 2) the flesh within the carpels, originating from the stylar column; and 3) the carpel walls, between (1) and (2).

One possible hypothesis to explain the presence of the abnormal types is that the expression of the pigment is caused by several different regulatory genes, one for each portion of the fruit. Thus, the mixed colorations would have been caused by recombination of these genes. Alternatively, tissue-specific expression of one of the regulatory genes in the pigmentation pathway may be the cause of the coloration patterns encountered.

Finally, the red and salmon yellow flesh colors in watermelon could be quantitative rather than qualitative traits. In our experiments and in our breeding work, we have not observed a clear quantitative distribution of pigmentation from one parental type to the other, the abnormal phenotype being intermediate levels. Should this trait be quantitative, the variation would be measurable for the intensity of the flesh color, rather than presence or absence of the pigments in different portions of the fruit.

Spotted Rind

The presence of spots on leaves (called stars) in crosses of 'Moon and Stars' with other three cultivars having solid green leaves segregated as expected under the control of the *Sp* gene (Poole, 1944; Rhodes, 1986).

The heterogeneity χ^2 for the F_2 and BC_1P_b data were non significant for the null hypothesis (2.39 and 0.16, respectively) (Table 6). In the F_1 generation, all plants in the three crosses had spotted leaves. The pooled F_2 segregated 253:84 spotted:solid green leaves, and the χ^2 was 0.01 (P-value=0.87) showing that the data were consistent with a 3:1 expectation. The leaves in the BC_1P_b generation (P_b having solid green leaves) segregated as expected, and the χ^2 for the 1:1 expectation was 0.15 (P-value=0.70).

Although linkage analysis confirmed that the spotted phenotype of the rind in the same crosses was also determined by the *Sp* gene (no significant recombination detected in all three families), the single gene hypothesis could not be confirmed using fruit data from the cross 'Moon and Stars' × 'King and Queen'. The identification of yellow spots on the light green background from 'King and Queen' was inaccurate, since the spots blended in with the background color. Therefore, distorted segregation ratios were measured for this family (Table 6). Nevertheless, pooled data from the other two crosses for the rind phenotype (spotted vs. normal) confirmed the expected segregation of the *Sp* gene. Green canopy segregated consistently with the inheritance of the previously named *Sp* gene. The heterogeneity χ^2 for the F_2 and BC_1P_b fruit data were 2.01 and 0.14, respectively (Table 6). In the pooled F_1 generation, all 69 plants had spotted fruit. The pooled F_2 segregated consistently with a 3:1 ratio, counting 181:63 spotted:normal fruit and a χ^2 of 0.09 (P-value=0.76). The fruit in the BC_1P_b generation (P_b having normal rind) segregated as expected with a χ^2 of 0.14 (P-value=0.70).

We concluded that the *Spotted* gene from 'Moon and Stars' produced the spotted phenotype in leaves and fruit of cultivars with solid dark green ('Black Diamond'), solid light green ('King and Queen'), and gray rind ('Charleston Gray') patterns. Nevertheless, the spotted type was inconsistently visible on the solid light green background, due to the lack of contrast between the two colors.

In this study, we observed the presence of F_2 and BC_1 plants with striped rind, thus suggesting that the dark green background of 'Moon and Stars' was generated by dark green stripes on a dark green background (data not shown). The segregation of the spotted gene on this fruit showed the potential for transferring the spotted type also to striped watermelons, where the spots would cover both the dark green stripes and the medium green background (Fig. 3).

During our experiments we did not find a consistent presence of the moons on the parental inbred 'Moon and Stars'. We observed moons a few centimeters in diameter to covering up to one third of the fruit surface. Moons were observed in the parental generation of the three crosses only in 21 of 57 P_aS_1 plants tested. On some fruit with moons we observed that stars close to the border of a moon tend to coalesce with the moon (Fig. 4). Based on this observation, and the random distribution of the stars on the fruit, we suggest that a moon was the result of a random event of coalescence of several stars. Alternatively, the moons could be larger spots caused by differential expression of the *Sp* gene, or moons could be controlled by a different gene. However, a separate gene for moons would not explain their inconsistent presence in the inbred parent having the spotted trait.

Conclusions

With these genetic studies we identified three new genes in watermelon. *Scarlet red flesh* (*Scr*) produced a higher intensity red color in the flesh of 'Dixielee' and 'Red-N-Sweet' compared to 'Angeleno Black Seeded', the type line for red flesh color in watermelon (Porter, 1937). *Scr* was inherited as a single dominant gene. *Yellow belly* (*Yb*) was classified as a single dominant gene changing the color of the ground spot in 'Black Diamond' from creamy white to dark yellow. The presence of intermittent vs. continuous stripes on the rind of 'Navajo Sweet' was explained by the action of a single recessive gene that we named *intermittent stripes* (*ins*), with the dominant allele present in 'Crimson Sweet'.

We confirmed the inheritance of the *C* gene for the canary yellow flesh as a single dominant gene. The inbred line NC-517, the canary yellow parent in our study, should be considered the homozygous public type line for the *C* gene, rather than the canary yellow F_1 hybrids available so far.

'Dixielee' and 'Red-N-Sweet', 'Black Diamond Yellow Belly', 'Navajo Sweet', and NC-517 will be kept in the gene mutant collection as type lines for the *Scr*, *Yb*, *ins*, and *C* genes, respectively, by the watermelon gene curators for the Cucurbit Genetics Cooperative (T.C. Wehner and S.R. King).

Our study highlighted a complex genetic background for the inheritance of red and salmon yellow flesh colors, previously attributed solely to the expression of the *Wf* and *y* genes. Based on our observations, we

discarded the hypothesis of a quantitative type of inheritance and suggested that different genes, or a tissue-specific expression of the same genes, might be involved in the pigmentation of different portions of the fruit.

A new possibility for the development of watermelon fruit with novel rind type results from our verification of the inheritance of the spotted phenotype (*Sp* gene) when transferred from 'Moon and Stars' to cultivars with gray and light green background. The spotted trait was more evident on the gray background, while it was undetectable on many fruit with light green rind. In addition, the presence of the small spots (stars) showed to have a random distribution on fruit and leaves, while the presence of large yellow blotches (moons) was inconsistent and may be determined by the coalescence of many stars. Moons were present only on fruit, even though in a single instance, large, irregular yellow areas were observed on the foliage.

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Table 1. Crosses and traits analyzed for qualitative inheritance of phenotypic traits in watermelon fruit during summer 2004 in Clinton and Kinston, North Carolina.[z]

	Trait of interest	
Cross (or Family)	Phenotype	Gene
Study of unknown genes		
'Angeleno Black Seeded' × 'Dixielee'	Scarlet red flesh color	*Scr*
'Angeleno Black Seeded' × 'Red-N-Sweet'	Scarlet red flesh color	*Scr*
'Dixielee' × 'Red-N-Sweet'	Allelism of scarlet red flesh color	*Scr*
'Black Diamond Yellow Belly' × 'Black Diamond'	Yellow belly (ground spot)	*Yb*
'Navajo Sweet' × 'Crimson Sweet'	Intermittent stripes	*ins*
Verification of known genes		
'Cream of Saskatchewan' × NC-517	Canary yellow flesh color	*C*,
'Golden Honey' × 'Cream of Saskatchewan'	Salmon yellow flesh color	*y*
'Charleston Gray' × 'Cream of Saskatchewan'	White flesh color	*Wf*
'Moon and Stars' × 'Black Diamond'	Spotted dark green rind	*Sp*
'Moon and Stars' × 'King and Queen'	Spotted light green rind	*Sp*
'Moon and Stars' × 'Charleston Gray'	Spotted gray rind	*Sp*

z Six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) for each family were developed using the greenhouses at North Carolina State University in Raleigh, North Carolina

Table 2. Single locus goodness-of-fit-test for scarlet red flesh color in watermelon.[z]

Generation	Total	Scarlet red[y]	Coral red[x]	Expected[w]	χ^2	df	P-value
'Angeleno Black Seeded' × 'Dixielee'							
P_aS_1[v]	20	0	20				
P_bS_1[u]	20	20	0				
F_1	40	40	0				
F_2	116	89	27	3:1	0.18	1	0.66
BC_1P_a	44	23	21	1:1	0.09	1	0.76
BC_1P_b	49	49	0				
'Angeleno Black Seeded' × 'Red-N-Sweet'							
P_aS_1[v]	20	0	20				
P_bS_1[u]	20	20	0				
F_1	33	33	0				
F_2	105	80	25	3:1	0.08	1	0.77
BC_1P_a	47	22	25	1:1	0.19	1	0.66
BC_1P_b	49	47	2	1:0	0.04	1	0.83

Table 2. Continued. [z]

Generation	Total	Scarlet red [y]	Coral red [x]	Expected [w]	χ^2	df	P-value
Pooled							
P_aS_1 [v]	40	0	40				
P_bS_1 [u]	40	40	0				
F_1	73	73	0				
F_2	221	169	52	3:1	0.25 [t]	1	0.61
BC_1P_a	91	45	46	1:1	0.01 [t]	1	0.91
BC_1P_b	98	96	2	1:0	0.04 [t]	1	0.83

z Data are ratings from two families of *Citrullus lanatus* var. *lanatus*: 'Angeleno Black Seeded' × 'Dixielee' and 'Angeleno Black Seeded' × 'Red-N-Sweet; data are presented by family and pooled over families

y Scarlet red was the standard red flesh color

x Coral red was the mutant red flesh color

w Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

v P_a was the carrier of the recessive gene (coral red)

u P_b was the carrier of the dominant gene (scarlet red)

t Heterogeneity $\chi^2_{(0.05;\,1)}$: $F_2 = 0.01$, $BC_1P_a = 0.27$, $BC_1P_b = 0.0$

Table 3. Single locus goodness-of-fit-test for yellow belly color in watermelon.[z]

Generation	Total	Yellow[y]	White[x]	Expected[w]	χ^2	df	P-value
'Black Diamond Yellow Belly' × 'Black Diamond'							
P_aS_1[v]	17	17	0				
P_bS_1[u]	20	0	20				
F_1	9	9	0				
F_2	123	91	32	3:1	0.07	1	0.79
BC_1P_a	22	22	0				
BC_1P_b	53	27	26	1:1	0.02	1	0.89

z Data are ratings from one family of *Citrullus lanatus* var. *lanatus*: 'Black Diamond Yellow Belly' × 'Black Diamond'

y Yellow was the mutant belly color

x White was the standard belly color

w Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

v P_a was the carrier of the dominant gene (yellow belly)

u P_b was the carrier of the recessive gene (white belly)

Table 4. Single locus goodness-of-fit-test for intermittent stripes in watermelon.[z]

Generation	Total	Continuous[y]	Intermittent[x]	Expected[w]	χ^2	df	P-value
'Navajo Sweet' × 'Crimson Sweet'							
P_aS_1[v]	20	0	20				
P_bS_1[u]	20	20	0				
F_1	34	34	0				
F_2	207	154	53	3:1	0.04	1	0.84
BC_1P_a	60	32	28	1:1	0.27	1	0.60
BC_1P_b	58	58	0				

z Data are ratings from one family of *Citrullus lanatus* var. *lanatus*: 'Navajo Sweet' × 'Crimson Sweet'

y Continuous was the standard stripe type

x Intermittent was the mutant stripe type

w Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

v P_a was the carrier of the recessive gene (intermittent stripes)

u P_b was the carrier of the dominant gene (continuous stripes)

Table 5. Single locus goodness-of-fit-test for canary yellow flesh color in watermelon.[z]

Generation	Total	Canary[y]	White[x]	Expected[w]	χ^2	df	P-value
'Cream of Saskatchewan' × NC-517							
P_aS_1[v]	20	0	20				
P_bS_1[u]	20	20	0				
F_1	34	34	0				
F_2	184	135	49	3:1	0.26	1	0.60
BC_1P_a	56	27	29	1:1	0.27	1	0.60
BC_1P_b	51	51	0				

z Data are ratings from one family of *Citrullus lanatus* var. *lanatus*: 'Cream of Saskatchewan' × NC-517

y Canary was the mutant flesh color

x White was the standard flesh color

w Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

v P_a was the carrier of the recessive gene (canary yellow flesh)

u P_b was the carrier of the dominant gene (white flesh)

Table 6. Single locus goodness-of-fit-test for spotted rind and leaves in watermelon.[z]

Generation	Total	Spotted[y]	Normal[x]	Expected[w]	χ^2	df	P-value
'Moon and Stars' × 'Black Diamond' - *Leaf and rind rating*							
P_aS_1[v]	20	20	0				
P_bS_1[u]	20	0	20				
F_1	34	34	0				
F_2	203	147	56	3:1	0.72	1	0.39
BC_1P_a	54	54	0				
BC_1P_b	56	26	30	1:1	0.29	1	0.59
'Moon and Stars' × 'Charleston Gray' - *Leaf and rind rating*							
P_aS_1[v]	17	17	0				
P_bS_1[u]	20	2	20				
F_1	35	35	0				
F_2	41	34	7	3:1	1.37	1	0.24
BC_1P_a	52	52	0				
BC_1P_b	56	28	28	1:1	0.00	1	1.00
'Moon and Stars' × 'King and Queen' - *Leaf rating*							
P_aS_1[v]	20	20	0				
P_bS_1[u]	20	0	20				
F_1	34	34	0				
F_2	93	72	21	3:1	0.29	1	0.59
BC_1P_a	57	57	0				
BC_1P_b	59	29	30	1:1	0.02	1	0.89

Table 6. Continued. [z]

Generation	Total	Spotted [y]	Normal [x]	Expected [w]	χ^2	df	P-value
'Moon and Stars' × 'King and Queen' - *Rind rating*							
P_aS_1 [v]	20	20	0				
P_bS_1 [u]	20	0	20				
F_1	34	34	0				
F_2	93	55	38	3:1	12.48	1	0.00
BC_1P_a	57	57	0				
BC_1P_b	59	19	40	1:1	7.47	1	0.01
Pooled - *Leaves rating*							
P_aS_1 [v]	57	57	0				
P_bS_1 [u]	60	0	60				
F_1	103	103	0				
F_2	337	253	84	3:1	0.01 [t]	1	0.87
BC_1P_a	163	163	0				
BC_1P_b	171	83	88	1:1	0.15 [t]	1	0.70
Pooled - *Rind rating*							
P_aS_1 [v]	37	37	0				
P_bS_1 [u]	40	0	40				
F_1	69	69	0				
F_2	244	181	63	3:1	0.09 [s]	1	0.76
BC_1P_a	106	106	0				
BC_1P_b	112	54	58	1:1	0.14 [s]	1	0.70

Table 6. Continued. [z]

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z Data are ratings from three families of *Citrullus lanatus* var. *lanatus*: 'Moon and Stars' × 'Black Diamond', 'Moon and Stars' × 'King and Queen', and 'Moon and Stars' × 'Charleston Gray'; data are presented by family and pooled over families, where the segregation confirmed the single gene hypothesis

y Spotted was the mutant rind and leaf type

x Normal was the standard rind and leaf type

w Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

v P_a was the carrier of the dominant gene (spotted phenotype)

u P_b was the carrier of the recessive gene (uniform phenotype)

t Heterogeneity $\chi^2_{(0.05;\,1)}$: $F_2 = 2.39$, $BC_1P_b = 0.16$

s Heterogeneity $\chi^2_{(0.05;\,1)}$: $F_2 = 2.01$, $BC_1P_b = 0.14$

Figure 1. Intermittent stripes in 'Navajo Sweet' (fruit on the left) and continuous stripes in 'Crimson Sweet' (fruit on the right).

Figure 2. In order to keep families, generations, and plants separate for data collection, each plant was manually trained each week into a spiral shape by turning all the vines in a clockwise circle around the crown until about 70% of the plants in the field set fruit.

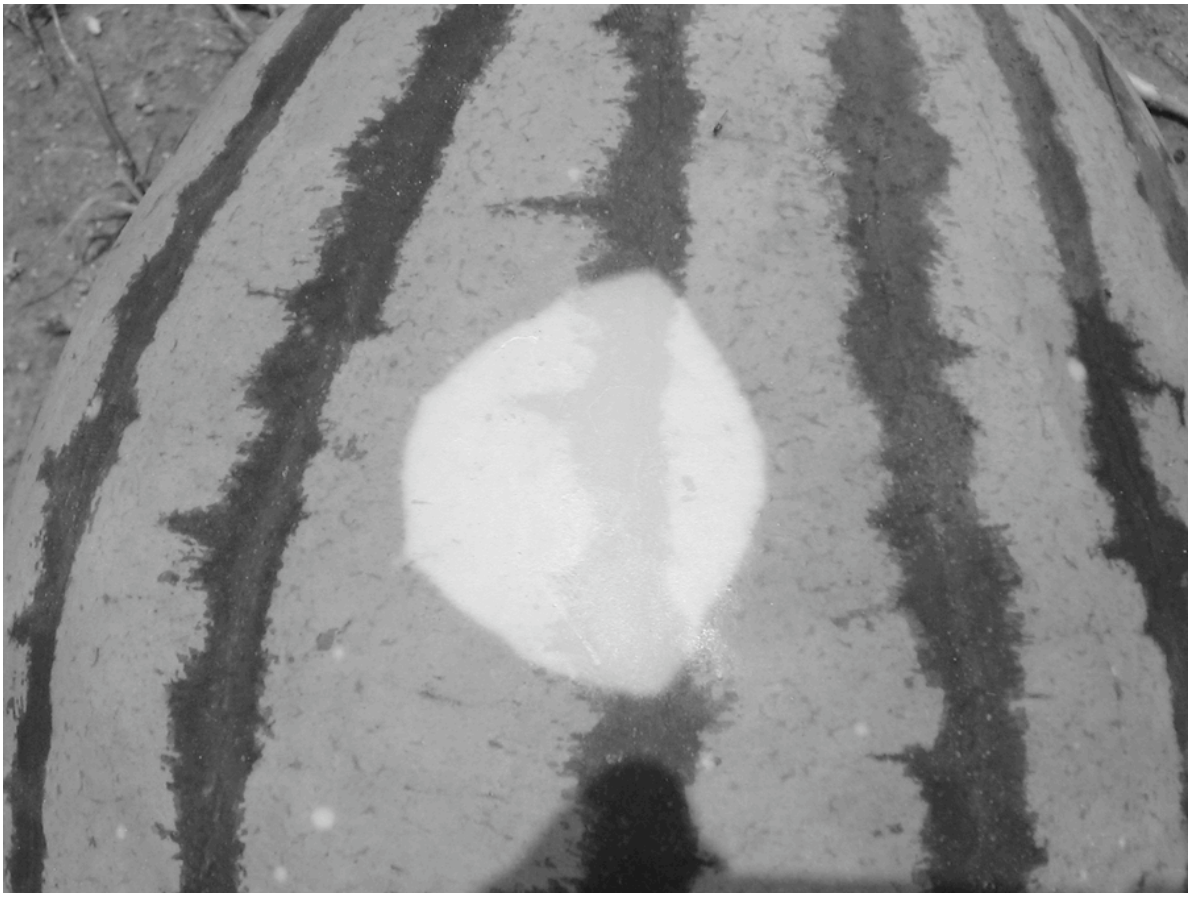

Figure 3. Moons and stars induced on watermelon by the *Sp* gene may be recovered on striped fruit, overlapping both dark green stripes and light green background.

Figure 4. Stars (small yellow spots) close to the border of a moon (large yellow spot) tend to coalesce with the moon in 'Moon and Stars' and in watermelon progenies segregating for the *Sp* gene.

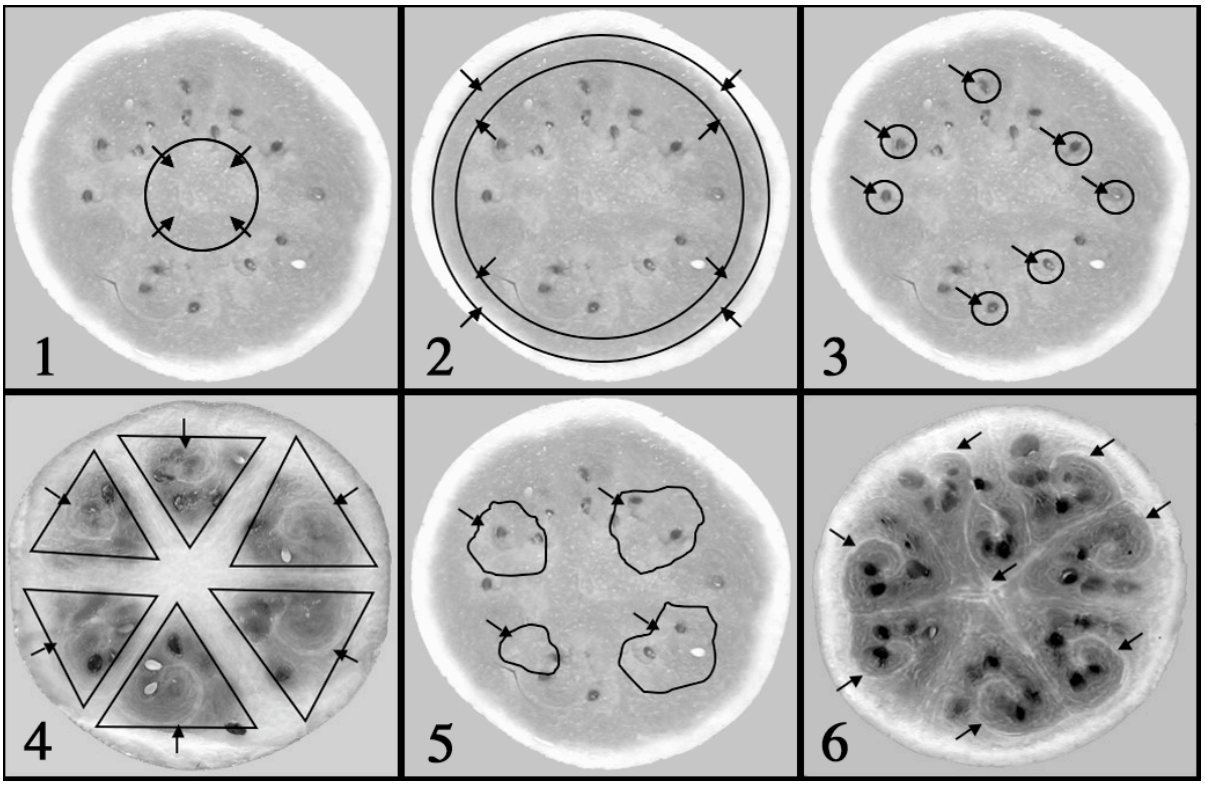

Figure 5. Examples of unexpected flesh colors in the progenies of the crosses 'Golden Honey' × 'Cream of Saskatchewan' and 'Charleston Gray' × 'Cream of Saskatchewan'. Arrows indicate areas of differential coloration of the flesh.

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CHAPTER THREE

FOUNDATIONS OF YIELD IMPROVEMENT IN WATERMELON

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Abstract

High yield is a major goal for watermelon breeders. The objective of this study were to measure yield in a diverse set of watermelon cultivars to identify high yielding germplasm for use in breeding programs. Phenotypic variation for fruit yield in a diverse set of 80 watermelon cultivars (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) was studied in the field in North Carolina. Yield was evaluated in replicated experiments of three environments (combinations of two years and two locations), and two to four replications per environment. Plots were harvested one to two times, depending on the average maturity of the fruits at the first harvest. The highest yield overall was obtained from 'Mountain Hoosier' and from 'Starbrite' among the modern elite hybrids. Some of the new, elite hybrid cultivars were in the top yielding group, but there were old, inbred cultivars in the top group as well. Consistent and significant yield differences among the 80 cultivars across environments suggests genetic variation for the trait. In addition, high yielding cultivars for use in breeding programs were identified. Watermelon breeders interested in developing new, high yielding cultivars should make use of top performers in this study in their breeding programs.

Introduction

High yield is a major goal for watermelon breeders (Mohr, 1986). Earlier efforts in watermelon breeding involved development of new cultivars of different types with good quality and early maturity in the late 1800s. By 1900 'Angeleno', 'Chilean', 'Florida Favorite', 'Georgia Rattlesnake', 'Cole Early', 'Kleckley Sweet', and other open pollinated cultivars had been on the market for many years (Whitaker and Jagger, 1937). In the 20th century, high yielding cultivars became a major goal for public and private breeders. Hybrids were popular among private breeders for protection of intellectual property and because of the results of many studies, mainly in the 1950s and 1960s, showing heterosis in watermelon. The studies measured heterosis as well as general (GCA) and specific (SCA) combining ability in watermelon (Brar and Sidhu, 1977; Brar and Sukhija, 1977; Nandpuri et al., 1974; Nandpuri et al., 1975; Sidhu and Brar, 1977; Sidhu and Brar, 1985; Sidhu et al., 1977a; Sidhu et al., 1977b). Major problems with those studies were that heterosis was inconsistent over experiments, and that results were based on diallel or top crosses of elite inbreds, not on a random set of lines from a population. More recent studies of the effects of reciprocal crosses on yield components in watermelon

have been contradictory (Gill and Kumar, 1988; Rajendran and Thamburaj, 1993; Sachan and Nath, 1976). Often, the experiments included only a small number (N_{max}=10) of non-randomly chosen elite cultivars as parents, so the results are valid only for those specific crosses and are not generally applicable.

Taken as a group, the studies indicate the presence of heterosis in watermelon and the importance of GCA in the choice of parents for hybrid production. Ferreira et al. (Ferreira et al., 2002) substantiated these conclusions testing seven intercrossing populations with evaluation of reciprocal crosses. There were significant GCA, SCA, and reciprocal combination effects, along with additive effects for all yield traits, except for the number of days to first female flower and number of seeds per fruit. A second study evaluated GCA and SCA for tetraploid females crossed with diploid males for the production of triploid seeds (Souza et al., 2002). This study confirmed a higher magnitude of GCA effects than SCA effects and strong additive effects for yield components, except for earliness and some qualitative indexes (i.e. hollowheart incidence). Today, watermelon breeders are less interested in studying heterotic effects and combining ability as reasons to prefer hybrids to inbreds for cultivar release. Hybrids have proven their advantage for protection of valuable parent lines. Furthermore, seedless cultivars are in high demand and can be produced only as triploid hybrids. However, in the future it might be possible to develop transgenic diploid seedless watermelons. In that case, the question of the advantage in using heterotic hybrids vs. inbred cultivars will still be important.

