ABSTRACT

KUMAR, RAKESH. Inheritance of Fruit Yield and other Horticulturally important Traits in Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai]. (Under the direction of Todd C. Wehner, Ph.D.)

Watermelon is a diverse crop in terms of qualitative genetic traits, with many different fruit types among the cultivars. We were interested in determining the optimum method for improving yield, and measuring the heritability and rate of natural outcrossing within elite populations. The rate of natural outcrossing is dependent upon the distance between plants, as well as other factors such as location and cultivar. The objective of this study was to 1) estimate narrow-sense heritability for yield using parent-offspring regression in two watermelon populations (NCHYWI and NCHYW2); 2) determine the inheritance of fruit yield, rind pattern, and fruit shape in six generations ($P_aS_1$, $P_aS_2$, $F_1$, $F_2$, $BC_1P_a$ and $BC_1P_b$) of three families; and 3) determine the rate of natural outcrossing effected by in-row spacing and cultivars. Field trials were conducted at two locations in North Carolina (Clinton and Kinston) to determine the narrow-sense heritability for yield in NCHYW1 and NCHYW2 watermelon populations using parent-offspring regression. Low estimates of narrow-sense heritability were recorded for total fruit weight (0.04-0.12), marketable fruit weight (0.06-0.15), total fruit number (0.04-0.16), fruit size (0.18-0.19), and percent culls (0.02-0.09) for NCHYW1 and NCHYW2 populations, respectively. Only low gain in yield can be made due to single-plant selection, based on the populations used. Strong positive genotypic correlations were observed between total fruit weight and marketable fruit weight; total fruit weight and marketable fruit weight with fruit size, and negative correlation was recorded

between total fruit number and fruit size, and total fruit weight and marketable fruit weight with percent culls. In the second experiment consisting of three families and six generations, a low to intermediate level of heritability was reported for total fruit weight, total fruit number, and fruit size. Recurrent selection is recommended to improve populations for these traits to accumulate favorable genes.

The family 'Mountain Hoosier' x 'Calsweet' did not fit the expected segregation ratios for the single dominant gene for solid dark green rind pattern (*G*) (solid dark green vs. wide stripe), or for the single incompletely dominant gene for elongate fruit shape (*O*) (elongate vs. round) in the $F_2$ and backcross, different from previous reports. Deviation from expected ratios was also observed for the single dominant gene controlling solid dark green rind (*G*) and light green rind (*g*) in the families 'Mountain Hoosier' x 'Minilee' and 'Early Arizona' x 'Minilee'. However, segregation ratios showed that light green rind was controlled by two recessive genes, *g-1* and *g-2*, by duplicate dominant gene action when crossed to a line having solid dark green rind.

The rate of natural outcrossing was determined by using split plot in randomized complete block design in two locations: Kinston and Clinton; eight in-row spacing as whole plots: 0.6, 1.2, 1.8, 2.4, 3.0, 3.7, 4.3, and 4.9 m; and two cultivars as sub plots: 'Allsweet' and 'Mickylee'. 'Moon and Stars' was used as the pollen donor of marker gene to track the rate of natural outcrossing. Location and cultivar, and all interactions were not significant. Closer in-row spacing (0.6 m and 1.2 m) had a significantly higher rate of natural outcrossing

(11.0% and 16.9%, respectively) than wide in-row spacing (> 4.3 m), which had a low rate of outcrossing (1.8 %). In conclusion, watermelon appears to act more like a self-pollinated crops when spaced >5 m apart. The rate of natural outcrossing should also be taken in to account while estimating heritability and genetic variance in watermelon populations.

# Inheritance of Fruit Yield and other Horticulturally important Traits in Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai].

by
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## DEDICATION

When tillage begins, other arts follow. The farmers, therefore, are the founders of human civilization.

- Daniel Webster

I, sincerely and respectfully, dedicate the work and efforts to pursue this research to the farming community of world who feeds billions of people.

## BIOGRAPHY

Rakesh Kumar was born on October 25, 1978 in Hamirpur, Himachal Pradesh 'The land of snowy mountains', India, to Sh. Dharam Singh and Smt. Leela Devi. Rakesh Kumar has caring elder brother, Surjit Singh, and loving younger sister, Nisu Devi. He grew up in his native village, Baloh, where he also went to get his elementary school education at Govt. Primary School, Panyali, Hamirpur from 1983 to 1989. Rakesh played 'Hide and Seek' with his friends on mango trees on his school premises. Growing up in the lofty Himalayas with Rolling Meadows and racy rivers, he developed strong association with nature. Going for bird watching, trekking, and listening soothing music of flowing water with friends is still fresh in his memories. He went to boarding school, Jawahar Navodaya Vidyalaya, Tarkwari; when he was 10, to pursue his further school education from 1989 to 1996. Living away from his parents since his childhood, lead him to inculcate qualities like prompt decision making and independent thinking. He missed his family so much though.

Rakesh had strong inclination for science since his childhood especially biology. He wanted to pursue career in medicine but it proved too difficult for him to secure seat in medical college. Later, he turned to plants from humans and he went on to pursue his undergraduate degree in horticulture at University of Horticulture and Forestry, Solan, India from 1997 to 2001. Here, he was exposed to challenging field of plant sciences. Rakesh always enjoyed courses on plant genetics and breeding while pursuing his undergraduate degree. He decided to pursue higher education in this field. Rakesh was awarded 'Certificate of Honor' for his scholarly efforts in his undergraduate degree. Rakesh played cricket while

he was in university. Reginald C. Punnet and G.H. Hardy, two eminent geneticists, also played cricket, but it will be unfair for him to compare him with those innovative minds. Rakesh also started dedicated effort to bodybuilding and still sweats a lot in Carmichael gym.

