USING PLANT BREEDING, CULTIVAR EVALUATION, AND CULTIVATION STRATEGIES TO ADDRESS CHALLENGES IN SUSTAINABLE WATERMELON PRODUCTION

by

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(Under the Direction of George Boyhan)

ABSTRACT

Organic watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] constitutes 1% of the $450 million watermelon market in the U.S. Although most U.S. conventional watermelon is produced in the Southern region, only one-fourth of the nation's organic watermelon is grown here. Organic production is difficult in the humid, subtropical climate because of intense disease and weed pressure. A breeding program to develop watermelon cultivars specifically suited for organic production, that addresses challenges including field space, organic weed control, and repetitive harvests, was initiated. Because weed control is the most expensive aspect of organic watermelon production, a study to determine an optimal hand-weeding regime, estimate labor costs, and evaluate the compact trait for improved weeding efficiency was conducted. In addition, a characterization of the diversity of open-pollinated watermelon cultivars popular in organic and direct market production was used to better inform growers, seed savers, breeders and the commercial seed industry about cultivar maintenance and conservation of genetic diversity.

INDEX WORDS: Backcross breeding, *Citrullus lanatus*, conservation breeding, dwarf, genetic diversity, heirloom, organic breeding, organic weed control, SSR

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DEDICATION

To my husband, David, who walked this path with me while we lovingly held hands, and to my two sweet children, Daisy and James, who will always be our greatest accomplishment by far.

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CHAPTER 1

Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a warm-season annual vegetable crop that is grown on 3.5 million hectares worldwide (FAO, 2014). It is a member of the *Cucurbitaceae* family along with cucumber, squash, and melon. Watermelon originated in western Africa (Chomicki and Renner, 2015) then spread to the Middle East and then China by 900 AD (Dane and Liu, 2007). The fruit is grown for its edible endocarp, rind, and seed oil. The colored flesh, though 93% water, contains significant amounts of carbohydrates, vitamin A, and lycopene (Wehner, 2008).

Cultivars demonstrate a wide diversity in fruit size, flesh color, rind pattern, and sweetness. Each growing region has a set of cultivars that are widely grown and are suited for local environmental conditions (Wehner, 2008). Of these, hybrids predominate due to uniformity, vigor, and proprietary value. Seedless 'Tri-X-313' types are the most common cultivars found at grocery stores and seeded 'Allsweet' picnic types are becoming increasingly rare (Wehner, 2008). A market for "personal" mini-watermelons (<4.0kg) is quickly developing. Consumers tend to favor large fruits only during summer holiday seasons, so often grocers provide quartered and cut sections. A survey of direct-market Georgia farmers indicates that the most popular cultivars grown are seeded open-pollinated (OP) cultivars such as icebox-sized 'Sugar Baby', large round 'Crimson Sweet' and the yellow-mottled novelty 'Moon and Stars' (J.W. Gaskin, unpublished data).

In the U.S., watermelon is grown commercially on a large scale as a row crop. The sprawling vine plants are typically grown at 1.5 to 3.5 m^2 spacing per plant (Boyhan, 2000). The monoecious or bisexual flowers are bee pollinated. Mature plants perform best in dry conditions and sandy soils. Farmers may direct seed or transplant seedlings into widely spaced rows or hills. Sprawling vines make weed and disease control less efficient than traditional row crops. Furthermore, hand-harvesting watermelons at the appropriate level of ripeness provides considerable challenge to large-scale farm operations. Experienced field labor is used for harvest. Various methods for determining ripeness have been used such as checking for a brown tendril adjacent to the fruit, a yellow ground spot, or a flat sound when thumped. Maturity requires about 120 days, and production peaks May through August. The fruit can be stored up to 2-3 weeks post-harvest in cool, humid conditions. Cultivation of watermelon is generally successful in the Southern region as the crop has greater disease resistance than other members of *Cucubitaceae* (Wehner, 2008).

Production Value

Over 100 million tons of watermelon are produced annually worldwide (FAO, 2014). Seventy-five percent of global watermelon production takes places in China, followed by Turkey, Iran, Brazil, and Egypt (FAO, 2014). The U.S. ranks eighth in watermelon production.

In the U.S., watermelon ranks third in fresh market vegetable production behind onions and lettuce (USDA, 2016a). Approximately 52,600 hectares are grown annually, which generates about 35 million hundred-weight (cwt) of fruit valued at $480 million (USDA, 2016a).

The Southeast U.S. is the primary watermelon growing region and Georgia consistently ranks in the top four states in production. California and Texas are also major watermelon

producing states. Drier regions of the western U.S., particularly California, Arizona and Colorado, are important production regions for organic watermelon and seed multiplication. Watermelon is the second most valuable vegetable crop, behind onion, in Georgia. The southern GA counties Tift, Worth, Crisp, and Wilcox each produce about 1000 hectares of watermelon, valued more than $10 million (Wolfe and Stubbs, 2015). Because the crop thrives in sandy or sandy loam soils and prefer dry conditions towards maturity (Hankins, 2009), most large-scale watermelon production in Georgia takes place in the coastal plain region (Wolfe and Shepherd, 2012).

Organic watermelon constitutes approximately 1% of the national $450 million watermelon market (USDA, 2014). Although the Southeast produces most of U.S. conventional watermelon (USDA, 2016a), it only produces one-fourth the nation's organic watermelon (USDA, 2014). Georgia ranks 10th in organic watermelon production, producing certified watermelon on 7 hectares statewide, valued at $42,000 (USDA, 2014). This, however, is a 300% increase in organic production since the last Organic Census of Agriculture in 2008 (USDA, 2008). The demand for organic food continues to grow by double digits each year, with fresh fruits and vegetables as the top selling category (McNeil, 2016). Furthermore, most organic produce is purchased locally: 80% of organic products are sold within 500 miles of the farm and 50% sold within 100 miles (USDA, 2014).

Organic Production

Organic production in the southern region of the U.S. lags behind other growing regions for practical reasons. The humid, subtropical climate in the South makes disease and weed pressure more intense than in drier regions in the western U.S., where commercial organic

production is concentrated. Georgia's environmental factors are not generally considered conducive for organic agriculture for most crops. Only about 280 hectares are dedicated to organic vegetable production statewide (USDA, 2014), a small percentage of the nearly 40,500 hectares currently under conventional vegetable production in Georgia (USDA, 2016a). Fresh market farmers in the Southeast region interested in growing organic or low pesticide watermelon face several production challenges. According to a report prepared for the USDA's Sustainable Agriculture Research and Education (SARE) program, farmers desire research-based strategies for organic insect, disease, and weed management (Boyhan, 2003). Watermelon grown under protected organic conditions in Turkey has been shown to yield greater than conventional systems (Çürük et al., 2004). Research to improve the economic sustainability of organic production in the U.S., and particularly in the South, is needed.

Organic Weed Control

Weeds are the biggest threat to watermelon yields. Weeds compete with the crop for space, light, and nutrients; can promote disease and insect pressure; and impede pollination and harvest (MacDonald, 2000). Low planting density and delayed canopy closure increase the opportunity for weed growth. Large crabgrass [*Digitaria sanguinalis* (L.) Scop.] was shown to impact watermelon yield for up to 6 weeks after transplant (WAT) (Monks and Schultheis, 1998); smooth pigweed (*Amaranthus hybridus* L.), up to 3 WAT (Terry et al., 1997); and American black nightshade (*Solanum americanum* P. Mill.*)*, up to 4 WAT (Adkins et al., 2010). Densities as low as 2 plants/m^2 of yellow nutsedge (*Cyperus esculentus* L.) (Buker et al., 2003), goosegrass [*Eleusine indica* (L.) Gaertn.] (Wallinder and Talbert, 1983) or American black nightshade (Gilbert et al., 2008) have been shown to reduce watermelon yield.

Organic growers are not permitted to use the synthetic pre- and post-emergence herbicides (USDA, 2016b) which conventional watermelon growers utilize (Culpepper and Smith, 2016). Black polyethylene mulch and mechanical weed control has been shown to effectively control weeds and protect yield better than low-input, no-till organic practices (Davis et al., 2007). Organic weed control must employ an integrated approach of "many little hammers" (Liebman and Gallandt, 1997) that may include the following: cover cropping, crop rotation, stale seedbed preparation, competitive crop genotype selection, tillage, and mechanical weeding (Bàrberi, 2002). It has been suggested to focus attention on minimizing the seedbank in the top inch of soil which can germinate rapidly (Cohen and Rubin, 2007). Mechanical implements can be customized for many crops to remove weeds between and within rows (Bowman, 1997). Cultivation is not practical once watermelon plants begin to vine about 1-2 weeks after transplanting, which forces organic growers to rely on hand-weeding for the duration of the season.

Organic weed control is estimated to cost 20x that of a conventional herbicide program (Gianessi and Reigner, 2007) due to greater labor demands. Conventional weed controls costs about $20 to $100 per acre in watermelon (Fonsah et al., 2016; Miller, 2016). Klonsky (2012) reported 92%, 68%, and 10% increases in weed control costs in California for organic tomato, broccoli, and lettuce, respectively. No cost estimates for organic weed control in watermelon are currently available. The perfectly clean, 100% weed-free fields for which most conventional growers strive is impractical for most organic growers (Zimdahl, 2013). When surveyed, organic GA farmers reported that minimal effort is put into weed control except hand-pulling pigweed (R. Walker and J. Payne, personal communication).

Other Important Organic Management Practices

Watermelon diseases such as Fusarium wilt (caused by *Fusarium oxysporum* f. sp. niveum), anthracnose (caused by *Colletotrichum spp*.), and gummy stem blight (caused by *Stagonosporopsis* spp.) reduce yield in the Southern region (Kousik et al., 2016). The synthetic fungicides that are typically applied weekly in conventional watermelon production (Dutta, 2017) are not permitted by organic standards (USDA, 2016b). Instead, organic growers are recommended to use resistant cultivars, crop rotation, and careful removal of diseased plant material. Infected debris should be excluded from the compost pile. Five to seven year crop rotations and cover crop green manures (Himmelstein et al., 2014) may reduce soil-borne diseases like Fusarium wilt. Keinath (1996) found a significant reduction in gummy stem blight incidence when watermelon was rotated with cabbage and the soil was amended with the cabbage residue. Drip irrigation, rather than overhead irrigation, may also reduce the spread of splash-dispersed diseases like gummy stem blight. Certified seed ensures that disease is not present at the start of the season. Cultivars resistant to gummy stem blight are currently not available, although non-elite germplasm indicates a potential source of resistant genetic material for breeders (Wehner, 2008). For greenhouse production, decreasing humidity and trellising combat favorable conditions for fungal growth. Once the watermelons are infected, a modest application of copper or sulfur fungicides is the only effective means to avoid rogueing plants (Hankins, 2009).

Aphids, including melon aphid, *Aphis gossypii*; green peach aphid, *Myzus persicae;* cowpea aphid, *Aphis craccivora*, create significant crop loss of watermelon in Georgia (Hankins, 2009). These pests feed on the plants' sap, promoting mold growth and transmitting viral diseases, such as Papaya Ringspot Virus, Watermelon Mosaic Virus, and Zucchini Yellow

Mosaic Virus. An aphid population mounts quickly over the growing season: adults migrate from milder climates in Florida or overwintering eggs hatch on nearby host plants, females reproduce sexually or asexually as needed, winged and wingless forms persist, and all forms can survive on primary and secondary hosts. Cucumber beetles, *Diabrotica undecimpunctata* and *Acalymma vittatum*, also cause problems by feeding on watermelon plants (Lyon and Smith, 2012). Organic growers can use reflective plastic mulch or living mulches, floating row covers, oils and pyrethrins, and biological controls to mitigate insect pests.

Organic production requires special considerations of soil fertility as well. As in all organic production, composted soil amendments will enhance the physical, chemical, and biological profile of the sandy to sandy-loam soil inherent to coastal plain Georgia (Boyhan et al., 2014). Compost consisting of cow or poultry manure with straw can be applied and integrated into the field prior to sowing (Hankins, 2009). Banding applications of fertilizer has been shown to limit weed growth better than broadcast applications (Santos et al., 2004). In the off-season, cover crops can be used to replenish the soil's available nitrogen. Austrian winter pea (*Pisum sativum* L. ssp. *sativum* var. *arvense*), hairy vetch (*Vinca villosa* Roth), and crimson clover (*Trifolium incarnatum*) can be planted in Georgia in September and flail-mowed before seed set in April (Hankins, 2009).

Watermelon should be planted in the well-drained areas of the field, as its roots cannot withstand long-term moisture and fruit set is actually encouraged by dry conditions (Hankins, 2009). Significant yield increase was noted by Wu et al. (2009) when a particular bio-organic fertilizer was applied to watermelon; the soil amendment resulted in increased enzyme activity of the plants that thwarted Fusarium wilt when compared to conventional production methods.

Optimized soil fertility will promote healthy plant growth, which may in turn allow watermelon to tolerate disease more effectively than stressed plants.

Breeding to Improve Organic Production

A major barrier to the widespread adoption of organic agriculture is its pervasive yield deficit. Organically grown fruits and vegetables yield on average 30% lower than their conventional counterparts (Seufert et al., 2012). The direct comparison of organic and conventional yield is problematic because an estimated 95% of commercially grown cultivars were bred specifically for conventional, high input conditions (Lammerts van Bueren et al., 2011). The top performing cultivars in conventional systems are not always the top performing cultivars in organic systems (Campion et al., 2014; Murphy et al., 2007). Yield can be increased on organic farms by simply selecting cultivars that are productive under organic and low pesticide conditions. This may involve breeding for improved nitrogen efficiency (Baresel et al., 2008), root structure (Melo, 2003), or weed competitiveness (Hoad et al., 2008). Conditions vary from organic farm to farm, so plant breeding efforts may need to focus on local and regional adaptation as well (Dawson et al., 2011).

The first step in organic breeding is trialing existing cultivars to evaluate performance and stability on organic farms (Myers et al., 2012; Swegarden et al., 2016). A small but growing list of trial reports is published in eOrganic's Variety Trial Database (Zystro, 2014). The next step is to develop new cultivars specifically suited for organic production. Selection under organic conditions has been shown to improve performance better than selection under conventional conditions (Burger et al., 2008; Murphy et al., 2007; Serpolay et al., 2011). The initiation of breeding programs specifically targeting organic production challenges requires a

significant resource investment but may effectively close the yield gap in organic agriculture (Lammerts van Bueren et al., 2011).

Organic growers are often proponents of heritage or heirloom cultivars due to their cultural significance, improved eating or cooking traits, or their unique genetic background (Phillips, 2016). However, these cultivars lack modern disease resistance packages and thus may not improve the economic sustainability of organic production. The organic community also favors OP cultivars over F_1 hybrids whose genetic resources are owned by private industry (Navazio et al., 2012). Mild heterosis in watermelon (Gusmini and Wehner, 2005) results in no clear advantage to F_1 hybrid cultivars over OP cultivars, although specific crosses have produced superior cultivars. Increasing yield in watermelon is achieved by selecting cultivars that produce larger fruit, but this strategy may be counter to customers' preference for small or personal-sized watermelon. Based on this information, breeding watermelon for traits that are specific to organic production and direct market preferences, rather than yield per se, may be warranted.

Compact watermelon

Dwarf plant architecture is a non-typical watermelon trait that may be useful in organic production. Six recessive dwarf genes have been described in watermelon. A spontaneous dwarf of 'Desert King', carrying a mutant form of *dw-1* gene, has short internodes as a result of shorter cells (Loy and Liu, 1975; Mohr, 1956). Mutant *dw-1* plants have thick, brittle, twisted vines; large leaves with a warped margin; 2 to 3 lateral branches; and poor floral organ development (Liu and Loy, 1972; Mohr, 1963). A dwarf of 'Somali Local' had intermediate vine length due to a gene that is allelic to *dw-1* and thus designated *dw-1*^{s} (Dyutin and Afanas'eva, 1987). A spontaneous dwarf of Japanese cultivar 'Asahi Yamato' (synonymous with WB-2 in the U.S.; Mohr and Sandhu, 1975) carrying a mutant form of *dw-2* has short

internodes as a result of fewer cells, normal vine and leaf morphology, 5 to 11 lateral branches, and normal floral organ development (Liu and Loy, 1972). 'Dwarf Male-Sterile Watermelon' plants with *dw-3* have short internodes, reduced lobes in leaves, and abortive staminate flowers, which may prove useful in hybrid seed production (Huang et al., 1998). Recently, two additional dwaft mutants have been described that have short internode length and normal floral organ development: a spontaneous dwarf of '5-6y' with putative *dw-4* (Yang et al., 2008) and spontaneous dwarf of 'Hanxuan Lvyuan' with putative *dsh* gene (Li et al., 2016). Extreme dwarfs resulting from the cross of *dw-1* mutants with *dw-2* mutants, named 'Kengarden', have a 90% reduction in vine lenth compared to normal cultivars (Liu and Loy, 1972). 'Bush Sugar Baby' and 'Bush Charleston Gray', which are derived from the *dw-1* mutant, are the only dwarf cultivars that are commercially available in the U.S.