Overall, watermelon yield in the United States has been increasing during the last four years from 24 Mg ha^{-1} in 1998 to 29 Mg ha^{-1} in 2002 (USDA-ARS, 2003). Part of the increase in yield might be due to more reliable production practices and to the availability of more effective pesticides (Maynard, 2001). The impact of environmental factors such as irrigation or general water availability on yield was important in contrasting inbred cultivars vs. hybrids in Florida in 1985. The hybrids outyielded inbred cultivars only in irrigated fields, while in dry conditions yield was the same for both groups, although fruit quality was higher among the inbred cultivars (Rhodes, 1985).

Many watermelon yield trials are run each year in the United States and few differences among the experimental entries in the trial are usually observed. Our question was whether that was due to a lack of genetic variation for yield in the crop species, or a lack of genetic variation for yield among the new experimental entries being tested. Genetic diversity among currently grown watermelon cultivars in the United

States appears to be narrow, with many derived from 'Allsweet'. Therefore, a diverse set of obsolete inbred cultivars that do not trace to 'Allsweet' and that represents as wide an array of cultivars as possible, were included in this study.

The objective of this study was to measure yield in a diverse set of watermelon cultivars. In addition, we were interested in identifying high yielding cultivars for use in breeding programs.

Materials and Methods

The experiment was conducted at the Horticultural Crops Research Station at Clinton, North Carolina (2001 and 2002) and at the Cunningham Research Station at Kinston, North Carolina (2002). The experiment at Clinton was a randomized complete block with four replications, 80 cultivars, and two years. At Kinston the experiment had two replications and 80 cultivars. All 80 cultivars were evaluated for all traits, except 'Weeks NC Giant' which had a low emergence rate at Clinton in 2001.

Field rows were direct seeded on raised, shaped beds on 3.1 m centers. Plots were 3.7 m long, with 0.6 m between hills, and 2.5 m alleys at each end of the plot. At Kinston, rows were covered with black polyethylene mulch and drip irrigated. The experiment was conducted using horticultural practices recommended to the growers by the North Carolina Extension Service (Sanders, 2001). Soil type at Clinton was an Orangeburg loamy sand (Fine-loamy, kaolinitic, thermic Typic Kandiudults). Soil type at Kinston was a Norfolk sandy loam (Fine-loamy, kaolinitic, thermic Typic Kandiudults).

Field preparation at Clinton included the soil incorporation of a 10-8.3-4.4 (N-P-K) fertilizer applied at 561 kg ha^{-1}. Fertilizer application for the remainder of the growing season consisted of 224 kg ha^{-1} of 13.5-0-19.8 (N-P-K) and 112 kg ha^{-1} of calcium along with 15.5-0-0 (N-P-K). Kinston field preparation included soil incorporation of a 10-16.6-8.8 (N-P-K) fertilizer applied at 336 kg ha^{-1} and the fumigant Telone C-17 (1,3-Dichloropropene + chloropicrin) applied at a rate of 60 L ha^{-1}. At transplanting, 20-16.6-8.8 (N-P-K) fertilizer was applied (less than 5.6 kg ha^{-1} or a water diluted equivalent of a 1-0.83-0.44 (N-P-K) fertilizer). The differences in cultural practices between the two locations reflected the two most common production systems adopted by watermelon growers, bare ground and overhead irrigation vs. black polyethylene mulch and drip irrigation.

A total of 80 cultivars were evaluated for fruit yield and quality. There were 72 obsolete cultivars obtained from seed companies, the Seed Savers exchange, and the National Seed Storage Laboratory (Fort Collins, Colorado). Eight elite hybrid cultivars were included as checks ('Starbrite', 'Stars-N-Stripes', 'Legacy', 'Sangria, 'Fiesta', 'Sultan', 'Regency', and 'Royal Flush').

Plots were harvested twice (26 July and 9 August) at Clinton in 2001, once (25 July) at Clinton in 2002, and twice (23 July and 6 August) at Kinston in 2002 for fruit yield and quality measurements. Fruit were determined to be ripe by looking for a dried tendril nearest the fruit, a light colored ground spot, and a dull sound of the fruit when thumped (Maynard, 2001). In addition, the sugar content of a test sample was measured at harvest. Individual cull and marketable fruit were weighed to the nearest pound for each plot. Numbers of cull and marketable fruit were also recorded. Yield was calculated as total and marketable weight (Mg ha^{-1}) and number (th ha^{-1}) of fruit by summing plot yields over the harvests.

Measurements of fruit quality were fruit length and diameter, hollowheart, rind pattern, flesh color, and soluble solids. Quality evaluations were not a major focus in this study, but meant to better describe the cultivars for future breeding efforts. Therefore, quality data were recorded only in Clinton in 2001. Fruit length and diameter were measured in millimeters. The total number of fruit with hollowheart were counted and the width of the defect was recorded in millimeters. Rind pattern was evaluated using a scale of 0-9 (0=special rind patterns of solid light green, irregular striping, or yellow spotting; 1=gray; 2-3=narrow stripe; 4–6=medium stripe; 7-8=wide stripe; 9= solid dark green). The stripes were considered to be the dark green area over a light or medium green background (Maynard, 2001). Flesh color was noted as red, orange, salmon yellow, or canary yellow. Soluble solids were measured in °brix using a refractometer that was dipped three times into the flesh in the center of the fruit.

Data were analyzed using the MEANS, CORR, and GLM procedures of SAS-STAT Statistical Software Package (SAS Institute, Cary, NC). We measured repeatability of ranking among replications at the same location and in the same year by comparing the rankings for each replication with the others. We also recorded the frequency of ranking in the top 20% for each cultivar in each replication as an indicator of variability. The analysis of variance was performed on a balanced dataset including three year-location combinations referred to as environments (Clinton 2001, Clinton 2002, Kinston 2002). Datasets were balanced

by using only two replications from Clinton, which had four rather than two. The analysis was performed after discarding different replications to determine whether there was a significant effect; there was none. The regression model used was Y = Environment + [Replication (Environment)] + Cultivar + (Cultivar × Environment) + Error. The term [Replication (Environment)] was used as the error to perform the F-test on Environment. The analysis of variance was not performed on quality traits measured in only one environment (length : diameter ratio and hollowheart percentage).

Results and Discussion

The analysis of variance (Table 1) showed a large and significant effect of environment for all traits (total weight, total fruit number, fruit size, and soluble solids), except for percentage of marketable weight. The large environment effect was expected in our experiment due to the different cultural practices, which resulted in higher weed incidence with bare ground and overhead irrigation vs. polyethylene mulch and drip irrigation. Watermelon yield is reduced by the presence of some species of weeds in the field (Maynard, 2001). In 2002, mean yield at Kinston was 110 Mg ha^{-1} vs. 61 Mg ha^{-1} at Clinton. Cultivation on bare ground at Clinton also promoted a higher growth rate of the fruit that were on average 0.45 kg heavier than at Kinston. Nevertheless, the Range/LSD ratio was similar for the two locations (4.9 at Clinton and 5.1 at Kinston), indicating that the test at both locations was effective in separating the cultivars for average fruit size. The Clinton fields were intended to simulate growers using bare ground and overhead irrigation, while the Kinston location simulated growers using black polyethylene mulch and drip irrigation, both common production systems worldwide for this crop. Our interest was more generally to determine the possibility of improving yield in watermelon regardless of cultural practices.

In the analysis of variance, replications within environment had a large effect only on total fruit weight. For all parameters, the repeatability index for ranking indicated consistency of performance of the cultivars across replications in each environment.

The effect of the cultivars tested was strong and significant for all traits, indicating the useful parents for improvement of yield in watermelon. Furthermore, cultivar by environment interactions were small and

mostly non-significant, indicating that cultivars ranked similarly in the three environments, and permitting the use of the mean over environments for cultivar summaries.

The correlations of total vs. marketable fruit weight, total vs. marketable fruit number, percentage marketable fruit number vs. weight, and total vs. marketable average fruit weight were high and significant. Therefore, among these traits only total yield, percentage marketable weight, and average marketable fruit weight are presented (Table 2).

The highest yielders (Table 2) were the inbreds 'Mountain Hoosier', 'Hopi Red Flesh', 'Early Arizona', 'Stone Mountain', 'AU-Jubilant', 'Sweetheart', 'Calhoun Gray', 'Big Crimson', 'Moon and Stars', 'Cole Early', 'Yellow Crimson', and 'Blacklee', and the F_1 hybrids 'Legacy', 'Starbrite', and 'Stars-N-Stripes'. These high yielders included cultivars producing an intermediate number of fruit of medium size, except 'Early Arizona', 'Stone Mountain', 'Sweetheart', and 'Cole Early', which had small size fruit. Small fruit were those weighting 6-9 kg, and medium were those weighing 9-12 kg. However, the correlation between total weight and single fruit weight was low (r=0.13, P-value=0.0002).

In general, the cultivars had high fruit quality, with 81 to 99 % marketable fruit (except for 'Weeks North Carolina Giant', with 69%). Hollowheart was unacceptably high in 'Hopi Red Flesh', with 55% of the fruit affected. However, hollowheart incidence was recorded only at Clinton in 2001 and, would probably be lower in most years. Old cultivars often have hollowheart, possibly because they are not adapted to the modern fertilization and irrigation regimes (Maynard, 2001). Also, rainy, hot and humid conditions at Clinton favored hollowheart formation in that year. 'Klondike Striped Blue Ribbon' was developed in California and had 38% hollowheart. 'Florida Favorite' was introduced by Girardeau in 1887 from a cross between 'Pierson' and 'Georgia Rattlesnake' (Whitaker and Jagger, 1937) with adaptation to the Southeast, and had only 9% hollowheart. Its parent 'Georgia Rattlesnake' had 0% hollowheart.

For soluble solids content, the highest yielding group of cultivars represented a wide range of sweetness, with 7.1 to 11.2 °brix. Soluble solids content was not correlated with total weight (r=0.09, P-value<0.01) or total number of fruit (r=0.01, P-value=0.85). Soluble solids content was similar at the two locations, with a mean of 10.0 at Clinton (σ=1.26) and 10.9 at Kinston (σ=0.99). An intermediate correlation was observed between cultivar means by location (r=0.67, P-value=0.0001) and by year at Clinton (r=0.62, P-

value=0.0001), indicating that cultivars changed rank somewhat for soluble solids content. A similar scenario was recorded in comparison by year at Clinton, with a mean of 10.0 in 2001 (σ=1.19) and 10.0 in 2002 (σ=1.32).

The highest yielding cultivars ranged in fruit shape from round to elongate, making it easier for plant breeders to develop high yielding cultivars having a particular fruit type. The length/diameter ratio measured on a vertical section of the fruit (L/D) ranged between 1.1 and 2.3. 'Yellow Crimson' was a high yielder in the yellow flesh group, but the color was salmon yellow (*y* gene) rather than canary yellow (*C* gene), the preferred color because of its improved appearance. The top yielders can be grouped by rind pattern for use by plant breeders interested in developing high yield with parents close to the target fruit type as follows. Dark solid cultivars were 'Mountain Hoosier', 'Hopi Red Flesh', 'Early Arizona', and 'Blacklee'. Gray cultivars were 'Sweetheart' and 'Calhoun Gray'. Striped cultivars were 'Stone Mountain', 'Stars-N-Stripes', 'Legacy', 'Yellow Crimson', 'Starbrite', and 'Big Crimson'. The only spotted cultivar was high yielding as well: 'Moon and Stars'. Surprisingly, one of the most popular cultivars, 'Sugar Baby', was the lowest yielding among the dark solid group, although its popularity is probably based on high fruit quality (deep red and crispy flesh of sweet and distinctive flavor).

The modern cultivars were generally not the highest yielders. 'Sangria' has been the leading cultivar in the southeastern United States for the last decade, but many obsolete cultivars outyielded it in this study. Of course, successful cultivars must have traits other than high yield. High fruit quality is of major importance, and includes bright flesh color, firm flesh texture, high sugar content, and proper fruit shape. Fruit quality of the obsolete cultivars was lower than that of the modern cultivars.

The market in the United States is currently oriented towards the 'Allsweet' type, but there is also a demand for other types. This is shown by the popularity of the mini watermelons (up to 3.5 kg per fruit), seedless yellow-flesh type, and seedless dark solid rind type. In this study, the obsolete cultivars had a wide range of types for fruit type, including shape, size, and flesh characteristics, all with medium to high soluble solids content. However, modern hybrids often yielded less than the obsolete cultivars tested, but had higher quality and uniformity.

Many of the yield trials run each year around the United States show few differences for yield among the experimental entries being evaluated. The screening of a diverse set of watermelon cultivars for fruit yield presented herein showed that there is variation for yield, and that sources of high yield are available. Furthermore, yield was strongly dependent on cultivar, even though influenced by environmental factors (including cultural practices), and probably could be improved through plant breeding. It is now apparent that the lack of genetic variation and the slow improvement in yield often mentioned by watermelon breeders may most likely be a result of the greater emphasis on traits other than yield, as well as the lack of diversity for yield among the modern cultivars. In any case, there is a need to identify sources of high yield, both as fruit weight and fruit number, and to use those sources to develop high yielding, but adapted lines for use by plant breeders. Important traits such as fruit quality and disease resistance should be incorporated into those high yielding lines before they are used to develop new cultivars. This should also result in an increase in the genetic diversity of modern cultivars.

Significant genetic diversity in RAPD markers has been observed among watermelon accessions from different geographical areas and from related species such as *C. colocynthis* (Levi et al., 2001). Now that phenotypic variability for yield in watermelon has been demonstrated, and high yielding cultivars identified, the next step would be to evaluate the USDA-ARS germplasm collection for fruit yield at several locations around the United States, including accessions originating from different areas of the world.

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Table 1. Analysis of variance (degrees of freedom and mean squares) for yield and relevant quality data of the 80 cultivars evaluated in three environments[z].

Source of variation	df	Total fruit yield		Percentage of marketable weight	Fruit size	Soluble solids
		Weight	Number			
Environment	2	145715.7 **	2853.8 **	233.3 NS	114.3 *	21.7 *
Rep. (Env.)	3	1723.1 *	33.5 **	48.6 NS	4.7 NS	1.4 NS
Cultivar	79	1715.5 ***	61.1 ***	121.8 *	43.2 ***	3.8 ***
Cult. × Env.	158	790.5 NS	14.7 ***	74.8 NS	2.9 **	0.7 NS
Error	236	649.9	7.8	87.9	1.9	0.7

z Environments: Clinton 2001, Clinton 2002, and Kinston 2002 (year × location)

Table 2. Yield and quality means obtained across environment(s) for the 80 cultivars tested in 2001 and 2002 at Clinton (four replications) and in 2002 (two replications) at Kinston, North Carolina, and flesh (Fl) and rind (Rn) color descriptors.

	Yield[z]			Fruit size		Quality[v]		Color[u]	
Cultivar	Mg ha^{-1}	th ha^{-1}	%mk[y]	kg[z]	L/D[xw]	%HH[x]	%SS[z]	Fl	Rn
Mountain Hoosier	114.2	10.2	90	10.2	1.1	21	10.6	R	DS
Hopi Red Flesh	113.9	10.0	93	11.2	1.3	55	10.4	R	DS
Early Arizona	108.4	16.2	88	6.8	1.2	22	9.8	R	DS
Starbrite F_1	107.1	9.8	96	11.5	1.6	26	10.8	R	MD
Stone Mountain	103.4	12.6	99	8.2	1.2	6	7.4	R	WD
Stars-N-Stripes F_1	102.7	10.6	94	10.0	2.1	0	10.7	R	WD
AU-Jubilant	101.5	9.1	92	11.5	2.1	0	10.0	R	NR
Sweetheart	99.7	14.5	95	7.2	1.3	18	7.7	R	GR
Calhoun Gray	98.7	10.5	95	9.9	2.3	0	10.4	R	GR
Big Crimson	98.3	9.5	97	10.8	1.1	7	10.1	R	NR
Moon and Stars	94.4	10.0	85	10.2	2.1	13	9.8	R	SP
Cole Early	94.1	12.2	94	8.8	1.3	6	7.1	R	MD
Legacy F_1	92.7	9.0	98	10.8	1.8	7	11.2	R	WD
Yellow Crimson	91.4	10.1	96	9.2	1.1	10	11.0	S	WD
Blacklee	90.4	9.8	98	10.2	2.0	0	10.1	R	DS
Charleston Gray	89.2	8.9	95	10.8	2.0	13	11.0	R	GR
Tom Watson	89.0	11.5	82	8.7	2.2	0	8.3	R	DS
King and Queen	88.8	19.6	97	4.9	1.1	12	9.2	R	LS

Table 2. Continued.

	Yield[z]			Fruit size		Quality[v]		Color[u]	
Cultivar	Mg ha^{-1}	th ha^{-1}	%mk[y]	kg[z]	L/D[xw]	%HH[x]	%SS[z]	Fl	Rn
Desert King	88.4	10.0	99	9.0	1.2	16	9.9	S	GR
Charlee	88.1	8.9	96	10.3	2.0	8	10.4	R	GR
Long Crimson	87.9	9.4	94	10.0	1.6	29	9.6	R	WD
Jubilee	87.9	7.4	93	12.9	2.0	8	10.7	R	MD
Sangria F_1	87.0	7.4	81	10.0	2.1	8	11.2	R	WD
Fiesta F_1	85.2	10.6	96	8.4	1.7	0	10.6	R	WD
Tendergold	85.1	9.0	96	9.6	1.7	19	10.4	S	WD
Sugarloaf	84.5	17.6	98	5.0	1.0	7	10.0	R	LS
Princeton	83.9	8.9	95	9.7	1.7	8	9.6	R	WD
Navajo Sweet	83.9	16.4	95	5.8	1.1	13	10.7	R	LS
Kleckley Sweet	83.1	9.2	79	9.1	2.0	5	8.9	R	DS
Black Diamond Yellow Flesh	83.1	10.1	98	8.8	1.3	27	10.3	S	DS
Verona	82.8	8.3	94	10.1	1.2	29	10.1	R	DS
Blackstone	81.9	9.4	97	9.3	1.2	46	10.0	R	DS
Sultan F_1	81.1	8.3	92	10.0	1.5	14	11.1	R	MD
Regency F_1	80.6	8.7	97	9.7	1.5	7	10.9	R	WD
RedNSweet	79.3	8.5	97	10.3	1.2	43	10.8	R	NR
Royal Flush F_1	79.3	9.2	97	9.0	1.8	0	11.2	R	WD
Fairfax	79.2	7.5	93	10.9	2.2	39	10.0	R	MD
Cobbs Gem	78.2	5.4	91	15.7	1.2	22	9.8	R	WD

Table 2. Continued.

	Yield[z]			Fruit size		Quality[v]		Color[u]	
Cultivar	Mg ha^{-1}	th ha^{-1}	%mk[y]	kg[z]	L/D[xw]	%HH[x]	%SS[z]	Fl	Rn
Yellow Shipper	77.9	8.2	95	9.7	1.7	29	10.1	S	WD
Crimson Sweet	77.4	7.8	90	10.3	1.3	10	10.2	R	WD
Super Sweet	76.8	10.0	98	7.9	1.1	35	10.7	R	MD
Klondike Striped Blue Ribbon	76.1	10.8	93	8.2	1.6	38	10.9	R	NR
Table 2. Continued.									
Peacock Shipper	75.7	9.3	90	6.6	1.6	17	10.2	R	DS
AU-Producer	75.6	8.1	98	10.0	1.1	9	10.6	R	MD
Wills Sugar	75.5	15.2	97	5.2	1.1	0	9.5	R	DS
Dixielee	74.7	7.8	94	10.1	1.1	0	10.9	R	NR
Golden Honey	72.4	10.6	98	7.0	1.3	56	10.3	S	MD
Dixie Queen	72.4	7.3	92	9.8	1.3	19	10.3	R	NR
New Winter	71.0	17.5	98	4.9	1.1	0	10.2	R	LS
Tastigold	70.5	8.5	96	8.4	1.1	18	10.3	S	GR
Georgia Rattlesnake	69.5	6.3	89	11.5	2.1	0	10.6	R	NR
Louisiana Sweet	69.2	7.7	97	9.5	1.1	30	10.9	R	NR
Florida Favorite	67.3	10.1	92	7.4	1.9	9	9.9	R	NR
Honey Red	66.5	11.5	99	6.2	1.1	3	10.3	R	DS
Mickylee	66.1	14.6	96	4.9	1.2	8	10.4	R	GR
Chubby Gray	66.1	6.2	92	11.0	1.4	19	9.9	R	GR
Allsweet	65.9	6.8	94	9.8	1.9	13	10.7	R	WD

Table 2. Continued.

	Yield[z]			Fruit size		Quality[v]		Color[u]	
Cultivar	Mg ha^{-1}	th ha^{-1}	%mk[y]	kg[z]	L/D[xw]	%HH[x]	%SS[z]	Fl	Rn
Picnic	65.3	9.2	96	7.2	1.6	0	10.5	R	DS
Black Diamond Yellow Belly	64.9	6.5	98	11.6	1.2	18	10.6	R	DS
Carolina Cross #183	64.1	4.0	84	19.3	1.5	44	9.5	R	MD
Garrisonian	61.9	5.7	96	10.7	2.0	0	10.6	R	NR
Sugarlee	59.9	7.5	96	8.6	1.1	21	11.1	R	NR
Perola	59.9	8.4	98	7.1	1.2	4	10.1	R	GR
Rhode Island Red	59.7	8.2	96	7.8	1.4	35	10.2	R	NR
Champion #2	59.7	7.9	93	8.3	1.5	3	10.6	R	GR
Giza	54.9	12.1	96	4.7	1.1	0	10.4	R	DS
Graybelle	54.7	9.3	95	6.1	1.2	0	10.9	R	GR
Congo	54.3	5.3	98	10.6	1.1	11	10.2	R	MD
Early Canada	53.1	10.7	95	5.0	1.1	34	9.5	R	GR
Black Boy	51.5	8.9	96	6.3	1.1	37	10.5	R	DS
Quetzali	49.1	9.2	99	5.7	1.2	23	11.1	R	MD
Weeks North Carolina Giant	48.8	3.1	69	17.3	n/a	n/a	9.1	R	MD
Sun Gold	48.6	7.3	99	6.9	1.1	5	10.5	C	NR
Peacock WR-60	47.9	7.6	97	6.9	1.8	0	9.8	R	DS
Sweet Princess	47.2	5.0	98	9.2	2.2	7	10.9	R	GR
Golden	46.1	9.0	91	5.2	1.1	48	10.5	C	NR
Sugar Baby	45.8	9.9	98	4.9	1.1	18	10.1	R	DS

Table 2. Continued.

	Yield [z]			Fruit size		Quality [v]		Color [u]	
Cultivar	Mg ha^{-1}	th ha^{-1}	%mk [y]	kg [z]	L/D [xw]	%HH [x]	%SS [z]	Fl	Rn
Tendersweet Orange Flesh	45.5	5.4	93	9.5	1.6	75	9.2	O	WD
Minilee	45.1	12.2	96	3.6	1.2	8	10.8	R	GR
Calsweet	36.4	4.4	90	8.6	1.6	0	9.8	R	WD
Statistics									
Mean	75.5	9.4	94	9.0	1.5	16	10.2		
Maximum	114.2	19.6	99	19.3	2.3	75	11.2		
Minimum	36.4	3.1	69	3.6	1.0	0	7.1		
LSD (α=0.05)	24.2	2.9	9	1.4	0.3	28	0.8		
Range/LSD	3.2	5.7	3	11.2	4.3	3	5.1		
Ranking Repeatability [t]	0.31	0.43	0.39	0.51	-	-	0.30		
Correlation (tot. weight vs. market. weight)				0.92 ***					
Correlation (tot. no. fruits vs. market. fruit no.)				0.97 ***					
Correlation (% market. weight vs. % market. fruit no.)				0.91 ***					
Correlation (tot. weight vs. average market. weight)				0.13 ***					

z Data averaged over two harvests, two to four replications, and three environments

y Percentage of marketable over total yield measured as weight (Mg ha^{-1})

x Data averaged over two harvests, four replications, one location, and one year; descriptive information to support the choice of interesting high-yielding cultivars for future breeding efforts

w L/D = length/diameter ratio measured in mm on a vertical section of the fruit from the peduncle to the blossom-end

v %HH = percentage of fruit with hollowheart over total yield measured as fruit number

%SS = percentage of soluble solids (measured by refractometer)

u Fl = flesh color (R = red; O = orange; S = salmon yellow; C = canary yellow)

Rn = rind pattern and color (NR, MD, WD = narrow, medium, and wide dark green stripes on light green background, respectively; GR = gray; LS = light solid green; DS = dark solid green; SP = yellow spots on solid green background)

t Ranking Repeatability = average frequency of cultivars included in the first 20% of the ranking in different replications, within year and location

CHAPTER FOUR

HERITABILITY AND GENETIC VARIANCE ESTIMATES FOR FRUIT WEIGHT IN WATERMELON

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Abstract

The cultivated watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] has fruit that may weigh from 1 kg to over 100 kg. In recent years, preference of consumers has shifted towards fruit of smaller size than the large types traditionally used for parties and picnics. This trend has produced increased interest in the genetics of fruit weight, especially among watermelon breeders. The genetics of fruit weight in watermelon has not been studied widely and there is little published information available to help watermelon breeders in choosing the proper breeding techniques for working with fruit weight. The objectives of this study were to determine the inheritance of fruit weight. Six adapted cultivars having the largest and smallest fruit weight we could find were crossed in a half diallel. We made controlled pollinations to produce F_1, F_2, and BC_1 generations for testing in 2004 at two locations in North Carolina. Generation means and variances were calculated from single-fruit weights. Giant-fruited parents had higher phenotypic variance than small-fruited parents. Environmental variance was higher than genetic at Kinston, where the field was less uniform than Clinton due to poor drainage conditions. At Clinton, genetic and environmental variance were similar for 67% of the families. Narrow- and broad-sense heritability were low to intermediate and consistent across locations. In addition, a high number of effective factors was estimated to influence fruit weight in watermelon. Based on these results, watermelon breeders will have to use quantitative methods to change fruit weight in the development of new cultivars. Nevertheless, the improvement of qualitative traits in small-fruited germplasm may be a more successful approach than reduction of the size of large-fruited, high quality cultivars.