In 2001, Rakesh secured prestigious 'Junior Research Fellowship' awarded by Indian Council of Agricultural Research to pursue his master degree in horticulture at University of Agricultural Sciences, Bangalore, India. He did his research on radish seed production. He always appreciated diversity in languages and cultures while studying at Bangalore and made a group of friends. After completing his masters, Rakesh was employed as Senior Research Fellow at Indian Institute of Horticultural Research, Bangalore where he worked with onion breeding program. His professional goals were still unfulfilled. Rakesh always wanted to be medical doctor since his childhood which did not come true. However, he decided to be a plant doctor and desired to pursue his Ph.D. in the field of plant breeding and genetics. In May 2006, he enrolled in the graduate program of the department of horticultural science at North Carolina State University, Raleigh, North Carolina to pursue his Ph.D. degree in plant breeding and genetics under the direction of Dr Todd C. Wehner. He has been working as graduate research assistant in cucurbit breeding program. He always tried to keep his 'knife' and 'pen drive' with him which was prerequisite to get a degree.

Following completion of his Ph.D, Rakesh plans to pursue his professional career in plant breeding and genetics. He also intends to earn MBA degree at some of time in his life.

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INHERITANCE OF FRUIT YIELD, RIND PATTERN, AND FRUIT SHAPE USING SIX RELATED GENERATIONS IN WATERMELON

# GENERAL INTRODUCTION

# REVIEW OF WATERMELON GENETICS AND BREEDING

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## Origin and taxonomy

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] belongs to the family *Cucurbitaceae* and subtribe *Benincasinae* (Wehner, 2008). Other members of the *Cucurbitaceae* are cucumber, melon, pumpkin, and gourd. The genus *Citrullus* has been divided taxonomically into four species: *C. lanatus* (*Syn. C. vulgaris*), *C. ecirrhosus*, *C. colocynthis*, and *C. rehmii*. Diploid watermelon has 22 chromosomes (2*n*=22, *x*=11) (Shimotsuma, 1963) with a genome size of 420 million base pairs (Arumuganathan and Earle, 1991; Guner and Wehner, 2004). Watermelon is indigenous to tropical Africa where it grows wild (De Candolle, 1882). It is native to southern Africa, mainly the Kalahari Desert area (Bailey, 1949). The secondary center of origin is China. Watermelon can be found growing wild in various parts of western hemisphere, particularly in India (Pangalo, 1930, 1944, 1955; Peter, 1998) and in the Mediterranean region, including Iran and Egypt.

## Area and production

The principal watermelon producing countries (Table 1) are China, Turkey, Iran, United States, and Egypt (FAO, 2002), making the United States the fourth largest producer of watermelon in world. Yields are highest in China and United States and somewhat lower in the other major producing countries. Watermelon is a major vegetable crop in the U.S., and increasing in popularity (Table 2). The total area has changed from 76 thousand hectare in 1998 to 65 thousand hectares in 2007 (U.S. Department of Agriculture, 2007). Production has increased from 1.7 million Mg in 1998 to 1.9 million Mg in 2007 (U.S. Department of Agriculture, 2007). At present, the total value of watermelon production in United States is

$476 million. Over 80% of the watermelon production is concentrated in Arizona, Florida, Georgia, Texas, California, and North Carolina where temperature are warmer and growing season are longer than states located in northern latitudes (Hassel et al., 2007) (Table 2).

## Brief history of watermelon breeding and genetics

Watermelon has been bred for thousands of years, but formal watermelon breeding programs in the U.S. did not start until the late 1800s. By 1900, 'Angeleno', 'Chilean', 'Florida Favorite', 'Georgia Rattlesnake', 'Cole Early', 'Kleckley Sweet', and other open-pollinated cultivars were in the market (Whitaker and Jagger, 1937). Planned improvement was started in the late 19$^{th}$ and early 20$^{th}$ century in both the public and private sectors (Maynard et al., 2007). In 1954, C.F. Andrus released 'Charleston Gray' with elongate fruit, gray rind, and red flesh. It was resistant to Fusarium wilt, anthracnose, and sunburn. In 1970, C.V. Hall developed 'Allsweet' with similar resistance to 'Charleston Gray', but higher in quality. 'Allsweet' had elongate fruit shape and rind with wide, dark green stripes. J. M. Crall released 'Dixielee', an alternative to 'Allsweet' for its different fruit type and superior quality, and 'Minilee' and 'Mickylee', the first icebox (< 5.5 kg/fruit) cultivars adapted to southeastern U.S. Cultivars that dominated the market in the mid 20$^{th}$ century were open-pollinated ones such as Charleston Gray, Jubilee, Crimson Sweet, and Sugar Baby. By the end of 20$^{th}$ century, hybrids had replaced open-pollinated cultivars for the commercial market. 'Sangria' was the first hybrid developed by T.V. Williams of Rogers NK (now Syngenta) in 1985. The most important change in the watermelon industry is the production of seedless cultivars. O.J. Eigsti released the first seedless watermelon, 'Tri-X-313', in 1962.

However, seedless watermelon did not become commercially important until the 1990s due to poor fertility of tetraploid parents used in triploid hybrid seed production. In the U.S., three quarters of the total production is seedless (USDA Economic Research Service, 2005), and 'Tri-X-313' is still popular. A recent advance in watermelon breeding was the introduction of mini watermelons that are seedless in the early 21st century. X. Zhang developed the first cultivars, sold under the PureHeart™ brand in the U.S. and Solinda™ brand in Europe (Maynard et al., 2007). These watermelons became popular because of their good flavor, crisp texture, small size, and seedlessness.