Promoting Genetic Diversity

Organic and direct-market growers wish to enhance on-farm biodiversity, which they feel can be acheived by using OP cultivars and preserving heritage cultivars from extinction (Navazio et al., 2012; Phillips, 2016). A current debate as to whether the disappearance of heirlooms and a transition to modern cultivars has reduced biodiversity is active (Mendum and Glenna, 2010), despite evidence that industrial seed model has had a neutral effect (Heald and Chapman, 2012).

Cultivar diversity is pertinent to watermelon production because the crop contains less genetic variation than maize, soybean, and rice (Guo et al., 2013). The limited diversity in cultivated watermelon (Levi et al., 2001a) is likely the result of a severe bottleneck during domestication (Nimmakayala et al., 2014a). Low diversity in watermelon has been consistently estimated using a variety of marker systems, including random amplified polymorphic DNAs (RAPDs) (Levi et al., 2001b), amplified fragment length polymorphisms (AFLPs) (Che et al.,

2003; Levi et al., 2004), restriction fragment length polymorphisms (RFLPs) (Dane and Liu, 2007), single sequence repeats (SSRs) (Huayu et al., 2016; Joobeur et al., 2006; Levi et al., 2009; Zhang et al., 2012), and single nucleotide polymorphism (SNPs) (Nimmakayala et al., 2014b; Yang et al., 2016). However, intracultivar diversity, which is likely the primary interest of the organic community, has not yet been estimated in watermelon. Furthermore, a direct comparison between the genetic diversity of heirloom versus modern cultivars has not been conducted. Genetic analysis on this level may better inform cultivar selection so that organic growers can meet biodiversity goals.

Research Objectives

Research is needed to improve watermelon production in the Southeast region, for organic systems and non-certified direct-market farms. The first objective of this research was to develop cultivars specifically suited for organic and direct-market production. A compact growth trait was used to address field space restrictions, labor-intensive organic weed control, and repetitive harvest limitations that these growers experience. Because weed control is the most expensive aspect of organic watermelon production, the second objective was to determine an optimal hand-weeding regime, estimate labor costs, and evaluate the compact trait for improved weeding efficiency. Finally, the third objective was to characterize the diversity of OP watermelon cultivars popular in organic and direct market production. The characterization of diversity within and among cultivars, with explicit attention given to heirloom versus modern cultivars, will enable seed savers, the commercial seed industry, and breeders to better maintain and conserve OP watermelon cultivars.

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CHAPTER 2

THE IMPACT OF WEEDING REGIME, PLANTING DENSITY, AND GROWTH HABIT ON WATERMELON YIELD IN AN ORGANIC SYSTEM[1]

[1] Stone, S.P., G.E. Boyhan, and W.C. Johnson, III. To be submitted to *Weed Technology*.

Abstract

The southeastern U.S. produces half of U.S. conventional watermelon but only 7% of U.S. organic watermelon. Weeds are a major threat to watermelon yield in the Southeast, and organic weed control is estimated to cost 20x more than conventional herbicide programs. The objectives of this study were to determine the optimal weeding regime to reduce hand-weeding costs while maintaining yield and to evaluate two watermelon growth habit genotypes for improved weed management in an organic system. In 2014 and 2015, watermelon plots were randomly assigned to the following treatments in a three-way factorial arrangement: (1) vine or compact growth habit; (2) 1.0 m or 0.5 m in-row spacing; and (3) hand-weeding once per week for 0, 4, or 8 weeks after transplant (WAT). Average total weed density in 2014 and 2015 in non-weeded treatments were 86.6 and 87.0 plant/m^2 and in 4 WAT treatments were 26.4 and 7.0 plants/m^2, respectively. Weeding 4 WAT resulted in similar watermelon yield as weeding 8 WAT in both years. This partial season weeding regime reduced labor costs in 2014 by 67% and 63% for vine and compact plants, respectively, and in 2015 by 43% across both growth habit types. Watermelon plants with a compact growth habit required less time to weed than vine-types when control was performed for 8 WAT but there was no advantage detected when weed control was performed only 4 WAT. The cost of weeding 4 WAT was estimated at $1,488 to $3,007/ha. The organic watermelon yield in response to weeding 4 WAT exceeded the national and state farmer-reported average for conventional watermelon, indicating the potential of this cropping system in the Southeast despite the hand-weeding expense.

Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) production is a $215 million industry in the southeastern U.S. This region produces half of U.S. conventional watermelon yet only 7% of U.S. organic watermelon (USDA, 2014, 2016a). Because organic food sales have increased more than 80% since 2007, wholesale prices of organic watermelon is twice that of conventional, and 80% of organic products are sold within 500 miles of the farm (USDA, 2014) there is an unmet market potential for organic watermelon in the Southeast. Research to support and improve organic watermelon production in the region is lacking.

Weed pressure is severe in the humid, subtropical Southeast and is a major threat to watermelon yield in both conventional and organic systems. Weeds compete with the crop for space, light, and nutrients; can promote disease and insect pressure; and impede pollination and harvest (Macdonald, 2000). Typically, watermelons are transplanted 3-4 weeks after sowing and harvested 8-10 weeks later. Approximately 1-2 weeks after transplant (WAT), vines begin to enter row middles, making chemical or mechanical weed control nearly impossible. Though vigorously vining in nature, watermelon plants are actually poor weed competitors: plants must be sown or transplanted approximately 2 m^2 apart and require 6 to10 weeks to create a closed canopy with adjacent plants, allowing ample opportunity for weeds to emerge. Various weeds, even at low densities, have been shown to impact watermelon yield throughout its growing season. Large crabgrass [*Digitaria sanguinalis* (L.) Scop.] was shown to impact watermelon yield for up to 6 WAT (Monks and Schultheis, 1998); smooth pigweed (*Amaranthus hybridus* L.), up to 3 WAT (Terry et al., 1997); and American black nightshade (*Solanum americanum* P. Mill.), up to 4 WAT (Adkins et al., 2010). Densities as low as 2 plants/m^2 of yellow nutsedge (*Cyperus esculentus* L.) (Buker et al., 2003), goosegrass [*Eleusine indica* (L.) Gaertn.]

(Wallinder and Talbert, 1983), or American black nightshade (Gilbert et al., 2008) have been shown to reduce watermelon yield.

Conventional watermelon production in the Southeast relies on synthetic pre- and post-emergence herbicides (Culpepper and Smith, 2016), which are not permitted in organic production (USDA, 2016b). Organic weed control must employ an integrated approach that may include the following: cover cropping, crop rotation, stale seedbed preparation, competitive crop genotype selection, tillage, and mechanical weeding (Bàrberi, 2002). Cultivation is not practical once watermelon plants begin to vine about 1-2 weeks after transplanting, which forces organic growers to rely on hand-weeding for the duration of the season.

Weed control in organic production is a serious barrier to economic sustainability because hand-weeding is estimated to cost growers 20x more than conventional herbicide programs (Gianessi and Reigner, 2007). Klonsky (2012) reports 192%, 168%, and 110% increases in weed control costs in California for organic tomato, broccoli, and lettuce, respectively. Identifying cost-effective organic weed control strategies would greatly benefit organic growers. This study is the first to our knowledge to determine the cost of weed control in organic watermelon production.

A long-term approach to sustainable weed management in organic production may be breeding and selecting competitive crop genotypes. To breed for weed competitiveness, the qualities that maximize watermelon yield in weedy conditions or under a specific weeding regime must be determined (Pester et al., 1999). At this time, no studies relating watermelon plant architecture to weed competitiveness are available. We hypothesized that short-internode genotypes, which have a compact growth habit, are well-suited for weed management in an organic system: compact plants develop 1) a denser leaf canopy that may shade out competing

weeds and 2) non-sprawling vines that may be easier to hand-weed than a traditional vine-type variety. The compact cultivar selected for this study, 'Companion', has high level of lateral branching and reduced vine length (Fig. 3.1A) compared to a traditional, sprawling vine-type watermelon cultivar (Fig. 3.1B). Although it was bred to be a non-harvested pollinizer for seedless watermelon production and is not grown commercially, 'Companion' was included in this study to determine if its unique compact growth habit trait conferred improved weed management in an organic system, which could then be exploited in plant breeding.

The two objectives of this study were 1) to determine the optimal weed control regime to reduce hand-weeding cost while maintaining yield in an organic system and 2) to evaluate compact growth habit, a non-typical watermelon trait, for weed competitiveness or improved hand-weeding efficiency.

Materials and Methods

The study was conducted in 2014 and 2015 on certified organic land at the University of Georgia Durham Horticulture Farm in Watkinsville, GA (lat. 33°55'N, long. 83°25'W), which has been organically managed since 2007. The soil type was a Cecil sandy loam (Fine, kaolinitic, thermicTypic Kanhapludults) with a soil organic matter of 1.5%. The study area rotated between similarly managed neighboring plots within an ongoing 4-year rotation scheme and followed USDA National Organic Program standards. Nature Safe® organic fertilizer (10N–0.9P– 6.6K, Nature Safe, Irving, TX) was broadcast and tilled into the soil two weeks prior to transplanting at a rate of 265 kg of nitrogen/ha. Seeds of 'Companion' were obtained from Seminis (Oxnard, CA) and seeds of 'AU-Producer' were obtained from Reimer Seed (Saint Leonard, MD). Plants were self-pollinated in the greenhouse to generate untreated seed. Seeds were sown in organic potting soil (Organic Fafard 3B, Sun Grow Horticulture, Agawam, MA) in

greenhouse flats on 11 Apr. 2014 and 11 May 2015 and transplanted to the field on 7 May 2014 and 11 June 2015. Beds were prepared 1.8 m on centers. No mulched was used. All plots were weed-free at the time of watermelon transplant by using a stale seedbed technique, in which surface weed seeds were allowed to germinate for 2 weeks and then removed by shallow tillage prior to transplanting, and by hand-pulling the day of transplanting. Fields were irrigated with overhead sprinklers as needed to approximately 2.5 cm/wk. Marketable fruit were harvested once in 2014 on 9 July and daily in 2015 from 31 July to 13 Aug. due to crow predation pressure. No pesticides were applied during the study.

The experimental design was a randomized complete block with three replications and each experimental unit contained 10 watermelon plants. Treatments were applied in a three-way factorial arrangement: (1) vine or compact growth habit; (2) 1.0 m or 0.5 m in-row spacing; and (3) weeding once per week for 0, 4 or 8 WAT.

The cultivars selected for the study to represent vine and compact growth habit were 'AU-Producer' and 'Companion', respectively. 'AU-Producer' produces 11 to 15 kg Crimson Sweet-type fruit and demonstrates good disease resistance. 'Companion' produces 4 to 5.5 kg round-blocky fruit.

Plots were weeded by the same 3 individuals each week using stirrup hoes and hand-pulling. The man-hours required to weed each plot was measured and converted to cost using the labor wage of $10/hour (Fake et al., 2009). Weeds were counted by species within 2 randomly-placed 0.5 m x 0.5 m quadrats per plot each week on the day prior to weeding treatments.

An additional study was conducted in 2015 to investigate a finer scale of weeding regime. The experimental design was a randomized complete block with 3 replications, using

'AU Producer' at 1 m in-row spacing under the same organic management system described above. The following treatments were randomly applied to ten-plant plots: weeding once per week for 1, 2, 3, 4, or 8 weeks, or a non-weeded control.

Weed density, watermelon yield, fruit count, and man-hours spent weeding each plot were measured. Data were converted to per-area estimates to account for different plot sizes that resulted from the in-row spacing factor. Analysis of variance was performed and means were separated using Fisher's protected least significant difference at $P \leq 0.05$ (STATA 14.1, StataCorp LC, College Station, TX). Weed pressure was greater and watermelon yield was lower in 2014 than in 2015. Because these magnitude differences result in many *treatment* × *year* interactions, all response variables were analyzed separately by year.

Results

Weed Pressure. Large crabgrass, Johnsongrass, and goosegrass were the most common monocot weeds and smooth pigweed and carpetweed (*Mollugo verticillata* L.) were the most common dicot weeds in the study areas both years. At the end of the growing season, grasses were denser than dicot weeds. Weed density was affected by weeding regime, but not growth habit or in-row spacing, both years (Table 2.1). In 2014, crabgrass density was low and variable among treatments; in 2015, crabgrass density in the non-weeded treatment exceeded 23 plants/m^2 and was 0.4 plants/m^2 or less in both weeded treatments. In 2014, Johnsongrass density in the non-weeded treatment exceeded 11 plants/m^2 but was less than 2 plants/m^2 in both weeded treatments. In 2015, Johnsongrass density was less than 2 plants/m^2 for all treatments. In 2014, goosegrass was affected by weeding regime, averaging the highest for plots weeded 4 WAT as compared to plots weeded for 8 WAT. In 2015, goosegrass density was 1.5x higher for compact versus vine-type non-weeded treatments, but averaged < 2 plants/m^2 in both weeded

treatments. Smooth pigweed density > 30 plants/m^2 in non-weeded treatments in 2014 but did not exceed 2.5 plants/m^2 in 2015. Carpetweed density was low and variable in 2014; in 2015, its density was significantly greater in 4 WAT weeding regime, likely as a consequence of taller weeds becoming predominant in non-weeded plots.

It should be noted that although weed densities in the 8 WAT weeding regime were high, those weeds likely had no effect on yield because they were only present for one week prior to harvest and were only in the seedling stage. A visual estimate of percent weed coverage was conducted to account for both weed density and biomass. Percent weed coverage was impacted by weeding regime in 2014 and weeding regime and growth habit in 2015 (data not shown). In 2014, weed coverage was 30% greater in the 4 WAT weeding regime than in the 8 WAT weeding regime. In 2015, the difference in weed coverage between 4 and 8 WAT weeding regime was just 2%.

These results suggest that the impact of weed density on yield depends both on the weed species and duration of weed interference. The variation in weed density and percent coverage between years and among treatments illustrates the necessity of knowing weed biology, timely scouting for weeds, and considering weed ecology when applying integrated weed management.

Watermelon Yield and Fruit Count. Weed control is essential to watermelon production: not weeding resulted in 62% to 93% yield loss depending on year and overall weed density (Table 2.2). However, the 4 WAT weeding regime resulted in similar yield and fruit count to the 8 WAT weeding regime. In the present experiment, weeds that were allowed to grow the second half of the season, 4 to 8 WAT, did not reduce yield despite average weed density of 26 plants/m^2 and 7 plants/m^2 in 2014 and 2015. This suggests that controlling weeds

up to the point of fruit set, which peaks around 4 WAT, may be an effective weed control strategy for organic watermelon growers to preserve yield. These results are consistent with a 3-6 WAT critical period for weed control observed in conventional watermelon studies (Adkins et al., 2010; Monks and Schultheis, 1998; Terry et al., 1997). The impact of a partial season weeding regime on the weed seedbank was not investigated in the present study, but should be considered in an integrated weed management program.

'AU-Producer' had greater yield but lower fruit count than 'Companion' in weeded plots both years, which is consistent with the differences in these cultivars' performance known prior to the present study. Denser in-row watermelon spacing increased fruit count in both years and yield in 2014 but not in 2015. The impact of cultivar selection and plant spacing factors on watermelon yield and fruit count are well-established in the literature and were not research objectives of the present study; these factors are of interest exclusively for their impact on weeding cost.