Introduction

The fruit of the cultivated watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] may vary in weight from 1 kg to over 100 kg. In the United States, commercial fruit are usually classified into four categories: icebox (<5.5 kg), small or pee wee (5.5-8.0 kg), medium (8.1-11.0 kg), large (11.1-14.5 kg), and giant (>14.5 kg) (Maynard, 2001).

The smallest cultivated watermelons are typically produced by 'New Hampshire Midget', from the University of New Hampshire in 1951. This cultivar has fruit with oval shape, gray rind, red flesh, black seeds, and thin rind, and produces very early fruit of icebox size (Wehner, 2002). Fruit of smaller size (<0.5 kg) can

be found in wild relatives of the cultivated watermelon, such as *C. colocynthis*, and are typically used in Africa as forages.

Historically, watermelon cultivars bearing giant fruit of 100 kg or more have been bred specifically for fruit competitions at State and local agricultural fairs. 'Cobbs Gem', 'Carolina Cross #183', 'Florida Giant', and 'Weeks NC Giant' are some of the popular cultivars. Their fruit have very thick rind (Gusmini and Wehner, 2004), low sugar content, fibrous flesh, and susceptibility to hollowheart and shape defects. The Guinness book of records (Matthews, 1993) reports the largest watermelon grown to be one of ten giant fruit weighing 119 kg harvested in 1990 in Arrington, Tennessee, by B. Carson.

In recent years, consumers in the United States have been increasingly interested in seedless fruit weighing 7 to 10 kg. In 2003, a new fruit type was introduced under the name of mini watermelon. Cultivars produce fruit that are round, have a thin rind, and weigh between 1.5 and 4.0 kg. Leading cultivars among those currently available are 'Petite Perfection', 'Precious Petite' (Syngenta Seeds - Rogers Brand), and cultivars of the Bambino trademark (Seminis Vegetable Seeds), as well as other cultivars being tested for release (Molinar and Mueller, 2004; Schultheis et al., 2005) in the next few years. Even though mini watermelons occupy a small portion of the market, their introduction and appreciation by consumers has increased the interest of watermelon breeders in cultivars having reduced fruit size.

Fruit weight in watermelon production is an important descriptor of fruit type, although it can also be considered a yield component. Yield is defined as the total weight per production unit: in the United States, growers expect to harvest at least "one load of fruit" per acre of land, corresponding to 50.52 Mg/ha of marketable fruit (Maynard, 2001). Marketable fruit must be free of defects, and fall into the weight classes most desired by the consumers. Currently, smaller sizes are preferred over the traditional large watermelon as a dessert for parties and picnics. In addition, the average American family, composed of three to four persons, eats watermelon only occasionally and may prefer watermelons that can be eaten in a single meal.

The genetics of watermelon have been studied widely, and several genes have been characterized (Cucurbit Gene List Committee, 1979; Cucurbit Gene List Committee, 1982; Henderson, 1991; Henderson, 1992; Rhodes and Dane, 1999). However, single genes or quantitative trait loci have not been identified for watermelon fruit weight. In two preliminary studies on the inheritance of fruit weight, significant additive,

dominance, and epistatic effects were reported, dominance and dominance-by-dominance being the largest gene effects (Brar and Nandpuri, 1974; Sharma and Choudhury, 1988).

Several methods of estimating heritability and predicting selection response are available. Primarily, these methods partition the total variance into genetic and environmental variances, and the genetic variance into additive and dominance components and inter-allelic interaction effects, whenever the population structure and composition allows (Holland et al., 2003; Nyquist, 1991). Among others, a design based on the measure of variance from six generations (P_a, P_b, F_1, F_2, BC_1P_a, BC_1P_b) can be used to estimate environmental, genetic, and additive variances. The variance of the F_2 provides an estimate of phenotypic variance, while the mean variance of the non-segregating generations (P_a, P_b, and F_1) gives an estimate of environmental effects (Wright, 1968). The additive variance is derived by subtracting the variances of the backcrosses from twice the phenotypic (F_2) variance, as an extension of the single locus model under the hypothesis of absence of linkage and genetic by environment interactions (Warner, 1952). The broad- and narrow-sense heritability and the predicted gain from selection can then be calculated from the available estimates of genetic, additive, and phenotypic variances.

The objective of this experiment was to estimate the heritability and genetic variances of fruit weight in watermelon using a set of crosses of giant by icebox type inbreds, using measures of variances of six generations for each cross.

Materials and Methods

Germplasm and Crosses

In the experiment we used nine families developed from nine crosses of *Citrullus lanatus* var. *lanatus* inbred cultivars. Each of the three cultivars with giant fruit 'Weeks NC Giant', 'Cobbs Gem', and 'Carolina Cross #183' was crossed with the three cultivars with small fruit 'Petite Sweet', 'Minilee', and 'NH Midget' (Fig. 1). For each family we developed six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) in the greenhouse at North Carolina State University in Raleigh, North Carolina.

Cultural Practices

Seeds from all six generations of each family were sown in 72-square-cell plug polyethylene flats in the greenhouse at North Carolina State University in Raleigh, North Carolina. An artificial soilless growing medium was used (Canadian sphagnum peat moss, perlite, vermiculite, processed pine bark). The medium was moistened to capacity after seeding and held in the greenhouse at constant temperature (25-30 °C) until full emergence. Transplants were moved to an open cold frame at the field site for acclimation for two weeks prior to transplanting. The seedlings were transplanted by hand at the two-true-leaf stage and missing or damaged transplants were replaced a week after transplanting.

Field rows were made up with drip tubing and covered with black polyethylene mulch. The experiment was conducted using horticultural practices recommended by the North Carolina Extension Service (Sanders, 2004). Soil type was an Orangeburg loamy sand at Clinton, and a Norfolk sandy loam at Kinston.

Each plant was manually trained each week in a spiral by turning all the vines in a clockwise circle around the crown until about 70% of the plants in the field had set fruit (Fig. 2). Plant training allowed accurate identification of each fruit and avoided duplication or misclassification of plants, generations, and families.

Experimental design and Data Analysis

The field test was run in the summer of 2004 at two locations: the Horticultural Crops Research Station in Clinton, and the Cunningham Research Station in Kinston, North Carolina. At each location, all six generations of the same family were planted. Single plants were transplanted into rows in the following order and number: P_aS_1, (10), P_bS_1 (10), BC_1P_a, (30) BC_1P_b (30), F_1, (20) F_2 (100) at Clinton and P_aS_1 (10), P_bS_1 (10), F_1 (20), BC_1P_a (30), BC_1P_b (30), F_2 (110) at Kinston. At Clinton, each field was 0.4 ha with eight rows 60 m long and each family occupied four rows. At Kinston, each field was 0.4 ha with six rows 85 m long and each family occupied three rows. The fields had raised, shaped beds (rows) on 3.1 m centers with single hills 1.2 m apart.

The fields were harvested when more than 90% of the fruit were ripe. Fruit were determined to be ripe by looking for a dried tendril nearest the fruit, a light colored ground spot, and a dull sound of the fruit when

thumped (Maynard, 2001). Weights were recorded by approximation to the nearest pound. The data were transformed to kilograms before statistical analysis.

The fruit of the giant-fruited parents in the family 'Weeks NC Giant' × 'Minilee' at Kinston were smaller than expected, possibly due to the presence of problems of water drainage in the field during fruit development. Therefore, the data from those families were presented in the tables , but were considered missing data for the calculation of means by location and overall means.

We tested the F_2 data for homogeneity of variances using Bartlett's method (Ostle and Malone, 1988; Steel et al., 1997). Since the variances were heterogeneous, we analyzed the data by family and location.

Phenotypic (P), environmental (E), genotypic (G), and additive (A) effects were estimated from generation variances as follows (Warner, 1952; Wright, 1968):

$$\sigma^2(P) = \sigma^2(F_2) \qquad \sigma^2(E) = \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}$$

$$\sigma^2(G) = \sigma^2(PH) - \sigma^2(E) \qquad \sigma^2(A) = \left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_a)\right]$$

Negative estimates for genetic variances are possible with the experimental design adopted. Negative estimates should be considered equal to zero (Robinson et al., 1955), but should be reported "in order to contribute to the accumulation of knowledge, which may, in the future, be properly interpreted" (Dudley and Moll, 1969). We considered negative estimates equal to zero for the calculation of the mean estimates over families or locations. When a negative estimate was derived from another negative value (narrow-sense heritability and gain from selection, calculated from additive variance), it was considered close to zero and omitted.

The number of effective factors was estimated using the following five methods (Lande, 1981; Mather and Jinks, 1982; Wright, 1968):

Lande's method I:
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}\right\}}$$

Lande's method II:
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_a)\right]\right\}}$$

Lande's method III:
$$\frac{\left[\mu(P_b)-\mu(P_a)\right]^2}{\left\{8\times\left[\sigma^2(BC_1P_a)+\sigma^2(BC_1P_a)-\sigma^2(F_1)\right]\right\}-\frac{\left[\sigma^2(P_a)+\sigma^2(P_b)\right]}{2}}$$

Mather's method:
$$\frac{\frac{\left[\mu(P_b)-\mu(P_a)\right]^2}{2}}{\left[2\times\sigma^2(F_2)\right]-\left[\sigma^2(BC_1P_a)+\sigma^2(BC_1P_a)\right]}$$

Wright's method:
$$\frac{\left[\mu(P_b)-\mu(P_a)\right]^2\times\left\{1.5-\left[2\times\frac{\mu(F_1)-\mu(P_a)}{\mu(P_b)-\mu(P_a)}\times\left(1-\frac{\mu(F_1)-\mu(P_a)}{\mu(P_b)-\mu(P_a)}\right)\right]\right\}}{8\times\left\{\sigma^2(F_2)-\frac{\sigma^2(P_a)+\sigma^2(P_b)+\left[2\times\sigma^2(F_1)\right]}{4}\right\}}$$

The possible gain from selection per cycle was predicted as $h_n^2\times\sqrt{\sigma^2(P)}$ multiplied by the selection differential in standard deviation units k for selection intensities of 5%, 10%, or 20% (Hallauer and Miranda, 1988). The statistical analysis was performed using the SAS-STAT statistical package (SAS Institute, Cary, North Carolina).

Results and Discussion

In our experiments, watermelon fruit weight was a quantitative trait, with normal distribution of the F_2 data for all the families tested (data not shown). Due to the large and continuous variation of the data, it was not possible to generate classes of weight and analyze the data according to a Mendelian model.

The field at Clinton had fewer problems with standing water during fruit development than at Kinston, providing a better environment for growth for those families. Overall, the crop at Clinton appeared more vigorous and uniform than at Kinston, even though cultural practices were similar, and there was no problem with biotic or abiotic diseases at either location. The overall mean weights were higher at Clinton than at Kinston (12.2 vs. 8.7 kg, respectively). Furthermore, the fruit weight of the parental inbred lines were closer to the expected weights at Clinton. Nevertheless, we were interested in variance estimates and not in mean performance across environments. Thus, we presented the data from both locations. The measured variances were not homogeneous across locations and families, based on Bartlett's test (Ostle and Malone, 1988; Steel et al., 1997). Therefore, the data were analyzed separately by location and family.

The giant-fruited parents had a larger variance than the small-fruited ones (13.15 vs. 1.16, respectively) at both locations (Table 1). The giant-fruited cultivars also had large differences in variance among families for the same cultivar. For example, different families where 'Carolina Cross #183' was used as one parent had a variance of 29.83, 16.73, and 9.16 at Clinton, or 23.23, 6.34, and 8.29 at Kinston. On the other hand, the small-fruited parents had similar variances among families and locations (1.12 at Clinton vs. 1.16 at Kinston).

The environmental variance was larger than the genetic variance in the majority of the families at both locations (8.52 vs. 6.41, respectively) (Table 2). At Clinton, genotype had similar or larger effect than environment for 78% of the families. Thus, a uniform environment (Clinton) was more favorable to control for environmental variance than testing at multiple locations.

Additive genetic effects were estimated, but a comparison with dominance effects was not possible. With our experimental design, dominance variance could be estimated by subtraction of genetic and additive variances from the phenotypic, but such an indirect estimate would not be precise. Additive genetic components had the largest effect for some families and locations (i.e., 'Carolina Cross #183' × 'Petite Sweet' at Clinton), and the smallest for others (i.e., 'Cobbs Gem' × 'Minilee' at Clinton) (Table 2). The relative importance of additive effects among locations and within family also varied greatly, which accounts for the differences in narrow-sense heritability among locations within family. The narrow-sense heritability was not estimated for those families with negative estimates of additive variance and it should be considered close to zero (Robinson et al., 1955). The broad-sense heritability estimates were higher at Clinton than at Kinston (0.49 vs. 0.33, respectively) (Table 2). At Clinton the broad-sense heritability was intermediate, ranging from 0.53 to 0.61, for 67% of the families. Similarly, at Kinston the estimates of broad-sense heritability spanned a narrow range (0.29 to 0.50) for 67% of the families. The estimates varied greatly among locations within family.

The heritability of fruit weight was low to intermediate. The narrow-sense heritability was larger than the broad-sense heritability, even though the additive variance estimates were not consistent among families. Therefore, additive components may play an important role in the improvement of fruit weight in watermelon, but further studies that allow direct estimation of additive and dominance effects in multiple and uniform environments may be needed for a correct quantification of these effects.

The number of effective factors varied from two to seven at Clinton, with the exception of the family 'Cobbs Gem' × 'NH Midget' that had 25 estimated effective factors, and the family 'Weeks NC Giant' × 'Minilee' that had less than one (Table 3). At Kinston, the range of estimates was larger, but this may depend on the higher variability and lower mean weights recorded at that location. These estimates should be considered only indicative and possibly biased by undetermined dominance and epistatic effects.

The possible gain from selection was largely different among families, even when they shared a common parent (Table 3). For example, data from Clinton predicted a possible gain in families from 'Carolina Cross #183' × 'Petite Sweet' of 9.9 to 14.6 kg, and of 0.4 to 3.0 kg in the two other crosses. In addition, the predicted gain differed more for some families than others among locations, due to the high variability in additive variance estimates.

Based on our experiments, it should be possible to vary the size of watermelon fruit in few generations of selection, with greater changes under high selection intensities (4.9 kg predicted at k = 5%). Breeding schemes that would allow high recombination rates may help in combining all the effective factors needed to obtain a desired fruit weight and break unfavorable linkages. Recurrent selection for population improvement seems to be a valid breeding method, even though lower gain per cycle would be obtained, due to the lower selection intensity (typically equal to 20%).

Recurrent selection programs in watermelon would require large isolated intercrossing blocks, due to the large size of the plants. The environmental variation and the generally intermediate to low heritability observed for this trait may require self pollination of the half-sib families and trialing in progeny rows at multiple locations for effective selection. In addition, it may be easier to introgress desired qualitative traits into breeding lines of desired fruit weight by pedigree or backcross breeding, rather than trying to change the fruit weight of otherwise acceptable cultivars.

Our study showed limited potential to reduce fruit weight in watermelon. Low heritability, quantitative inheritance, and high environmental variance are important limiting factors that may greatly reduce the realized gain from selection in populations of cultivated watermelon.

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Table 1. Phenotypic variances by generation for the watermelon families tested for fruit weight in 2004 at Clinton and Kinston, North Carolina [z].

Pedigree	$\sigma^2(P_a)$	$\sigma^2(P_b)$	$\sigma^2(F_1)$	$\sigma^2(F_2)$	$\sigma^2(BC_1P_a)$	$\sigma^2(BC_1P_b)$
Clinton						
'Carolina Cross #183' × 'Petite Sweet'	29.83	2.59	10.45	28.62	12.81	6.60
'Carolina Cross #183' × 'Minilee'	16.73	0.07	12.07	22.89	37.14	7.30
'Carolina Cross #183' × 'NH Midget'	9.16	1.56	6.57	12.80	17.75	2.70
'Cobbs Gem' × 'Petite Sweet'	16.95	3.15	31.64	23.52	16.30	11.25
'Cobbs Gem' × 'Minilee'	5.84	0.38	5.67	11.22	36.98	7.45
'Cobbs Gem' × 'NH Midget'	35.39	0.10	4.41	13.09	10.12	5.72
'Weeks NC Giant' × 'Petite Sweet'	18.72	1.35	11.21	24.32	21.85	8.34
'Weeks NC Giant' × 'Minilee'	4.32	0.74	5.11	17.86	11.17	16.73
'Weeks NC Giant' × 'NH Midget'	9.63	0.16	4.66	11.70	14.07	3.43
Mean	16.29	1.12	10.20	18.45	19.80	7.72

Table 1. Continued.

Pedigree	$\sigma^2(P_a)$	$\sigma^2(P_b)$	$\sigma^2(F_1)$	$\sigma^2(F_2)$	$\sigma^2(BC_1P_a)$	$\sigma^2(BC_1P_b)$
Kinston						
'Carolina Cross #183' × 'Petite Sweet'	23.23	3.57	8.21	15.27	8.25	9.82
'Carolina Cross #183' × 'Minilee'	6.34	0.62	14.49	14.24	28.31	8.10
'Carolina Cross #183' × 'NH Midget'	8.29	0.05	8.12	10.62	13.95	2.58
'Cobbs Gem' × 'Petite Sweet'	2.44	2.29	11.22	13.68	9.32	10.58
'Cobbs Gem' × 'Minilee'	6.70	0.29	8.41	9.13	3.27	17.86
'Cobbs Gem' × 'NH Midget'	9.31	0.14	5.31	7.15	7.93	2.73
'Weeks NC Giant' × 'Petite Sweet'	22.08	2.52	12.01	14.63	17.02	7.03
'Weeks NC Giant' × 'Minilee'	24.96	0.69	12.79	9.17	6.40	18.29
'Weeks NC Giant' × 'NH Midget'	1.65	0.05	8.70	6.49	22.77	2.69
Mean [y]	10.01	1.19	9.56	11.40	13.85	7.67
Overall mean	13.15	1.16	9.88	14.93	16.83	7.70

z Data are single-fruit weights (kg) from nine families of giant- by mini-fruited cultivars of *Citrullus lanatus* var. *lanatus*. Single plants were transplanted as follows: P_aS_1, (20), P_bS_1 (20), F_1 (40), BC_1P_a, (60) BC_1P_b (60), and F_2 (210)

y Not including 'Weeks NC Giant' × 'Minilee'

Table 2. Variance and heritability estimates for the watermelon families tested for fruit weight in 2004 at Clinton and Kinston, North Carolina[z].

Pedigree	$\sigma^2(P)$[y]	$\sigma^2(E)$[x]	$\sigma^2(G)$[w]	$\sigma^2(A)$[v]	H^2_B[u]	h^2_n[t]
Clinton						
'Carolina Cross #183' × 'Petite Sweet'	28.62	13.33	15.29	37.83	0.53	1.32
'Carolina Cross #183' × 'Minilee'	22.89	10.24	12.65	1.33	0.55	0.06
'Carolina Cross #183' × 'NH Midget'	12.80	5.97	6.83	5.15	0.53	0.40
'Cobbs Gem' × 'Petite Sweet'	23.52	20.84	2.68	19.50	0.11	0.83
'Cobbs Gem' × 'Minilee'	11.22	4.39	6.83	-21.99	0.61	--[r]
'Cobbs Gem' × 'NH Midget'	13.09	11.08	2.01	10.33	0.15	0.79
'Weeks NC Giant' × 'Petite Sweet'	24.32	10.62	13.70	18.46	0.56	0.76
'Weeks NC Giant' × 'Minilee'	17.86	3.82	14.04	7.82	0.79	0.44
'Weeks NC Giant' × 'NH Midget'	11.70	4.78	6.92	5.90	0.59	0.50
Mean	18.45	9.45	8.99	9.37	0.49	0.64

Table 2. Continued.

Pedigree	σ^2(P) [y]	σ^2(E) [x]	σ^2(G) [w]	σ^2(A) [v]	H^2_B [u]	h^2_n [t]
Kinston						
'Carolina Cross #183' × 'Petite Sweet'	15.27	10.80	4.46	12.47	0.29	0.82
'Carolina Cross #183' × 'Minilee'	14.24	8.99	5.26	-7.93	0.37	-- [r]
'Carolina Cross #183' × 'NH Midget'	10.62	6.15	4.48	4.72	0.42	0.44
'Cobbs Gem' × 'Petite Sweet'	13.68	6.79	6.88	7.45	0.50	0.55
'Cobbs Gem' × 'Minilee'	9.13	5.95	3.18	-2.87	0.35	-- [r]
'Cobbs Gem' × 'NH Midget'	7.15	5.02	2.13	3.64	0.30	0.51
'Weeks NC Giant' × 'Petite Sweet'	14.63	12.15	2.47	5.21	0.17	0.36
'Weeks NC Giant' × 'Minilee'	9.17	12.81	-3.64	-6.34	-- [r]	-- [r]
'Weeks NC Giant' × 'NH Midget'	6.49	4.78	1.72	-12.48	0.26	-- [r]
Mean [s]	11.40	7.58	3.82	4.19	0.33	0.54
Overall mean	14.93	8.52	6.41	6.78	0.41	0.59

z Data are single-fruit weights (kg) from nine families of giant- by mini-fruited cultivars of *Citrullus lanatus* var. *lanatus*. Single plants were transplanted as follows: P_aS_1, (20), P_bS_1 (20), F_1 (40), BC_1P_a, (60) BC_1P_b (60), and F_2 (210)

y σ^2(P) = phenotypic variance = $\sigma^2(F_2)$

x σ^2(E) = environmental variance = $\dfrac{\sigma^2(P_a)+\sigma^2(P_b)+\left[2\times\sigma^2(F_1)\right]}{4}$

w σ^2(G) = genetic variance = $\sigma^2(P)-\sigma^2(E)$

v σ^2(A) = additive variance = $\left[2\times\sigma^2(F_2)\right]-\left[\sigma^2(BC_1P_a)+\sigma^2(BC_1P_a)\right]$

u H^2_B = broad-sense heritability

t h^2_n = narrow-sense heritability

s Not including 'Weeks NC Giant' × 'Minilee'

r Negative estimate from a negative estimate of additive variance

Table 3. Estimates of number of effective factors and predicted gain from selection under different selection intensities for the watermelon families tested for fruit weight in 2004 at Clinton and Kinston, North Carolina[z].

	Effective Factors			Gain from Selection[y]		
Pedigree	Wright[x]	Lande I[w]	Mean	5%	10%	20%
Clinton						
'Carolina Cross #183' × 'Petite Sweet'	2.4	2.3	2.35	14.6	12.4	9.9
'Carolina Cross #183' × 'Minilee'	3.7	3.1	3.40	0.6	0.5	0.4
'Carolina Cross #183' × 'NH Midget'	7.0	7.0	7.00	3.0	2.5	2.0
'Cobbs Gem' × 'Petite Sweet'	5.3	5.3	5.30	8.3	7.1	5.6
'Cobbs Gem' × 'Minilee'	3.0	2.9	2.95	--[u]	--[u]	--[u]
'Cobbs Gem' × 'NH Midget'	26.5	23.5	25.00	5.9	5.0	4.0
'Weeks NC Giant' × 'Petite Sweet'	2.7	2.7	2.70	7.7	6.6	5.2
'Weeks NC Giant' × 'Minilee'	0.8	0.3	0.55	3.8	3.3	2.6
'Weeks NC Giant' × 'NH Midget'	5.7	5.6	5.65	3.6	3.0	2.4
Mean	6.3	5.9	6.1	5.9	5.1	4.0

Table 3. Continued.