Much of the breeding work in watermelon over the past 100 years has been concentrated on disease resistance and fruit quality traits controlled by single genes. There are numerous published reports on the genes of watermelon, many of which have been used in cultivar improvement (Cucurbit Gene List Committee, 1979; Cucurbit Gene List Committee, 1982; Guner and Wehner, 2004; Henderson, 1991, 1992; Rhodes and Dane, 1999; Rhodes and Zhang, 1995; Wehner and Guner, 2004).

## Breeding for high yield

Many studies have been done on qualitative traits in watermelon, but there have been relatively few on quantitative traits such as yield. The yield goal for growers is to harvest at least one load per hectare (51 Mg/ha). Hybrids became popular in 1950s and 1960s. However, heterosis is not a large factor in yield, but some studies do show inconsistencies with some estimates, with some estimates approximately 10% over the high yield parent (Brar and Sidhu, 1977; Brar and Sukhija, 1977; Nandpuri et al., 1974; Sidhu and Brar, 1977,

1985; Sidhu et al., 1977a, 1977b). This small amount of heterosis makes it unnecessary to develop hybrid cultivars since inbred lines would have similar performance (Wehner, 2008). However, hybrids are popular in the seed industry because they provide both a protection for intellectual property and novel traits, such as triploid cultivars. For example, diploid (seeded) and triploid (seedless) hybrids are available, which include example such as the popular diploid hybrids 'Sangria', 'Royal Sweet', 'Fiesta', 'Mardi Gras', and 'Regency' and the popular triploid hybrids 'Tri-X-313', 'Summer Sweet 5244', 'Millionaire', 'Genesis', and 'Tri-X-Shadow' (Tetteh, 2008). It is also possible to produce diploid seedless fruit using growth regulators.

Many yield trials of new watermelon cultivars are run every year in the U.S., but often there are few differences among the entries (Gusmini and Wehner, 2005a). The question arises as to whether that is due to a lack of genetic diversity for yield in the species, or just among the elite, new experimental entries. In the U.S., genetic diversity among watermelon cultivars is narrow because most of them have been derived from just a few original germplasm sources, which includes 'Allsweet'. Gusmini and Wehner (2005a) tested a diverse set of obsolete inbred cultivars that do not trace to 'Allsweet' type and found that cultivars differed in yield from 36.6 $Mg.ha^{-1}$ in Calsweet to 114.2 $Mg.ha^{-1}$ in Mountain Hoosier. This indicates that genetic variation for yield does exist in the germplasm pool made up of diploid inbred cultivars. Since sources of high yield have been identified, it is important to develop populations using the high yielding cultivars and then use those populations to produce even higher yield.

Yield is a complex quantitative trait, and such traits are typically controlled by many genes, each often having a small effect. In order to improve such traits, it is important to get estimates of heritability, number of genes and gene action. There are several published estimates of broad-sense heritability for yield in watermelon, which are easy to calculate (Gill and Kumar, 1986; Prasad et al., 1988; Vashistha et al., 1983). However, in order to develop new inbred lines from segregating populations, it is important to estimate narrow-sense heritability in those populations.

Currently consumers prefer to have a choice of watermelon fruits from a variety of sizes. Fruit size is a component of yield in cultivated watermelon that is reported as fruit weight, ranging from 1 to 100 kg. Fruit sizes in watermelon are classified as icebox (<5.5 kg), small or pee-wee (5.5-8 kg), medium (8.1-11 kg), large (11.1-14.5 kg), and giant (>14.5 kg) (Maynard, 2001). Significant additive, dominant, and epistatic effects have been reported for fruit size, where dominance and dominance-by-dominance effect was largest (Sharma and Choudhury, 1988). Brar and Nandpuri (1974) found considerable heterosis for fruit size due to partial dominance and overdominance. Gusmini and Wehner (2007) recorded low to intermediate estimates of broad- and narrow-sense heritability for fruit size.

## Breeding for qualitative traits

Watermelon has many single genes that produce interesting differences for leaf shape (Mohr, 1953), fruit size and shape (Mackay, 1936; Poole and Grimball, 1945), seed color (Kanda, 1931), seed size (Poole et al., 1941), rind pattern (Poole, 1944), flesh color (Shimotsuma, 1963), and plant growth habit (Liu and Loy, 1972). Watermelon fruit can be

round (spherical), oval, blocky, oblong, and elongate. Authors have reported different genes for fruit shape. Dominance of oval fruit shape over spherical (Kang et al. (2000) and dominance of round over elongate in egusi melon (Ogbonna and Ubi, 2005) has been reported. Tanaka et al. (1995) reported that the single allele *O* ($O^s$ and $O^+$ for spherical and oval fruit shape, respectively) controls fruit shape in incompletely dominant fashion, where $O^s$ is incompletely dominant over $O^+$. However, the most accepted study of fruit shape is that the single incompletely dominant gene *O* determines elongate fruit shape. Thus the genotype of elongate fruit shape is *OO*, oval is *Oo,* and round is *oo* (Poole and Grimball, 1945; Weetman, 1937). Fruit shape can be predicted by the ovary shape at anthesis, thus ovary shape is a useful phenotypic marker for fruit shape (Warid and Abd el Hafez, 1976).

Watermelon has a diverse array of rind patterns. The more common patterns on cultivars include solid green (dark, medium, and light), striped (narrow, medium, and wide), and gray (medium green lines on light green background). Three alleles are reported to control rind pattern. *G,* $g^s$, and *g* confer dark green, striped, and light green rind color, respectively (Weetman, 1937). During the 1900s, inbred cultivars with interesting rind patterns were released by watermelon breeders in the United States and Japan, but have been lost over the years as they are not grown anymore. 'Japan 6' had inconspicuous and penciled lines on the rind (*p* allele), 'China 23' had a medium green colored network on the striped rind (*P* allele), and 'Long Iowa Belle' and 'Round Iowa Belle' had randomly-distributed and irregularly-shaped greenish-white spots on a mostly solid dark green rind (*m* gene) (Gusmini and

Wehner, 2005b). However, many interesting mutations have been maintained in current cultivars.