Weeding Cost. The high labor costs associated with hand-weeding is a critical barrier to the adoption of organic watermelon production. This study provides a much-needed estimation of hand-weeding costs for organic watermelon growers.

The cost of weeding increases with time invested; based on the comparable yield response 4 and 8 WAT weeding regimes discussed in the previous section, the most cost effective weeding treatment is clearly partial season weeding regime, which took place 1 to 4 WAT (Table 2.2). Under this weeding regime, labor costs were reduced in 2014 by 67% and 63% for vine and compact plants, respectively; and in 2015, by 43% across both growth habits.

The cost of the 4 WATweeding regime ranged from $1,488/ha to $3,007/ha depending on weed density, growth habit, and in-row spacing, which makes it a substantial but prudent input.

A secondary objective of this study was to determine if compact growth habit conferred a lower weed control cost. Plant spacing was included in the factorial design to account for differences in space requirements for compact and vine-type watermelon.

In 2014, the interactions of *habit* × *weeding regime* and *in-row spacing* × *weeding regime* significantly impacted weeding cost (Table 2.2). Compact plants required less time to hand-weed than vine-type plants in the 8 WAT weeding regime. However, the advantage of compact plants was not detected in the 4 WAT weeding regime. Simliarly, watermelon spaced 1 m apart required less time to weed than watermelon spaced 0.5 m apart for the 8 WAT weeding regime; however, no spacing advantage was detected for the 4 WAT weeding regime. Weeding regime and in-row spacing significant affected weeding cost in 2015. The 4 WAT weeding regime reduced labor costs by 43% compared to the 8 WAT weeding regime. One-meter in-row spacing reduced labor costs by 18% compared to 0.5 m in-row spacing. This result indicates that more plants per area will not reduce weeding costs; however, if a grower used higher density planting to reduce total area planted with watermelon, then weeding cost may also be reduced. There was no detectable difference in weeding cost for the planned comparison of vine-type watermelon spaced 1 m apart versus compact-type watermelon spaced 0.5 m apart (P=0.614 in 2014 and P=0.649 in 2015).

It was hypothesized that compact plants are better suited for hand-weed control than plants with sprawling vines. Compact plants were quicker than vine types to hand-weed in the 8 WAT weeding regime, but no advantage was detected when weeding was applied for only 4 WAT. Unfortunately, because the experimental design included only single-row plots, the

potential benefit of row middles free of sprawling vines was not clearly investigated. Further evaluation of these growth habit genotypes under various row-middle cultivation and mulching strategies is warranted.

Additional Study on Weeding Regimes. The effect of weeding once weekly for 1, 2, 3, or 4 WAT on watermelon yield was compared to non-weeded and weed-free control plots in an additional study in 2015. The treatments were applied to 10-plant plots of 'AU-Producer' at 1 m in-row spacing. Consistent with the primary study in 2015, weeding 4 WAT resulted in similar watermelon yield as the weed-free control treatment. In fact, the results indicate that weeding for only 2 or 3 WAT results in similar yield and fruit count to weeded controls, respectively. The average fruit size was not significantly different among the weeding treatments (data not shown). Weeding 3 WAT reduced weeding cost by 62% compared to full season weeding. These findings suggest that weeding less than 3 WAT may be effective in preserving yield and fruit count for organic watermelon and further investigation is warranted.

Conclusion

The results indicate that organic growers may weed once per week for 4 WAT to reduce labor costs while maintaining watermelon yield. This partial season weeding regime can reduce weeding costs to approximately $1500 to $3000/ha. Applying the wholesale price of U.S. organic watermelon of $0.58/kg during the study period, the value for 'AU-Producer experimental yield (Table 2.2) was approximately $27,000 in 2014 and $32,000 in 2015. This indicates a profit potential for the cropping system despite the hand-weeding costs. The improved weeding efficiency of compact plants in some treatments suggests that this non-typical growth habit may be a viable cultivar type for organic watermelon production.

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Table 2.1. Significant main and interaction effects of growth habit, spacing, and weeding regime on weed density in organic watermelon plots 8 weeks after transplant (WAT) in 2014 and 2015.

Main effect or interaction[z,y]	Total weeds 2014	Total weeds 2015	Crabgrass 2015	Johnson-grass 2014	Goosegrass 2014	Goosegrass 2015	Smooth pigweed 2014	Smooth pigweed 2015	Carpet-weed 2015
Weeding regime main effect									
0 WAT[x]	86.6 c	87.0 b		11.2 b	0.0 a		30.3 b		0.0 a
4 WAT	26.4 b	7.0 a		0.7 a	6.0 c		0.9 a		1.4 b
8 WAT	12.5 a	2.2 a		1.8 a	2.2 b		0.2 a		0.3 ab
Interactions									
Habit × weeding regime									
Vine, 0 WAT						37.3 b		0.1 b	
Vine, 4 WAT						1.3 a		0.0 a	
Vine, 8 WAT						0.3 a		0.0 a	
Compact, 0 WAT						56.7 c		2.5 c	
Compact, 4 WAT						1.3 a		0.0 a	
Compact, 8 WAT						0.0 a		0.0 a	
Habit x spacing x weeding regime									
Vine, 1 m, 0 WAT			44.0 a						

Vine, 1 m, 4 WAT	0.0 c
Vine, 1 m, 8 WAT	0.0 c
Compact, 1 m, 0 WAT	23.5 b
Compact, 1 m, 4 WAT	0.0 c
Compact, 1 m, 8 WAT	0.0 c
Vine, 0.5 m, 0 WAT	25.9 b
Vine, 0.5 m, 4 WAT	0.4 c
Vine, 0.5 m, 8 WAT	0.0 c
Compact, 0.5 m, 0 WAT	32.6 ab
Compact, 0.5 m, 4 WAT	0.0 c
Compact, 0.5 m, 8 WAT	0.0 c

[z]Within each main or interaction effect, means followed by different letters are significantly different at $P \leq 0.05$.

[y]Weed density is reported as plants/m^2.

[x]Weeks after transplant (WAT).

Table 2.2. Main and interaction effects of growth habit, spacing, and weeding regime on organic watermelon yield and weeding labor cost.

Main Effects or Interaction[z]	Yield (kg/ha)		Fruit count (no./ha)		Weeding cost ($/ha)	
	2014	2015	2014	2015	2014	2015
Main effects						
Habit						
Vine		54,850 a				
Compact		25,390 b				
Spacing						
1 m	23,040 b		7,080 b	9,080 b		$2,480 a
0.5 m	29,030 a		11,900 a	13,360 a		$3,020 b
Weeding regime						
0 WAT[y]		19,050 b				$0 a
4 WAT		51,650 a				$3,010 b
8 WAT		49,650 a				$5,240 c
Interactions						
Habit × weeding regime						
Vine, 0 WAT	7,360 c		5,650 c	8,290 cd	$0 a	

Vine, 4 WAT	46,450	a	10,480	b	11,570	bc	$1,910	b
Vine, 8 WAT	47,420	a	9,840	b	10,480	c	$5,740	d
Compact, 0 WAT	1,860	c	3,920	c	4,650	d	$0	a
Compact, 4 WAT	25,930	b	13,480	a	15,670	ab	$1,610	b
Compact, 8 WAT	27,180	b	13,570	a	16,670	a	$4,400	c
Spacing × weeding regime								
1 m, 0 WAT							$0	a
1 m, 4 WAT							$1,490	b
1 m, 8 WAT							$4,340	c
0.5 m, 0 WAT							$0	a
0.5 m, 4 WAT							$2,030	b
0.5 m, 8 WAT							$5,800	d

[z] Within each main or interaction effect, means followed by different letters are significantly different at $P \leq 0.05$.

[y] Weeks after transplant (WAT).

Table 2.3. The effect of weeding regime on weed density, watermelon yield, fruit count, and weeding cost in an organic system as an additional study in 2015.

Weeding Regime[z]	Weed Density (plants/m^2)		Yield (kg/ha)		Fruit Count (fruit/ha)		Weeding Cost ($/ha)	
0 WAT[y]	88.6	c	13,930	d	3,280	d	$0	a
1 WAT	64.7	c	37,390	c	5,290	bc	$750	a
2 WAT	20.7	b	51,260	b	6,020	ab	$1,710	b
3 WAT	13.1	ab	63,110	a	7,660	a	$3,110	c
4 WAT	7.1	ab	71,410	a	8,200	a	$4,880	d
8 WAT	2.3	a	64,740	a	8,200	a	$8,150	e

[z] Means followed by different letters are significantly different at $P \leq 0.05$.

[y] Weeks after transplant

(A) (B)

Figure 3.1. Vine-type watermelon plants at 1 m in-row spacing (A) and compact watermelon plants at 0.5 m in-row spacing (B) were evaluated for weeding efficiency in an organic production system. Internal size standard = 1 m^2.

CHAPTER 3

INTER- AND INTRACULTIVAR VARIATION OF HEIRLOOM AND OPEN-POLLINATED WATERMELON CULTIVARS[1]

[1] Stone, S.P., C.E. McGregor, and G.E. Boyhan. To be submitted to *HortScience*.

Abstract

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] cultivars exhibit diverse phenotypic traits yet are derived from a narrow genetic base. Heirloom cultivars, and to a lesser extent modern open-pollinated (OP) cultivars, are perceived to contain vital genetic variation that is critical for maintaining biodiversity and crop improvement. The objective of this study was to characterize the diversity of six heirloom and open-pollinated watermelon cultivars that are popular among U.S. organic, direct-market, and home growers. An additional evaluation was conducted to determine if significant phenotypic and genotypic variation existed among seed lots sourced from different commercial seed vendors. Important horticultural traits such as days to germination, days to first flower, yield, and fruit quality were measured over two field seasons. Genetic diversity was estimated using 32 simple sequence repeat (SSR) markers. No significant difference in horticultural traits among seed lots was observed both years, except in days to germination and first male flower, which may be a consequence of vendor differences in seed storage and quality control. Heirloom 'Moon and Stars' and modern OP 'Sugar Baby' were the most genetically distinct from the other cultivars and heirloom 'Georgia Rattlesnake' was determined to be highly related to the modern OP 'Charleston Gray'. The two heirloom cultivars were observed to have lower average gene diversity than the modern cultivars. Heirloom 'Moon and Stars' contained significant genetic variation among seed lots, yet heirloom 'Georgia Rattlesnake' contained none. These findings suggest that genetic variation can be more accurately attributed to pedigree and foundation seed maintenance practices than to the "heirloom" designation per se. The variation reported in this study can be used to inform conservation and breeding efforts.

Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] is a warm-season annual vegetable crop that is grown on 3.5 million hectares worldwide (Faostat, 2014). Cultivars express a wide range of phenotypes including fruit size, flesh color, rind pattern, disease resistance and sweetness.

Despite geographic and phenotypic diversity, the genetic variation of cultivated watermelon is limited (Levi et al., 2001b). Analysis of genome-wide diversity revealed that cultivars from Asia, Europe and America are derived from one of three subsets of sweet watermelon accessions from Africa (Nimmakayala et al., 2014b). As such, estimates of genotypic variation among cultivars have been low. The genetic diversity among 130 edible-type accessions sampled throughout the world was estimated at 5% (Nimmakayala et al., 2014a). Levi et al. (2001b) found that 46 American cultivars varied by 0.4 to 8%. East Asian and American cultivar types were found to be genetically similar by some analyses (Nimmakayala et al., 2014a; Reddy et al., 2015) but as distinct ecotypes in others (Guo et al., 2013; Zhang et al., 2012). The resequencing of 20 watermelon accessions shows that watermelon is less genetically diverse than maize, soybean, and rice (Guo et al., 2013). In all, these findings are consistent with a severe genetic bottleneck during domestication and the development of elite cultivars.

Conservation of genetic variation is critical to crop improvement through plant breeding. Farmer-maintained landraces are favorable sources of genetic variation because they are more adapted to agricultural production than wild relatives (Villa et al., 2005). By their nature, open-pollinated (OP) cultivars maintain greater population-level genetic diversity than hybrid seed types, which are derived from the cross-pollination of two inbred parental lines. A benefit to the grower is that seed of OP cultivars can be saved from year to year, unlike hybrid seed that does

not grow true-to-type in subsequent generations and thus must be purchased from seed companies each season. Due to these realized and perceived benefits, organic, direct-market, and home growers have inspired a renewed interest in OP cultivars (Phillips, 2016).

Today, farmer-maintained landraces in industrialized countries are quite rare (Thomas et al., 2011). In America, the designation "heirloom" is considered by some as analogous to landrace in that heirlooms are perceived to be locally adapted and genetically diverse. In this study and in present-day seed catalogues, "heirloom" is defined as a cultivar that was introduced before the advent of modern breeding techniques (the year 1942 is a commonly-used temporal threshold) by farmers or non-professional breeders (Demuth, 1998). However, the development of the modern seed industry has made the term ambiguous. If one considers that commercially distributed heirloom varieties are maintained and multiplied in a similar fashion to modern OP cultivars within a "certified seed" model (Parlevliet, 2007), rather than maintained through on-going recurrent selection by end-users, then the public perception of heirloom cultivars as more diverse than modern OP cultivars is questionable.

Nonetheless, the discovery of within-cultivar variation, whether in heirloom or modern cultivars, is of practical relevance to the seed industry and scientific community. Within-cultivar variation is an essential genetic resource in the maintenance and improvement of elite cultivars in a changing climate (Berry et. al, 2014). Numerous studies report that significant variation of many agronomic traits was observed within inbred S_5 to S_{20+} lines from different seed stock sources (reviewed in Tokatlidis (2015)), which is invaluable information to breeders. In these cases, cultivars and inbred lines assumed to be pure lines undergo changes when they are regenerated and/or maintained in separate locations; when properly characterized, this variation can be used for cultivar improvement and the conservation breeding of elite cultivars.

The OP cultivars featured in this study are not covered by plant variety protection (PVP) and thus foundation seed maintenance is unregulated and likely decentralized (M. Colley, personal communication). The term "foundation seed" in the present study refers to seed stock from which commercial seed is multiplied, but does not imply the formal designation associated with state-certified seed programs. It is expected that seed multiplied from independent foundation seed stocks, particularly in an unregulated model, is more likely to be genetically differentiated than certified seed covered by PVP. Significant variation among seed lots of cultivars sourced from different seed companies should be considered in conservation and breeding efforts. For example, Candole et al. (2012) identified differentiated levels of disease resistance among seed lots from different seed companies in the heirloom pepper 'California Wonder', which had long been used as a standard in pathological experiments. This finding helped scientists select a more reliable cultivar against which to judge other cultivars in disease screens. A seed lot with greater genetic diversity or with novel alleles may prove more useful in breeding programs than genetically uniform seed lots.

What role, then, does commercial seed production play in the conservation of genetic diversity of heirloom and modern OP cultivars? A balance between the maintenance of cultivar integrity and the conservation of genetic diversity must be sustained.

Cultivars must satisfy distinctness, uniformity, and stability (DUS) standards (Upov, 2002) and are perceived to be uniform genotypes. Consequently, in commercial seed production, emphasis is placed on rogueing off-type and diseased plants to maintain uniform and high-quality seed (Parlevliet, 2007). Guidelines for isolation distance and minimum population size during multiplication and maintenance of foundation seed vary by crop (George, 2013). These practices safeguard against genetic drift and gene flow between cultivars. The extent to which

cultivar purity strategies, both during foundation seed maintenance and multiplication, are practiced by each commercial seed company is unknown.

Nonetheless, OP cultivars are *not* genetically uniform, which is a reflection of the abundant mechanisms in place to ensure adaptability of genomes. Genetic variation is inherent to cultivars via natural processes, including (1) heterogeneity in the progenitor gene pool, (2) *de novo* mutation, (3) genetic drift, and (4) environmentally-triggered alterations to the genome. Artificial forces also affect intracultivar diversity during commercial seed propagation, including (1) unintentional gene flow during seed propagation, (2) bottlenecks during establishment of foundation or multiplication seed stocks, and (3) unintentional selection as a result of environmental conditions or management practices. Although most genetic variation is derived from the progenitor gene pool, *de novo* variation has been estimated to be up to 18% in single-plant derived soybean lines (Yates et al., 2012). Genetic variation is not necessarily a condition to be avoided, but in fact is an essential mechanism to exploit for longterm maintenance of cultivars and in breeding improved cultivars. Useful intracultivar genetic variation has been documented maize (Gethi et al., 2002), soybean (Yates et al., 2012), rice (Olufowote et al., 1997), cotton (Hinze et al., 2012), sunflower (Zhang et al., 1995), olive (Caruso et al., 2014) and mango (Singh et al., 2009) and the selection of superior lines has been used to improve performance or quality of the cultivars. Therefore, although unadapted germplasm is often regarded as the prime source of novel alleles for crop improvement, there is actually a great deal of incremental progress that can be made when plant breeders exploit the variation within elite cultivars (Rasmusson and Phillips, 1997).