Pedigree	Effective Factors			Gain from Selection [y]		
	Wright [x]	Lande I [w]	Mean	5%	10%	20%
Kinston						
'Carolina Cross #183' × 'Petite Sweet'	4.2	4.2	4.20	6.6	5.6	4.5
'Carolina Cross #183' × 'Minilee'	0.6	0.6	0.60	-- [u]	-- [u]	-- [u]
'Carolina Cross #183' × 'NH Midget'	9.7	9.6	9.65	3.0	2.5	2.0
'Cobbs Gem' × 'Petite Sweet'	0.6	0.6	0.60	4.2	3.5	2.8
'Cobbs Gem' × 'Minilee'	0.4	0.0	0.20	-- [u]	-- [u]	-- [u]
'Cobbs Gem' × 'NH Midget'	3.4	2.9	3.15	2.8	2.4	1.9
'Weeks NC Giant' × 'Petite Sweet'	7.7	7.6	7.65	2.8	2.4	1.9
'Weeks NC Giant' × 'Minilee'	-4.1	-4.0	-4.05	-- [u]	-- [u]	-- [u]
'Weeks NC Giant' × 'NH Midget'	11.6	10.7	11.15	-- [u]	-- [u]	-- [u]
Mean [v]	4.8	4.5	4.7	3.9	3.3	2.6
Overall mean	5.6	5.2	5.4	4.9	4.2	3.3

z Data are single-fruit weights (kg) from nine families of giant- by mini-fruited cultivars of *Citrullus lanatus* var. *lanatus*. Single plants were transplanted as follows: P_aS_1, (20), P_bS_1 (20), F_1 (40), BC_1P_a, (60) BC_1P_b (60), and F_2 (210)

y $k \times h_n^2 \times \sqrt{\sigma^2(P)}$

x $$\frac{\left[\mu(P_b)-\mu(P_a)\right]^2 \times \left\{1.5-\left[2\times\frac{\mu(F_1)-\mu(P_a)}{\mu(P_b)-\mu(P_a)}\times\left(1-\frac{\mu(F_1)-\mu(P_a)}{\mu(P_b)-\mu(P_a)}\right)\right]\right\}}{8\times\left\{\sigma^2(F_2)-\frac{\sigma^2(P_a)+\sigma^2(P_b)+\left[2\times\sigma^2(F_1)\right]}{4}\right\}}$$

w $$\frac{\left[\mu\left(P_b\right)-\mu\left(P_a\right)\right]^2}{8\times\left\{\sigma^2\left(F_2\right)-\frac{\sigma^2\left(P_a\right)+\sigma^2\left(P_b\right)+\left[2\times\sigma^2\left(F_1\right)\right]}{4}\right\}}$$

v Not including 'Weeks NC Giant' × 'Minilee'

u Negative estimate from a negative estimate of additive variance

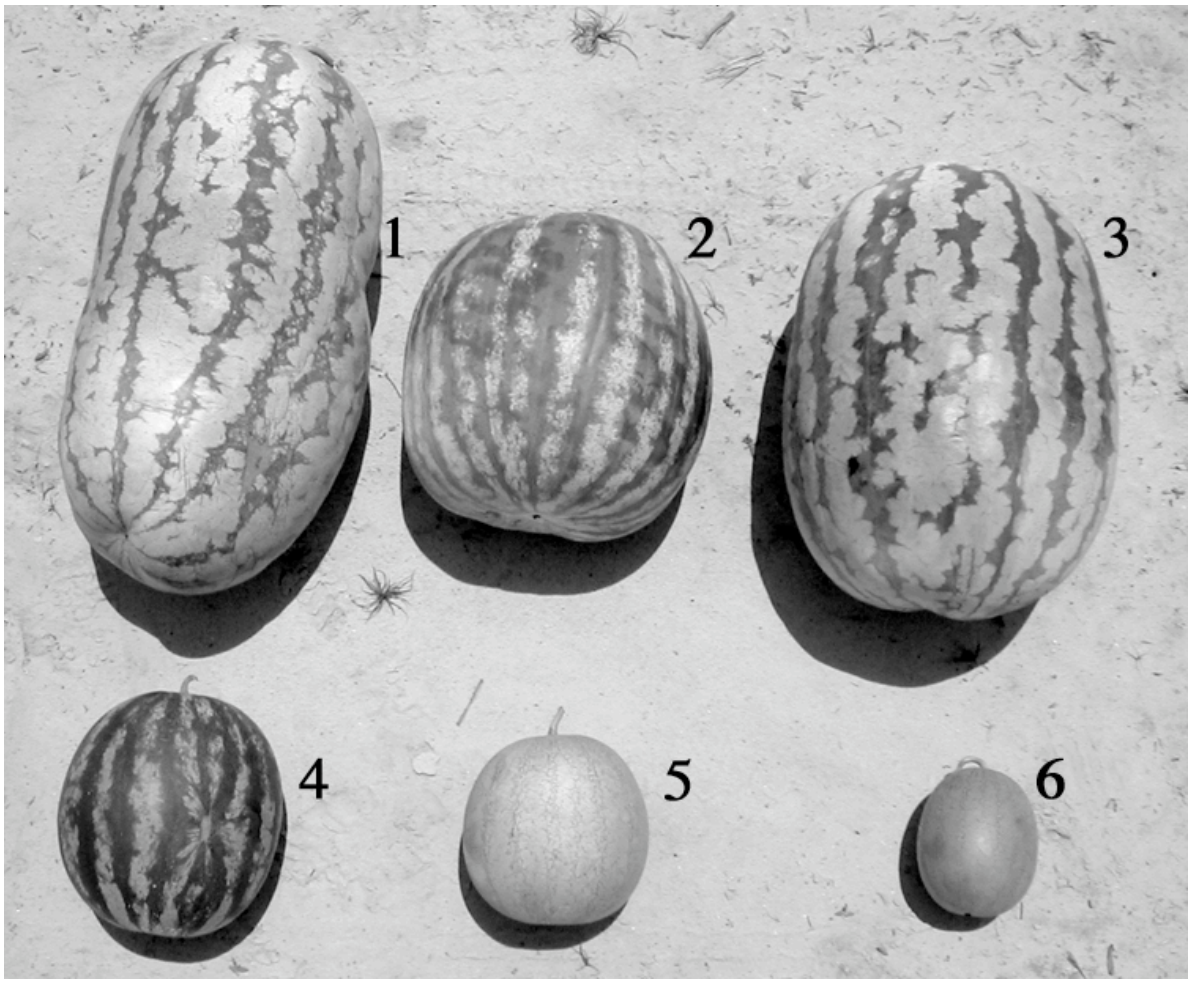

Figure 1. Each of the three cultivars with giant fruit 'Carolina Cross #183' (1), 'Cobbs Gem' (2), and 'Weeks NC Giant' (3) was crossed with the three cultivars with small fruit 'Petite Sweet' (4), 'Minilee' (5), and 'NH Midget' (6) to estimate the variance components and heritability of fruit-weight in watermelon.

Figure 2. In order to keep families, generations, and plants separate for data collection, each plant was manually trained each week into a spiral shape by turning all the vines in a clockwise circle around the crown until about 70% of the plants in the field set fruit.

CHAPTER FIVE

HERITABILITY AND GENETIC VARIANCE ESTIMATES FOR RESISTANCE TO GUMMY STEM BLIGHT IN WATERMELON

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Abstract

Gummy stem blight, caused by *Didymella bryoniae* (Auersw.) Rehm, is a major disease of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Our study explains the inheritance of resistance to gummy stem blight in watermelon. Four families of six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) were produced from four crosses of resistant plant introduction (PI) accessions by susceptible cultivars. Each family was tested in 2003 and 2004 in North Carolina under field and greenhouse conditions for resistance to gummy stem blight. Artificial inoculation was used to induce uniform and strong epidemics. The effect of the Mendelian gene for resistance, *db*, was tested. Failure of the data to fit the single gene model suggested that resistance should be regarded as a quantitative trait. Therefore, generation variances were measured and genetic parameters estimated (genetic variances, heritability, number of effective factors, and possible gain from selection). Genetic effects were greater than environmental effects. Broad- and narrow-sense heritability and additive variance were large. Few effective factors were estimated to regulate resistance, and the estimated gain from selection indicated possible improvement of three or more points of resistance (on a nine-point scale) per cycle of selection.

Introduction

Didymella bryoniae (Auersw.) Rehm [=*Mycosphaerella citrullina* (C.O.Sm.) Gross. and *Mycosphaerella melonis* (Pass) Chiu & Walker] and its anamorph *Phoma cucurbitacearum* (Fr.:Fr.) Sacc. [=*Ascochyta cucumis* Fautrey & Roum] (Keinath et al., 1995) are the perfect and imperfect stages of the fungal pathogen causing the disease known as gummy stem blight. Gummy stem blight was first observed in 1891 by Fautrey and Roumeguere in France on cucumber (*Cucumis sativus* L.) (Chiu and Walker, 1949; Sherf and MacNab, 1986). In 1917, gummy stem blight was reported for the first time in the United States, affecting watermelon fruit from Florida (Sherbakoff, 1917), where it is still an important limiting factor for the watermelon industry (Keinath, 1995; Schenck, 1962). Severe economic losses have been reported in the field (Power, 1992) and in storage (Leupschen, 1961; Norton, 1978; Sowell and Pointer, 1962). Gummy stem blight on watermelon plants is evident as crown blight, stem cankers, and extensive defoliation, with symptoms observed on the cotyledons, hypocotyls, leaves, and fruit (Maynard and Hopkins, 1999). *D. bryoniae* is a

fungus that is seed-borne (Lee et al., 1984), air-borne (van Steekelenburg, 1983), or soil-borne (Bruton, 1998; Keinath, 1996).

Adequate control of gummy stem blight through fungicide applications (Keinath, 1995; Keinath, 2000) and good cultural practices (Keinath, 1996; Rankin, 1954) is difficult, particularly during periods of frequent rainfall when relative humidity remains high for a long period. In addition, there is a growing concern among pathologists and breeders for the acquired resistance of *D. bryoniae* to fungicides (Kato et al., 1984; Keinath and Zitter, 1998; Malathrakis and Vakalounakis, 1983; Miller et al., 1997; van Steekelenburg, 1987). Genetic resistance to gummy stem blight has received attention since the 1970s as a possible alternative to chemical control by fungicide applications (Norton et al., 1993; Norton et al., 1995; Norton et al., 1986).

Genetic differences for gummy stem blight resistance among commercial cultivars of watermelon had been previously reported: 'Congo' was the least susceptible, 'Fairfax' was intermediate, and 'Charleston Gray' was the most susceptible (Schenck, 1962). Screening experiments with artificial inoculation of watermelon plants with spore suspensions of *D. bryoniae* indicated that PI 189225 and PI 271778 were the most resistant accessions available in the USDA-ARS watermelon germplasm collection (Sowell, 1975; Sowell and Pointer, 1962). In crosses with susceptible 'Charleston Gray', the single recessive gene *db* was indicated as the resistance gene in PI 189225 (Norton, 1979). Resistant watermelon cultivars were developed from two crosses ('Jubilee' × PI 271778, 'Crimson Sweet' × PI 189225) by selecting disease-resistant seedlings from backcrossed families that produced high yield of excellent quality fruit (Norton et al., 1986). 'AU-Jubilant', 'AU–Producer' (Norton et al., 1986), 'AU-Golden Producer' (Norton et al., 1993), and 'AU-Sweet Scarlet' (Norton et al., 1995) were released with moderate to high resistance to gummy stem blight. However, they were much less resistant to gummy stem blight than the resistant parents PI 189225 and PI 271778.

To date, no cultivars of watermelon (Sumner and Hall, 1993), melon (McGrath et al., 1993), or cucumber (Wehner and Shetty, 2000; Wehner and St. Amand, 1993) have been released that have high resistance to natural epidemics of gummy stem blight in the field.

The watermelon industry in the southeastern United States and the increasing losses due to gummy stem blight epidemics in the last decade promoted a new set of studies for the use of genetic resistance to control gummy stem blight in watermelon. The watermelon breeding program at North Carolina State

University has developed an efficient screening method of watermelon germplasm (Gusmini and Wehner, 2002; Song et al., 2004), including systems for mass production of inoculum of *D. bryoniae* for large field screening experiments (Gusmini et al., 2003), and a disease assessment scale for rating foliar and stem lesions (Gusmini et al., 2002). Between 1998 and 2001, all the available plant introduction (PI) accessions (1,274) from the USDA-ARS watermelon germplasm collection, along with 51 adapted cultivars, were tested to identify new genetic sources of resistance to gummy stem blight (Gusmini et al., 2005). New accessions (59) were identified that had higher resistance to field and greenhouse epidemics of gummy stem blight than PI 189225 and PI 271778.

The objective of this study was to describe the inheritance of resistance to gummy stem blight in watermelon. In addition, we verified the Mendelian inheritance of the *db* gene and estimated genetic variances and heritability of resistance.

Materials and Methods

Germplasm and Crosses

In the experiment, we used four families developed from the four crosses PI 189225 × 'NH Midget', PI 482283 × 'NH Midget', PI 482283 × 'Calhoun Gray', and PI 526233 × 'Allsweet'. 'Allsweet', 'Calhoun Gray', 'NH Midget', and PI 526233 were *Citrullus lanatus* var. *lanatus*. PI 189225 and PI 482283 were *Citrullus lanatus* var. *citroides*. PI 189225, PI 482283, and PI 526233 were the resistant parents. 'Allsweet', 'Calhoun Gray', and 'NH Midget' were the adapted susceptible parents.

The inbred cultivars were obtained from commercial seed stocks and the PI accessions were obtained from the Southern Regional Plant Introduction Station at Griffin, Georgia. For each family we developed six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) in the greenhouses at North Carolina State University in Raleigh, North Carolina.

Inoculum Preparation

Originally, the isolate of *D. bryoniae* was obtained from diseased cucumber tissues harvested from naturally infected plants in Charleston, South Carolina in 1998. In the fall of 2001, we reisolated the strains of *D. bryoniae* from watermelon plants that were artificially inoculated with the isolates in our greenhouses using

the technique described herein. Pycnidia were identified with a dissecting microscope (20×) and transferred to Petri plates containing potato dextrose agar (PDA) (25 mL/Petri plate). Isolates were selected from the first subculture on PDA based on macroscopic observations: colonies dark in color and showing concentric circles of growth were kept and transferred to fresh PDA. Cultures that did not appear contaminated by other fungi or bacteria, were transferred to a medium containing 25% PDA to stimulate abundant sporulation. Finally, we observed pycnidia/pseudothecia and spores to verify that their shape and size matched those of *D. bryoniae* as published (Zitter et al., 1996). For long-term storage (Dhingra and Sinclair, 1995), we transferred the fungus onto a disk of sterile filter paper (Whatman #2, 70 mm diameter) sitting over a layer of PDA in a Petri plate, subcultured the fungus for 2 to 4 weeks, dehydrated the filter paper disk and the mycelium for 12 to 16 hours at room temperatures (24±3 °C) under a sterile laminar-flow hood, cut the filter paper into squares (5×5 mm), and stored them in sterile test tubes in a refrigerator (3±1 °C) in the dark.

D. bryoniae was grown in Nalgene autoclavable pans (420×340×120 mm) containing 1,000 mL of 50% PDA (Gusmini et al., 2003). We incubated infested Nalgene pans for 2 to 4 weeks at 24±2 °C under alternating periods of 12 hours of fluorescent light (40 to 90 $\mu mol \bullet m^{-2} \bullet sec^{-1}$ PPFD) and 12 hours of darkness until pycnidia formed. For all inoculations, we prepared a spore suspension by flooding the culture plates with 10 mL of sterile, distilled water, and gently scraping the surface of the agar with an L-shaped sterile glass-rod to remove the spores from the mycelia. We filtered the liquid from each pan through four layers of sterile cheesecloth to remove dislodged agar and some mycelia. The final pH of the inoculum was not adjusted. We measured spore concentration with a hemacytometer and adjusted to a concentration of 5×10^5 $spores \bullet mL^{-1}$ by adding deionized water. Tween 20 (0.06 $g \bullet L^{-1}$) was added to the inoculum to keep the spores well dispersed in the inoculum solution (Song et al., 2004).

Cultural Practices and Inoculation Methods

In the greenhouse, we seeded directly in plastic pots (100×100 mm size, 600 mL volume) filled with a soilless mix (Canadian sphagnum peat moss, perlite, vermiculite, processed pine bark). We used two seeds per pot to ensure a good plant stand, and then thinned the seedlings to one per pot. In the field, seeds were sown on

raised, shaped beds on 3.1 m centers in single hills, 1.2 m apart. Border rows of the susceptible 'Charleston Gray' and 'Calhoun Gray' were planted around each test.

In the greenhouse, we inoculated plants at the second true leaf stage, after damaging the trichomes on the leaf surface by brushing the plants with a wooden stake 200 mm long and 20 mm wide. The sprayer was a hand-pumped spray bottle. Immediately before inoculation, we moved the plants into a humidity chamber made of clear polyethylene on the sides and top. The top was kept open during the summer and closed during the winter to keep the internal temperature close to 24°C, the optimum for *D. bryoniae*. We used humidifiers in the chamber running continuously for the treatment time (one day before inoculation through three days after inoculation) to keep the relative humidity close to 100% day and night. Plants were watered daily using overhead sprinklers, except when humidifiers were running.

In the field, we inoculated plants when they reached the fourth-true-leaf stage, after irrigating with about 12 mm of water during the two previous days to promote guttation on the day of inoculation, and damaging the trichomes on the leaf surface by brushing the plants with a wooden stake 200 mm long and 20 mm wide mounted on an aluminum handle 600 mm long. Plants were inoculated four times at two week intervals by spraying the inoculum onto all upper leaf surfaces. We delivered the inoculum as a fine mist using a backpack-sprayer operated at a pressure of 200 to 275 kP (30 to 40 psi). In the late afternoon of the day of inoculation, we irrigated with approximately 12 mm of water to promote disease development with high relative humidity at night. Artificial inoculation is not a required practice in the field in North Carolina, since the gummy stem blight is endemic to the region. However, natural epidemics of gummy stem blight on watermelon plants grown in the greenhouse are rare. We needed to use a similar testing technique both in the field and in the greenhouse. Thus, we chose to inoculate artificially at both sites.

Disease Assessment

Plants were rated for disease severity when symptoms appeared on the leaves and stems of the susceptible checks. Instead of the interval Horsfall-Barratt scale, we adopted an ordinal disease assessment scale (Gusmini et al., 2002), with 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem; 6 to 8 = symptoms on leaves and stems;

9 = plant dead. Plants with a disease rating greater than 5 had lesions on the stem, thus being prone to death from subsequent development of the disease. Plants with a disease rating of 5 or less had lesions only on the leaves (no stem lesions). Leaf ratings are important, because plant yield and survival is affected by leaf area, which is reduced by severe disease outbreaks. Stem ratings are important, because large, localized lesions can kill the plant, especially if located near the crown (base) of the plant. Nevertheless, our rating scale allowed a quantitative assessment of the amount of disease present on each plant, because stem lesions are typically accompanied by major leaf damage and appear after leaf lesions during the epidemic.

Experimental Design and Data Analysis

We conducted all our tests in the greenhouses at North Carolina State University in Raleigh, North Carolina, and in the field at the Horticultural Crops Research Station at Clinton, North Carolina. The two families 'Allsweet' × PI 526233 and 'Calhoun Gray' × PI 482283 were tested in 2002, while the other two were tested in 2003. The experiment had two sets in 2002 and four sets in 2003 (equally divided in field and greenhouse tests), each set including all six generations.

The inheritance of the *db* gene from Norton (1979) was tested after classifying each plant as susceptible or resistant based on their rank relative to the mean value of the disease assessment scale adopted (4.5). We performed segregation analysis and goodness-of-fit tests with the SAS-STAT statistical package (SAS Institute, Cary, NC) and the SASGene 1.2 program (Liu et al., 1997). All χ^2 tests were performed at the 95% confidence level. Since there was strong evidence against the single gene hypothesis, we verified the distribution of the F_2 data for each family using the UNIVARIATE procedure of SAS-STAT and by plotting the disease ratings against their frequency, prior to analyzing resistance to gummy stem blight as a quantitative trait.

We tested the F_2 data for homogeneity of variances using the Bartlett's method (Ostle and Malone, 1988; Steel et al., 1997). Since variances were homogeneous only among tests (field vs. greenhouse) within family, we pooled the data by family. We also analyzed the data for each family and test, to highlight possible differences among tests.

Phenotypic (P), environmental (E), genotypic (G), and additive (A) variances were estimated from generation variances as follows (Warner, 1952; Wright, 1968):

$$\sigma^2(P) = \sigma^2(F_2) \qquad \sigma^2(E) = \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}$$

$$\sigma^2(G) = \sigma^2(PH) - \sigma^2(E) \qquad \sigma^2(A) = \left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_a)\right]$$

Negative estimates for genetic variances are possible with the experimental design adopted. Negative estimates should be considered equal to zero (Robinson et al., 1955), but should be reported "in order to contribute to the accumulation of knowledge, which may, in the future, be properly interpreted" (Dudley and Moll, 1969). We considered negative estimates equal to zero for the calculation of the mean estimates over families or locations. When a negative estimate was derived from another negative value (narrow-sense heritability and gain from selection, calculated from additive variance), it was considered close to zero and omitted.

The number of effective factors was estimated using the following methods (Lande, 1981; Mather and Jinks, 1982; Wright, 1968):

Lande's method I:
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}\right\}}$$

Lande's method II:
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_a)\right]\right\}}$$

Lande's method III:
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{\left\{8 \times \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_a) - \sigma^2(F_1)\right]\right\} - \frac{\left[\sigma^2(P_a) + \sigma^2(P_b)\right]}{2}}$$

Mather's method:
$$\frac{\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{2}}{\left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_a)\right]}$$

Wright's method:
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2 \times \left\{1.5 - \left[2 \times \frac{\mu(F_1) - \mu(P_a)}{\mu(P_b) - \mu(P_a)} \times \left(1 - \frac{\mu(F_1) - \mu(P_a)}{\mu(P_b) - \mu(P_a)}\right)\right]\right\}}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}\right\}}$$

The possible gain from selection per cycle was predicted as $h_n^2 \times \sqrt{\sigma^2(P)}$ multiplied by the selection differential in standard deviation units k for selection intensities of 5%, 10%, or 20% (Hallauer and Miranda, 1988). The statistical analysis was performed using the SAS-STAT statistical package (SAS Institute, Cary, North Carolina).

Results and Discussion

In our study, resistance to gummy stem blight in watermelon was not inherited as a single gene, as previously described by Norton in PI 189225 (Table 1). The expected segregation ratios for the inheritance of the *db* gene were not observed in the F_2 and backcross generations, when PI 189225 was crossed with the susceptible 'NH Midget'. Similar results were obtained in greenhouse and field tests for the other three families tested, involving PI 482283 and PI 526233 as resistant parents.

The lack of fit to the single gene hypothesis suggests that gummy stem blight resistance in watermelon could be inherited as a quantitative trait locus (QTL). Most likely, multiple QTLs could be involved in the complete expression of resistance. Nevertheless, the distribution of our F_2 data was strongly skewed towards susceptibility (Fig. 1) and far from the expected bell-shaped (normal) distribution for quantitative traits. This distribution pattern would suggest the presence either of a single gene or a QTL with high environmental variation, or of QTLs regulating the expression level of a major gene.

A similar distribution was recorded in all four families, with the exception of the field test of the family PI 526233 × 'Allsweet'. Higher variability in the field than in the greenhouse tests and low correlation among tests is commonly found when screening for resistance to gummy stem blight (Gusmini and Wehner, 2002) and may be caused by differences in microclimate in the field.

In our analysis, the variances of the six generations tested were generally consistent across families. Larger differences in variance estimates among families and within generation were found in the field test, compared to the greenhouse test (Table 2). Genetic variance was larger than environmental variance in three of the four crosses (Table 3). The larger environmental variance in the cross PI 482283 × 'Calhoun Gray' was determined solely by the field test. A large genetic component was found also for this cross in the greenhouse

test. The large genetic variance found in our study indicates that the hypothesis of a quantitative trait is more likely than the hypothesis of a single gene with large environmental variation.

Additive genetic effects were estimated, but a comparison with dominance effects was not possible. With our experimental design, dominance variance could be estimated by subtraction of genetic and additive variances from the phenotypic, but such an indirect estimate would not be precise. Additive effects in our experiment were large in the greenhouse tests (mean = 4.45), but small in the field tests (mean = 1.32).

The broad-sense heritability was high for field and greenhouse tests (0.68 vs. 0.73, respectively). In the family PI 482283 × 'Calhoun Gray' the broad-sense heritability was largely different among tests (0.21 in the field test vs. 0.82 in the greenhouse test). The narrow-sense heritability was much larger in the greenhouse than in the field tests (1.13 vs. 0.54, respectively), except for the family PI 526233 × 'Allsweet' (1.14 vs. 1.93, respectively).

Our data indicated that broad-sense heritability for resistance to gummy stem blight in watermelon can be high, indicating more importance of genetic than environmental variability in many tests. Nevertheless, greenhouse testing should be used to capitalize on the higher additive components and increase the narrow-sense heritability for population improvement. In addition, the overall large heritability estimates confirm that the genotype has a larger effect than the testing environment, even though the use of more uniform and controlled environments, as in greenhouse tests, helps to enhance the genotypic effect and to allow more precise selections of resistant parents for the next generation.

Our analysis could not estimate dominance and epistatic effects. Thus, the estimates of the minimum number of effective factors (genes) for resistance may be biased. We used five estimates, but only those most consistent among families are presented (Table 4). These estimates indicate that few genetic factors may be involved in the inheritance of resistance to gummy stem blight in watermelon.

Our analysis showed that almost no progress can be done by field selection. On the contrary, selection based on greenhouse data could lead to a gain of at least three points (on a 10 point scale) per generation even under the lower selection intensities (i.e., 20%) typically used in recurrent selection programs.

Based on our data, Norton may not have used a large enough population during the development of the AU-series of resistant watermelon cultivars. In addition, escapes from disease testing might have been selected as resistant plants.

Conclusions

Resistance to gummy stem blight in watermelon has been previously described as dependent solely on the inheritance of the recessive gene *db* (Norton, 1979). Watermelon cultivars have been improved by introgression of the *db* gene, but they were less resistant than the resistant parents in the field. Thus, no cultivar so far has been released with an acceptable degree of resistance for field production of watermelon during gummy stem blight epidemics.

Our study indicated that resistance to gummy stem blight in watermelon should be regarded as a quantitative trait. Few QTLs may be involved in the expression of resistance and the *db* gene may be a QTL with a major effect or a single Mendelian gene, under epistatic influence of other regulatory QTLs. In addition, we measured a large heritability and high additive variance for resistance.

Watermelon breeders interested in the development of resistant cultivars should use breeding techniques that make the best use of additive variance, such as recurrent selection. Greenhouse testing of the breeding material would be preferred to field testing, based on the larger heritability for the greenhouse test. Field testing may be used at later stages of selection to confirm resistance of improved material under field conditions. Multiple locations and replications, and large plots to simulate commercial production fields should be used. A precise assessment of the causal agent for the loss of plants also would be necessary before classifying them as susceptible. Field resistance could be tested by relying on natural sources of inoculum in areas where gummy stem blight is an endemic disease. Resistance to natural epidemics in the field could ultimately be confirmed with severe artificial inoculations in the greenhouse.