The cross 'Japan 6' x 'China 23' was used by Weetman (1937) to study the inheritance of solid light green vs. striped rind and penciled vs. netted rind. Weetman confirmed his hypothesis of two independent genes regulating the presence of stripes and penciled vs. netted rind, recovering four phenotypic classes in a 9:3:3:1 ratio (striped netted: striped penciled: nonstriped netted: nonstriped penciled) in $F_2$ and 1:1:1:1 ratio in the backcross. However, these genes were not named at the time. Kang et al. (2000) reported that green or bright green fruit rind was dominant to yellow color, and black rind color was incompletely dominant to green or bright green. In egusi type melon, blue rind color was reported to be dominant over white rind color (Ogbonna and Obi, 2005). Gusmini and Wehner (2006) reported that a single dominant gene controls yellow belly (*Yb*), and intermittent striped rind pattern is controlled by single recessive gene *ins*. Of course, these results were obtained from specific crosses and do not apply to all cultivars. Not much is known about the inheritance of light (gray) rind color and furthermore, inheritance of stripe width (narrow, medium, and wide) should be studied to determine inheritance.

**Rate of natural outcrossing and its implications in watermelon breeding**

Knowledge of the reproductive system of a species or population is essential in a plant breeding program. The breeding strategy applied to self-pollinated crops is distinct from that for cross-pollinated crops. The genetic structure of plant populations is determined in part by the rate of natural outcrossing. However, consideration of the rate of self-pollination is also

important to calculate precise estimates of genetic variances and heritability. In general, individuals within a family in allogamous (cross-pollinated) crops are assumed to be half-sibs, but that is not necessarily the case if self-pollination occurs. As a result of inbreeding, coancestry among half-sibs is greater than expected (Ferreira et al., 2002). Due to self-pollination, variability within families decreases and variability among families increases. The way plants reproduce depends on their sex expression. This is important in cucurbits because of their different types of sex expression, such as monoecious (staminate and pistillate flowers on the same plant) and andromonoecious (staminate and perfect flowers on same plant) (Robinson et al., 1976). Sex expression in cucurbits besides being genetically controlled is also highly affected by environment (temperature, humidity, light, and nutrition). A single pair of alleles determines the sex expression in watermelon. Andromonoecious gene *a* controls monoecious (*AA*) vs. andromonoecious (*aa*) sex expression (Guner and Wehner, 2004; Rhodes and Dane, 1999; Rhodes and Zhang, 1995; Rosa, 1928). Watermelon is considered allogamous because both andromonoecious and monoecious sex forms encourage cross-pollination. At the same time, both sex forms show varying degrees of self-pollination. Andromonoecious sex form promotes autogamy because of the presence of hermaphroditic flowers where as monoecious plants are closer to allogamy. Allard (1960) reported that domesticated cucurbits are more autogamous than allogamous because they originated from few individuals during domestication. Furthermore, because of their viny growth habit crossing among related individuals may be common,

increasing inbreeding levels, leading to purging of many deleterious recessive genes, which may explain why inbreeding depression in watermelon is low.

There are many factors that can influence the rate of natural outcrossing in watermelon including density of insect pollinators, plant spacing, genotype of cultivar and climatic conditions. Insect pollinators are directional in movement (Cresswell et al., 1995; Handel, 19982; Walters and Schultheis, 2009) and can carry pollen up to 2 to 3 m. Thus, the rate of natural outcrossing is higher in closely spaced plants. In watermelon, the rate of natural outcrossing (measured between-row only) was near zero for rows separated by 6 m or more (Rhodes et al., 1987; Walters and Schultheis (2009). Thus, it is important to know the effect of in-row spacing on the rate of natural outcrossing in watermelon.

## Objectives

The objectives of this research were to 1) study the narrow-sense heritability of yield and its components in two watermelon populations using parent-offspring regression; 2) study the inheritance of yield, fruit shape, and rind pattern using six generations in three watermelon families; and 3) study the effect of environment, genotype, and in-row spacing on the rate of natural outcrossing in watermelon.

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Table 1. Major watermelon producing countries, 2002.

| Country | Production (Mt) | Crop area (1,000 ha) | Average yield (kg/ha) |
|---|---|---|---|
| China | 69,315 | 2,221 | 31,203 |
| Turkey | 3,800 | 2,137 | 27,737 |
| Iran | 2,150 | 100 | 21,500 |
| United States | 1,669 | 55 | 31,139 |
| Egypt | 1,500 | 62 | 24,193 |

Source: FAOSTAT.

Table 2. Major watermelon producing states in United States, 2008.

| State | Production '000 Mg | Crop area '000 ha | Average yield Mg/ha |
|---|---|---|---|
| Georgia | 466. | 16 | 31 |
| Florida | 365 | 10 | 37 |
| California | 308 | 6 | 57 |
| Texas | 190 | 11 | 21 |
| Arizona | 151 | 3 | 46 |
| Indiana | 120 | 3 | 40 |
| Total ( US) | 1,929 | 65 | 32 |

# CHAPTER ONE

# INHERITANCE OF FRUIT YIELD IN WATERMELON

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## Inheritance of fruit yield in watermelon