The forces described above that drive intracultivar variation should be explicitly addressed during the production of breeder and foundation seed for long-term cultivar

maintenance. Tokatlidis (2015) recommends that breeder seed be maintained through ultra low-density plantings with periodic intense selections and consecutive mild selection. The intense selection period requires the selection of top performing sister lines to be evaluated by progeny testing. Wide plant spacing ensures that competition is minimized and the true genotypic character of each individual is expressed, so that effective evaluation can take place. Beyond the typical rogueing for off-types and diseased seed that takes place in traditional commercial seed propagation, this method ensures that genetic degradation is avoided, high-quality and uniform stocks are maintained, the cultivar can be incrementally improved to meet the demands of long-term environmental changes, and that interesting selections can be funneled into alternative breeding pipelines. Small to medium scale companies that serve organic, direct-market, and home growers may be limited in their ability to follow these conservation breeding recommendations; nonetheless, not explicitly addressing foundation seed maintenance may directly conflict with their customers' desire to grow genetically diverse and adapted cultivars.

The objective of this study was to characterize the diversity of heirloom and modern OP watermelon cultivars popular among U.S. organic, direct-market and home growers. Phenotypic variation, genetic differentiation, and within-cultivar gene diversity were measured. For each cultivar, variation among seed lots sourced from various commercial vendors was also investigated. Important horticultural traits, such as days to germination, days to first flower, yield, and fruit quality were measured over two field seasons. Despite the low genetic diversity among watermelon cultivars worldwide, the American cultivars that are the focus of this study have been successfully differentiated using a variety of marker systems (Levi et al., 2001b; Levi et al., 2009; Yang et al., 2016). The current study used 32 simple sequence repeat (SSR) markers to estimate genetic diversity. Because SSR have a high mutation rate and are multi-allelic, they

are an ideal marker choice for studying highly related populations. SSRs have been shown to be more informative than SNPs in their ability to detect rare genotypes and to discern genetic distance over a short time span (Hamblin et al., 2007), although as the cost of SNPs per locus continues to decrease, this advantage diminishes. This study is the first attempt to detect intracultivar variation in watermelon and aims to characterize the level of genetic diversity maintained at the commercial seed level to inform conservation efforts. Plant breeders may also benefit from exploiting divergent seed lots for cultivar improvement. A direct comparison of heirloom versus modern OP diversity serves to clarify the role of both cultivar types in the promotion of biodiversity.

Materials and Methods

Plant Material. Seeds of six heirloom and modern OP cultivars were obtained from various commercial seed vendors for a total of 24 seed lots (Table 3.1). The following pedigrees were obtained from Wehner and Mou (2013). 'Sugar Baby' is a modern OP cultivar developed by M. Hardin in 1955 by inbreeding a selection of 'Tough Sweets' for 13 years. 'Crimson Sweet' is a modern OP cultivar developed by Charles V. Hall at Kansas State University in 1963 using pedigree selection of ('Miles' x 'Peacock') x 'Charleston Gray'. 'Moon and Stars' is an heirloom cultivar with unknown parentage, developed by an unknown farmer in Colorado and released by Peter Henderson and Company in 1926. 'Charleston Gray' is a modern OP cultivar developed by the Southeastern Vegetable Breeding Laboratory in 1954 as a pedigree selection of ((('Africa 8' x 'Iowa Belle') x 'Garrison') x 'Garrison') x (('Hawkesbury' x 'Leesburg') x 'Garrison'). 'Georgia Rattlesnake' is an heirloom cultivar developed by M.W. Johnson in 1870 using unknown parentage. 'Congo' is a modern OP cultivar bred by the Southeastern Vegetable Breeding Laboratory in 1949 as a pedigree selection of ('African' x 'Iowa Belle') x 'Garrison'.

Seeds from each seed lot were sown in the greenhouse on March 26, 2014 and March 27, 2015, into two seedling trays each with Fafard 3B potting mix (Conrad Fafard, Inc., Agawam, MA) in a completely randomized design. Seedlings were transplanted to polyethelyne-covered beds at the Durham Horticultural Farm in Watkinsville, GA on April 23, 2014 and May 8, 2015. Each seed lot was randomly assigned to a 10-plant plot in a randomized complete block design with 4 replications. Seedlings were transplanted 1.2 m apart in-row and 1.8 m apart between rows. Fertilizer was applied to plots at the rate of 67 kg of N per hectare as pre-plant granular fertilizer (10N–0.9P–6.6K) and 12 kg of N per hectare of soluble fertilizer (15N–0P–12.5K) applied weekly via drip irrigation. Plants were irrigated 3 times per week as needed to accumulate approximately 2.5 cm water per week. Leaf samples were collected in the field from the 3rd, 5th, and 7th plant per plot, immediately frozen in liquid nitrogen, and stored at –80°C until further processing.

***Horticultural Traits*.** Days to germination was recorded in the greenhouse for each seed based on the criteria of full cotyledon expansion. Days to first male and female flower were recorded for each plant in the field from 1 May to 15 June in 2015 and from 15 May to 30 June in 2015. Marketable fruit from each plot were harvested, weighted, and counted on 27 June and 7 July in 2014 and on 17 July and 21 July in 2015. Two representative fruit from each plot were weighed individually and cut to measure fruit length, width, and rind thickness. Firmness of flesh was measured at 2 locations per fruit, off-centered in the endocarp heart, using a handheld penetrometer with 10 mm solid probe (Certified Material Testing Products, Palm Bay, FL). Soluble solids content was measured for 1 teaspoon of watermelon juice using a handheld refractometer (Spectrum Technologies, Plainfield, IL). Analysis of variance was conducted

using Stata version 13 (StataCorp, College Station, TX) and means were separated using Fisher's protected least significant difference at $P \leq 0.05$.

DNA Extraction and SSR analysis. Twelve individuals per seed lot were genotyped. Frozen leaf samples collected in 1.5 mL microtubes were ground using 5 mm steel beads in a TissueLyser (Qiagen, Inc., Valencia, CA) for 30 seconds then DNA was extracted using the E-Z 96® Plant DNA Kit (Omega Bio-Tek, Norcross, GA). Extracted DNA was quantified using the Tecan Infinite M200 Pro (Tecan, Morrisville, NC) and diluted to 20 ng/μL. Thirty-eight SSR loci reported as variable among commercial cultivars (Joobeur et al., 2006; Ren et al., 2012; Zhang et al., 2012) were tested and 32 polymorphic loci that were evenly distributed among watermelon chromosomes were selected (Table 3.2) for genetic analysis. Polymerase chain reaction (PCR) was conducted using the M13 universal primer system (Schuelke, 2000) in which the M13 sequence (5'-TGTAAAACGACGGCCAGT-3') was appended to the 5' end of the forward primer sequence, the reverse primer sequence was unaltered, and an M13 primer labeled with FAM, TAM, or HEX fluorescent dye was added to each reaction. PCR was conducted separately for each locus with reactions containing 20 ng DNA template, 1x standard Taq buffer (New England Biolabs, Ipswich, MA), 0.1mM dNTP (Qiagen, Inc.), 0.1 μM M13-appended forward primer, 0.4 μM reverse primer, 0.4 μM dye-labeled M13 primer, 0.6 U Taq DNA polymerase (New England Biolabs) in a 20 μL total volume. PCR was conducted using MyCycler (Bio-Rad, Hercules, CA) with the following program: 90 sec initial denaturation at 95°C; 10 cycles of 15 sec denaturation at 95°C, 20 sec annealing at 53°C (-1°C each subsequent cycle), and 30 sec of extension at 72°C; 35 cycles of 15 sec denaturation at 95°C, 20 sec annealing at 43°C and 30 sec of extension at 72°C; and 15 min final extension at 72°C. PCR

products were diluted 2 to 4x depending on agarose band, pooled into sets with each of the 3 unique fluorescent dyes present, and added to formamide with a GeneScan-500 ROX internal-lane size standard (ABI; Applied Biosystems by Life Technologies Corporation, Carlsbad, CA). Product fragment lengths were measured on the Applied Biosystems 3730xl 96-capillary DNA Analyzer (ABI) at the Georgia Genomics Facility (Athens, GA).

Alleles were interpreted from fluorescent peaks using Geneious version R8.1 (Kearse et al., 2012). Power analysis was conducted by estimating genetic diversity (mean expected heterozygosity) versus number of loci via 1000 random permutations of the data using MultiLocus version 1.3b (Agapow and Burt, 2001). Genetic parameters were analyzed using GenAlEx version 6.502 (Peakall and Smouse, 2012). Confidence intervals for gene diversity were obtained by 1000 bootstraps using the PopGenKit package (Paquette, 2012) in R (Team, 2017). Polymorphism information content (PIC) was calculated using the formula $PIC = 1-\sum P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus (Botstein et al., 1980). Analysis of Molecular Variance (AMOVA) and F-statistics were calculated for codominant allelic distance in a 2N x 2N matrix and tested using 999 standard permutations and significance threshold of $P \leq 0.05$ (Michalakis and Excoffier, 1996). Nei's standard genetic distance (Nei et al., 1983) was used to construct a Principle Coordinate Analysis (PCoA) in GenAlEx and infer an unrooted neighbor-joining (uNJ) tree (Saitou and Nei, 1987) using MEGA version 4 (Tamura et al., 2007).

Results

***Horticultural traits*.** Intercultivar variation was apparent for all horticultural traits as expected (Table 3.3A – C). However, variation among seed lots of each cultivar was detected for only a few horticultural traits (Table 3.4). Analysis was conducted separately for each year due to a by-year interaction. Days to germination differed among seed lots of 'Sugar Baby' in

2014; ‘Georgia Rattlesnake’ in 2015; and ‘Crimson Sweet’, ‘Moon and Stars’, and ‘Congo’ in a consistent pattern both years. Days to first male flower differed among seed lots of ‘Georgia Rattlesnake’ in 2014; ‘Moon and Stars’ in 2015; and ‘Crimson Sweet’, ‘Charleston Gray’ and ‘Congo’ both years. Intracultivar variation was detected for days to first female flower in ‘Sugar Baby’ and ‘Georgia Rattlesnake’ in 2014 only. ‘Sugar Baby’ seed lots varied in yield and fruit count in 2014 but not 2015. Fruit count differed among ‘Crimson Sweet’ seed lots in 2014 only. Variation in rind thickness was found among ‘Congo’ seed lots in 2015. Finally, soluble solids content, a proxy for sweetness, differed among ‘Moon and Stars’ and ‘Charleston Gray’ seed lots in 2014.

Because flowering time may be a consequence of days to germination, Pearson’s correlation was used to assess the relationship between the traits. In 2014, there was a strong correlation between days to germination and days to first male flower (r=0.548, $P<0.001$). The correlation between days to first female flower and days to germination was moderate (r=0.334, $P=0.001$). In 2015, the correlation between days to germination and first male flower was moderate (r=0.443, $P=0.000$) but the correlation with days to first female flower was not significant. Because days to germination may be influenced by an assortment of environmental factors, such as seed storage conditions and age of seed, rather than genetic variation alone, it is possible that the intracultivar variation detected in days to germination and flowering time are a consequence of the vendors’ seed quality practices. While quality control is an important component of commercial seed production, it is beyond the scope of our study.

Overall, the presence of phenotypic variation for horticultural traits among seed lots was limited, and the detection of variation consistently both years was rarer still; this indicates that for most horticultural traits, there is not strong evidence of phenotypic divergence among seed

lots during commercial seed production. Seed companies are responsible for enforcing maintenance selection during seed multiplication (Parlevliet, 2007). Traits such as fruit size, rind pattern, and sugar content are relatively easy to maintain through rogueing off-types. Traits such as yield and disease resistance are harder to maintain due to *genotype x environment* interactions. For example, variation of such traits may not be noticeable unless a particular environmental condition is present. Also, divergence of seed lots for environmentally influenced traits may occur when multiplication is performed in disparate growing conditions that significantly differ in selective pressure. The limited occurrence of significant intra-cultivar variation of horticultural traits in the present study indicates that companies are sufficiently maintaining cultivar DUS.

Genetic parameters of the SSR loci. A total of 104 putative alleles across 32 SSR polymorphic loci were detected (Table 3.5). The average alleles per locus of 3.3, with a range of 2 to 7 alleles per locus, is lower than typical diversity studies but not unexpected given the narrow genetic diversity of commercial cultivars (Levi et al., 2001b). The average diversity (PIC) per locus was 36%, which indicates low frequency of minor alleles. Of the 32 loci examined, 10 were very diverse (PIC>0.50), 11 were minimally diverse (PIC<0.25), and the remaining 11 were intermediately diverse. Locus BVWS00287 was the least diverse (PIC=0.003) and locus BVWS00177 was the most diverse (PIC=0.744). The 32 selected loci demonstrate a wide range in allele number and PIC. Taken together, these genetic parameters reflect the known features of U.S. watermelon cultivars: low genetic diversity and high homozygosity (Levi et al., 2001a).

A NJ tree constructed from Nei's standard distance (Fig. 3.1) shows that all seed lots were correctly assigned into cultivar groups and is consistent with previous studies that used other marker systems (Huayu et al., 2016; Levi et al., 2004; Levi et al., 2009; Yang et al., 2016; Zhang et al., 2012). The tree configuration is further supported by available pedigree information, which includes shared parentage of 'Congo', 'Crimson Sweet', and 'Charleston Gray' and no shared parentage of 'Sugar Baby'. These findings are consistent with the Reddy et al. (2015) study, which found that 'Crimson Sweet' and 'Georgia Rattlesnake' clustered together in a group of African ancestry, 'Congo' and 'Moon and Stars' clustered together in a group of European and American ancestry, and 'Sugar Baby' was placed in a second, genetically distinct group of European and American ancestry; as well as additional studies that describe 'Sugar Baby' as the least related among commercial cultivars (Reddy et al., 2015; Yang et al., 2016).

Interestingly, the parentage of 'Georgia Rattlesnake' and its use in the pedigree of modern cultivars is undocumented in published cultivar descriptions (Elmstrom et al., 2010). The genetic distance estimated in this study (Fig. 3.1) revealed that it is closely related to 'Charleston Gray' and may be a progenitor parent. This result prompted a through review of historical literature, which uncovered a biographical account of Ruben F. Kolb using 'Georgia Rattlesnake' to develop 'Kolb Gem' in 1885 (Rogers, 1958). 'Kolb Gem' is a documented progenitor of 'Charleston Gray', thus its relatedness to 'Georgia Rattlesnake' is now confirmed.

A post-hoc power analysis was conducted to determine the optimal number of loci necessary to estimate genetic diversity using 1000 random permutations of the data (Fig. 3.2). For 'Sugar Baby', 'Charleston Gray', and 'Congo', ninety-five percent of genetic diversity can be explained by 22 to 23 loci and the genetic information gained upon adding additional loci

begins to plateau. A similar plateau pattern occurs in 'Crimson Sweet', 'Moon and Stars', and 'Georgia Rattlesnake' at 26 to 27 loci. From this analysis, it can be inferred that additional loci beyond the 32 selected for this study would have provided diminishing returns in information gained.