Further studies on the inheritance of resistance should include the development of molecular marker maps and QTL mapping. The identification of major QTLs in greenhouse tests and the verification of their effect in field tests would be the most suitable approach to take advantage of the large additive variance and heritability available. The identification of molecular markers for the major QTLs for resistance would allow

plant breeders to reduce the number of replications needed to test breeding lines in field and greenhouse, and to confirm the transfer of the QTLs to the progeny. In crosses of adapted by wild germplasm in watermelon, it is difficult to recover a usable cultivar with high fruit quality. Linkage drag of deleterious fruit characteristics from the resistant wild germplasm (resistant PI accessions) and the reduction of the number of backcrosses needed to recover the adapted parental fruit type would be additional advantages of a molecular assisted breeding program (Frisch et al., 1999; Stuber et al., 1999). Negative effects of linkage drag in these crosses have been experienced by watermelon breeders using pedigree and backcross breeding strategies. Furthermore, recurrent selection programs, even though requiring more resources, may be more useful in breaking linkage of resistance and fruit quality.

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Table 1. Single locus goodness-of-fit-test for the *db* gene for resistance to gummy stem blight in watermelon.[z]

Generation	Total	Susceptible[y]	Resistant[x]	Expected[w]	χ^2	df	P-value
PI 189225 × 'NH Midget'							
Field test							
P_aS_1[v]	18	3	15				
P_bS_1[u]	12	12	0				
F_1	25	25	0				
F_2	110	74	36	3:1	3.50	1	0.06
BC_1P_a	46	32	14	1:1	7.04	1	0.01
BC_1P_b	56	44	12	1:0	2.57	1	0.10
Greenhouse test							
P_aS_1[v]	40	0	40				
P_bS_1[u]	40	40	0				
F_1	60	60	0				
F_2	360	242	118	3:1	11.61	1	0.00
BC_1P_a	140	98	42	1:1	22.40	1	0.00
BC_1P_b	140	136	4	1:0	0.11	1	0.73

Table 1. Continued. [z]

Generation	Total	Susceptible [y]	Resistant [x]	Expected [w]	χ^2	df	P-value
PI 482283 × 'NH Midget'							
Field test							
P_aS_1 [v]	16	0	16				
P_bS_1 [u]	15	15	0				
F_1	18	18	0				
F_2	124	64	60	3:1	36.17	1	0.00
BC_1P_a	44	25	19	1:1	0.82	1	0.36
BC_1P_b	58	41	17	1:0	4.98	1	0.02
Greenhouse test							
P_aS_1 [v]	40	0	40				
P_bS_1 [u]	40	40	0				
F_1	60	46	14				
F_2	400	248	152	3:1	36.05	1	0.00
BC_1P_a	140	38	102	1:1	29.26	1	0.00
BC_1P_b	140	140	0				

Table 1. Continued. [z]

Generation	Total	Susceptible [y]	Resistant [x]	Expected [w]	χ^2	df	P-value
PI 482283 × 'Calhoun Gray'							
Field test							
P_aS_1 [v]	3	0	3				
P_bS_1 [u]	3	3	0				
F_1	7	3	4				
F_2	61	50	11	3:1	1.58	1	0.20
BC_1P_a	14	8	6	1:1	0.29	1	0.59
BC_1P_b	11	11	0				
Greenhouse test							
P_aS_1 [v]	3	1	2				
P_bS_1 [u]	3	3	0				
F_1	6	6	0				
F_2	75	45	30	3:1	9.00	1	0.00
BC_1P_a	21	18	3	1:1	10.71	1	0.00
BC_1P_b	12	12	0				

Table 1. Continued. [z]

Generation	Total	Susceptible [y]	Resistant [x]	Expected [w]	χ^2	df	P-value
PI 526233 × 'Allsweet'							
Field test							
P_aS_1 [v]	4	1	3				
P_bS_1 [u]	1	1	0				
F_1	2	2	0				
F_2	75	72	3	3:1	17.64	1	0.00
BC_1P_a	19	19	0	1:1	19.00	1	0.00
BC_1P_b	11	11	0				
Greenhouse test							
P_aS_1 [v]	3	0	3				
P_bS_1 [u]	3	3	0				
F_1	6	5	1				
F_2	75	31	44	3:1	45.34	1	0.00
BC_1P_a	21	15	6	1:1	3.86	1	0.05
BC_1P_b	12	12	0				

z Data are ratings from four families of resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *lanatus* and *citroides*. Disease assessment scale adopted for evaluating watermelon for resistance to gummy stem blight: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem,; 6 to 8 = symptoms on leaves and stems; 9 = plant dead

y Susceptible plants had a disease rating > 4.5

x Resistant plants had a disease rating < 4.5

w Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

v P_a was the hypothetic carrier of the recessive gene (*dbdb*)

u P_b was the hypothetic carrier of the dominant gene (*DbDb*)

Table 2. Phenotypic variances by generation for the four watermelon families screened for resistance to gummy stem blight in greenhouse and field tests in North Carolina (2002-2003) [z].

Pedigree	$\sigma^2(P_a)$	$\sigma^2(P_b)$	$\sigma^2(F_1)$	$\sigma^2(F_2)$	$\sigma^2(BC_1P_a)$	$\sigma^2(BC_1P_b)$
Field test						
PI 189225 × 'NH Midget'	0.97	0.08	1.12	3.37	3.18	4.35
PI 482283 × 'NH Midget'	0.46	0.35	1.23	4.22	3.39	4.66
PI 482283 × 'Calhoun Gray'	0.33	0.00	8.57	5.52	10.23	0.00
PI 526233 × 'Allsweet'	0.67	0.00	0.00	2.12	0.05	0.09
Mean	0.61	0.11	2.73	3.81	4.21	2.27
Greenhouse test						
PI 189225 × 'NH Midget'	0.95	0.05	1.52	4.20	2.70	1.63
PI 482283 × 'NH Midget'	0.25	0.34	3.19	4.28	2.61	1.08
PI 482283 × 'Calhoun Gray'	0.33	1.00	0.80	4.09	2.05	0.88
PI 526233 × 'Allsweet'	0.33	0.33	1.37	3.16	2.35	0.39
Mean	0.46	0.43	1.72	3.93	2.43	0.99
Pooled						
PI 189225 × 'NH Midget' [y]	1.03	0.06	1.39	4.00	2.80	2.51
PI 482283 × 'NH Midget' [x]	0.34	0.34	2.83	4.31	2.99	2.27
PI 482283 × 'Calhoun Gray' [w]	1.47	1.60	5.41	5.58	5.11	0.79
PI 526233 × 'Allsweet' [v]	1.24	0.30	2.70	6.39	4.48	0.58
Mean	1.02	0.57	3.08	5.07	3.84	1.54

z Data are ratings from four families of resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *lanatus* and *citroides*. Disease assessment scale adopted for evaluating watermelon for resistance to gummy stem blight: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms

on leaves only; 5 = some leaves dead, no symptoms on stem,; 6 to 8 = symptoms on leaves and stems; 9 = plant dead

y F_2 Bartlett's $\chi^2 = 1.94$; P-value = 0.16

x F_2 Bartlett's $\chi^2 = 0.01$; P-value = 0.92

w F_2 Bartlett's $\chi^2 = 1.49$; P-value = 0.22

v F_2 Bartlett's $\chi^2 = 2.95$; P-value = 0.09

Table 3. Variance and heritability estimates for the four watermelon families screened for resistance to gummy stem blight in greenhouse and field tests in North Carolina (2002-2003)[z].

Pedigree	$\sigma^2(P)$[y]	$\sigma^2(E)$[x]	$\sigma^2(G)$[w]	$\sigma^2(A)$[v]	H^2_B[u]	h^2_n[t]
Field test						
PI 189225 × 'NH Midget'	3.37	0.83	2.55	-0.79	0.76	--[s]
PI 482283 × 'NH Midget'	4.22	0.82	3.40	0.39	0.81	0.09
PI 482283 × 'Calhoun Gray'	5.52	4.37	1.15	0.82	0.21	0.15
PI 526233 × 'Allsweet'	2.12	0.17	1.95	4.09	0.92	1.93
Mean	3.81	1.55	2.26	1.32	0.68	0.54
Greenhouse test						
PI 189225 × 'NH Midget'	4.20	1.01	3.19	4.07	0.76	0.97
PI 482283 × 'NH Midget'	4.28	1.74	2.54	4.87	0.59	1.14
PI 482283 × 'Calhoun Gray'	4.09	0.73	3.36	5.25	0.82	1.28
PI 526233 × 'Allsweet'	3.16	0.85	2.31	3.60	0.73	1.14
Mean	3.93	1.08	2.85	4.45	0.73	1.13
Pooled						
PI 189225 × 'NH Midget'[r]	4.00	0.97	3.03	2.70	0.76	0.67
PI 482283 × 'NH Midget'[q]	4.31	1.58	2.72	3.36	0.63	0.78
PI 482283 × 'Calhoun Gray'[p]	5.58	3.47	2.11	5.25	0.38	0.94
PI 526233 × 'Allsweet'[o]	6.39	1.73	4.65	7.70	0.73	1.21
Mean	5.07	1.94	3.13	4.75	0.63	0.90

z Data are ratings from four families of resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *lanatus* and *citroides*. Disease assessment scale adopted for evaluating watermelon for resistance to gummy stem blight: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms

on leaves only; 5 = some leaves dead, no symptoms on stem,; 6 to 8 = symptoms on leaves and stems; 9 = plant dead

y σ^2(P) = phenotypic variance = $\sigma^2\left(F_2\right)$

x σ^2(E) = environmental variance = $\dfrac{\sigma^2\left(P_a\right)+\sigma^2\left(P_b\right)+\left[2\times\sigma^2\left(F_1\right)\right]}{4}$

w σ^2(G) = genetic variance = $\sigma^2\left(P\right)-\sigma^2\left(E\right)$

v σ^2(A) = additive variance = $\left[2\times\sigma^2\left(F_2\right)\right]-\left[\sigma^2\left(BC_1P_a\right)+\sigma^2\left(BC_1P_a\right)\right]$

u H^2_B = broad-sense heritability

t h^2_n = narrow-sense heritability

s Negative estimate from a negative estimate of additive variance

r F_2 Bartlett's χ^2 = 1.94; P-value = 0.16

q F_2 Bartlett's χ^2 = 0.01; P-value = 0.92

p F_2 Bartlett's χ^2 = 1.49; P-value = 0.22

o F_2 Bartlett's χ^2 = 2.95; P-value = 0.09

Table 4. Estimates of number of effective factors and predicted gain from selection under different selection intensities for the four watermelon families screened for resistance to gummy stem blight in greenhouse and field tests in North Carolina (2002-2003).

	Effective Factors			Gain from Selection[y]		
Pedigree	Wright[x]	Lande I[w]	Mean	5%	10%	20%
Field test						
PI 189225 × 'NH Midget'	1.5	1.4	1.45	--[v]	--[v]	--[v]
PI 482283 × 'NH Midget'	1.3	1.2	1.25	0.4	0.3	0.3
PI 482283 × 'Calhoun Gray'	4.8	4.8	4.80	0.7	0.6	0.5
PI 526233 × 'Allsweet'	2.4	1.6	2.00	5.8	4.9	3.9
Mean	2.5	2.3	2.37	1.5	1.3	1.0
Greenhouse test						
PI 189225 × 'NH Midget'	1.6	1.5	1.55	4.1	3.5	2.8
PI 482283 × 'NH Midget'	1.9	1.8	1.85	4.9	4.1	3.3
PI 482283 × 'Calhoun Gray'	0.4	0.3	0.35	5.3	4.6	3.6
PI 526233 × 'Allsweet'	2.0	1.9	1.95	4.2	3.6	2.8
Mean	1.5	1.4	1.42	4.6	4.0	3.1
Pooled						
PI 189225 × 'NH Midget'[u]	1.5	1.4	1.45	2.8	2.4	1.9
PI 482283 × 'NH Midget'[t]	1.7	1.7	1.70	3.3	2.8	2.3
PI 482283 × 'Calhoun Gray'[s]	0.8	0.8	0.80	6.3	5.4	4.3
PI 526233 × 'Allsweet'[r]	1.3	1.3	1.30	4.6	3.9	3.1
Mean	1.3	1.3	1.31	4.3	3.6	2.9

z Data are ratings from four families of resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *lanatus* and *citroides*. Disease assessment scale adopted for evaluating watermelon for resistance to gummy stem blight: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem,; 6 to 8 = symptoms on leaves and stems; 9 = plant dead

y $k \times h_n^2 \times \sqrt{\sigma^2(P)}$

x $$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2 \times \left\{1.5 - \left[2 \times \frac{\mu(F_1) - \mu(P_a)}{\mu(P_b) - \mu(P_a)} \times \left(1 - \frac{\mu(F_1) - \mu(P_a)}{\mu(P_b) - \mu(P_a)}\right)\right]\right\}}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}\right\}}$$

w $$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]}{4}\right\}}$$

v Negative estimate from a negative estimate of additive variance

u F_2 Bartlett's $\chi^2 = 1.94$; P-value = 0.16

t F_2 Bartlett's $\chi^2 = 0.01$; P-value = 0.92

s F_2 Bartlett's $\chi^2 = 1.49$; P-value = 0.22

r F_2 Bartlett's $\chi^2 = 2.95$; P-value = 0.09

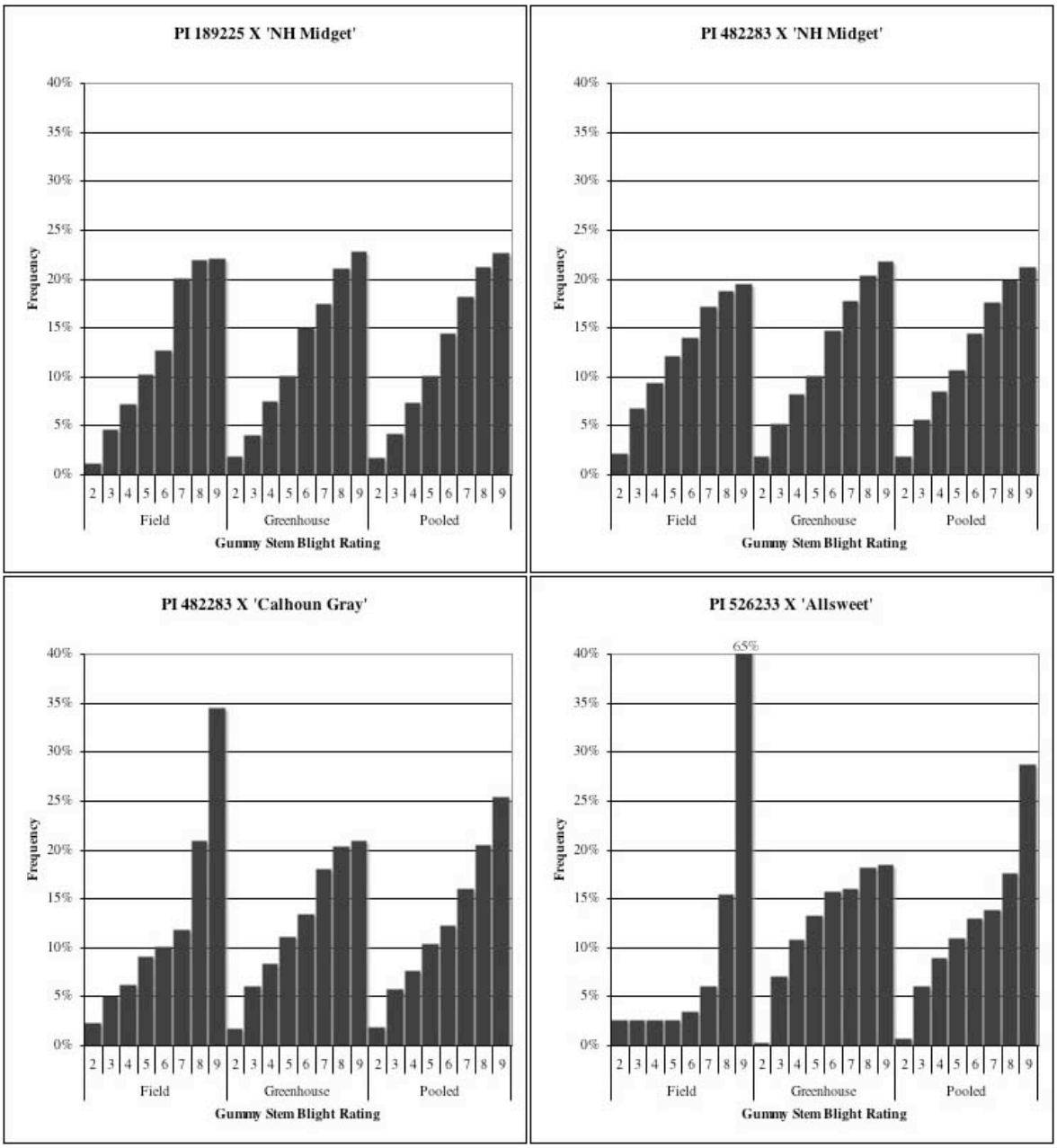

Figure 1. Frequency distribution plots of F_2 data for the four watermelon families (resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *lanatus* and *citroides*) screened for resistance to gummy stem blight in greenhouse and field tests in North Carolina (2002-2003). Disease assessment scale adopted for the screening: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem,; 6 to 8 = symptoms on leaves and stems; 9 = plant dead.

CHAPTER SIX

PRELIMINARY STUDY OF MOLECULAR MARKERS LINKED TO RESISTANCE TO GUMMY STEM BLIGHT IN WATERMELON

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Abstract

Gummy stem blight, caused by *Didymella bryoniae* (Auersw.) Rehm (anamorph: *Phoma cucurbitacearum* (Fr.:Fr.) Sacc.), is a major disease of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. Watermelon breeders have had little success in transferring resistance to adapted lines because of difficult testing procedures. The objective of this study was to optimize methodologies and protocols for the identification of molecular markers linked to resistance to gummy stem blight in watermelon to allow molecular assisted breeding. A fast and reliable protocol for the extraction of DNA from watermelon leaves was developed. Several types of molecular markers were tested to identify polymorphism among the resistant and susceptible parents of F_2 populations, segregating for resistance to gummy stem blight. Polymorphic markers were tested for linkage to resistance in two F_2 populations derived from the crosses PI 189225 × 'NH Midget' and PI 482283 × 'NH Midget'. The phenotypic value of F_2 plants was determined by progeny testing of (F_2 derived) F_3 families in replicated greenhouse tests. Artificial inoculations and controlled environmental conditions reduced replication effects and improved genotypic differences among families. Simple sequence repeat (SSR) markers produced a higher frequency of polymorphic bands than other marker types. Nevertheless, none of the SSR markers analyzed was linked to resistance to gummy stem blight in our populations. However, one random amplified polymorphic DNA (RAPD) primer produced a dominant molecular marker linked to resistance from PI 189225. The UBC338-600 RAPD marker was estimated to be 35-42 cM from the locus conferring resistance.

Introduction

Didymella bryoniae (Auersw.) Rehm [=*Mycosphaerella citrullina* (C.O.Sm.) Gross. and *Mycosphaerella melonis* (Pass) Chiu & Walker] and *Phoma cucurbitacearum* (Fr.:Fr.) Sacc. [=*Ascochyta cucumis* Fautrey & Roum] (Keinath et al., 1995) are the perfect and imperfect stages of the fungal pathogen causing the disease known as gummy stem blight. Gummy stem blight on watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is evident as crown blight, stem cankers, and extensive defoliation, with symptoms observed on the cotyledons, hypocotyls, leaves, and fruit (Maynard and Hopkins, 1999). *D. bryoniae* is a

fungus that is seed-borne (Lee et al., 1984), air-borne (van Steekelenburg, 1983), or soil-borne (Bruton, 1998; Keinath, 1996).

It is difficult to get adequate control of gummy stem blight through fungicide applications (Keinath, 1995; Keinath, 2000) and good cultural practices (Keinath, 1996; Rankin, 1954), particularly during periods of frequent rainfall when relative humidity remains high for a long period. Genetic resistance to gummy stem blight has received attention since the 1970s as an alternative to chemical control (Norton et al., 1993; Norton et al., 1995; Norton et al., 1986). Resistant watermelon cultivars were developed by selecting disease resistant seedlings from backcrossed families that produced high yield of excellent quality fruit (Norton et al., 1986). From the program, 'AU-Jubilant' and 'AU–Producer' (Norton et al., 1986), 'AU-Golden Producer' (Norton et al., 1993), and 'AU-Sweet Scarlet' (Norton et al., 1995) were released with moderate to high resistance to gummy stem blight. However, they were much less resistant than the resistant parents PI 189225 and PI 271778.

We have developed an efficient screening method for testing watermelon lines (Gusmini and Wehner, 2002; Song et al., 2004), including systems for mass production of inoculum of *D. bryoniae* for large field screening experiments (Gusmini et al., 2003), and a disease assessment scale to rate for the presence of foliar and stem lesions (Gusmini et al., 2002). Between 1998 and 2001, all available accessions (1,274) from the watermelon germplasm collection of the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), along with 51 adapted cultivars, were tested to identify new genetic sources of resistance to gummy stem blight (Gusmini et al., 2005b). Additional efforts to study the inheritance of resistance are underway and our results indicate that resistance to gummy stem blight may be under the genetic control of one or more quantitative trait loci (QTLs). The *db* gene conferring resistance to PI 189225 (Norton, 1979) may actually be either the QTL with a major effect, or a single Mendelian gene whose expression may be regulated by minor genes.

It is difficult to test watermelon for resistance to gummy stem blight. The high variability associated with the tests requires the use of multiple years, locations, and replications of progeny rows (Song et al., 2004). The need for large replicated tests does not fit well in watermelon breeding programs. Plant breeders need testing methods that allow early selection, preferably at the seedling stage, to make targeted crosses only among plants with high resistance. In addition, in segregating generations single-plant selection would be more

convenient than progeny selection, which requires an additional self-pollination and testing of the next generation. We were interested to determine whether molecular markers could be found for resistance to gummy stem blight.

Molecular markers linked with resistance to gummy stem blight are not currently available. Nevertheless, linkage maps of watermelon have been constructed and several molecular markers are available (Hashizume et al., 2003; Hawkins et al., 2001; Levi et al., 2002; Zhang et al., 2004). Hawkins et al. (2001) constructed two partial maps from an F_2 and F_3 population, respectively, of the cross 'NH Midget' × PI 294361-*FR*. The two short maps were 112.9 centimorgans (cM) and 139 cM long and included 26 and 13 RAPD (random amplified polymorphic DNA) markers, respectively. In 2002, Levi et al. published a linkage map where 171 RAPD markers, 27 ISSR (inter-simple sequence repeats) markers, and one SCAR (sequence-characterized amplified region) marker were assigned to 24 linkage groups, the haploid number for watermelon being 11. The linkage map by Hashizume et al. (2003) included RAPD, RFLP (restriction fragment length polymorphism), and ISSR markers that mapped 554 loci in 11 linkage groups, for a total length of 2,384 cM. Finally in 2004, Zhang et al. constructed a linkage map from a population of 117 recombinant inbred lines (RILs) using a total of 104 RAPD, SCAR, and ISSR markers. The map covered a total distance of 1,027 cM and included 15 linkage groups.

Additional molecular markers have been made available, but not mapped, for use in watermelon (Guerra-Sanz, 2002; Jarret et al., 1997; Katzir et al., 1996; Poleg et al., 2001). Katzir et al. (1996) identified SSR (simple sequence repeat) markers from DNA sequences of melon (*Cucumis melo* L.), watermelon, and cucumber (*Cucumis sativus* L.) that amplified DNA fragments also in cucurbit species different from the one of origin. In 1997, Jarret et al. used seven SSR markers to measure diversity among 32 watermelon lines. Poleg et al. (2001) designed 24 SSR markers from a genomic library of melon DNA, four from a cucumber cDNA library, and six from database sequences of cucumber and melon. Guerra–Sanz (2002) designed 19 primer pairs from watermelon ESTs (expressed sequence tag) containing SSR markers.

Currently, the database of the National Center for Biotechnology Information (NCBI) contains 722 nucleotidic sequences (mostly ESTs) for watermelon (NCBI, 2004). These sequences can be also used to design additional primers for amplification and screening of candidate sequence-based markers. A similar

approach was successful in assembling a linkage map for *Medicago* spp., using 288 markers, half of them designed from database ESTs (Choi et al., 2004).

The development of a fast, easy, and cheap technique for the deployment of molecular markers in marker assisted selection (MAS) should start with efficient DNA extraction protocols that would reduce the number of steps and the amount of reagents needed to process each sample. In addition, the quality (freedom from polysaccharides and proteins) of the DNA should be compatible with the marker technique that will be used. For example, ISSR, RAPD, and SSR markers do not require the DNA purity needed for AFLP™ (amplified fragment length polymorphism) and RFLP markers (de Vienne, 2002). The extraction of DNA from watermelon leaves may result in poor yields and co-isolation of highly viscous polysaccharides, if proper techniques are not used (Levi and Thomas, 1999). Levi and Thomas designed a specific protocol for the isolation of high quality DNA, requiring several steps and high quantities of leaf tissue per sample (approximately 5 g).

The use of smaller quantities of tissue (<0.5 g), faster DNA extraction techniques, and fast PCR-based (polymerase chain reaction-based) techniques for molecular screening would permit plant breeders to use MAS between seeding and transplanting (or pollination) stages in the selection program. In order to achieve this, it would be necessary for researchers to develop marker techniques based on PCR amplification of the marker locus and direct screening of the amplification products by electrophoresis (possibly on agarose gels). SSR, ISSR, EST-based, and RAPD markers do not need additional steps between amplification and gel electrophoresis, nor do they require high quality DNA samples, so those would be useful techniques for MAS in watermelon. In addition, microsatellite (SSR and ISSR) and EST-based markers may provide codominant bands, thus allowing the distinction of dominant homozygotes from heterozygotes at the marker locus.