### Abstract

Elite cultivars of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) are high in fruit quality but they may not be the highest yielders. There are obsolete cultivars that are high yielding but are poor in fruit quality that can be used as a source of high yield in breeding programs. The objective of this study was to estimate heritability of, and genotypic and phenotypic correlations among traits related to in two watermelon populations developed from crosses between obsolete cultivars with high yield and elite modern cultivars. Traits measured were total fruit weight, marketable fruit weight, total fruit number, fruit size, and percent culls. Field trials were conducted at two locations in North Carolina (Clinton and Kinston). The data were analyzed by regressing $S_{0:1}$ progeny data over $S_0$ parent data to estimate narrow-sense heritability. Narrow-sense heritability estimates were low for all trait measured (total fruit weight (0.04-0.12), marketable fruit weight (0.06-0.15), total fruit number (0.04-0.16), fruit size (0.18-0.19), and percent culls (0.02-0.09) in NCHYW1 and NCHYW2 populayion, respectively). Estimates of broad-sense heritability were higher than, and realized heritability were close approximation of narrow-sense heritability for all traits in both the populations. Genotypic and phenotypic correlations among traits showed some desirable relationships for use in indirect selection. Total fruit weight and marketable fruit weight had a highly significant positive correlation. Total fruit number had a significant positive correlation with marketable fruit weight in NCHYW2. Fruit weight and percent culls were not correlated. Marketable fruit weight and fruit size used as a single selection criterion

were predicted to give best correlated response in total fruit weight in NCHYW1 and only marketable fruit weight in NCHYW2. Total fruit number and fruit size were negatively correlated. Our data indicate that watermelon breeders should use multi-environment trials and replicated progeny rows in order to make gain in selection. Selection for larger fruit size should be practiced to get higher yield. Breeders can also select for more fruit number to get higher yield based on simulated response to indirect selection.

## Introduction

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a major vegetable crop in the U.S., and increasing in popularity. The total planted area has changed from 76 thousand ha in 1998 to 65 thousand ha in 2006 (U.S. Dept. Agriculture, 2007), which has contributed to corresponding production increases from 1.7 million Mg in 1998 to 1.9 million Mg in 2006. The total value of watermelon production is currently $435 million.

High yield is a major breeding objective in many crops. Increase in watermelon yield in the last decade has been attributed to improved production techniques, as well as the use of cultivars that are resistant to biotic and abiotic stress (Maynard, 2001). In the U.S., growers expect to harvest 51 Mg/ha of marketable fruit (Maynard, 2001), but average yield of watermelon in 2008 in the U.S. was only 32 Mg/ha. Until now, breeders were interested only in improving quality traits (TSS, flesh color, and fruit shape). Though most cultivars have high quality and uniformity, higher yield would be of great interest to growers. Gusmini and Wehner (2005) reported large differences in yield among a diverse array of obsolete watermelon cultivars tested in 3 environments with 4 replications. Fruit quality of the

obsolete cultivars was low, and cultivars must have good fruit quality in order to be successful in the market. ‘Sangria’ has been the leading cultivar in the southeastern U.S. for the last decade in the seeded market. We have developed populations by crossing 6 elite cultivars and 6 high yielding obsolete cultivars for use in recurrent selection and the production of inbred lines with high yield and fruit quality.

New cultivars have been developed with high fruit quality, excellent shipping characteristics, and disease resistance by different breeding programs across U.S. Uniform hybrids, seedless triploids, and high sugar content, dark red flesh, 20 lb picnic watermelons, and 7 lb mini watermelons are examples of successful innovations. However, yield has remained about the same during the time in which these cultivars were developed. Watermelon breeders test for yield during the development of new cultivars, but unfortunately yield increases are difficult to make. Proper evaluation requires replicated plots with multiple hills per plot, and there are many traits besides yield that are important to select for during cultivar development. Most watermelon breeders in the U.S. run field trials using 1-row or 3-row plots 10 to 16 m long with 10 to 20 plants per plot harvested 1 to 3 times (Neppl and Wehner, 2001). In yield studies, a 6-plant plot 3.7 m long was more efficient than a single-plant hill 2.4 m long, or a 12-plant plot 7.3 m long (Neppl and Wehner, 2001). In addition, 3-row plots were not necessary to reduce competition from different genotypes in bordering rows (Neppl et al., 2003).

In the last century, quality and pest resistance were major goals for watermelon breeders. Hybrids were of major interest to private breeders because of intellectual property right for

parental lines, and along with a small amount of heterosis for yield (Gusmini and Wehner, 2005). Researchers reported heterosis, and measured general combining ability (GCA) and specific combining ability (SCA) (Bansal et al., 2002a, 2002b; Brar and Sidhu, 1977; Brar and Sukhija, 1977; Gopal et al., 1996). Hybrids have proven their advantage for protection of parental lines, and seedless watermelons, produced using method of triploid hybrids, have become extremely important (Gusmini and Wehner, 2005). Eventually, it may also become economically viable option to produce diploid seedless watermelon using growth regulators.

Complex traits such as yield are often controlled by multiple genes referred to as polygenes or quantitative trait loci (QTLs). Planning an efficient breeding program requires estimates of heritability, number of effective factors (genes) and gene action. Researchers have estimated genetic variability and broad-sense heritability for yield in watermelon over the last century (Gill and Kumar, 1986; Prasad et al., 1988; Vashistha et al., 1983). However, broad-sense heritability estimates are of limited use to inbred line development programs because they are influenced by non-additive components of variance that are not fixable. Estimates of narrow-sense heritability would be more applicable in such cases. Heritability can be expressed on the basis of a single plant, individual plots, and entry mean measurements (Holland et al., 2003; Nyquist, 1991). If the narrow-sense heritability for yield in watermelon is high, elite inbreds can be improved for yield by backcrossing them to high yielding lines. However, if yield has low heritability, recurrent selection or other long-term breeding approaches will be needed to develop high yielding lines. Recurrent selection of diverse populations has been used in cross-pollinated crops such as maize to improve the

germplasm base (Lamkey, 1992; Weyhrich et al., 1998). An improved population can be used as a starting point for the development of improved inbred lines.