Together, the NJ tree and post-hoc power analysis suggest that the 32 loci selected for the present study provided sufficient power to address the research objectives. Furthermore, a number of loci were observed as fixed for different alleles between cultivars (Table 3.6). 'Sugar Baby' was fixed at loci BVWS00225 and BVWS000233 for alleles that no other cultivar in the study contained. Likewise, 'Moon and Stars' was fixed at loci BVWS00333 and BVWS02205 for alleles that no other cultivar in the study contained. These diagnostic markers can be used to distinguish cultivars in other genetic applications, such as to confirm crosses or in the support of distinctness, uniformity, and stability (DUS) claims under the Plant Variety Protection Act (UPOV, 2002). The number of SSR makers used in DUS evaluations differs by crop, ranging from 7 in rose to 60 in maize parental lines (Upov, 2011). The diagnostic markers listed in Table 3.6 may prove useful in the support of watermelon DUS claims in the future.

***Genetic diversity among cultivars*.** Intercultivar diversity was detected for all genetic parameters (Table 3.7). 'Sugar Baby' was polymorphic at the most loci (%P=75.0) and contained the greatest average number of alleles per locus (Na=1.91). 'Georgia Rattlesnake' was polymorphic for the fewest loci (%P=43.8) and had the least average number of alleles per locus (Na=1.50). 'Crimson Sweet', 'Moon and Stars', 'Charleston Gray', and 'Congo' were each polymorphic for 59.4% of loci, though only 50% of loci were polymorphic for all 4 cultivars. Average gene diversity (Fig. 3.3), which accounts for both number and frequency of alleles, is a

useful estimation for describing within-cultivar variation. 'Sugar Baby' had the greatest gene diversity; 'Charleston Gray' and 'Congo' were intermediate; and 'Crimson Sweet', 'Moon and Stars', and 'Georgia Rattlesnake' had the lowest. 'Sugar Baby' has significantly greater gene diversity than 'Moon and Stars (P=0.0461). Low gene diversity estimates indicate that one allele per loci is predominant and alternative alleles occur at very low frequencies. One-third of loci were polymorphic across all six cultivars (data not shown); this variation may be attributed to residual variation from the progenitor gene pool, genetic drift, and *de novo* mutation.

Major patterns of variation in a pairwise *individual x individual* genetic distance matrix were calculated and plotted using PCoA (Fig. 3.4). The arrangement of cultivar groups parallels the NJ tree (Fig. 3.1) and additionally reveals the variation among individuals in each cultivar as the spread of points in the group. 'Sugar Baby and 'Moon and Stars' are distantly related to the other cultivars, whereas overlap occurs among the other cultivar groups, particularly 'Charleston Gray' and 'Georgia Rattlesnake'. This is further evidence that Georgia Rattlesnake was involved in the pedigree of 'Charleston Gray' and that additional diagnostic loci would be needed to completely separate the two cultivars in the PCoA plot. As expected, AMOVA revealed that more variation exists among cultivars (F_{ST}=65%) than within cultivars and individuals (F_{IS}=35%; Table 3.8).

Private alleles, which occur in one cultivar and no other, may be of interest to conservationists and breeders aiming to preserve and exploit diversity. As expected based on previous genetic distance estimates, 'Sugar Baby' has the most private alleles (PA=14; Fig. 3.5). 'Crimson Sweet' and 'Moon and Stars' have 8 and 7 private alleles, respectively. 'Charleston Gray', 'Georgia Rattlesnake' and 'Congo' have relatively fewer private alleles, likely because of overlapping pedigrees. The abundant private alleles observed in 'Crimson Sweet' despite its

close relation to other cultivars in the study may be attributed to the portion of its progenitor gene pool that excludes the other cultivars.

Genetic diversity among seed lots. A second level of analysis was conducted to characterize the extent of genetic variation among seed lots of each cultivar sourced from different commercial seed vendors. For this within-cultivar evaluation, each cultivar was analyzed as a separate dataset.

There are differences in percentage of polymorphic loci and average number of alleles among seed lots in each cultivar (Table 3.9), though gene diversity is fairly consistent (Fig. 3.6). One exception was a significant difference (P=0.021) in gene diversity between 'Charleston Gray' seed lot #16 (D=0.13) and seed lot #17 (D=0.08). PCoA reveals no obvious clustering of individuals by seed lot (Fig. 3.7A– F). However, a significant difference among seed lots of 'Moon and Stars' is detected via AMOVA (Table 3.10). There was zero variation detected among seed lots of 'Georgia Rattlesnake'. For the remaining cultivars, 1% variation was detected between seed lots. Pairwise estimates of F_{ST} indicate that most seed lots are genetically similar (F_{ST}<0.05) and in each cultivar group, there was at least one pair of seed lots that differed by less than 0.1% (Table 3.11). Differentiation among seed lots of 'Moon and Stars' can mostly be attributed to the significant variation of seed lot #15 (F_{ST}>0.05) from the others.

Though significant variation among seed lots was uncommon, almost all seed lots contained private alleles, defined as those alleles that occur in no other seed lot (Fig. 3.8). However, private alleles were observed in only one sampled individual in most cases (gray bars; Fig. 3.8), thus their practical use in breeding programs is limited.

Heirloom versus modern OPs. The prevailing view of the grassroots "seed savers" movement is that heirloom cultivars are bastions of genetic diversity and are at risk of being lost and replaced by genetically narrow modern varieties, though evidence points to the contrary (Heald and Chapman, 2012). Breeders and professionals involved with formal germplasm maintenance require genetic evaluations to effectively prioritize conservation efforts. With these concerns in mind, an *a priori* comparison was used to determine if heirlooms 'Moon and Stars' and 'Georgia Rattlesnake' exhibit more genetic diversity than the modern cultivars featured in this study.

Intercultivar analysis revealed that 'Moon and Stars' is genetically distinct from the other cultivars, whereas 'Georgia Rattlesnake' is relatively similar to modern cultivar 'Charleston Gray' (Fig. 3.4). Thus, for these particular cultivars, genetic variation is most accurately attributed to pedigree rather than the "heirloom" designation per se. Furthermore, 'Moon and Stars' and 'Georgia Rattlesnake' exhibited the lowest average gene diversity (Fig. 3.3), which is an effective estimator for within-cultivar diversity. When exploring genetic diversity among seed lots, significant variation was observed in 'Moon and Stars', yet zero variation was detected in 'Georgia Rattlesnake' (Table 3.10). This result echoes the previous conclusion that the "heirloom" designation does not consistently correlate with genetic variation. Instead, variation among seed lots is likely a consequence of foundation seed maintenance practices. For example, 'Moon and Stars' is an heirloom of high consumer demand; it is possible that the sampled seed lots were multiplied from independent foundation seed stocks. However, there is a lower demand for 'Georgia Rattlesnake' seed and thus the seed lots sampled in this study may be derived from a single foundation seed stock or even from a single multiplication plot. Unfortunately, requests from the seed companies for origin information that would confirm these

inferences were largely unfulfilled. Nonetheless, our results suggest that both pedigree information and foundation seed maintenance practices should be considered when targeting cultivars and seed lots for conservation efforts rather than an heirloom designation.

Conclusion

The present investigation sought to characterize the genetic diversity of cultivars popular among U.S. organic, direct-market, and home growers, at both the cultivar and seed lot level, to better inform conservation and breeding efforts. Cultivars that contain distinct genetic resources, such as 'Sugar Baby' and 'Moon and Stars', should be prioritized over cultivars that carry the "heirloom" designation per se. Breeders can use within-cultivar variation to maintain and improve elite cultivars for a changing climate (Tokatlidis, 2015). In this conservation breeding strategy, seed lots with above-average gene diversity, such as 'Charleston Gray' seed lot #16, should be prioritized over more genetically uniform seed lots, such as 'Charleston Gray' seed lot #17 (Fig. 3.6). Furthermore, when significant genetic differentiation occurs among seed lots, as was observed in 'Moon and Stars' in the present study, cultivar diversity is not fully captured by conserving one seed lot. Currently, the U.S. National Plant Germplasm System maintains a seed lot of 'Moon and Stars' deposited by Seed Savers Exchange in 2004. 'Moon and Stars' seed lot #15 was found to be significantly differentiated from the others (Table 3.11), yet was phenotypically similar to other seed lots for major horticultural traits (Table 3.4). Therefore the conservation of this differentiated seed lot, both via independent foundation stock maintenance and formal germplasm bank deposit, is warranted. In the case of heirloom 'Georgia Rattlesnake', no genetic differentiation was observed among seed lots. This suggests that one foundation seed stock is likely the source for commercial seed and special attention should be given to conserve and properly maintain this stock. The active maintenance and protection of genetic variation

among seed lots may prove essential in the long-term conservation of these beloved heirloom cultivars.

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Table 3.1. Heirloom (H) and modern open-pollinated (OP) watermelon cultivars and seed vendors used for phenotypic and genotypic analysis.

Cultivar	Type	Seed Lot	Vendor	Germination (%) 2014	2015
Sugar Baby	OP	1	Johnny's Selected Seeds[z]	89	93
		2	Clifton Seed Company[y]	94	94
		3	High Mowing Organic Seeds[x]	96	98
		4	Harris Seeds[w]	93	95
		5	NE Seed[v]	93	98
Crimson Sweet	OP	6	Baker Creek Heirloom Seeds[u]	90	91
		7	Johnny's Selected Seeds	89	98
		8	Clifton Seed Company	86	88
		9	High Mowing Organic Seeds	83	87
		10	Harris Seeds	90	97
Moon and Stars	H	11	Seed Savers Exchange[t]	85	90
		12	NE Seed	81	83
		13	High Mowing Organic Seeds	89	96
		14	Sow True Seed[s]	90	62
		15	Sustainable Seed Company[r]	68	71
Charleston Gray	OP	16	Baker Creek Heirloom Seeds	83	95
		17	NE Seed	93	82
		18	Sow True Seed	89	88
Georgia	H	19	Baker Creek Heirloom Seeds	92	90

Rattlesnake		20	Clifton Seed Company	85	85
		21	Sow True Seed	89	84
Congo	OP	22	Baker Creek Heirloom Seeds	72	71
		23	Clifton Seed Company	74	65
		24	Sustainable Seed Company	88	89

[z]Johnny's Selected Seeds, 955 Benton Avenue, Winslow, Maine 04901

[y]Clifton Seed Company, P.O. Boc 206, Faison, NC 28341

[x]High Mowing Organic Seeds, 76 Quarry Road, Walcott, VT 05680

[w]Harris Seeds, 355 Paul Road Rochester, NY 14624

[v]NE Seed, 122 Park Ave, Building H, East Hartford, CT 06108

[u]Baker Creek Heirloom Seeds, 2278 Baker Creek Road, Mansfield, MO 65704

[t]Seed Savers Exchange, 3094 North Winn Road, Decorah, Iowa 52101

[s]Sow True Seed, 146 Church Street, Asheville, NC 28801

[r]Sustainable Seed Company, 355 East 20th Street, Chico, CA 95928

Table 3.2. Simple sequence repeat (SSR) primers used for genetic analysis of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars.

Primer Name		Sequence (5' --> 3')	Chromosome	Start Location[z]	Stop Location[z]	Reference
BVWS01836	Fwd	AGAGGAGTCCAAAGGTGCAA	1	1185151	1185437	Ren et al. (2012)
	Rev	CACTTGGTTCTGCATTGAGG				
BVWS00948	Fwd	TCAAACCGACTGCCATATCA	1	22668108	22668377	Zhang et al. (2012)
	Rev	AGCTTGTCTTCCTGGCCTTT				
BVWS00155	Fwd	TGGATCATTTGACAGATTTAGCGA	1	30213324	30213162	Zhang et al. (2012)
	Rev	CATCACAGTTAACGATCACAAGGC				
BVWS01911	Fwd	CCTTCTCTGCTGCAGGTTCT	2	16239566	16239832	Ren et al. (2012)
	Rev	AAGAAGAAACCACCGATCCC				
BVWS00314	Fwd	GAGGAGAATCGGTTCTTGGACATA	2	23078022	23078158	Zhang et al. (2012)
	Rev	TTGAGCATCCTTGGGACTATCATT				
BVWS00297	Fwd	ACAACTTTGATTGATTGCACGATG	2	34241669	34241531	Zhang et al. (2012)
	Rev	AAGTGAAAGACCCTTTTCCCAAAC				
BVWS00244	Fwd	GCTACAAGAAAGCAGTTTGGATTTTC	3	3416523	3416669	Ren et al. (2012)
	Rev	GCATGGATTGTATCAAACAAATGCT				
BVWS01199	Fwd	ATTGGCAACACCTCCAACTC	3	12292958	12293157	Ren et al. (2012)
	Rev	AAAAGATGTCTCCTTCTCCCAA				
BVWS00048	Fwd	TCAAAAGGTTTGCCCTAAATGAAA	3	27914506	27914682	Zhang et al. (2012)
	Rev	TGCTGATCTCCCATTCTTAACCTC				
BVWS02428	Fwd	TTGGATGGGGAACTGAAGAG	4	7356764	7356879	Ren et al. (2012)
	Rev	GGCATGAACTTCTTTCACCC				
BVWS00102	Fwd	TGTCCATCAATTTTCAACCTCAGA	4	15377126	15376994	Ren et al. (2012)
	Rev	GGACAGGTGGGGTTTATTCAAGTA				
BVWS00208	Fwd	GCAAAGATTGTCTATGAAGCAGCA	4	18760155	18760327	Zhang et al. (2012)

	Rev	GCTCATTGGCTTCTTGAATCTGTT				
BVWS00215	Fwd	GGCTCTCCGTAACTTTGTCTTTGA	5	3566759	3566639	Ren et al. (2012)
	Rev	CATAAAGGAAGCTGAGTCCTCGAC				
BVWS00441	Fwd	TGGTTGAAATCAATAAAAAGTGAA	5	12518305	12518477	Zhang et al. (2012)
	Rev	TGGATGTTTTTGGCATTTGA				
BVWS00106	Fwd	TGGCCTAGAAGATTATTGAGCTGC	5	29257732	29257588	Zhang et al. (2012)
	Rev	CATTATCACATGGCAGATAATGGAAA				
BVWS00287	Fwd	TTTAGCATTACAGGTAGACTTTGTAGCA	6	17381037	17381191	Ren et al. (2012)
	Rev	ATGTAACAATTTTGGTCCATGTATTTT				
BVWS00233	Fwd	AAACCATGATTTTACAGGGGATCA	6	23367900	23367738	Zhang et al. (2012)
	Rev	TTTCTGTCTTCTTTTGACCAATGC				
MCPI-5	Fwd	ATTTCTGGCCCCAGTGTAAG	6	26786254	26786441	Joobeur et al. (2006)
	Rev	GAACAACGCAACCACGTATG				
BVWS00225	Fwd	TGAATTTCAATGAGAAGTCTGTTTTCTA	7	2785791	2785950	Ren et al. (2012)
	Rev	GCATGATGAAACTGATTTGTTCCT				
BVWS00433	Fwd	TCTTTTAAGTTTTGAGGGAGAGC	7	4151409	4151009	Zhang et al. (2012)
	Rev	TTCCCAAGCTAGCCTTTTCA				
BVWS02453	Fwd	CCAAATTGGACCAGAACCAC	7	25558609	25558908	Ren et al. (2012)
	Rev	AAGCCGTCAGTCTCGGTTAG				
BVWS00522	Fwd	GCAAAGCAATATCGGGAAAA	8	8496014	8495740	Ren et al. (2012)
	Rev	TTCCTTCGCCATTTTCATTC				
BVWS01001	Fwd	TGGTTTGTTGGATTTTGTGAA	8	11630160	11629919	Ren et al. (2012)
	Rev	ATATTATCCCAGCAGCCACG				
BVWS00177	Fwd	TTCAACCAAGCAGTTCTTAACACAA	9	4023989	4024182	Ren et al. (2012)
	Rev	GATGCATTAAGATTTTCGTTTCGC				
BVWS00333	Fwd	TGTTGAGATTCTTTGATTTCAACTGT	9	21608401	21608274	Zhang et al. (2012)
	Rev	TGGGTCAAAGTATTTTTGCTTTTT				
BVWS00209	Fwd	TGCTTCAAAATCTATTCACAATTTGC	9	34063455	34063581	Zhang et al. (2012)
	Rev	TTCTTGGTTTCGGGTTTCTTTACA				
BVWS00236	Fwd	CTTGAGCATTTGGCTTCCTAGTGT	10	1007583	1007748	Zhang et al. (2012)

	Rev	GTCAAAATGTCCTTTGATTCCCAA				
BVWS02048	Fwd	TCTGTGTGGATGCAAATGGT	10	15916596	15916844	Zhang et al. (2012)
	Rev	GCTAATCGAGCCCAGTTACG				
BVWS02205	Fwd	CAAAATTGTGGGAGGAAAAA	10	26080606	26080721	Ren et al. (2012)
	Rev	TGGAGTGGTAGCGACTAAAACA				
BVWS00067	Fwd	GCCCAAAGTAAAGCCCAATTTTAC	11	1947675	1947549	Ren et al. (2012)
	Rev	TCATTTAAGTAGGCCCCCAAGTTT				
BVWS00839	Fwd	TTCCACACCAAGGAGGTAGG	11	10534583	10534832	Zhang et al. (2012)
	Rev	CATGTCATTCGATAAAGCAGAAA				
BVWS00228	Fwd	GGAAGAGTGAGGTGATAAATCAATATGT	11	23231725	23231570	Zhang et al. (2012)
	Rev	AATTGGCCCAAATATCCATATGAC				

[z]Chromosome, start, location and stop location based on Guo et al. (2013) genome sequence, reported in base pairs (bp).