The development and deployment of molecular markers on large segregating populations for the construction of linkage maps or the identification of genes or testing of markers developed on other populations can require high inputs (time and resources), sometimes with few results (identification of none to few polymorphic molecular markers). In 1991, Michelmore et al. proposed a new methodology, called bulk segregant analysis (BSA), that allowed the comparison of genotype at a specific marker locus of two bulks of DNA from the phenotypic extremes of the segregating population (Michelmore et al., 1991). BSA reduced the

number of amplification reactions needed and allowed an initial screen of polymorphic markers for linkage analysis. Once polymorphic markers were identified among the DNA bulks, they could be used for a mapping experiment on the entire segregating populations, thus increasing the chances of finding linked markers for the trait of interest with fewer reactions. Even though this technique was developed for RFLP markers, it can be easily adapted to any molecular marker.

The objective of this study was to optimize methodologies and protocols for the identification of molecular markers linked to resistance to gummy stem blight in watermelon. In addition, we tested 355 primers (176 SSR, 15 ISSR, 68 EST-based, and 96 RAPD) for polymorphism among the resistant and susceptible parents of our F_2 populations, segregating for resistance to gummy stem blight. We used BSA to identify candidate molecular markers linked to resistance, and mapped them on our F_2 populations.

Materials and Methods

Germplasm and Crosses

In the experiment, we used two families developed from the two crosses PI 189225 × 'NH Midget' and PI 482283 × 'NH Midget'. PI 189225 and PI 482283 (resistant parents) were *C. lanatus* var. *citroides*. 'NH Midget' (susceptible parent) was *C. lanatus* var. *lanatus*.

'NH Midget' was obtained from commercial seed stocks and the plant introduction (PI) accessions were obtained from the Southern Regional Plant Introduction Station at Griffin, Georgia. For each family, we developed four generations (P_aS_1, P_bS_1, F_1, F_2, F_3) in the greenhouses at North Carolina State University in Raleigh, North Carolina.

Inoculum Preparation

Originally, the isolate of *D. bryoniae* was obtained from diseased cucumber tissues harvested from naturally-infected plants in Charleston, South Carolina in 1998. In the fall of 2001, we reisolated the strains of *D. bryoniae* from watermelon plants that were artificially inoculated with the isolates in our greenhouses using the technique described here. Pycnidia were identified with a dissecting microscope (20×) and transferred to Petri plates containing potato dextrose agar (PDA) (25 ml/Petri plate). Isolates were selected from the first

subculture on artificial medium based on macroscopic observations: colonies dark in color and showing concentric circles of growth were kept and transferred to fresh PDA. Cultures that did not appear contaminated by other fungi or bacteria were transferred to a medium containing 25% PDA to stimulate abundant sporulation. Finally, we observed pycnidia, pseudothecia and spores to verify that their shape and size matched those of *D. bryoniae* as published (Zitter et al., 1996). For long-term storage (Dhingra and Sinclair, 1995), we transferred the fungus onto a disk of sterile filter paper (Whatman #2, 70 mm diameter) sitting over a layer of PDA in a Petri plate, subcultured the fungus for 2 to 4 weeks, dehydrated the filter paper disk and the mycelium for 12 to 16 hours at room temperatures (24±3 °C) under a sterile, laminar flow hood, cut the filter paper into squares (5×5 mm), and stored them in sterile test tubes in a refrigerator (3±1 °C) in the dark.

D. bryoniae was grown in Petri plates containing 25 mL of 50% PDA. We incubated infected Petri plates for two to four weeks at 24±2 °C under alternating periods of 12 hours of fluorescent light (40 to 90 $\mu mol \bullet m^{-2} \bullet sec^{-1}$ PPFD) and 12 hours of darkness until pycnidia formed. For the inoculations, we prepared a spore suspension by flooding the culture plates with 10 mL of sterile, distilled water, and gently scraping the surface of the agar with an L-shaped sterile glass rod to remove the spores from the mycelia. We filtered the liquid from each pan through four layers of sterile cheesecloth to remove dislodged agar and some mycelia. The final pH of the inoculum was not adjusted. We measured spore concentration with a hemacytometer and adjusted to a concentration of $5 \bullet 10^5$ spores$\bullet mL^{-1}$ by adding deionized water. Tween 20 (0.06 $g \bullet L^{-1}$) was added to the inoculum to keep the spores well dispersed in the inoculum solution (Song et al., 2004).

Cultural Practices

Seeds were planted in 72-square-cell plug flats for all generations and families. Seedlings from the F_2 seeds were transplanted into the greenhouse in black polyethylene potting bags (vol ≈ 11 L). We used a soilless mix (Canadian sphagnum peat moss, perlite, vermiculite, processed pine bark) and fertigated as needed using a 10:10:10 NPK soluble fertilizer. F_2 plants in the pollination greenhouse were sprayed with fungicide and insecticide as needed, starting only after the collection of leaves for DNA extraction. For each of the F_2 plants, we grew a single runner on trellises and, starting after the collection of leaves for DNA extraction, we removed laterals and self-pollinated open flowers daily by hand. Fruit were harvested 40 days after pollination. Seeds

from each fruit were extracted by hand, sterilized with a 10% solution of chlorine (10 minutes), and dried at a temperature close to 27°C in a forced air dryer (Wehner and Humphries, 1994).

Inoculation Methods

Immediately prior to inoculation, we moved the plants into a humidity chamber made of clear polyethylene on the sides and top. The top was kept open during the summer and closed during the winter to keep the internal temperature close to 24°C, the optimum for *D. bryoniae*. We used humidifiers in the chamber running continuously for the treatment time (one day before inoculation through three days after inoculation) to keep the relative humidity close to 100% day and night. We inoculated seedlings of the P_aS_1, P_bS_1, F_1, and F_3 generations at the second true leaf stage, after damaging the trichomes on the leaf surface by brushing the plants with a wooden stake (200 mm long and 20 mm wide) to stimulate fungal growth and infection. The sprayer was a hand-pumped spray bottle. Plants were watered daily using overhead sprinklers, except when humidifiers were running.

Disease Assessment

Plants were rated for disease severity when severe symptoms appeared on the leaves and stems of the susceptible checks (typically three weeks after inoculation). Instead of the interval Horsfall-Barratt scale, we adopted an ordinal disease assessment scale (Gusmini et al., 2002), with 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem; 6 to 8 = symptoms on leaves and stems; 9 = plant dead. Plants with a disease rating of 5 or less, had lesions only on the leaves (no stem lesions). Leaf ratings are important, because plant yield and survival is affected by leaf area, which is reduced by severe disease outbreaks. Stem ratings are important, because large, localized lesions can kill the plant, especially if located near the crown (base) of the plant.

Leaf Collection and DNA Extraction

We collected four to six young (1 to 4 days old) leaves from each plant. Each sample was stored in a polyethylene Easy Zipper Ziploc® Bag, resistant to freezing temperatures, in a -80°C freezer.

We extracted DNA using an extraction solution containing 0.1 M Tris Hydroxymethyl Aminomethane (Tris-base), 0.5% N-Lauroylsarcosine (Sarcosyl), 1.4 M NaCl, 20.0 mM EDTA-Disodium, 2.5% Hexadecyltrimethyl-ammonium Bromide (CTAB), 1% Polyvinylpyrrolidone, molecular weight 40,000 (Soluble PVP or PVP-40), 1% Polyvinylpolypyrrolidone (Insoluble PVP or PVPP), and 2% β-Mercaptoethanol. The extraction solution was heated at 60°C prior to use. For each sample we used 50 to 100 mg of leaf tissue, and we homogenized it in a 1.5 mL microcentrifuge tube using a Kontes™ Pellet Pestle™, in presence of 700 µL of extraction buffer. The DNA was phase-separated from proteins, sugars, and cell debris with Chloroform:Isoamyl Alcohol (24:1), precipitated and incubated for 20 minutes at -20 °C in Isopropanol, and pelleted by centrifugation for 15 minutes at 12,500 rpm. The DNA pellet was rinsed in 70% ethanol, dried at room temperature, and suspended in 100 µL of 0.1× TE. DNA samples were stored in a -80°C freezer (Gusmini et al., 2005a).

Design and Sources of PCR Primers

We purchased SSR and EST-based primers from Integrated DNA Technologies (Coralville, Iowa). SSR primer sequences were obtained from previous publications (Guerra-Sanz, 2002; Jarret et al., 1997; Katzir et al., 1996; Poleg et al., 2001). We designed additional SSR primers from watermelon genomic sequences (available from R.L. Jarret) and clones of a melon Bacterial Artificial Chromosome (BAC) library (available from R.A. Dean and T. Joobeur). We designed EST-based primers from public (NCBI, 2004) and private (available from I. Garcia) EST sequences. For the design of all primers we used the Primer3 software, through the GenoMax application (InforMax, 2002). SSR and EST-based primers (the forward, or reverse, if shorter) had the adaptor AAC AGC TAT GAC CAT GA at the 5' end for fluorescent labeling of the PCR amplification products. We purchased RAPD primers (decamers) and ISSR primers (13 to 19 nucleotides) from the University of British Columbia, Biotechnology Center (Vancouver, British Columbia, Canada).

Polymerase Chain Reaction Amplifications

We performed all PCR amplification reactions in Perkin Elmer 9700®-Thermalcyclers (Perkin Elmer, Wellesley, Massachusetts). We performed PCR amplification reactions for SSR, EST-based, and ISSR markers

in a 10 µL reaction mixture containing 20.0 mM Tris Hydroxymethyl Aminomethane (Tris-HCl, pH 8.8), 0.1% Triton-X-100, 2.0 mM $MgSO_4$, 10.0 mM KCl, 10.0 mM $(NH_4)_2SO_4$, 250µM dATP, dCTP, dGTP, and dTTP (Promega U.S., Madison, Wisconsin), 1.25 µM primer, one unit of *Taq* DNA Polymerase (New England Biolabs®, Beverly, Massachusetts), and ~15 ng of template DNA. PCR amplification reactions were repeated for 40 cycles for SSR and EST-based markers and 35 cycles for ISSR markers (SSR/EST-based: 15 s denaturation at 92.0 °C, 15 s annealing at 52.0 °C, 120 s elongation at 72.0 °C; ISSR: 25 s denaturation at 94.0 °C, 60 s annealing at 50.0 °C, 120 s elongation at 72.0 °C).

The 25 µL reaction mixture used for PCR amplification reactions of RAPD markers contained 20.0 µM NaCl, 50.0 mM Tris Hydroxymethyl Aminomethane (Tris-HCl, pH 9.0), 1% Triton-X-100, 0.01% gelatin, 1.6 mM $MgCl_2$, 200 µM dATP, dCTP, dGTP, and dTTP (Promega U.S., Madison, Wisconsin), 0.2 µM primer, seven units of *Taq* DNA Polymerase (New England Biolabs®, Beverly, Massachusetts), and ~25 ng of template DNA. PCR amplification reactions were repeated for 50 cycles (40 s denaturation at 93.5 °C, 70 s annealing at 48.0 °C, 120 s elongation at 72.0 °C).

Gel Electrophoresis

We separated amplification products by electrophoresis on agarose gel (1.5% agarose in 1× Tris-Acetate-EDTA (TAE) for SSR and EST-based markers, 2.0% agarose in 1× TAE for ISSR and RAPD markers), using 1× TAE as running buffer. The gel contained ethidium bromide (0.67 µg/mL) to stain the DNA fragments. We visualized DNA fragments on a UV trans-illuminator. We calculated the molecular weights of the amplification products using the 100 bp DNA ladder (Promega U.S., Madison, Wisconsin).

Amplification products of SSR and EST-based markers that did not show polymorphism on agarose gel were also analyzed by electrophoresis in acrylamide gel. An ABI PRISM ® 377 DNA Sequencer (Applied Biosystems, Foster City, California) was used. The acrylamide gel used was the Long Ranger ® Singel ® Pack (Cambrex Bio Science, Rockland, Maine). We fluorescently labeled DNA fragments with the 6-FAM (fluorescein) fluorophore. 6-FAM had the adaptor AAC AGC TAT GAC CAT GA at the 3' end. 6-FAM was incorporated in the PCR reaction buffer (0.25 µM). We calculated the molecular weights of the amplification

products using the Genescan ® -500 ROX Size Standard (Applied Biosystems, Foster City, California). Electrophoresis was performed at 3,000 V, 60 mA, 200 W of power, with a collection time of 2.5 hours. The acrylamide gel was preheated and maintained throughout the run at the 51 °C of constant temperature. Fluorescence was detected with a laser operating at 40 mW of power. We analyzed the outputs with the software Genescan 3.1 ® (Applied Biosystems, Foster City, California).

Experimental design and Data Analysis

We determined the phenotypic value of F_2 plants by progeny testing of (F_2 derived) F_3 families. The cross PI 189225 × 'NH Midget' had 112 F_3 families, while the cross PI 482283 × 'NH Midget' had 125. All the F_3 families were tested for resistance to artificial inoculations of *D. bryoniae* in a randomized complete block experiment with subsampling, including the three parental lines and their F_1 hybrids as checks. For each family, three plants per replication were tested. The experiment had four replications, but some F_3 families were represented only in three replications, due to low fertility of the F_2 mother plants. The experiment was replicated over time (replications = runs) in the same humidity chamber and F_3 families were randomized within each replication (replications = blocks). F_3 data were standardized to a new population of ratings with a reference mean of 4.5 and a standard deviation of 1.5, to remove experimental error due to replication over time. F_2 plants were classified as resistant or susceptible depending on the phenotypic ratings of the single F_3 plants and the within-family variation. F_3 plants were considered resistant or susceptible if they had a standardized rating lower or higher than 4.5, respectively. We performed statistical analyses using the MEAN, STANDARD, and GLM procedures of the SAS-STAT statistical package (SAS Institute, Cary, North Carolina). We performed the analysis of variance for F_3 single-plant data adopting the random model for hypothesis testing. Genotypic effects (due to F_3 data) were considered random, because there was no control on the genotype of single plants from self pollinated F_2 plants.

Initially, we tested 355 primers (Table 1) for their ability to amplify DNA fragments during PCR amplifications and for polymorphism among the parental lines used in the experiment. The marker bands were scored as monomorphic, codominant, or dominant, and their molecular weight was calculated.

We performed BSA for the polymorphic markers. For each cross, we used two bulks (one resistant and one susceptible) of DNA of F_2 plants. The bulks from the cross PI 189225 × 'NH Midget' had DNA from seven resistant or four susceptible plants, respectively. The bulks from the cross PI 482283 × 'NH Midget' had DNA from eight resistant or eight susceptible plants, respectively.

Following BSA, we mapped the polymorphic markers from BSA on the F_2 populations. Genotypic data were tested for linkage to phenotypic values using Mapmaker 2.0 for Macintosh, adopting the Kosambi mapping function. In our experiment, some families had most of the single-plant ratings close to the mean of the rating scale (4 to 6). This could have led to a misclassification of the F_2 genotypic value and we controlled for this effect by excluding those families from the linkage analysis.

Finally, one RAPD marker was found to be possibly linked to resistance to gummy stem blight from PI 189225. To verify the consistency of this marker among other resistant PI accessions, we tested this marker among resistant and susceptible PI accessions and cultivars of diverse geographical origin and level of resistance (Gusmini et al., 2005b). We used 37 resistant PI accessions (PI 189225, PI 195771, PI 211915, PI 227203, PI 244019, PI 247398, PI 249009, PI 271982, PI 274035, PI 277979, PI 296332, PI 357677, PI 482257, PI 482260, PI 482283, PI 482293, PI 482294, PI 482297, PI 482307, PI 482315, PI 482326, PI 482342, PI 482343, PI 482357, PI 482374, PI 482379, PI 490375, PI 490376, PI 490384, PI 500312, PI 500323, PI 508443, PI 512361, PI 512388, PI 512398, PI 526233, and PI 542123), and 42 susceptible PI accessions (PI 113326, PI 167124, PI 169237, PI 169285, PI 169286 , PI 171581, PI 173669, PI 173888, PI 175662, PI 175665, PI 176495, PI 176916, PI 177320, PI 179885, PI 179886, PI 183398 , PI 207472, PI 214044, PI 222775, PI 223764 , PI 226445 , PI 226445, PI 226459, PI 234287, PI 266028, PI 277991, PI 357660, PI 357689, PI 357735, PI 357741, PI 368510, PI 381734, PI 435282, PI 512373, PI 512399, PI 525084 , PI 525086, PI 525087, PI 525091, PI 536460, PI 537465, PI 595203). In addition, we included a set of four susceptible cultivars ('Allsweet', 'Charleston Gray', 'Congo', and 'NH Midget').

Results and Discussion

The DNA extraction protocol developed during our experiments allowed us to obtain DNA suited for PCR screening with SSR, ISSR, and RAPD primers. The average absorbance ratios 260/230

(DNA/Polysaccharides) and 260/280 (DNA/Proteins) for 20 random samples measured 1.75 (optimum = 1.8±0.2) and 2.1 (optimum ≥2.0), respectively. Thus, the quality of the DNA was considered good and consistent across samples (Fig. 1). Nevertheless, the complete protocol suggested by Levi and Thomas (1999) should be considered the most appropriate for the extraction of large quantities of high quality DNA from watermelon leaves. Our modified procedure should be adopted for faster extraction of small quantities of DNA from a large number of samples. Furthermore, the full procedure by Levi and Thomas would be more appropriate for studies of molecular markers requiring highly purified DNA (i.e., AFLP™ and RFLP markers).

Of the 355 primers tested (Table 1), 30% did not produce any PCR amplification product. Thus, 248 primers produced bands that could be compared in order to identify polymorphism among the parental lines of our populations segregating for resistance to gummy stem blight. The most efficient primers in producing positive PCR amplifications were those designed for ISSR markers (100%), followed by EST-based markers (81%), RAPD markers (68%), and SSR markers (64%). However, RAPD primers yielded the highest number of polymorphic marker bands (97%), followed by ISSR primers (60%), SSR primers (48%), and EST-based primers (20%). The RAPD markers were all dominant, as expected. The codominant:dominant marker ratio was 1.25 for ISSR, 0.57 for EST-based, and 2.00 for SSR primers.

In summary, all the marker types that we used had medium to high efficiency in amplifying watermelon DNA from a *C. lanatus* var. *lanatus* cultivar and two *C. lanatus* var. *citroides* PI accessions. Primers often used for fingerprinting (RAPD and ISSR) were the most efficient in identifying polymorphism among parental lines. SSR primers had a medium efficiency in polymorphism detection, but allowed the identification of twice the number of codominant versus dominant markers. EST-based markers were mainly monomorphic in our experiments, so we discontinued their use. For future studies, we would use primarily SSR markers, due to their high efficiency in amplifying codominant polymorphic bands in our populations. Nevertheless, we would continue to use RAPD and ISSR markers as a fast and efficient means of identification of polymorphism.

The analysis of variance for the phenotypic values of the F_3 plants showed that replication effects were non-significant, and most of the variation was due to the genotype of the families (Table 2). Thus, we pooled

the data from each replication over family and used 9 to 12 data points (F_3 plants) per family to predict the genotype of each F_2 plant.

Through BSA of the molecular markers polymorphic among the parental lines, we identified five SSR markers and one RAPD marker as candidates for mapping on the segregating populations (Table 3). Since the difference between the segregating bands in BSA for the three SSR markers was not always clear, we verified their polymorphism on the DNA of the individual plants composing the bulks, prior to mapping on the entire populations. Based on the results of this additional analysis, we discarded four of the five markers, and mapped the fifth [C.l.1-20-a from Jarret et al. (1997)] on the F_2 population from the cross PI 482283 × 'NH Midget'. We found that this marker was not linked to resistance to gummy stem blight. The RAPD marker UBC 338 (University of British Columbia, Biotechnology Center) in BSA produced a band (molecular weight ≈ 600 bp) segregating among the parental lines of the cross PI 189225 × 'NH Midget'. The band was a candidate dominant marker, linked to the resistant phenotype. Thus, we mapped this marker on the F_2 (Fig. 2).

The segregation of the 600 bp band of the RAPD marker UBC 338 (hereafter referred to as marker UBC 338-600) in the F_2 population was compared to the F_2 phenotypic values for resistance to gummy stem blight, as inferred by progeny testing of the F_3 families. Based on our linkage analysis performed with Mapmaker 2.0 (Kosambi function), the molecular and the phenotypic markers for resistance had a recombination frequency of ~35%, estimating a map distance of ~42 cM.

We would suggest that the UBC 338 marker might be linked to either a major QTL for resistance or to the *db* gene. Under both hypotheses, the high variation of the trait due to its quantitative genetic components may have contributed to estimate a longer mapping distance between the molecular and phenotypic marker in our experiment.

When we tested the marker UBC 338-600 on resistant and susceptible PI accessions and cultivars, we could find a band of similar molecular weight in 21.6% of the resistant PI accessions, compared to 4.8% of the susceptible PI accessions, and in none of the four cultivars (Fig. 3). For our test, the UBC 338-600 was not converted to a sequence-characterized amplified region (SCAR) marker. Thus, we cannot confirm that the 600 bp band recovered in some of the PI accessions tested was the same marker previously identified in PI 189225.

Future Research

Based on our preliminary results on the inheritance of resistance to gummy stem blight in our populations, we suggest that QTL mapping be the most reliable way to: 1) identify molecular markers closely linked to resistant genes (or QTLs), and 2) clarify the genetics of resistance. The identification of QTLs and the estimation of their average effect could be useful for the development of a proper MAS breeding strategy for this trait in watermelon. In addition, markers and QTLs identified through greenhouse testing of the segregating populations could be verified in field tests for linkage to resistance to natural epidemics of gummy stem blight on adult plants.

The success of a QTL mapping experiment depends mostly by the heritability of the trait tested in specific populations, by the number of QTLs involved, and by their relative contribution to the variation for the trait (Goodman, 2004). In our preliminary studies, the heritability of resistance in our F_2 populations was high in the greenhouse (97 to 100%) and low in the field (0 to 9%). In addition, only one to two effective factors were estimated to regulate the expression of resistant genes both in greenhouse and field tests. Thus, the populations that we developed would be appropriate material for QTL mapping experiments, particularly for resistance to greenhouse inoculations. Furthermore, the use of F_2 populations would allow us to estimate both additive and dominance effects for the QTLs. Dominance effects would be of great importance to watermelon breeders, since almost all the modern cultivars are F_1 hybrids. Thus, mapping based on populations that do not allow estimation of dominance effects, such as RIL populations, would not be appropriate.

The availability of codominant SSR markers in watermelon is still limited, making it difficult to saturate linkage maps easily and to do fine QTL mapping by random pairing of markers. Therefore, we would first continue to screen available SSR markers from other cucurbit species among the parental lines of our populations to identify polymorphism at the molecular level.

The UBC 338-600 marker could be mapped on *C. melo*, where several well distributed SSR markers are available (Perin et al., 2002; Poleg et al., 2001). They could be used to identify other candidate SSR markers to be tested on our populations. Nevertheless, the low frequency of amplification of this marker among resistant PI accessions would discourage us from this approach and suggest a greater investment of resources into the development and screening of a greater number of SSR markers.

The goal of our QTL mapping experiment would be to identify at least two markers flanking each QTL that explains large portions of the variation associated with resistance. Alternatively, should the single gene *db* be regulated by minor QTLs, a single molecular marker linked to *db* would be a useful tool to plant breeders for MAS. Ideally, the identification of a marker tightly linked to *db* and other markers detecting the presence of the minor QTLs would provide enough information to predict the phenotype of the progenies of each cross. Nevertheless, it could be difficult to achieve the correct mapping of these minor QTLs, due to the possibility that each of them explains too little of the total variation.

Based on the results of this investigation, plant breeders could integrate MAS for the *db* gene and field and greenhouse testing to select the progenies with the highest expression of resistance under natural and artificial epidemics of gummy stem blight. Thus, minor QTLs could be selected directly by observation of the level of resistance in phenotypic testing. Field testing would be necessary for yield and quality traits, so that multiple years of trialing for disease resistance would be possible, prior to release of the best germplasm.

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Table 1. Segregation of molecular markers among watermelon inbred lines resistant or susceptible to gummy stem blight.[z]

	Primers		Molecular markers		
Type	Tested	Non effective[y]	Monomorphic	Codominant	Dominant
SSR[x]	176	63	64	36	18
EST-based[w]	68	13	44	4	7
ISSR[v]	15	0	7	5	4
RAPD[u]	96	31	30	-	63
Total	355	107	145	45	92

z PI 189225 and PI 482283 (resistant parents) were *Citrullus lanatus* var. *citroides*. 'NH Midget' (susceptible parent) was *Citrullus lanatus* var. *lanatus*

y Primers that did not amplify any fragment

x Simple Sequence Repeats

w Expressed Sequence Tags based

v Inter-Simple Sequence Repeats

u Random Amplified Polymorphic DNA

Table 2. Analysis of variance for the F_3 families tested for resistance to gummy stem blight in the greenhouse in 2003 and 2004. [z]

Sources of variation	df	Mean Squares	F-Ratio	Expected Mean Squares [y]
Replication	5	0.74	0.44NS	$\sigma^2+2.38*\sigma^2_{RG}+213.62*\sigma^2_R$
Genotype [x]	223	6.08	3.58***	$\sigma^2+2.35*\sigma^2_{RG}+7.37*\sigma^2_G$
Experimental error [w]	452	1.74		$\sigma^2+2.54*\sigma^2_{RG}$
Sampling error [v]	1,063	1.20		σ^2

z Data are ratings from F_3 families of resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *citroides* and *lanatus* tested in four replications of three plants each. Disease assessment scale adopted for evaluating watermelon for resistance to gummy stem blight: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem,; 6 to 8 = symptoms on leaves and stems; 9 = plant dead

y From SAS-STAT (GLM procedure, adopting the random model for hypothesis testing)

x F_3 families

w Genotype * Replication

v Plants (Genotype * Replication)

Table 3. F_3 phenotypic data and F_2 genotypic (molecular marker) data for the two crosses of watermelon cultigens, segregating for resistance to gummy stem blight.