Now that efficient testing methods have been developed, high yielding lines have been identified, and high yielding populations have been developed, the next step is to estimate heritability for yield, and develop high yielding families for use in watermelon breeding. Parent-offspring regression has been one method used to obtain estimates of narrow-sense heritability in plants and animals. The relationship between parent and offspring is of specific interest in breeding programs where the direct resemblance is usefully applied to programs that include either mass selection or mass selection indices (Baker, 1986; Lynch and Walsh, 1998).

The objectives of this research are to: develop high yielding populations; measure the narrow-sense heritability of yield using the two populations on a single plant basis; and release the highest yielding families to industry for use in cultivar development.

## Materials and Methods

*Germplasm and Crosses.* In this experiment, two watermelon populations, North Carolina High Yielding Watermelon1 (NCHYW1) and North Carolina High Yielding Watermelon2 (NCHYW2) were developed using a diverse set of cultivars. Cultivars were divided in to two sets to reduce the number of crosses to be made. Cultivars that were chosen to develop the populations are high yielding obsolete cultivars and elite cultivars that have good flesh color, high total soluble solids, and are disease resistant (Table 1). Six cultivars were present in each set and they were crossed in half-diallel with total of 15 crosses. In

NCHYW2, only 4 cultivars were used in second set. An overview of the method used to develop the populations is presented in Table 2. High yielding cultivars were crossed with elite cultivars to develop $F_1$ individuals. $F_1$ individuals were self- and sib-pollinated in greenhouse to obtain $S_0$ seeds that represent parents for NCHYW1 population. To develop NCHYW2, $F_1$ individuals were planted in field as a single plant hills and were allowed to open pollinate to obtain $S_0$ seeds. Both populations were handled in similar way to determine narrow-sense heritability using parent-offspring regression. Three hundred-twenty $S_0$ seeds were picked randomly as parents and planted in field as single plant hills and measured for yield. Seeds were harvested from one fruit from each parent as $S_{0:1}$ progenies (offspring). $S_0$ plants were spaced 3.05 m apart. In another study, it was observed that the rate of outcrossing was 4% when plants were spaced 3.05 m apart in watermelon (Kumar, 2009). Thus, $S_{0:1}$ seeds were considered as a result of self-pollination in $S_0$ plants, since self-pollinated crop too experience low level of crossing (Fujita et al., 1997; Lesley, 1924).

*Cultural practices.* Field rows were direct seeded on raised, shaped beds on 3.1 m centers. Field rows were made up with drip tubing and covered with black polyethylene mulch. The experiment was conducted using horticultural practices recommended by the North Carolina Extension Service (Sanders, 2004). Soil type at Clinton was an Orangeburg loamy sand (fine-loamy, kaolintic, thermic Type, Kandiudults), and a Norfolk sandy loam (fine-loamy, kaolinitic, thermic Typic Kandiudults) at Kinston. Field preparation at Clinton included the soil incorporation of a 10.0-8.3-4.4 (N-P-K) fertilizer applied at 561 kg $ha^{-1}$. Fertilizer application for the remainder of the growing season consisted of 224 kg $ha^{-1}$ of

13.5-0-19.8 and 112 kg $ha^{-1}$ of calcium along with 15.5-0-0. Kinston field preparation included soil incorporation of a 10-16.6-8.8 fertilizer applied at 336 kg $ha^{-1}$ and the fumigant Telone C-17 (1, 3-Dichloropene + Chloropicrin) applied at a rate of 60 $L.ha^{-1}$.

Each parent plant was manually trained each week in a spiral by turning all the vines in a clockwise circle around the crown until fruit set began (Gusmini and Wehner, 2007). Plants growing in the offspring plots (6 plants per hill) were trained to turn the vines back before they intermingled with vines in other plots. Plant training allowed accurate identification of each fruit and plot and avoided duplication or misclassification of parents, offspring, and families. No disease problems were observed. Fruit were harvested when more than 90% of fruit were ripe. Fruit were determined to be ripe by looking for a dried tendril nearest the fruit, a light colored ground spot, and a dull sound of the fruit when thumped (Maynard, 2001).

*Locations.* The field tests to estimate narrow-sense heritability, broad-sense heritability, and realized heritability, and genotypic and phenotypic correlations were conducted in the summers of 2006 through 2008 at two locations: the Horticultural Crops Research Station in Clinton, and Cunningham Research Station in Kinston, North Carolina.

*Parental evaluation.* A large number (320) of $S_0$ parents were grown as single plant hills in summer of 2006 for NCHYW1 and 2007 for NCHYW2 at Clinton. There were 16 rows with 20 hills each. Each row was 65 m long with hills spaced at 1.22 m apart with 1.82 alleys between hills. The fields had raised rectangular shaped beds on 3.1 m centers. Seeds were

extracted from one fruit from each parent plant to be used as offspring ($S_{0:1}$ progenies) in following year. Seeds were washed, dried and packeted.

*Offspring evaluation.* The 240 $S_{0:1}$ progenies with enough seeds for progeny testing were selected at random to be planted as offspring in summer of 2007 for NCHYW1 and 2008 for NCHYW2 population at Clinton and Kinston. Progeny were randomized with one replication at each location. Thus location and replication has been used interchangeably in this study. Each $S_{0:1}$ progeny was planted to a density of 6 hills (plants) per plot. Plots were 3.7 m long, with 0.6 m between hills, and 2.5-m alleys at end of each plot (Fig. 1). Field was consisted of 24 blocks (rows) of 10 plots each.