Table 3.3. Mean phenotypic values of horticultural traits six heirloom and open-pollinated watermelon cultivars. Groups with a different lowercase letter have means that are significantly different at $P \leq 0.05$.

(A)

	Days to Germination		Days to First Male Flower		Days to First Female Flower	
Cultivar	2014	2015	2014	2015	2014	2015
Sugar Baby	7.3 bc	9.4 a	43.6 a	54.5 a	52.4 a	61.0 a
Crimson Sweet	7.4 bc	9.6 a	46.8 b	62.0 b	60.1 b	69.1 c
Moon and Stars	7.5 c	9.8 a	50.5 d	61.6 b	63.4 c	69.4 c
Charleston Gray	6.7 a	10.5 b	48.2 c	61.6 b	59.5 b	68.2 b
Georgia Rattlesnake	7.0 ab	11.3 c	48.6 c	63.1 c	62.4 c	69.6 c
Congo	10.8 d	13.1 d	56.0 e	65.1 d	65.3 d	69.8 c

(B)

	Yield (1000 kg/a)		Fruit Count (1000 fruit/ha)	
Cultivar	2014	2015	2014	2015
Sugar Baby	35.6 c	30.7 c	10.4 a	7.2 a
Crimson Sweet	45.3 ab	46.5 b	5.9 bc	5.5 bc
Moon and Stars	45.6 a	61.2 a	6.5 b	5.8 bc
Charleston Gray	38.1 abc	50.9 ab	4.9 cd	5.1 cd
Georgia Rattlesnake	37.7 bc	46.3 b	5.7 bcd	4.3 d
Congo	36.4 c	53.0 ab	4.4 d	6.5 ab

(C)

	Weight (kg)		Length (cm)		Width (cm)		Rind Thickness (cm)		SSC (°BRIX)		Firmness (kg/cm^2)	
Cultivar	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Sugar Baby	3.2 c	4.7 c	21.0 d	21.3 e	19.5 c	20.1 d	1.7 b	1.0 a	9.3 b	9.4 c	1.7 a	1.7 b
Crimson Sweet	7.2 ab	9.5 b	27.1 c	28.0 d	24.2 a	25.2 b	1.4 a	1.5 cd	10.7 a	11.5 a	1.7 a	1.2 a
Moon and Stars	6.4 b	11.8 a	26.5 c	29.6 c	23.7 a	26.8 a	1.4 a	1.6 d	8.6 c	9.4 c	2.0 b	1.2 a
Charleston Gray	7.3 ab	10.4 b	39.7 a	41.8 a	19.8 c	21.2 c	1.5 a	1.2 b	9.8 b	10.2 b	2.0 b	1.1 a
Georgia Rattlesnake	6.8 ab	10.5 b	39.4 a	41.8 a	19.9 c	21.2 c	1.4 a	1.3 bc	9.2 bc	10.6 b	1.9 ab	1.2 a
Congo	7.5 a	10.0 b	36.1 b	38.4 b	20.9 b	21.9 c	1.5 ab	1.3 b	9.0 bc	9.6 c	2.0 b	1.5 b

Table 3.4. Mean phenotypic values of horticultural traits of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars. Groups with a different lowercase letter have means that are significantly different at $P \leq 0.05$.

(A)

Cultivar	Seed Lot	Days to Germination		Days to First Male Flower		Days to First Female Flower	
		2014	2015	2014	2015	2014	2015
Sugar Baby	1	7.5 b	9.3	42.8	54.7	51.2 a	60.6
	2	7.3 ab	9.4	43.8	54.4	52.8 ab	60.6
	3	7.1 ab	9.6	44.1	55.1	53.7 b	62
	4	7.9 b	9.4	42.3	53.6	50.8 a	60.3
	5	6.6 a	9.2	45	54.7	53.8 b	61.1
	6	7 ab	9.3 b	46.8 ab	61 ab	59.9	68.8
Crimson Sweet	7	6.9 ab	8 a	46.5 ab	59.6 a	58.8	68.3
	8	7.1 b	9.9 b	47.3 b	62.5 c	59.9	69
	9	9.7 c	12.6 c	48.8 b	64.6 d	61.1	69

	10	6.4 a	8.5 a	44.8 a	62.4 bc	61.3	68.4
Moon and Stars	11	6.9 a	8.6 a	49	60.7 a	63.3	69.5
	12	8.1 b	11.4 c	51	61.2 a	64.3	68.4
	13	6.9 a	9.6 b	50.2	64.6 b	61.3	68.4
	14	7.8 b	9.5 b	51.9	60 a	64.1	67.7
	15	7.7 b	9.8 b	50.4	61.2 a	64.5	69.7
Charleston Gray	16	6.7	10.2	46.4 a	61.3 a	59.7	67.9
	17	6.6	10.6	50 b	63 b	59.1	68.1
	18	6.8	10.6	48.1 ab	60.3 a	59.6	67.3
Georgia Rattlesnake	19	7	12.4 c	50.1 b	63.6	64.8 b	69.6
	20	7.3	11.5 b	47.5 a	63.8	62.4 a	68.8
	21	6.9	9.7 a	48.1 ab	61.9	60.1 a	68.8
Congo	22	13 c	15 b	56.4 b	64.2 a	65	70.1
	23	11.4 b	14.6 b	54.5 ab	64.4 a	67.1	69.6

24	8.6 a	10.4 a	52.6 a	66.5 b	63.9	70.3

(B)

Cultivar	Seed Lot	Yield (1000 kg/ha) 2014	Yield (1000 kg/ha) 2015	Fruit Count (1000 fruit/ha) 2014	Fruit Count (1000 fruit/ha) 2015
Sugar Baby	1	39.5	26.6	12.5	6.2
	2	43.1	38.9	11.6	8.9
	3	31	28.6	9.7	6.2
	4	34.2	30.6	9.7	7.5
	5	30.2	28.9	8.3	7.2
Crimson Sweet	6	43.4	51.2	5.9	6.2
	7	49.1	46.7	6.6	5.5
	8	43.7	42.6	5.6	5.2
	9	42.4	49.7	4.5	5.8
	10	48	42.2	6.8	4.8
Moon and Stars	11	56.1	70.9	7.5	6.3
	12	48.5	68.1	6.4	6.4

	13	41.2	52.8	6.7	5.1
	14	39.3	64.9	5.9	6.3
	15	43.1	49.1	6.1	5
Charleston Gray	16	37	56.1	5.3	5.7
	17	38.4	48	4.5	4.8
	18	38.9	48.5	5	4.8
Georgia Rattlesnake	19	31.6	48.3	6.6	4.8
	20	40.9	31.2	5	2.9
	21	40.5	59.3	5.6	5.2
Congo	22	38.2	49.6	4.6	6.3
	23	26	60.7	3.4	7.1
	24	45.1	48.7	5.3	6

(C)

Cultivar	Seed Lot	Weight (kg)		Length (cm)		Width (cm)		Rind Thickness (cm)		SSC (°BRIX)		Firmness (kg/cm^2)	
		2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Sugar Baby	1	2.9	5.1	20.6	21.9	19.1	20.7	1.7	0.8	9.4	10.0	1.6	1.5
	2	3.4	4.7	21.7	21.2	20.0	19.5	1.8	1.0	9.8	9.2	1.5	1.8
	3	2.9	4.9	21.3	21.7	19.4	20.1	1.9	1.0	8.8	9.7	1.5	1.5
	4	3.3	4.6	21.5	21.2	20.2	20.3	1.4	1.0	9.3	9.0	2.1	1.7
	5	3.4	4.7	19.7	21.3	18.6	20.3	1.8	1.0	9.4	9.4	1.7	1.8
Crimson Sweet	6	6.8	10.0	27.7	28.4	23.9	25.9	1.4	1.5	11.0	11.4	1.7	1.2
	7	6.9	9.3	27.8	28.1	24.3	25.0	1.6	1.5	11.1	11.3	1.5	1.2
	8	7.2	9.7	26.2	27.5	24.1	25.3	1.3	1.6	10.1	11.4	1.7	1.1
	9	9	9.0	26.9	28.5	24.3	24.5	1.6	1.4	10.3	11.8	1.7	1.3
	10	6.5	9.5	27.0	27.8	24.3	25.5	1.4	1.5	11.1	11.5	1.8	1.1
Moon and Stars	11	6.8	12.2	25.9	30.6	23.4	26.7	1.3	1.6	8.5 b	9.6	1.8	1.2
	12	7	12.2	26.7	30.2	24.0	27.3	1.5	1.6	8.6 b	9.7	1.9	1.2

	13	5.9	11.2	27.1	28.8	23.7	26.8	1.2	1.6	8.6 ab	9.5	2.0	1.1
	14	6.1	12.0	25.9	28.8	23.6	26.5	1.3	1.7	8.1 b	9.0	2.0	1.3
	15	6.5	12.0	26.7	30.3	24.0	26.9	1.5	1.6	9.1 a	9.4	2.2	1.1
	16	6.5	11.3	38.7	42.6	20.3	22.1	1.5	1.3	8.3 b	10.4	2.0	1.0
Charleston Gray	17	8.2	8.1	41.3	37.5	19.8	19.7	1.4	1.1	10.9 a	9.9	2.0	1.1
	18	7.2	10.8	39.2	43.7	19.3	21.3	1.5	1.3	10.2 ab	10.2	1.9	1.2
	19	6.1	10.4	38.3	41.3	19.5	21.1	1.2	1.3	8.8	10.6	1.8	1.0
Georgia Rattlesnake	20	7.4	10.5	39.6	41.1	19.1	21.4	1.5	1.2	8.9	10.7	2.1	1.2
	21	7	10.8	40.4	43.0	21.1	21.4	1.4	1.5	9.9	10.6	1.9	1.3
	22	7.7	10.1	36.2	39.5	21.0	22.1	1.4	1.3 ab	9.4	9.2	1.7	1.4
Congo	23	7	11.0	37.0	39.3	21.9	22.6	1.7	1.5 b	9.6	10.2	2.0	1.4
	24	7.8	8.8	35.7	36.5	20.5	21.1	1.5	1.1 a	8.2	9.3	2.4	1.8

Table 3.5. Genotypic parameters for 32 simple sequence repeat (SSR) loci used to characterize genetic variation in six heirloom and open-pollinated watermelon cultivars.

Locus	Fragment Size Range (bp)[z]	Na[y]	PIC[x]
BVWS00048	177 - 179	2	0.44
BVWS00067	140 - 152	3	0.15
BVWS00102	148 - 152	2	0.06
BVWS00106	156 - 212	4	0.36
BVWS00155	171 - 185	6	0.54
BVWS00177	180 - 202	4	0.74
BVWS00208	150 - 190	5	0.64
BVWS00209	122 - 136	4	0.36
BVWS00215	134 - 140	2	0.05
BVWS00225	178 - 184	2	0.34
BVWS00228	156 - 202	7	0.54
BVWS00233	172 - 180	2	0.34
BVWS00236	187 - 194	2	0.35
BVWS00244	159 - 165	2	0.21
BVWS00287	169 - 173	2	0.00
BVWS00297	150 - 166	6	0.64
BVWS00314	148 - 154	2	0.09
BVWS00333	132 - 144	3	0.64
BVWS00433	269 - 296	3	0.57
BVWS00441	178 - 196	3	0.45
BVWS00522	279 - 289	2	0.14
BVWS00839	248 - 268	4	0.53

BVWS00948	278 - 286	2	0.49
BVWS01001	259 - 263	2	0.49
BVWS01199	217 - 226	3	0.02
BVWS01836	291 - 316	3	0.01
BVWS01911	262 - 276	3	0.06
BVWS02048	256 - 278	3	0.32
BVWS02205	152 - 186	6	0.65
BVWS02428	130 - 134	2	0.5
BVWS02453	229 - 316	3	0.09
MCPI-5	208 - 230	5	0.68
Mean		***3.3***	***0.36***
SE		*0.26*	*0.06*

[z]Base pairs (bp)

[y]Number of alleles (Na).

[x]Polymorphism information content (PIC) is $1-\sum P_{ij}^2$, where P_{ij} is the frequency of j^{th} allele of the i^{th} locus (Botstein et al., 1980).

Table 3.6. Simple sequence repeat (SSR) loci for which six heirloom and open-pollinated watermelon cultivars are fixed for different alleles.

Cultivar	Sugar Baby	Crimson Sweet	Moon and Stars	Charleston Gray	Georgia Rattlesnake	Congo
Crimson Sweet	BVWS00225 BVWS01001					
Moon and Stars	BVWS00048 BVWS00225 BVWS00333 BVWS02205	BVWS00048 BVWS00333 BVWS00948 BVWS01001 BVWS02205				
Charleston Gray	BVWS00225 BVWS00333	MCPI-5 BVWS00333	BVWS00048 BVWS00333 BVWS02205			
Georgia Rattlesnake	BVWS00225 BVWS00333 BVWS01001	BVWS00333	BVWS00048 BVWS00333 BVWS00938 BVWS01001 BVWS02205	MCPI-5		
Congo	BVWS00048 BVWS00225 BVWS00333 BVWS01001	BVWS0048 BVWS0333	BVWS00333 BVWS01001 BVWS02205	MCPI-5 BVWS00048	BVWS00048	

Table 3.7. Genetic diversity parameters of six heirloom and open-pollinated watermelon cultivars based on 32 simple sequence repeat loci.

Cultivar	%P[z]	Na[y]
Sugar Baby	75.0%	1.91
Crimson Sweet	59.4%	1.72
Moon and Stars	59.4%	1.72
Charleston Gray	59.4%	1.69
Georgia Rattlesnake	43.8%	1.50
Congo	59.4%	1.72
Mean	**59.4%**	**1.71**
SE	4.0%	0.05

[z]Percent polymorphic loci (%P).

[y]Number of alleles, averaged over loci (Na).

Table 3.8. Analysis of molecular variance (AMOVA) among seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars based on 32 simple sequence repeat markers.

Source	df[z]	Variation (%)[y]	F-statistics[x]	*P*-value[w]	Fst' [v]
Among cultivars	5	65%	0.649	0.001	0.772
Within cultivars	263	12%	0.337	0.001	
Within individuals	269	23%	0.767	0.001	

[z]Degrees of freedom (df).

[y]AMOVA was used to partition total variance into among group, within group, and within individual variance (Michalakis and Excoffier, 1996a).

[x]F-statistics calculated via AMOVA (Nei, 1977) were used to estimate differentiation among groups (F_{ST}), within groups (F_{IS}), and within individuals (F_{IT}) .

[w]The null hypothesis was tested using 999 random permutations of the data. F-statistics are significant at $P \leq 0.05$.

[v]F_{ST}' is F_{ST} standardized based on maximum F_{ST} possible in the data set (Meirmans, 2006).

Table 3.9. Genetic diversity parameters of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars based on 32 simple sequence repeat loci.