		Single plant phenotypic data[z]												Genotypic data[y]					
Smp.[x]	Cr.[w]	1	2	3	4	5	6	7	8	9	10	11	12	I	II	III	IV	V	VI
Parents and F_1 genotypic and phenotypic data																			
Ps	.	7	7	7	7	7	7	7	7	6	7	7	7	-	A	-	-	A	-
Pr	1	3	3	3	3	3	3	3	3	3	4	3	3	+	B	+	+	B	*
Pr	2	3	3	3	4	3	3	3	3	3	3	4	4	+	B	+	+	*	+
F_1	1	6	6	6	6	6	7	7	6	7	7	7	7	+	H	+	+	H	*
F_1	2	7	7	6	6	6	6	7	6	6	6	7	6	+	H	+	+	*	*
F_2 genotypic data and F_3 phenotypic data																			
019[v]	1	.	.	.	4	3	3	3	.	3	.	.	.	-	H	+	+	H	*
020[u]	1	6	5	7	7	6	7	7	7	6	.	.	.	+	A	+	-	B	*
021[v]	1	3	3	3	3	3	3	.	4	3	.	.	.	-	B	+	+	H	*
022[v]	1	4	3	3	3	4	3	4	4	2	.	.	.	-	H	+	+	H	*
023	1	4	5	3	4	4	4	5	3	3	.	.	.	*	*	*	*	H	*
024	1	6	7	7	4	5	3	3	3	4	.	.	.	*	*	*	*	H	*
025	1	2	4	.	4	7	7	3	3	5	.	.	.	*	*	*	*	A	*
026	1	.	4	5	4	3	4	.	6	.	.	.	.	*	*	*	*	A	*
027	1	6	3	3	4	3	6	3	.	6	.	.	.	*	*	*	*	H	*
028	1	5	5	5	4	3	3	3	3	3	.	.	.	*	*	*	*	A	*

Table 3. Continued.

029[v]	1	.	3	3	.	.	3	4	3	3	.	.	.	+	B	+	+	B	*
030[u]	1	5	6	5	6	6	5	6	.	.	.	.	.	+	H	+	-	H	*
031	1	3	.	3	4	.	.	.	.	4	.	.	.	*	*	*	*	H	*
032	1	3	3	.	4	3	6	3	6	.	.	.	.	*	*	*	*	U	*
033	1	3	.	3	4	3	3	3	4	4	.	.	.	*	*	*	*	B	*
034[u]	1	6	5	5	7	6	6	7	7	5	.	.	.	+	A	+	+	U	*
035[u]	1	5	6	.	.	.	.	.	.	6	.	.	.	+	.	-	+	H	*
036[u]	1	.	.	.	7	6	6	5	5	6	.	.	.	+	A	-	+	U	*
037	1	4	5	.	4	3	5	3	5	3	.	.	.	*	*	*	*	H	*
038[v]	1	3	3	3	4	3	4	3	3	3	.	.	.	-	H	-	+	U	*
039	1	.	5	3	4	3	4	6	6	6	.	.	.	*	*	*	*	A	*
040	1	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	U	*
041	1	4	3	3	4	.	4	.	.	3	.	.	.	*	*	*	*	B	*
042	1	5	3	.	4	3	.	5	6	6	.	.	.	*	*	*	*	A	*
043	1	4	4	3	4	3	4	4	4	.	.	.	.	*	*	*	*	B	*
044	1	3	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	A	*
045	1	3	.	3	4	3	5	5	4	5	.	.	.	*	*	*	*	B	*
046	1	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	A	*
047	1	5	.	.	6	6	.	.	.	.	.	.	.	*	*	*	*	B	*
048	1	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	A	*
049	1	4	3	5	4	4	3	7	4	3	.	.	.	*	*	*	*	A	*

Table 3. Continued.

050	1	5	3	3	.	.	.	.	.	.	.	.	*	*	*	*	H	*
051	1	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	A	*
052	1	3	.	.	4	3	3	5	3	3	.	.	*	*	*	*	H	*
053	1	5	5	.	4	4	5	4	5	3	.	.	*	*	*	*	B	*
054[u]	1	7	6	5	6	6	6	7	6	6	.	.	+	H	-	+	H	*
055	1	3	.	.	.	.	.	.	.	.	.	.	*	*	*	*	U	*
056[u]	1	5	5	5	7	.	.	.	.	.	.	.	-	A	+	-	H	*
057	1	4	3	3	7	6	6	7	5	6	.	.	*	*	*	*	H	*
058	1	7	3	3	4	6	6	.	.	3	.	.	*	*	*	*	A	*
059	1	.	.	.	.	3	4	3	4	.	.	.	*	*	*	*	B	*
060	1	3	3	3	.	.	.	.	.	.	.	.	*	*	*	*	U	*
061	1	4	3	4	4	3	4	4	4	.	.	.	*	*	*	*	B	*
062	1	.	.	.	6	6	3	5	4	6	.	.	*	*	*	*	H	*
063	1	3	3	.	7	6	5	6	4	3	.	.	*	*	*	*	H	*
064[v]	1	3	3	.	4	3	4	.	3	3	.	.	+	A	+	-	H	*
065	1	.	3	3	.	.	.	.	.	.	.	.	*	*	*	*	U	*
066	1	5	3	3	4	.	4	4	4	4	.	.	*	*	*	*	B	*
067	1	.	3	3	7	7	6	6	5	3	.	.	*	*	*	*	B	*
068	1	.	5	.	4	3	4	7	3	5	.	.	*	*	*	*	H	*
069	1	.	5	.	4	3	3	3	3	6	.	.	*	*	*	*	H	*
070	1	.	3	.	.	.	.	.	.	3	.	.	*	*	*	*	A	*

Table 3. Continued.

071[v]	1	3	.	3	4	3	3	3	.	3	.	.	.	-	H	+	+	H	*
072	1	3	6	3	3	.	.	.	.	6	.	.	.	*	*	*	*	A	*
073[v]	1	3	3	.	4	4	3	3	3	3	.	.	.	-	.	+	+	H	*
074	1	.	.	6	6	6	.	.	.	.	.	.	.	*	*	*	*	H	*
075	1	5	.	5	4	4	3	4	7	4	.	.	.	*	*	*	*	A	*
076	1	5	6	5	4	7	3	3	4	6	.	.	.	*	*	*	*	H	*
077	1	.	5	3	7	3	.	5	7	3	.	.	.	*	*	*	*	A	*
078	1	4	.	4	4	7	3	4	4	6	.	.	.	*	*	*	*	A	*
079	1	4	5	5	4	3	3	5	7	3	.	.	.	*	*	*	*	B	*
080	1	4	5	5	4	4	3	4	4	.	.	.	.	*	*	*	*	H	*
081	1	.	5	.	.	6	.	.	4	.	.	.	.	*	*	*	*	H	*
082	1	5	5	7	4	3	.	.	.	4	.	.	.	*	*	*	*	B	*
083	1	4	6	6	4	.	3	.	6	3	.	.	.	*	*	*	*	A	*
084	1	4	5	.	4	6	4	6	.	3	.	.	.	*	*	*	*	B	*
085	1	4	.	.	4	.	.	.	.	6	.	.	.	*	*	*	*	A	*
086	1	.	.	6	.	.	.	.	.	.	.	.	.	*	*	*	*	H	*
087	1	5	5	3	5	3	.	.	3	.	.	.	.	*	*	*	*	H	*
088	1	5	.	5	6	4	3	3	6	6	.	.	.	*	*	*	*	H	*
089	1	5	5	5	4	3	3	3	.	3	.	.	.	*	*	*	*	H	*
090[u]	1	5	.	7	.	.	.	.	.	.	.	.	.	-	H	+	-	A	*
091	1	4	.	5	.	.	.	.	.	.	.	.	.	*	*	*	*	H	*

Table 3. Continued.

092	1	4	3	4	.	.	.	.	.	.	.	.	.	*	*	*	*	H	*
093	1	.	6	.	4	3	4	7	.	3	.	.	.	*	*	*	*	A	*
094	1	7	3	.	.	6	5	.	5	5	.	.	.	*	*	*	*	H	*
095	1	.	.	.	4	4	6	5	6	5	.	.	.	*	*	*	*	U	*
096	1	7	5	5	6	.	7	.	.	.	.	.	.	*	*	*	*	B	*
097	1	5	.	.	.	4	3	3	.	2	.	.	.	*	*	*	*	U	*
098	2	.	4	4	4	3	7	6	.	6	.	.	.	*	*	*	*	*	.
099	2	.	4	4	.	.	3	3	.	3	.	.	.	*	*	*	*	*	.
100	2	.	3	3	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
101	2	4	3	5	4	3	.	3	.	3	.	.	.	*	*	*	*	*	-
102	2	6	3	7	4	7	8	3	3	6	.	.	.	*	*	*	*	*	+
103[t]	2	4	.	3	4	3	3	3	3	.	.	.	.	-	A	-	-	*	+
104	2	4	5	3	4	7	8	3	3	6	.	.	.	*	*	*	*	*	+
105[t]	2	3	3	3	4	3	3	3	3	.	.	.	.	-	.	-	+	*	+
106	2	6	3	3	4	3	6	3	4	3	.	.	.	*	*	*	*	*	+
107	2	3	3	.	.	.	.	.	3	5	.	.	.	*	*	*	*	*	+
108	2	5	5	5	.	3	3	4	4	4	.	.	.	*	*	*	*	*	+
109	2	4	6	4	7	7	6	.	6	6	.	.	.	*	*	*	*	*	+
110	2	.	7	.	4	3	.	.	3	.	.	.	.	*	*	*	*	*	-
111	2	4	3	3	5	3	4	5	4	5	.	.	.	*	*	*	*	*	-
112	2	4	7	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-

Table 3. Continued.

113	2	4	5	5	4	3	5	7	5	6	.	.	.	*	*	*	*	*	-
114	2	3	3	3	7	6	7	3	7	6	.	.	.	*	*	*	*	*	-
115	2	.	.	3	.	7	.	4	4	3	.	.	.	*	*	*	*	*	-
116	2	3	6	6	4	6	6	.	3	.	.	.	.	*	*	*	*	*	-
117	2	3	.	7	4	3	.	3	4	.	.	.	.	*	*	*	*	*	.
118[t]	2	3	3	3	4	.	3	.	3	3	.	.	.	-	H	+	+	*	-
119	2	3	.	.	.	.	.	6	6	6	.	.	.	*	*	*	*	*	-
120[t]	2	4	3	3	4	.	3	.	3	3	.	.	.	-	H	+	+	*	-
121	2	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-
122	2	5	.	7	7	.	4	3	4	.	.	.	.	*	*	*	*	*	-
123	2	3	3	7	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-
124	2	.	6	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
125	2	4	5	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
126	2	3	6	3	4	3	3	6	.	.	.	.	.	*	*	*	*	*	-
127	2	.	.	3	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-
128[s]	2	7	6	5	5	7	5	.	.	7	.	.	.	-	H	-	+	*	-
129[t]	2	3	3	3	4	.	3	3	.	.	.	.	.	-	A	+	-	*	+
130[s]	2	5	5	.	.	.	8	.	7	7	.	.	.	-	.	+	+	*	-
131	2	6	6	4	.	.	7	3	6	.	.	.	.	*	*	*	*	*	.
132	2	.	3	.	4	6	4	4	.	6	.	.	.	*	*	*	*	*	-
133	2	5	7	5	4	6	6	.	3	3	.	.	.	*	*	*	*	*	+

Table 3. Continued.

134[t]	2	4	.	3	4	4	4	3	3	.	.	.	.	-	A	+	+	*	-
135	2	5	5	5	7	3	4	3	3	6	.	.	.	*	*	*	*	*	-
136	2	6	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-
137	2	.	3	3	6	3	3	3	5	5	.	.	.	*	*	*	*	*	+
138	2	5	3	3	4	7	7	7	6	5	.	.	.	*	*	*	*	*	+
139	2	3	3	3	7	6	.	7	6	6	.	.	.	*	*	*	*	*	+
140	2	5	5	3	4	3	4	5	6	5	.	.	.	*	*	*	*	*	+
141	2	.	.	.	.	5	.	4	4	6	.	.	.	*	*	*	*	*	+
142	2	6	7	7	6	5	5	7	7	6	.	.	.	*	*	*	*	*	-
143	2	7	5	4	7	4	.	6	7	6	.	.	.	*	*	*	*	*	.
144[s]	2	7	6	7	.	6	6	7	7	6	.	.	.	-	H	+	-	*	-
145[s]	2	3	5	4	.	3	3	6	.	.	.	.	.	-	B	+	+	*	+
146	2	5	5	5	.	3	3	5	6	.	.	.	.	*	*	*	*	*	+
147	2	6	6	5	7	.	3	6	6	6	.	.	.	*	*	*	*	*	+
148	2	3	6	3	4	3	7	3	4	.	.	.	.	*	*	*	*	*	+
149	2	6	5	5	4	3	3	3	3	6	.	.	.	*	*	*	*	*	-
150	2	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	.
151	2	6	5	5	4	.	4	5	4	3	.	.	.	*	*	*	*	*	+
152	2	3	3	3	4	7	7	7	.	3	.	.	.	*	*	*	*	*	-
153	2	5	5	4	4	.	6	7	3	.	.	.	.	*	*	*	*	*	-
154	2	3	3	5	4	3	6	3	3	5	.	.	.	*	*	*	*	*	-

Table 3. Continued.

155	2	4	5	4	.	4	4	.	.	3	.	.	.	*	*	*	*	*	.
156	2	.	.	5	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-
157	2	.	.	5	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
158	2	4	6	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
159	2	5	5	5	.	.	.	.	5	3	.	.	.	*	*	*	*	*	-
160	2	6	3	5	4	3	4	.	6	6	.	.	.	*	*	*	*	*	+
161	2	3	7	3	4	4	3	3	3	6	.	.	.	*	*	*	*	*	.
162	2	3	3	5	8	7	6	7	8	6	.	.	.	*	*	*	*	*	.
163	2	.	3	.	.	3	.	.	.	3	.	.	.	*	*	*	*	*	-
164	2	4	4	5	.	.	.	.	6	.	.	.	.	*	*	*	*	*	-
165	2	7	.	4	5	.	.	5	.	5	.	.	.	*	*	*	*	*	-
166	2	5	6	6	7	4	7	5	.	.	.	.	.	*	*	*	*	*	-
167	2	5	7	6	5	4	6	4	6	6	.	.	.	*	*	*	*	*	+
168	2	5	6	4	7	3	3	5	7	6	.	.	.	*	*	*	*	*	-
169	2	.	5	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
170	2	3	5	7	4	.	3	3	3	.	.	.	.	*	*	*	*	*	+
171[t]	2	3	3	3	4	3	3	3	3	3	.	.	.	-	H	-	-	*	-
172	1	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	H	*
173	1	5	5	5	4	4	4	5	6	5	5	6	4	*	*	*	*	A	*
174	1	6	6	6	6	4	.	3	6	4	6	6	4	*	*	*	*	A	*
175	1	3	3	3	.	.	3	3	.	.	.	.	.	*	*	*	*	U	*

Table 3. Continued.

176	1	6	6	6	3	.	.	3	3	6	3	3	5	*	*	*	*	A	*
177	1	7	7	7	3	3	.	3	3	3	3	.	.	*	*	*	*	U	*
178	1	3	3	3	5	3	.	3	6	3	3	6	3	*	*	*	*	B	*
179	1	5	5	5	3	3	.	3	3	3	4	3	5	*	*	*	*	H	*
180	1	7	7	7	5	5	6	7	6	5	7	6	6	*	*	*	*	A	*
181	1	7	7	7	5	6	5	7	7	7	3	.	.	*	*	*	*	U	*
182	1	7	7	7	.	.	.	3	3	4	3	.	.	*	*	*	*	H	*
183	1	3	3	3	3	3	4	4	6	3	4	6	.	*	*	*	*	A	*
184	1	5	5	5	5	5	5	5	6	5	6	6	5	*	*	*	*	A	*
185	1	3	3	3	.	.	.	.	.	.	.	.	.	*	*	*	*	H	*
186	1	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	*
187	1	3	3	3	3	3	6	3	6	3	5	3	3	*	*	*	*	*	*
188	1	3	3	3	4	3	.	3	3	3	3	3	4	*	*	*	*	*	*
189	1	3	3	3	6	3	3	4	3	3	3	3	.	*	*	*	*	*	*
190	1	4	4	4	3	.	.	3	3	3	3	3	3	*	*	*	*	*	*
191	1	5	5	5	7	7	3	6	5	3	3	4	6	*	*	*	*	*	*
192	1	3	3	3	3	3	4	3	3	3	6	6	3	*	*	*	*	*	*
193	1	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	*
194	1	7	7	7	7	7	.	8	3	.	6	6	3	*	*	*	*	*	*
195	1	7	7	7	7	6	4	6	6	3	3	7	3	*	*	*	*	*	*
196	1	3	3	3	4	5	.	6	3	6	3	3	.	*	*	*	*	*	*

Table 3. Continued.

197	1	5	5	5	4	6	7	3	3	5	3	4	5	*	*	*	*	*	*
198	1	3	3	3	5	7	6	8	7	3	7	3	3	*	*	*	*	*	*
199	1	4	4	4	7	7	6	3	7	6	6	5	5	*	*	*	*	*	*
200	1	6	6	6	7	5	4	5	5	7	6	5	7	*	*	*	*	*	*
201	1	6	6	6	7	7	7	6	7	6	7	6	7	*	*	*	*	*	*
202	1	6	6	6	6	6	6	6	6	3	6	6	6	*	*	*	*	*	*
203	1	6	6	6	3	3	5	7	7	6	6	6	6	*	*	*	*	*	*
204	1	7	7	7	7	6	6	3	6	7	6	6	6	*	*	*	*	*	*
205	1	7	7	7	6	5	3	6	6	7	7	7	3	*	*	*	*	*	*
206	1	3	3	3	6	5	5	7	3	3	3	4	.	*	*	*	*	*	*
207	1	3	3	3	3	.	.	3	6	3	.	.	.	*	*	*	*	*	*
208	1	3	3	3	3	3	3	3	4	3	3	3	3	*	*	*	*	*	*
209	1	6	6	6	3	3	3	6	6	6	3	3	6	*	*	*	*	*	*
210	1	5	5	5	5	5	3	3	6	6	3	4	3	*	*	*	*	*	*
211	1	3	3	3	3	.	3	3	5	3	3	3	3	*	*	*	*	*	*
212	1	3	3	3	6	3	3	3	5	3	3	3	3	*	*	*	*	*	*
213	1	3	3	3	6	2	3	3	3	3	6	7	7	*	*	*	*	*	*
214	1	3	3	3	3	4	4	3	6	6	6	3	4	*	*	*	*	*	*
215	1	5	5	5	5	4	6	5	3	6	3	3	.	*	*	*	*	*	*
216	1	3	3	3	.	.	.	3	6	3	.	.	.	*	*	*	*	*	*
217	1	6	6	6	.	.	.	6	3	3	3	3	.	*	*	*	*	*	*

Table 3. Continued.

218	2	4	4	4	3	4	.	3	6	3	5	3	.	*	*	*	*	*	-
219	2	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
220	2	3	3	3	4	.	.	3	3	6	3	.	.	*	*	*	*	*	-
221	2	4	4	4	.	.	.	3	3	4	4	4	3	*	*	*	*	*	-
222	2	3	3	3	4	.	.	6	4	3	6	.	.	*	*	*	*	*	-
223	2	4	4	4	3	5	4	3	5	3	5	6	.	*	*	*	*	*	+
224	2	3	3	3	5	.	.	3	3	3	3	3	.	*	*	*	*	*	+
225	2	3	3	3	3	3	.	3	3	3	3	3	3	*	*	*	*	*	+
226	2	7	7	7	6	6	.	6	6	6	5	.	.	*	*	*	*	*	+
227	2	.	.	.	3	4	.	3	3	3	3	3	3	*	*	*	*	*	+
228	2	6	6	6	.	.	.	6	7	7	6	5	8	*	*	*	*	*	+
229	2	3	3	3	.	.	.	3	.	.	.	.	.	*	*	*	*	*	+
230	2	3	3	3	4	7	3	3	3	6	3	8	3	*	*	*	*	*	+
231	2	3	3	3	7	6	7	3	3	3	3	.	.	*	*	*	*	*	-
232	2	3	3	3	.	.	.	3	4	4	7	3	.	*	*	*	*	*	+
233	2	3	3	3	3	3	3	3	3	3	4	3	4	*	*	*	*	*	+
234	2	3	3	3	3	3	3	4	4	3	4	3	3	*	*	*	*	*	-
235	2	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	+
236	2	3	3	3	3	3	4	3	3	6	6	3	4	*	*	*	*	*	.
237	2	3	3	3	4	3	.	3	3	3	5	3	5	*	*	*	*	*	+
238	2	.	.	.	.	.	.	.	.	.	.	.	.	*	*	*	*	*	-

Table 3. Continued.

239	2	6	6	6	3	5	3	6	6	5	3	5	5	*	*	*	*	*	+
240	2	6	6	6	3	5	3	5	4	5	5	3	3	*	*	*	*	*	+
241	2	6	6	6	2	3	3	6	6	3	7	3	.	*	*	*	*	*	-
242	2	6	6	6	.	.	.	7	6	6	7	7	.	*	*	*	*	*	+
243	2	4	4	4	4	4	4	5	4	4	3	4	4	*	*	*	*	*	+
244	2	6	6	6	6	6	6	3	3	3	3	6	3	*	*	*	*	*	-
245	2	5	5	5	3	5	5	3	3	3	4	6	3	*	*	*	*	*	+
246	2	5	5	5	3	5	5	3	3	3	4	6	3	*	*	*	*	*	-
247	2	3	3	3	7	4	3	3	3	6	4	6	.	*	*	*	*	*	+
248	2	7	7	7	3	3	6	3	3	3	5	6	3	*	*	*	*	*	+
249	2	3	3	3	4	3	4	3	4	4	3	3	4	*	*	*	*	*	.
250	2	5	5	5	5	3	4	3	7	6	6	3	4	*	*	*	*	*	+
251	2	3	3	3	5	6	.	6	6	5	3	6	7	*	*	*	*	*	+
252	2	3	3	3	3	.	.	3	3	3	3	3	4	*	*	*	*	*	+
253	2	3	3	3	4	3	.	3	3	3	3	3	3	*	*	*	*	*	+
254	2	3	3	3	4	4	3	3	3	4	3	4	3	*	*	*	*	*	+
255	2	6	6	6	.	6	7	6	6	8	7	7	7	*	*	*	*	*	*

z Data are ratings of single plants of resistant PI accessions by susceptible cultivars of *Citrullus lanatus* var. *citroides* and *lanatus* tested in four replications of three plants each. Disease assessment scale adopted for evaluating watermelon for resistance to gummy stem blight: 0 = no disease; 1 = yellowing on leaves (suspect of disease only); 2 to 4 = symptoms on leaves only; 5 = some leaves dead, no symptoms on stem,;

6 to 8 = symptoms on leaves and stems; 9 = plant dead. Dominant markers: - = no band; + = band. Codominant markers: A, B, H = band A, band B, hemizygote, respectively. ∗ = data not available

y I = SAT (SSR - Forward: CCT TGT CAT TAC CGT AGA GC - Reverse: TAT CAT TCA ATA GCA TCC CC)

II = C.l.1-06-a (SSR - Forward: CAC CCT CCT CCA GTT GTC ATT CG - Reverse: AAG GTC AGC AAA GCG GCA TCG G)

III = CSWTA07 (SSR - Forward: TGC GAT TTG AGA CCA CCT ATT GAT - Reverse: AAA CAG GGA CAT AGC ATG GAT CTA)

IV = C.l.1-17 (SSR - Forward: TTG AAA GGG GTA ATC GGA GA - Reverse: GGG TCC CTC AAA TCT AAG CC)

V = C.l.1-20-a (SSR - Forward: CGC GCG TGA GGA CCC TAT A - Reverse: AGC AAT TGA GGC GGT TCT)

VI = UBC-338 (RAPD - CTG TGG CGG T - 600 bp band)

x Sample number (Ps = Parent susceptible; Pr = Parent resistant)

w Cross number (1 = PI 482283 × 'NH Midget'; 1 = PI 189225 × 'NH Midget')

v Part of BR1 (Resistant bulk #1)

u Part of BS1 (Susceptible bulk #2)

t Part of BR2 (Resistant bulk #1)

s Part of BS2 (Susceptible bulk #2)

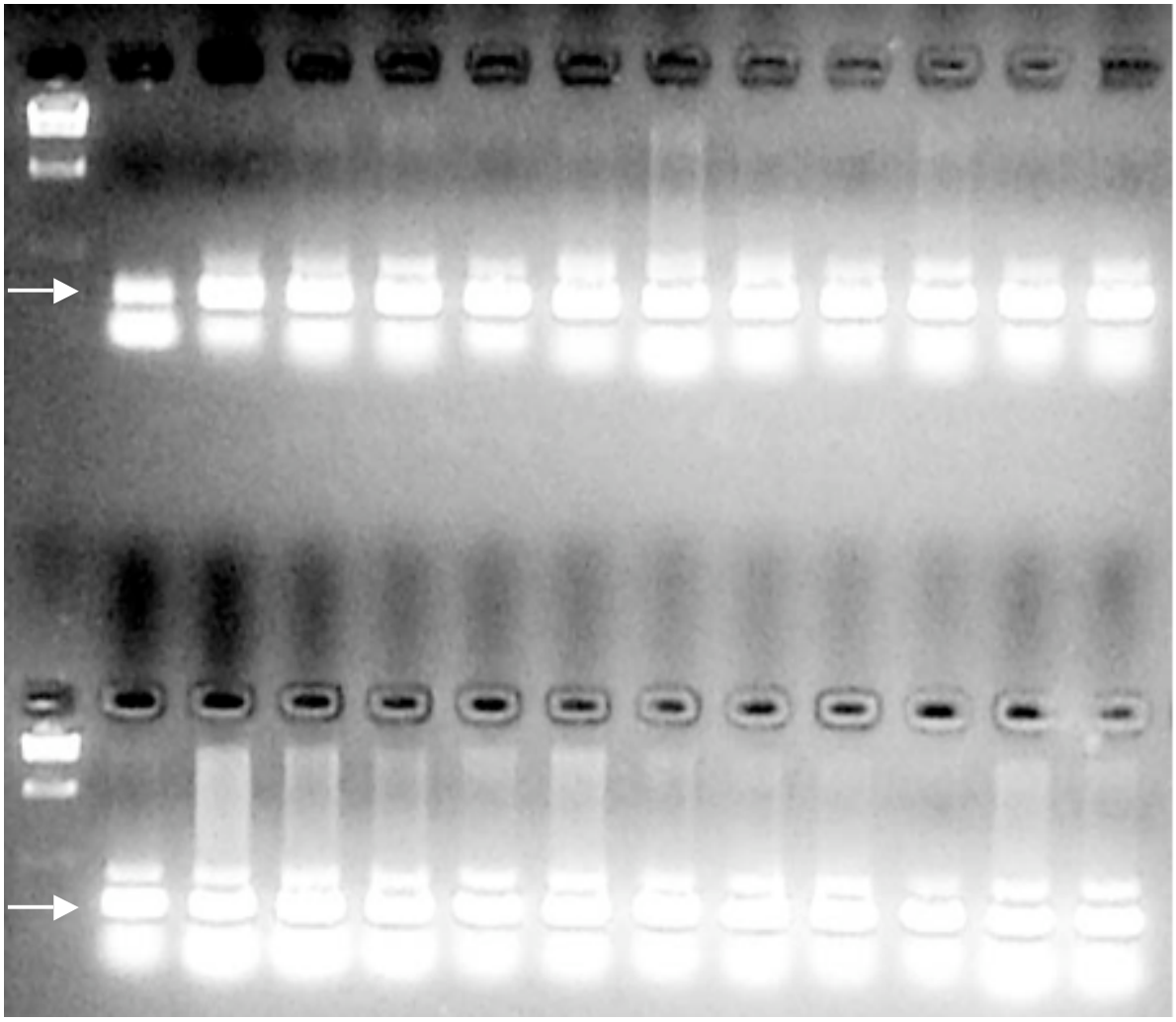

Figure 1. Agarose gel electrophoresis of PCR products of the monomorphic SSR marker URF1 (Forward: AGC AGC ACC TTG TCT TGT AT - Reverse: CAC AGA TCC CAC TCA ATC TT from 24 random samples of DNA extracted from watermelon leaves. Arrows indicate expected PCR products.