In parent-offspring heritability estimation, the unit of selection is determined in the parental generation. A parental phenotype can be based on measurement of single plants. The offspring phenotype can be based on a family of plants as was used in this experiment. Heritability was determined for total fruit yield (Mg/ha), marketable fruit yield (Mg/ha), total fruit number per hectare, fruit size (kg), and percent culls. Weight of fruit was measured by placing each fruit on weighing machine and approximated to nearest pound and converted to kilogram before analysis. Percent culls were calculated as percentage of cull fruit weight out of total fruit weight. All crooked-necked, bottle-necked, undersized, and deformed fruit were recorded as cull fruit.

*Narrow-sense heritability.* Estimates of heritability of yield traits in each population were made by regressing the mean $S_{0:1}$ family values on their $S_0$ parental values (Table 5). For this study, the inbreeding coefficient of the $S_0$ population was assumed to be zero as was

the case for a randomly mated population. In the case of F=0 in the $S_0$ generation where two equally frequent alleles exist, the single locus covariance is Cov $S_0/S_{0:1} = \sigma^2_A + (1/2)\ \sigma^2_D$ (Holland et al., 2003; Nyquist, 1991). This differs from the formulation given by Frey and Horner (1957), where Cov $S_0/S_{0:1}$ was equated to $\sigma^2_A + (1/4)\ \sigma^2_D$ ignoring epistasis. Smith and Kinman (1965) suggested a correction factor to account for inbreeding in such estimates, but Nyquist (1991) reported that it is incorrect. The regression coefficient or narrow-sense heritability is equal to $b_{S1:S0} = h^2_n = [\sigma^2_A + (1/2)\ \sigma^2_D + \sigma^2_{AA}]/\ \sigma^2_P$, where $\sigma^2_P$ is the phenotypic variance among $S_0$ plants (Holland et al., 2003). Estimates of narrow-sense heritability are biased upward due to dominant and epistatic genetic variances. Negative heritability estimates should be considered equal to zero according to Robinson et al., 1955. Alternatively, negative heritability estimates should be reported as such because they will avoid bias in future reviews (Dudley and Moll, 1969). The standard error of the estimated heritability was obtained by using the standard error of estimated regression slope. The t-test of the slope was used (Steel et al., 1997) to test the significance of heritability. Parents and progenies were grown in separate environments to reduce the potential bias due to correlation of genotype x environment interaction covariance between parent and offspring (Casler, 1982). Although there was independence of years, possible upward bias could occur at Clinton because both parents and progenies were grown at that location. However, they were grown in different fields, which might reduce the bias. The statistical analysis was performed using the SAS-STAT statistical package (SAS 9.1, SAS Institute, Cary (North Carolina). Offspring yield was regressed on parent yield to get the estimates of narrow sense heritability

using PROC REG procedure of SAS. Some of the parent plots did not produce any yields. Hence, only 225 and 200 $S_{0:1}$ progenies were used for regression analysis in NCHYW1 and NCHYW2, respectively. Distributions of $S_0$ and $S_{0:1}$ progenies were tested for normality using Shapiro-Wilk's statistics (Shapiro and Wilk, 1965) in PROC UNIVARIATE procedure of SAS-STAT.

*Broad-sense heritability (per-plot basis).* Broad-sense heritability was estimated as ratio of genotypic and phenotypic variance (Table 6). Variance components were calculated using *method of moments* (Milliken and Johnson, 1992) in PROC ANOVA procedure of SAS-STAT.

The following linear model was used for one trait, Y*i*:

$Y_{ijk} = \mu_i + E_{ji} + G_{ki} + \square_{ijk}$,

where $\mu_i$ is the mean effect on trait *i*, *Ej* is the effect of environment *j* on trait *i*, $G_{ki}$ is the effect of $S_{0:1}$ family *k* on trait *i*, and $\square_{ijk}$ is the experimental error effect associated with genotype *k* and environment *j* on trait *i*. Environment were treated as replication because $S_{0:1}$ families were not replicated within environment/location. Estimates of broad-sense heritability were inflated due to confounding of G x E component of variance in genotypic variance.

*Realized heritability.* There are several methods to estimate realized heritability (Nyquist, 1991). We estimated it as a ratio of observed response to selection to the observed selection differential (Table 6). The superior 10% of parents ($S_0$ individuals) were selected based on trait value. Selection differential was calculated by subtracting mean of selected

individuals in parental generation and overall parental populations mean. The difference between the performance of offspring of selected individuals and mean of all the progeny was recorded as response to selection.

*Genetic correlation and phenotypic correlation.* In addition to heritability, the genotypic and phenotypic correlations for paired traits were also estimated using multivariate restricted maximum likelihood estimation with SAS Proc MIXED (Holland, 2006) (Table 3 and Table 4). The linear model used is given under broad-sense heritability section.

Generally, '$r_g$' is defined as correlation between genetic effects for traits X and Y (Table 3). Genetic correlation ($r_g$) is calculated by Cov $G_xG_y$/ ($\sqrt{(\sigma^2G_x.\sigma^2G_y)}$, where, Cov $G_xG_y$ is the covariance between genetic effects of trait X and trait Y, $\sigma^2G_x$ is genetic variance of trait X, and $\sigma^2G_y$ is genetic variance of trait Y in $S_{0:1}$ progeny. Phenotypic correlation ($r_{ph}$) $M_{xy}$/ ($\sqrt{M_{xx}.M_{yy}}$), where $M_{xy}$ is the mean product of trait X and trait Y, and $M_{xx}$ and $M_{yy}$ are the mean squares for the traits X and Y in $S_{0:1}$ progeny. Genotypic and phenotypic correlations were also calculated by using parent-offspring relationship (Falconer and Mackay, 1996). Genetic correlation ($r_g$) is Cov $_{XY}$/ ($\sqrt{Cov_{XX}.Cov_{YY}}$), where, Cov $_{XY}$ is the 'cross covariance' for paired traits (parental value for trait X and mean offspring value for trait Y), and Cov $_{XX}$ and Cov $_{YY}$ are the offspring-parent covariance of each trait separately. The 'cross covariance' was calculated in two ways (X in parents and Y in offspring and vice versa). Two estimates were averaged to get the mean (Table 4). Phenotypic correlation ($r_{ph}$) is $M_{xy}$/

($\sqrt{M_{xx}}.M_{yy}$), where $M_{xy}$ is the mean product of trait X and trait Y, and $M_{xx}$ and $M_{yy}$ are the mean squares for the traits X and Y in parents ($S_0$) (Table 4).