Cultivar	Seed Lot	%P[z]	Na[y]
Sugar Baby	1	37.5	1.44
	2	46.9	1.53
	3	53.1	1.56
	4	59.4	1.59
	5	43.8	1.44
Crimson Sweet	6	31.3	1.31
	7	25.0	1.25
	8	34.4	1.38
	9	37.5	1.44
	10	28.1	1.28
Moon and Stars	11	34.4	1.41
	12	37.5	1.44
	13	31.3	1.38
	14	40.6	1.47
	15	34.4	1.38
Charleston Gray	16	40.6	1.47
	17	34.4	1.34

	18	46.9	1.50
Georgia Rattlesnake	19	28.1	1.28
	20	21.9	1.28
	21	37.5	1.44
Congo	22	43.8	1.50
	23	37.5	1.44
	24	40.6	1.41

[z]Percent polymorphic loci (%P).

[y]Number of alleles, averaged over loci (Na).

Table 3.10. Analysis of molecular variance (AMOVA) among seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars based on 32 simple sequence repeat markers.

Cultivar	Source	df[z]	Variation (%)[y]	F-statistic[x]	*P*-value[w]	F_{ST}'[v]
Sugar Baby	Among seed lots	4	1%	0.006	0.264	0.007
	Within seed lots	52	13%	0.131	0.002	
	Within individuals	57	86%	0.136	0.002	
Crimson Sweet	Among seed lots	4	1%	0.009	0.131	0.010
	Within seed lots	52	52%	0.526	0.001	
	Within individuals	57	47%	0.531	0.001	
Moon and Stars	Among seed lots	4	4%	0.037	0.001	0.043
	Within seed lots	51	32%	0.332	0.001	
	Within individuals	56	64%	0.357	0.001	
Charleston Gray	Among seed lots	2	1%	0.006	0.314	0.007
	Within seed lots	28	16%	0.159	0.004	
	Within individuals	31	84%	0.164	0.003	
Georgia Rattlesnake	Among seed lots	2	0%	0.000	0.538	0.000
	Within seed lots	32	42%	0.417	0.001	
	Within individuals	35	58%	0.416	0.001	
Congo	Among seed lots	2	1%	0.008	0.239	0.010
	Within seed lots	30	42%	0.426	0.001	

Within individuals	33	57%	0.430	0.001

[z]Degrees of freedom (df).

[y]AMOVA was used to partition total variance into among group, within group, and within individual variance (Michalakis and Excoffier, 1996).

[x]F-statistics calculated via AMOVA (Nei, 1977) were used to estimate differentiation among groups (F_{ST}), within groups (F_{IS}), and within individuals (F_{IT}) .

[w]The null hypothesis was tested using 999 random permutations of the data. F-statistics are significant at $P \leq 0.05$.

[v]F_{ST}' is F_{ST} standardized based on maximum F_{ST} possible in the data set (Meirmans, 2006).

Table 3.11. Pairwise estimates of genetic differentiation (F_{ST}) between seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars. F_{ST} was calculating according to Nei (1977).

Cultivar	Seed Lot	1	2	3	4	5
Sugar Baby	1					
	2	0.002				
	3	0.000	0.005			
	4	0.005	0.002	0.014		
	5	0.032	0.009	0.020	0.000	

Cultivar	Seed Lot	6	7	8	9	10
Crimson	6					
Sweet	7	0.042				
	8	0.000	0.003			
	9	0.023	0.002	0.006		
	10	0.004	0.014	0.018	0.000	

Cultivar	Seed Lot	11	12	13	14	15
Moon and	11					
Stars	12	0.011				
	13	0.022	0.027			
	14	0.001	0.022	0.032		
	15	0.062	0.077	0.067	0.058	

Cultivar	Seed Lot	16	17	18
Charleston	16			
Gray	17	0.039		
	18	0.001	0.000	

Cultivar	Seed Lot	19	20	21
Georgia	19			
Rattlesnake	20	0.017		
	21	0.000	0.006	

Cultivar	Seed Lot	22	23	24
Congo	22			
	23	0.027		
	24	0.000	0.000	

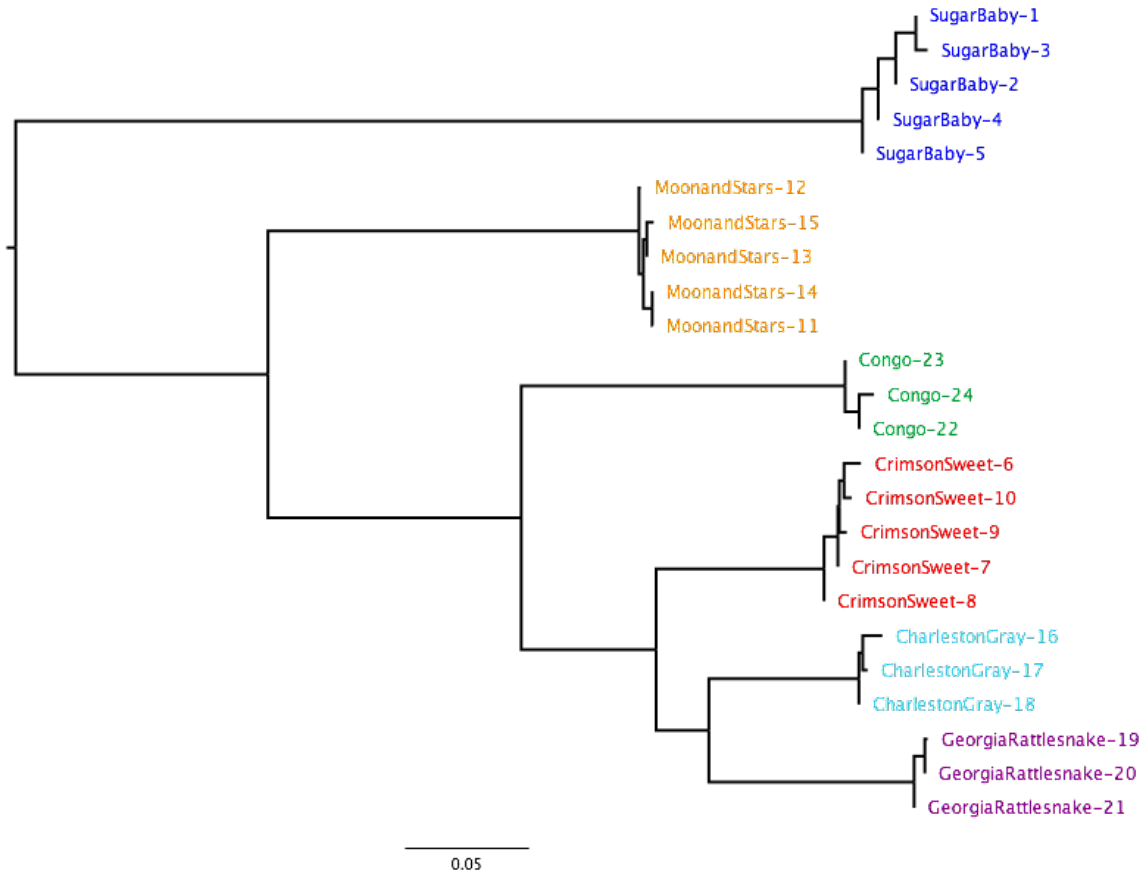


Figure 3.1. Neighbor-joining tree using Nei's standard distance (Nei, 1972) for seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars. The optimal tree with the sum of branch length = 1.12 is shown. The tree is drawn to scale.

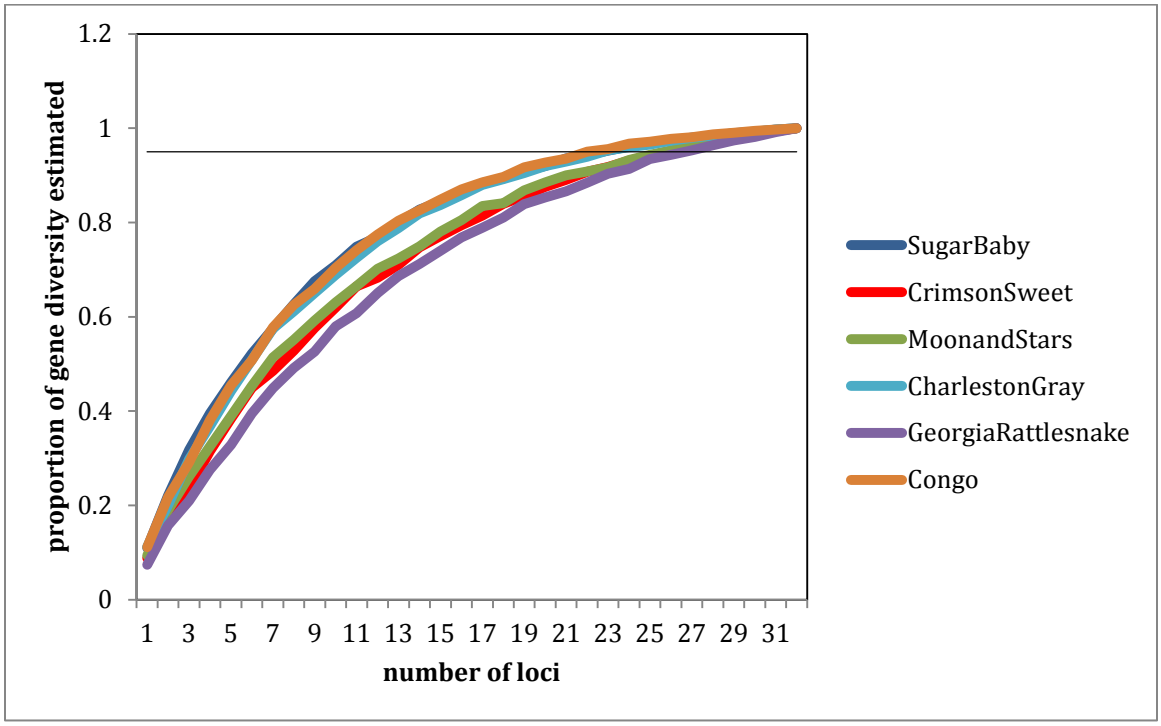


Figure 3.2. A power analysis of proportion of gene diversity estimated in six heirloom and open-pollinated watermelon cultivars versus number of loci, as generated by 1000 random permutations of the dataset of 32 simple sequence repeat loci.

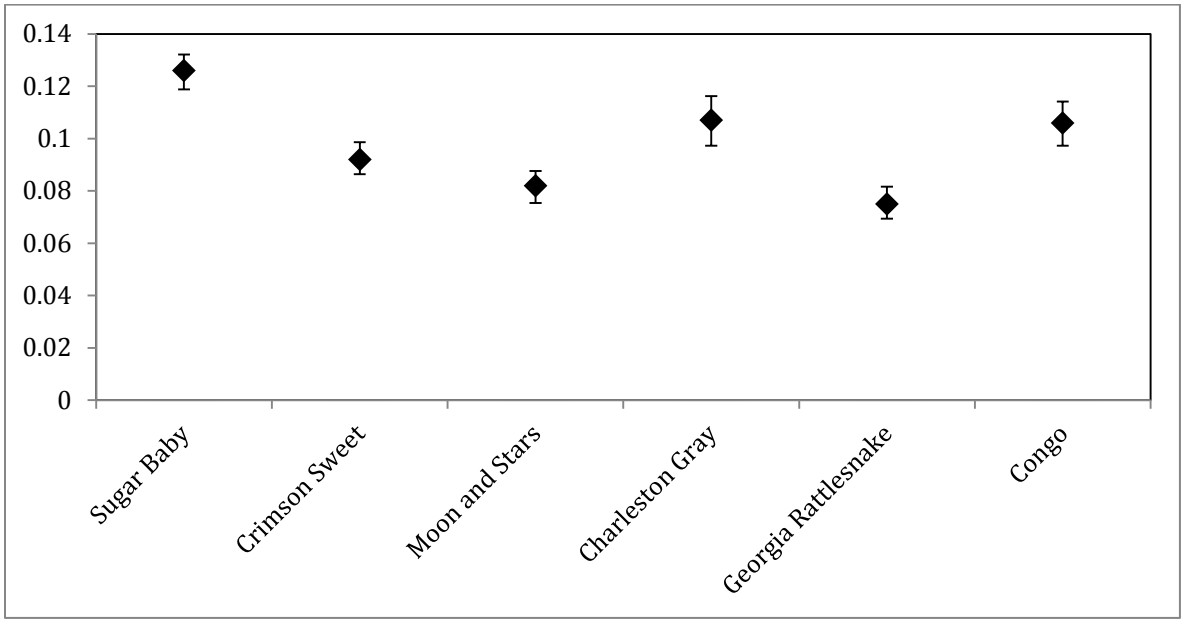


Figure 3.3. The average gene diversity of six heirloom and open-pollinated watermelon cultivars using 32 simple sequence repeat loci. Gene diversity was calculated for each loci using the formula $D = 1-\sum p_i^2$, where p_i is the frequency of i^{th} allele, then averaged over loci. Standard error bars were generated by bootstrapping the data 1000 times over loci.

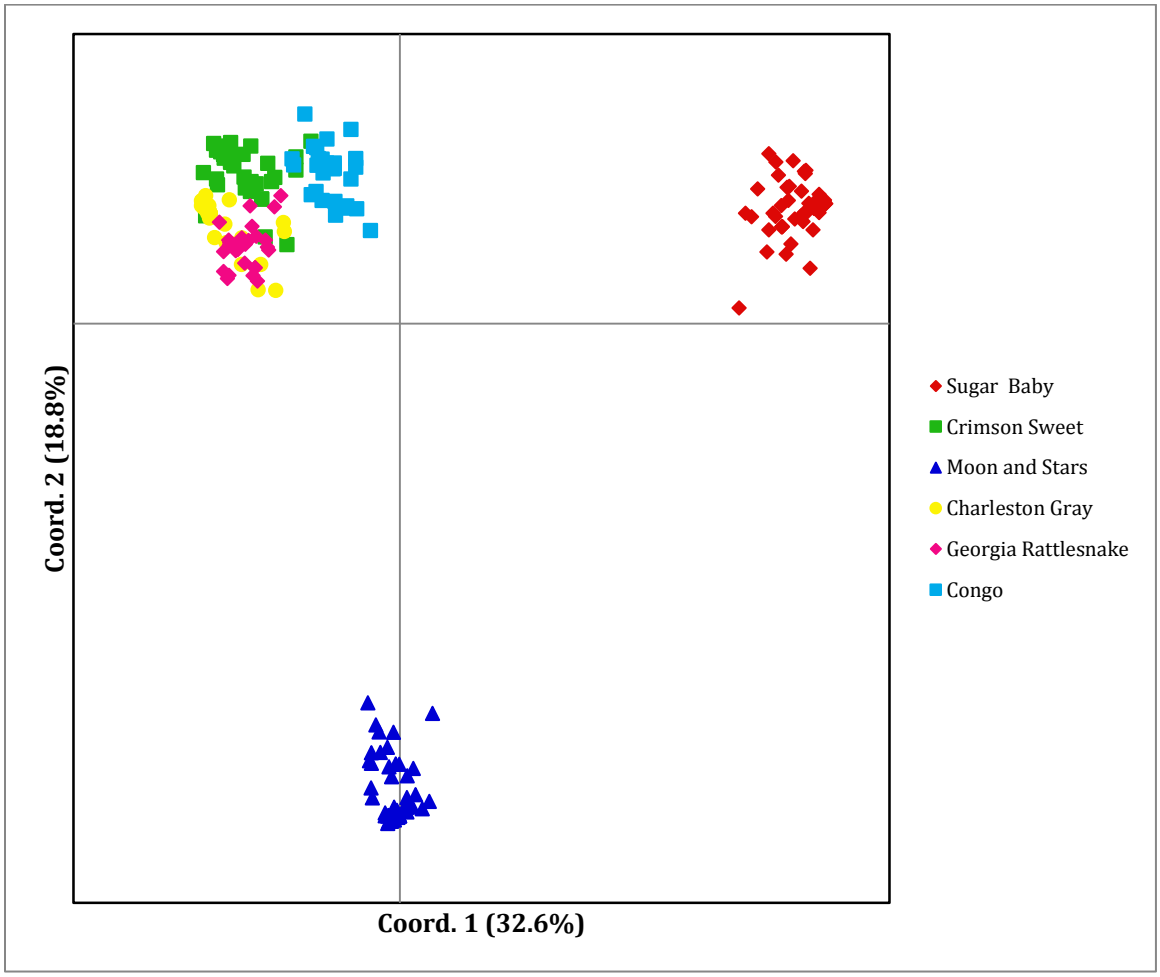


Figure 3.4. A principal coordinate analysis using genetic distance among individuals of six heirloom and modern open-pollinated watermelon cultivars.