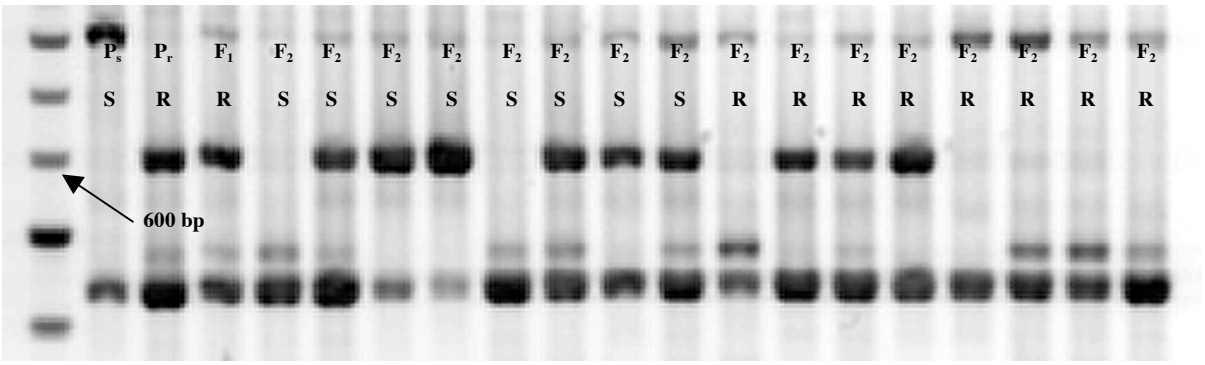

Figure 2. Example of agarose gel electrophoresis of PCR products of the polymorphic RAPD marker UBC-338 (CTG TGG CGG T; 600 bp band) in the F_2 population of the cross PI 189225 × 'NH Midget' (resistant × susceptible), segregating for resistance to gummy stem blight (S = susceptible, R = resistant).

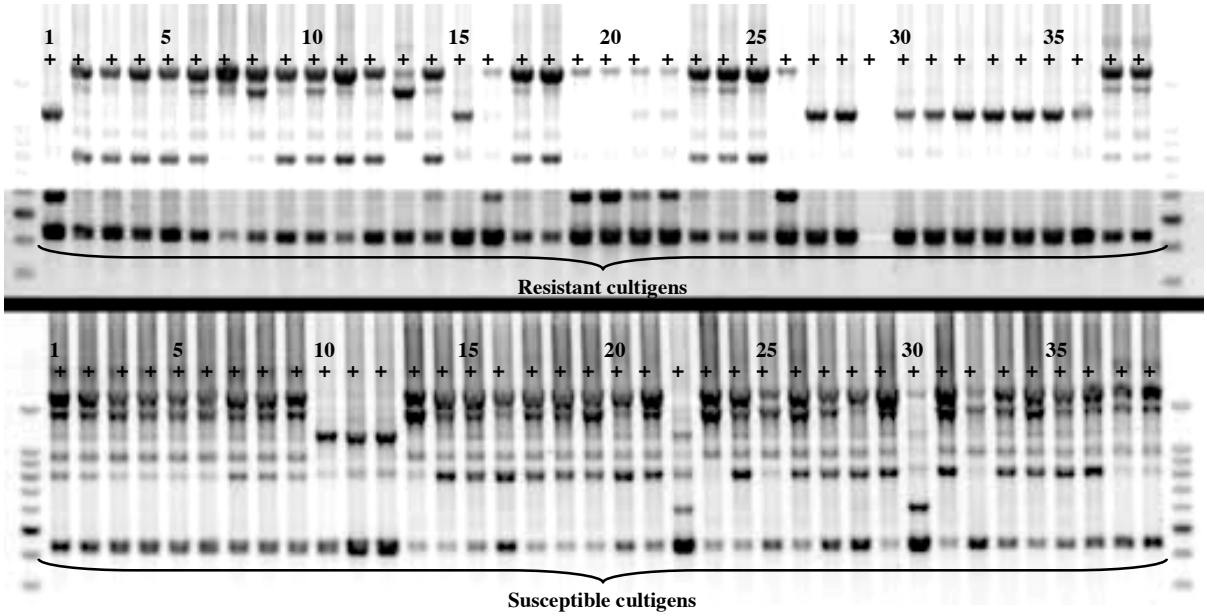

Figure 3. Agarose gel electrophoresis of PCR products of the polymorphism of the RAPD marker UBC-338 (CTG TGG CGG T; 600 bp band) among inbred cultigens of watermelon resistant or susceptible to gummy stem blight. Resistant cultigens (top half; 1-38): PI 189225, PI 195771, PI 211915, PI 227203, PI 244019, PI 247398, PI 249009, PI 271982, PI 274035, PI 277979, PI 296332, PI 357677, PI 482257, PI 482260, PI 482283, PI 482293, PI 482294, PI 482297, PI 482307, PI 482315, PI 482326, PI 482342, PI 482343, PI 482357, PI 482374, PI 482379, PI 490375, PI 490376, PI 490384, PI 500312, PI 500323, PI 508443, PI 512361, PI 512388, PI 512398, PI 526233, and PI 542123, respectively. Susceptible cultigens (bottom half; 1-38): PI 167124, PI 169237, PI 169285, PI 169286, PI 171581, PI 173669, PI 173888, PI 175662, PI 175665, PI 176495, PI 176916, PI 177320, PI 179885, PI 179886, PI 183398, PI 207472, PI 214044, PI 222775, PI 223764, PI 226445, PI 226445, PI 226459, PI 234287, PI 266028, PI 277991, PI 357660, PI 357689, PI 357735, PI 357741, PI 368510, PI 381734, PI 435282, PI 512373, PI 512399, PI 525084, PI 525086, PI 525087, and PI 525091.

GENERAL CONCLUSIONS

Summary of Findings Presented in the Dissertation

The studies presented herein are a contribution to the understanding of the genetics of rind pattern, flesh color, and resistance to gummy stem blight in watermelon.

We identified three new genes in watermelon. *Scarlet red flesh* (*Scr*) produced a higher intensity red color in the flesh of 'Dixielee' and 'Red-N-Sweet' compared to 'Angeleno Black Seeded', the type line for red flesh color in watermelon. *Scr* was inherited as a single dominant gene. *Yellow belly* (*Yb*) was classified as a single dominant gene changing the color of the ground spot in 'Black Diamond' from creamy white to dark yellow. The presence of intermittent vs. continuous stripes on the rind of 'Navajo Sweet' was explained by the action of a single recessive gene that we named *intermittent stripes* (*ins*), with the dominant allele present in 'Crimson Sweet'. We confirmed the inheritance of the *C* gene for the canary yellow flesh as a single dominant gene. The inbred line NC-517, the canary yellow parent in our study, should be considered the homozygous public type line for the *C* gene, rather than the canary yellow F_1 hybrids available so far. We highlighted a new possibility for the development of watermelon fruit with novel rind: the spotted phenotype (*Sp* gene) was successfully transferred from 'Moon and Stars' to cultivars with gray background. The spotted trait was undetectable on many fruit with light green rind. In addition, the presence of the small spots (stars) was shown to have a random distribution on fruit and leaves, while the presence of large yellow blotches (moons) was inconsistent and may be determined by the coalescence of many stars.

Our study highlighted a complex genetic background for the inheritance of red and salmon yellow flesh colors, previously attributed solely to the expression of the *Wf* and *y* genes. Based on our observations, we discarded the hypothesis of quantitative inheritance and suggested that different genes, or a tissue-specific expression of the same genes, might be involved in the pigmentation of different portions of the fruit. Our hypothesis should be further investigated. The tissue-specific identification of the pigments in the fruit of segregating populations by high performance liquid chromatography (HPLC) would indicate localized expression of genes and, possibly, indicate a model for pigment development in watermelon, by similarity with known pathways in other species. The same crosses used in our study could be investigated at first, while new crosses could be developed for future research.

The genetics of rind color (or pattern) should be further studied. Genes for the gray and medium green, or narrow- and wide-striped rind have not been identified so far. The study of gray rind should include the parental lines 'Charleston Gray' (gray rind), 'Crimson Sweet' (striped, netted rind), and 'King and Queen' (light green, non-netted rind). To investigate the inheritance of striped rind with different width of dark stripes, 'Dixielee' (narrow stripes), 'Crimson Sweet' (medium stripes), and 'Allsweet' (wide stripes) should be crossed in a half-diallel. Should a new type line for *pencilled* be identified, it should be included in the half-diallel as well. The cross 'Allsweet' × 'Georgia Rattlesnake' would clarify the inheritance of straight vs. "rattlesnake" (irregularly shaped) stripes. In addition, it is still not known whether the striping of the rind is determined by a background color covered by stripes (either dark green stripes on light green background or vice versa). The HPLC analysis of the pigments present in cells of the light and dark stripes could provide some insights on this issue.

High yield, measured as total weight per unit of land area, is a major goal for watermelon breeders. We studied yield in a diverse set of watermelon cultivars and identified high yielding germplasm for use in breeding programs. Consistent and significant yield differences among the 80 cultivars across environments indicates genetic variation for the trait. It is apparent from our study that the lack of genetic variation and the slow improvement in yield often mentioned by watermelon breeders most likely is a result of the greater emphasis on traits other than yield, as well as the lack of diversity for yield among the modern cultivars. In any case, there is a need to identify sources of high yield, and to use those sources to develop high yielding, but adapted lines for use by plant breeders. Important traits such as fruit quality and disease resistance should be incorporated into those high yielding lines before they are used to develop new cultivars. This also should result in an increase in the genetic diversity of modern cultivars. Now that phenotypic variability for yield in watermelon has been demonstrated, and high yielding cultivars identified, the next step would be to evaluate the USDA-ARS germplasm collection for fruit yield at several locations around the United States, including accessions originating from different areas of the world.

Our investigations of the genetics of fruit weight have indicated that it should be possible to vary the size of watermelon fruit in a few generations of selection, with greater changes under high selection intensities. Breeding schemes that would allow high recombination rates may help in combining all the effective factors

needed to obtain a desired fruit weight. Recurrent selection for population improvement seems to be a valid breeding method, even though lower gain per cycle would be obtained, due to the lower selection intensity (typically equal to 20%). Nevertheless, it may be easier to introgress desired qualitative traits into breeding lines of desired fruit weight by pedigree or backcross breeding, rather than trying to change the fruit weight of otherwise acceptable cultivars. Low heritability, quantitative inheritance, and high environmental variance are important limiting factors that may greatly reduce the realized gain from selection in populations of cultivated watermelon.

Resistance to gummy stem blight in our studies was shown to be determined by quantitative trait loci, rather than the inheritance of the single gene *db* from PI 189225, as previously described. Few QTLs may be involved in the expression of resistance, and the *db* gene may be a QTL with a major effect or a single Mendelian gene, under epistatic influence of other regulatory QTLs. In addition, we measured a large heritability and high additive variance for resistance. Watermelon breeders interested in the development of resistant cultivars should use breeding techniques that make the best use of additive variance, such as recurrent selection. Greenhouse testing of the breeding material would be preferred to field testing, based on the larger heritability for the greenhouse test. Field testing may be used at later stages of selection to confirm resistance of improved material under field conditions. Multiple locations and replications, and large plots to simulate commercial production fields should be used. A precise assessment of the causal agent for the loss of plants also would be necessary before classifying them as susceptible.

Based on the inheritance of resistance to gummy stem blight in our populations (few effective factors involved, and heritability high in the greenhouse and low in the field), we suggest that QTL mapping would be a reliable way to: 1) identify molecular markers closely linked to resistant genes (or QTLs), and 2) clarify the genetics of resistance. The identification of QTLs and the estimation of their average effect could be useful for the development of a proper MAS breeding strategy for this trait in watermelon. In addition, markers and QTLs identified through greenhouse testing of the segregating populations could be verified in field tests under natural epidemics of gummy stem blight on adult plants. The availability of codominant SSR markers in watermelon is still very limited, thus making it difficult to easily saturate linkage maps and proceed to fine QTL mapping by random pairing of markers. Our efforts to identify markers linked to resistance to gummy stem blight, so far,

were not successful, even though several molecular markers were found to be polymorphic among the parental lines of our populations. Therefore, it is necessary to screen available molecular markers from other cucurbit species to identify further polymorphic loci. In addition, we have found that SSR and ISSR markers can be successfully deployed, while EST-based markers identify little polymorphism in watermelon. RAPD markers were useful in our study, but the use of dominant markers could be a limitation to QTL mapping. Based on the results of the investigation that we suggest, plant breeders could integrate MAS for the *db* gene and field and greenhouse testing to select the progenies with the highest expression of resistance under natural and artificial epidemics of gummy stem blight. Thus, minor QTLs could be selected directly by observation of the level of resistance in phenotypic testing. Field testing would be necessary for yield and quality traits, so that multiple years of trialing for disease resistance would be possible, prior to release of the best germplasm.

Future Objectives for Watermelon Breeding and Genetics

Maintenance and Availability of Mutants

The information currently available to watermelon breeders and geneticists, as often highlighted throughout this dissertation, is incomplete, somewhat out of date, and in some instances incorrect and misleading. Scientists working on watermelon are currently making efforts to rectify the mistakes present in the literature and carried forward in the updates of the gene list for the crop. Nevertheless, a major issue arises from the loss of many mutants that were type lines for newly described genes at the time of their discovery.

Nowadays, lack of seeds or proper images of those mutants and poor descriptions of the mutant phenotypes make it sometimes impossible to correctly understand the usefulness of previously described genes. Thus, to avoid similar problems to future generations of scientists, all the mutants used to described the inheritance of new genes, or to study heritability and genetic effects of QTLs, should be submitted to the Cucurbit Genetics Cooperative watermelon gene curators (T.C. Wehner and S.R. King) for inclusion in the collection and maintenance. In addition, carefully detailed descriptions and color images of the mutations should be submitted for publication to refereed journals. To reduce the charge for publication of color images, a gray-scale version could be used for the print version and a color version could be linked in the electronic version of the article, as currently allowed by many publishers.

In order to increase the rate of discovery of new mutations, private breeders should submit those genetic mutants that are not of proprietary interest in seed companies to public geneticists for inheritance study and maintenance of the mutants. Typically every 10 to 15 years, private breeders dispose of germplasm that is not anymore interesting to their breeding program. Thus, it is likely that a very large portion of newly discovered mutants be lost every year.

Evaluation and Availability of the USDA-ARS Germplasm Collection

The USDA-ARS watermelon germplasm collection in Griffin, Georgia, lists over 1,500 accessions. Very little information is available on the characteristics of these lines. In addition, many lines are still open pollinated populations collected from wild environments or increased without proper isolation. Thus, the level of heterozygosity in the collection is still unknown.

There is a strong need for a closer survey of the entire germplasm collection to characterize all the accessions from a phenotypic and genotypic standpoint. The accessions should be tested to measure their homozygosity level, inbred several generations, tested for multiple traits, and finally screened for specific genes of interest.

Efforts are currently made by public breeders to screen the collection for specific disease resistance genes, but the information produced should be better coordinated and centralized. A database containing all the information available on each accession should be compiled. This database should allow the user to screen *in silico* the collection for specific traits of interest. Currently, the GRIN database (http://www.ars-grin.gov/npgs/searchgrin.html) by the National Plant Germplasm System of the USDA-ARS is the only public database available that contains some information on the watermelon germplasm collection. Nevertheless, the GRIN database allows the user to search information only by accession number, and not by trait of interest. Furthermore the time required to update the database with newly available data is very long. Geneticists and breeders should be able to submit screening data directly to the database on-line, thus making them readily available to other users.

Breeding Methods

Traits of interest to commercial watermelon breeders are disease resistance, yield, fruit size, and nutritional content (sugars, lycopene, etc.). The breeding techniques adopted for the improvement of these traits are limited to pedigree and backcross breeding. Even though these breeding schemes are useful to transfer single genes to high quality cultivars, they do not necessarily suit the requirements for the improvement of quantitative traits, such as yield, nutritional content, and resistance to certain diseases.

The development and release of recombinant populations for population improvement and inbred development is a major need to ensure successful improvement of quantitative traits. Certainly, the size of the watermelon plant has discouraged commercial breeders from this approach, but public breeders should be funded to pursue this objective. Populations improved for specific traits of interest and enriched of adapted germplasm would then be readily useful to private breeders for inbred development and hybrid testing.

In order to maximize the usefulness of these populations, a precise estimation of the intercrossing frequency in watermelon is needed. The design of proper field technique, including optimum plot size and field layout, is needed to favor intercrossing and reduce self-mating in the populations.

Breeding for Production of Seedless Fruit

One of the greatest advances in watermelon breeding during the last century has been the development of triploid seedless cultivars. This technique has provided a new product, highly appreciated by customers, and highly emphasized by private breeders. However, the almost complete shift of the breeding industry towards seedless types has greatly reduced breeding progress.

The development of triploid seed requires the separate and contemporary breeding of tetraploid and diploid lines with similar characteristics that can be combined during hybrid production. The breeding of tetraploid parents is greatly limited by the low fertility of the first generations after doubling of the chromosome number. Thus, only few lines can be successfully advanced through a tetraploid breeding program, resulting in higher emphasis in reselection within already available tetraploid germplasm, rather than breeding of new lines and populations.

As a consequence, the already narrow genetic base of the watermelon breeding populations currently used will become even narrower and the improvement of major traits, such as disease resistance and yield, will be strongly limited and delayed for many years.

Alternatives to breeding triploid hybrids for seedless production could come from biotechnology. Genes for parthenocarpy and gynoeciousness are not currently known in watermelon, but homologous genes are known in cucumber, Comparative studies of the two species may allow watermelon biotechnologists to isolate the genes involved in sex expression and seed development in watermelon. The knowledge of the genetics of these mechanisms may allow to produce seedless fruit from diploid plants. It could be possible to repress the production of seeds, or to overexpress the development of female flowers with parthenogenetic potential.

A strong limitation to the deployment of such a strategy would certainly come from customer acceptance of transgenic crops, but certainly this is a common issue to all the cultivated transgenic species. Even though it is difficult to forecast the future of customer preferences, history teaches us that initial response will be cautious and reluctant towards what is new, but it then becomes accepted and commonplace. It is possible that each generation somehow is needed to balance the mistakes of the previous and that the randomness of this scheme be the internal motor of progress. Should an example be needed, we could refer to the last century. Destruction and loss of lives, resources, and freedom came prior to World War II, then reconstruction and fast progress followed with a second industrial revolution in Europe, the end of the Cold War, the defeat of many dictators in Europe, Asia, Latin America, and Africa, and the end of colonialism. In art, music, and culture, Futurism and Cubism promoted the idea of the humankind projected into the future and in fast movement towards a better, but yet unknown, society. Yet, now we are folding back on ourselves, trying to slow down and fix the mistakes that have been unwittingly made during those years of great progress. It is useful to fix mistakes, but it is necessary to move forward again soon. Some repairs are necessary, but many may be overreaction to the unknown.

For watermelon breeding, certainly the deployment of biotechnology for the production of seedless diploid cultivars may be delayed by major ethical and sociological issues in biotechnology. Nevertheless, in absence of natural mechanisms for parthenocarpy and gynoeciousness in watermelon, and in light of the limitations that tetraploid breeding is posing to the genetic improvement of this crop, there is not a valid

alternative to biotechnology and tetraploid breeding must be substituted with a less limiting system of production of seedless fruit.

Molecular Genetics and Biotechnology

Watermelon is not a commodity producing large revenues for seed companies and growers, particularly when compared to horticultural crops such as tomato, pepper, potato, and melon. The little availability of funding for basic research in watermelon genetics has greatly limited the development of molecular tools to assist breeders in their activity. The study of traits of great importance, such as disease resistance and nutritional content, has been limited also by the lack of molecular information for this crop.

Cucurbit geneticists should concentrate their efforts in the development of molecular marker maps using marker technologies that could be easily adopted by breeders to screen their populations. Currently, SSR markers seem to be the most promising technique to be used and a linkage map of watermelon including several hundred SSR markers would be the basis for further marker development. It would also permit the study and improvement of complex traits (e.g. nutritional content, diploid seedlessness, or disease resistance), and for preliminary approaches to transformation of watermelon seedlings. In the development of this map, watermelon molecular geneticists should also make use of the information available in other crops, such as tomato and melon, and transfer them to the watermelon genome.

A reliable method for transformation in watermelon is not yet available, even though preliminary work has been done and should be continued to increase the efficiency of the system. Furthermore, the availability of a linkage map and markers linked to important traits would be fundamental to allow the selection of target loci for transformation experiments.

The availability of mapping and transformation tools would permit the exploration of the germplasm collection currently available to search for new traits of interest, or to evaluate the potential use of the wild relatives of cultivated watermelon in crop improvement. So far, major limitations in the use of wild germplasm for watermelon breeding has come from the negative effects of linkage drag, due to the wide phenotypic differences between fruit of wild lines and elite cultivars. Molecular assisted breeding would be useful in

controlling linkage drag, or in reducing the number of generations required to restore in the selected progeny the quality of the adapted parent by backcross breeding.

In order to facilitate and expedite progress in developing basic information and tools for molecular genetics and biotechnology in watermelon, a coordinated effort of breeders and molecular geneticists will be required. The objectives should be prioritized and a consortium should be created for the development and release of basic mapping and transformation tools. In this first phase, the involvement of private sponsors would be fundamental, but proprietary issues of the information produced may limit the availability of private funding.

Final Remarks

In conclusion, a greater support by governmental agencies and private seed companies will be necessary to increase the number of researchers involved in watermelon breeding and genetics. Furthermore, funding of public breeding programs of all horticultural crops should be a shared responsibility of public and private institutions. The nutritional value of horticultural crops to human nutrition is now widely recognized, but the support for research is still very limited.

Plant breeding has been a key player in the green revolution of the last century, which greatly reduced hunger in the world. Nevertheless, in the last decade the number of institutions and people involved in plant breeding and genetics has been declining constantly and rapidly. More funding is urgently needed to sustain what remains of public sector plant breeding. A common policy for training and employment of future plant breeders and geneticists is also strongly needed.

The ever-growing human population, and the reduction of land available to agriculture, together with the need for more environmentally-friendly cultural practices, require the development of new cultivars capable of producing higher outputs with lower inputs. Yet, public plant breeders are disappearing and those left are not well funded. Private breeders are focusing their efforts on fewer crops each year and are being consolidated into fewer seed companies that can support the cost of research, but cannot support research on long-term or low-payoff areas.

The solution to these problems is not obvious, but an open dialogue at the highest levels of the national government is needed and requires the direct involvement of the scientific community. A world-wide policy for the development and deployment of new cultivars is now more necessary than ever, and a shared international support for plant breeding could be the basis for the support of future generations.

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