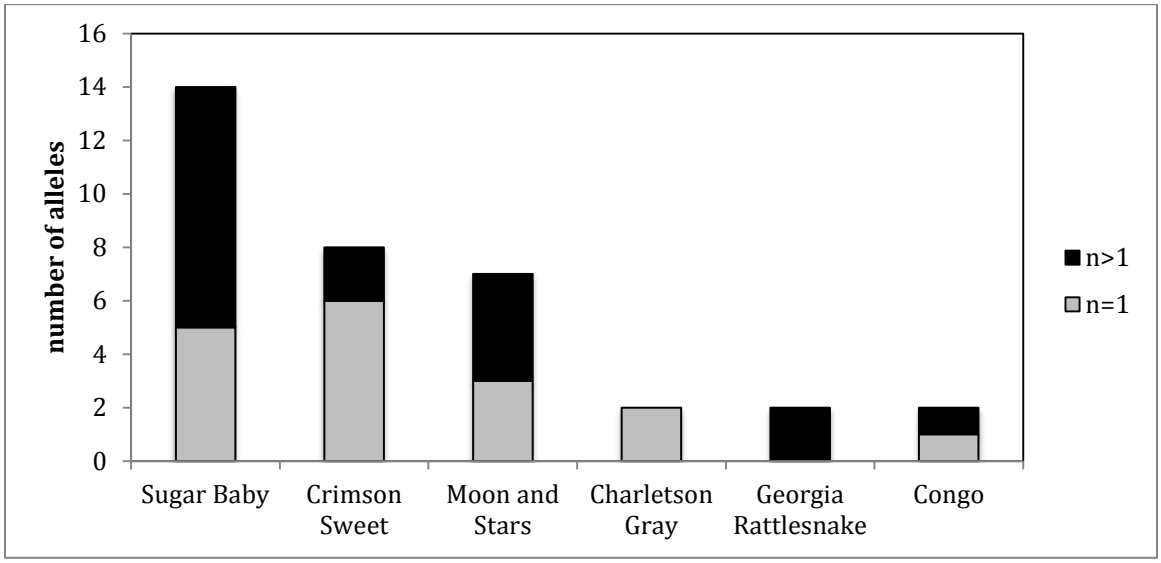


Figure 3.5. Number of private alleles observed in in six heirloom and modern open-pollinated watermelon cultivars generated from the genotyping of 32 simple sequence repeat loci. Gray bars indicate private alleles that were found in only one individual; black bars indicate private alleles that were found in more than on individual.

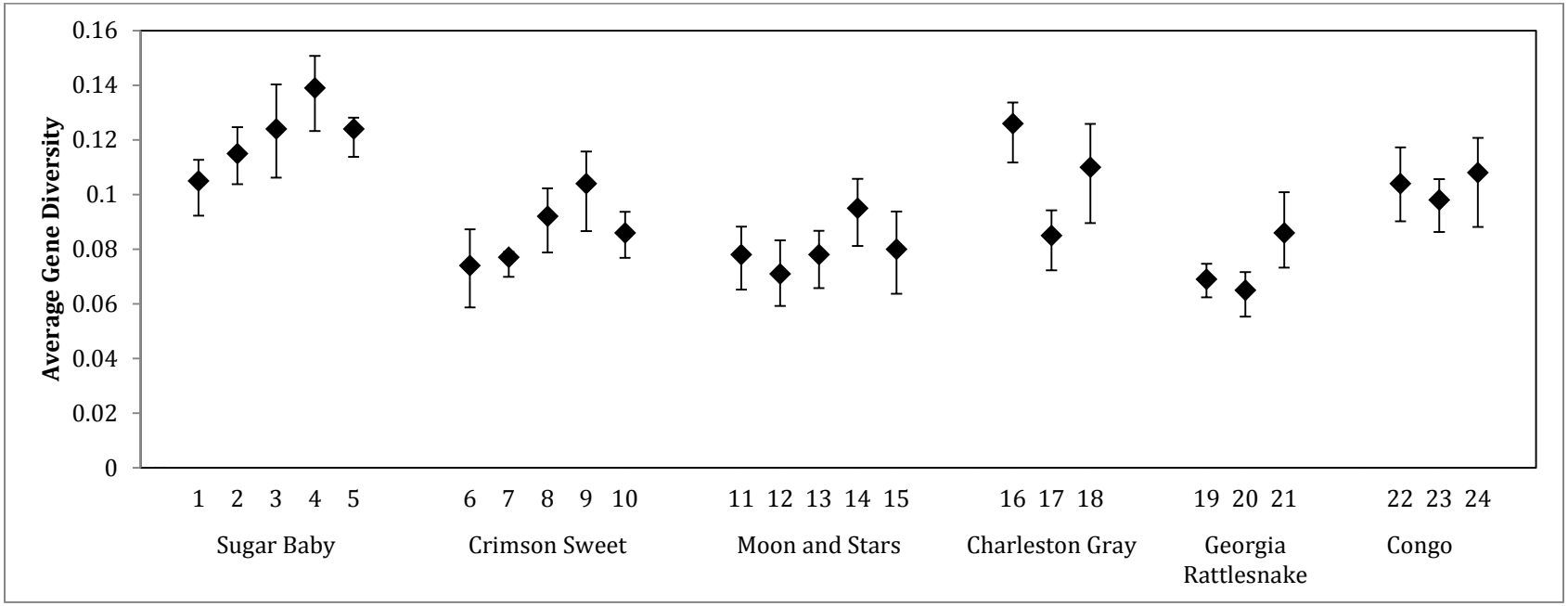
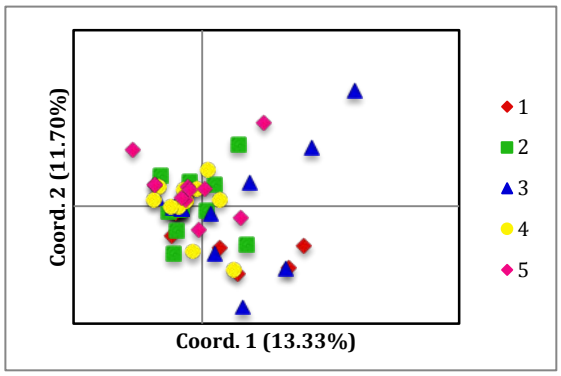
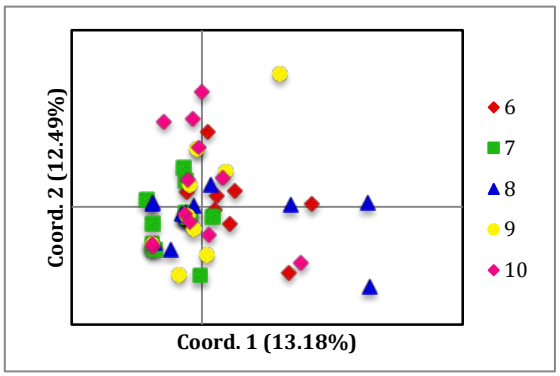


Figure 3.6. The average gene diversity of seed lots from various commercial seed vendors for six heirloom and open-pollinated watermelon cultivars using 32 simple sequence repeat loci. Gene diversity was calculated for each loci using the formula $D = 1-\sum p_i^2$, where p_i is the frequency of i^{th} allele, then averaged over loci. Standard error bars were generated by bootstrapping the data 1000 times over loci.

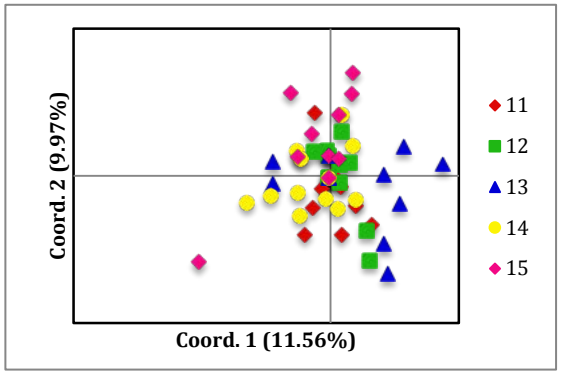
(A) ‘Sugar Baby’



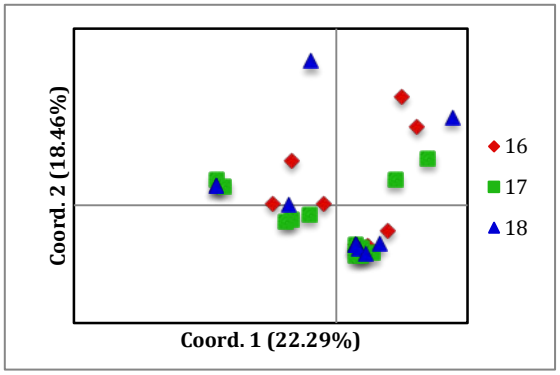
(B) ‘Crimson Sweet’



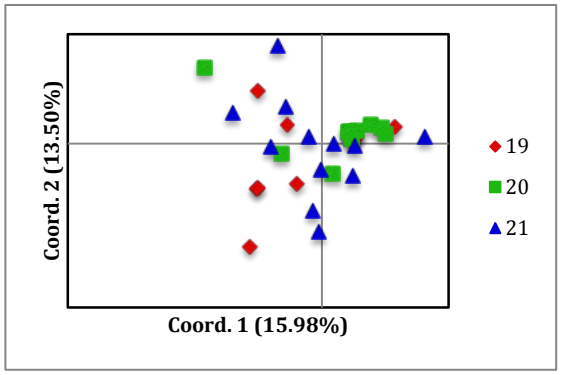
(C) ‘Moon and Stars’



(D) ‘Charleston Gray’



(E) ‘Georgia Rattlesnake’



(F) ‘Congo’

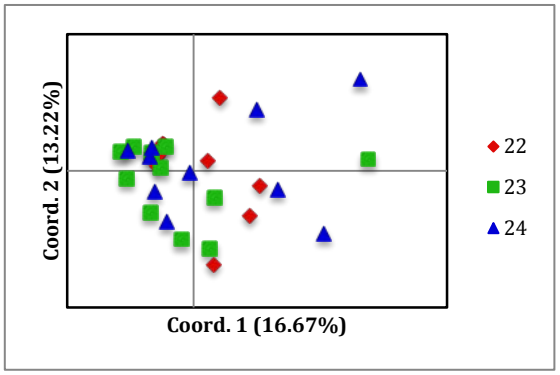


Figure 3.7. A principal coordinate analysis using genetic distance among individuals of seed lots from various commercial seed vendors for six heirloom and modern open-pollinated watermelon cultivars.

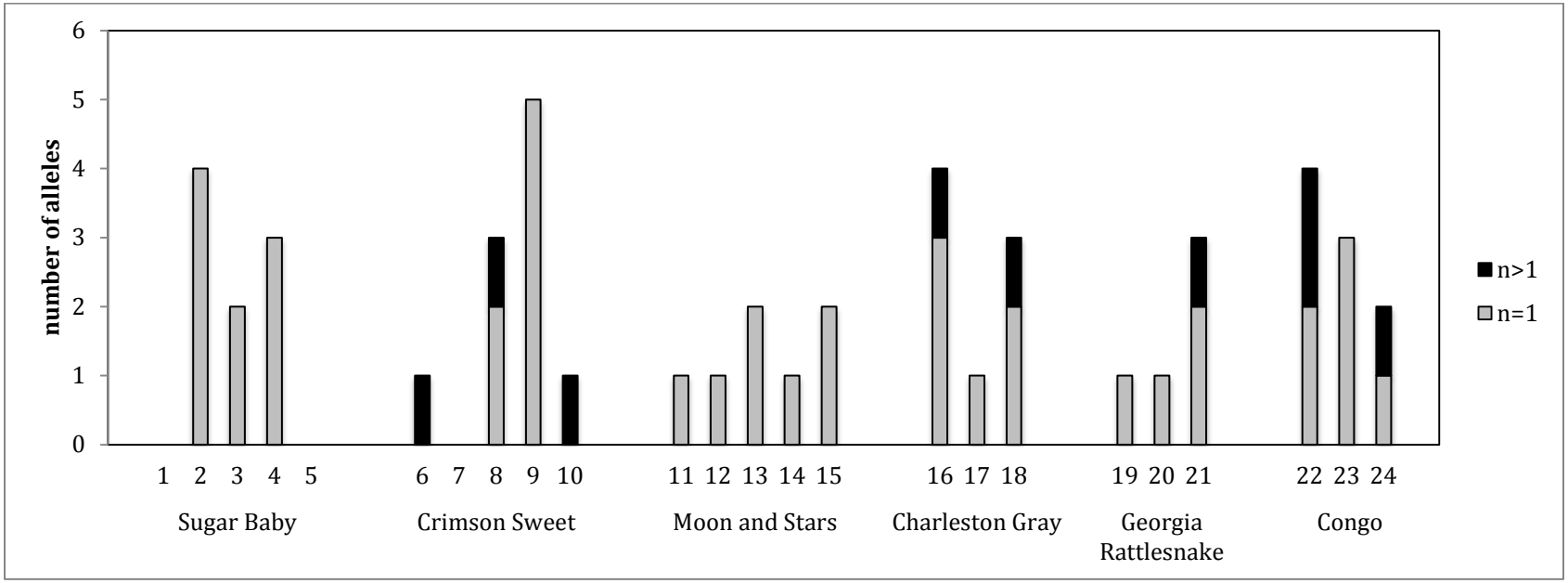


Figure 3.8. Number of private alleles observed in seed lots from various commercial seed vendors generated from the genotyping of 32 simple sequence repeat loci in six heirloom and modern open-pollinated watermelon cultivars. Gray bars indicate private alleles that were found in only one individual; black bars indicate private alleles that were found in more than on individual.

CHAPTER 4

Conclusion

An estimated 70% increase in crop production will be necessary to feed the world in 2050 (FAO, 2009). Consumer demands for organic and direct-market fresh produce in the U.S. grows by double digits each year (McNeil, 2016). However, organic yield continues to lag behind conventional yield for most crops (Seufert et al., 2012). Applied research is necessary to improve the sustainability of organic agriculture (Obach, 2015). Breeding crops that thrive in organic and low-input conditions may help bridge the yield gap (Lammerts van Bueren et al., 2011).

The objective of this research was to address challenges in organic and direct-market watermelon production. A breeding program to develop cultivars that are suited for limited field space, organic weed control, and repetitive harvests is ongoing. Compact plants also required less time to hand-weed than traditional vine types in full-season weeding regimes. These results suggest that compact watermelon may be better suited for organic production than vine-types. Additional studies on weeding and harvesting efficiency of compact versus vine-type plants are needed.

The most expensive aspect of organic watermelon production is the labor required for weed control (Gianessi and Reigner, 2007). An evaluation of hand-weeding regimes indicated that a partial season weeding regime, consisting of weeding once a week for four weeks after transplant, may be sufficient to preserve yield in an organic system. This study also provided watermelon growers with much-needed organic weeding cost estimates. The cost estimates

provide information that is critical in making the decision to transition from conventional to organic watermelon production. Further research to refine the critical period of weed interference and improve weed seedbank management under organic conditions is needed.

Finally, an analysis of the diversity of watermelon cultivars popular in organic and direct-market production was conducted. Growers and consumers in the organic community promote open-pollinated (OP) cultivars as a way to enhance on-farm biodiversity and sustainable seed systems (Navazio et al., 2012). Heirloom cultivars are perceived by some to be more diverse than modern OP cultivars (Heald and Chapman, 2012). The investigation herein of variation among and within cultivars demonstrates that in watermelon, pedigree and commercial seed practices are better predictors of genetic diversity than the heirloom designation per se. An additional study to determine the genetic differentiation of current commercial seed lots from original U.S germplasm deposits may reveal how much change has occurred to these OP cultivars over the past fifty years. Knowledge of the variation within and among seed lots can be used to improve commercial seed multiplication practices, inform conservation breeding efforts for elite cultivar maintenance, and prioritize genetic resource conservation.

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