*Predicted gain*. The predicted gain from selection per cycle was predicted as $h_n^2 \sqrt{\sigma^2_P}$ multiplied by the selection differential in standard deviation units k for selection intensity 10% (k= 1.76) (Hallauer and Miranda, 1988) (Table 6). Realized gains from selection were also calculated by substituting narrow-sense heritability ($h_n^2$) with realized heritability ($h_r^{2)}$ in above formula (Table 6).

*Correlated responses ($CR_Y$).* Correlated responses to gain from selection per cycle were calculated from the equation: $k\, h_x\, h_y\, r_g \sqrt{\sigma^2_P}$ (Falconer and Mackay, 1996), where $CR_Y$, the response in trait Y when selection was applied to trait X, equals multiple of k, selection differential at 10 % selection intensity; the square roots of heritability for trait X and Y; $r_g$, genetic correlation between trait X and Y based on $S_{0:1}$ progeny (Table3) and $\sqrt{\sigma^2_P}$, phenotypic standard deviation of Y in parents (Table 7). Response to indirect selection was also simulated using parent-offspring data (Table 8). Superior 10 % individuals in parental generation were selected for the trait and response for indirect selection was evaluated in offspring generation of selected individual for other traits.

## Results and discussion

High yield and fruit of different size are one of the focuses of public and private watermelon breeding programs. It is desirable to produce high marketable fruit yield with minimum of cull fruit yield. Estimated phenotypic and genotypic correlations among paired traits in Table 3 and Table 4 suggest several useful associations of importance to watermelon

breeders. In this study, the genotypic and phenotypic correlations among pairs of traits were consistent across the two populations (Table 3 and Table 4). Method of estimations (i.e. estimated from $S_{0:1}$ progeny and parent-offspring relationship) of genotypic and phenotypic correlations also provided similar estimates (Table 3 and Table 4). In several comparisons, genotypic and phenotypic correlations were significant. Total fruit weight showed significant and high positive genotypic and phenotypic correlation with marketable fruit weight, suggesting that most of fruit weight harvested was marketable. There was a negative genotypic correlation between total fruit weight and percent culls in both NCHYW1 and NCHYW2 populations. Total fruit weight and marketable fruit weight also showed appreciably high positive genotypic and phenotypic correlation with fruit size (weight per fruit). This suggests that selecting for bigger fruit size would produce higher total and marketable yields. Total fruit weight showed significant positive phenotypic correlation with total fruit number in both the populations. Correlation at genetic level was moderate between these traits.

In NCHYW2, marketable fruit weight showed significant positive genotypic and phenotypic correlation with total fruit number. Genotypic and phenotypic correlation between total fruit number and fruit size was significantly negative. This demonstrates the general trend that selecting for greater number is predicted to result in smaller size. Fruit number was negatively genetically correlated with percent culls. This indicated that if more fruit were produced, most of them would be marketable, resulting in a decrease in cull fruit. Marketable fruit weight showed strong negative correlation with percent culls at both

genotypic and phenotypic level. Selection for higher marketable fruit would result in lower yield of culls based on correlation estimates. Fruit size did not show any correlation with percent culls. Both genotypic and phenotypic correlations were consistent across two populations as both the populations were developed from similar cultivars. However, these estimates may vary for other populations as genotypic correlations are a function of gene frequencies (Bohren et al., 1966). These estimates are also dependent on the environment and the breeding design used.

The distribution of $S_0$ individuals and $S_{0:1}$ family mean did not show discrete classes (Fig. 2, 3, 4, and 5). Distribution of percent culls was strongly skewed to 0 to 20 % (not shown). In NCHYW2 population, marketable fruit weight and fruit size showed normal distribution in offspring generation based on Shapiro and Wilk's test (Appendix Table 1). Total fruit weight and marketable fruit weight in parents, and fruit size in progenies had normal distribution in NCHYW1 population. The other traits in the parent and offspring generations deviated from normal distribution. Distributions showed quantitative inheritance of yield in watermelon. Heritability estimates varied across locations and populations. The heritability of a trait, regardless of the method of estimation, is not a fixed value. It varies across populations, or within or across environments. Heritability is specific to the population on which it was measured, environments targeted in the experiment, and in the type of experimental units. Estimates of additive genetic variances vary from population to population as they are function of allelic frequencies. Hence, different values of heritability are produced. Phenotypes of single family can vary across environments due to genotypic x

environment interaction; while one can estimate genotypic x environment variance from set of environments targeted; this value is likely to change in a different set of environments (Casler, 1982). The experimental units used (e.g., single plants, mean of several plants, mean of whole plot, and the plot size) will impact the magnitude of error variance (Holland et al., 2003; Nyquist, 1991).

Within populations at both the locations, relatively higher estimates of heritability occurred for population NCHYW1 at Clinton and for NCHYW2 at Kinston (Table 5). Estimates of heritability are specific to environments. NCHYW2 had higher estimates of heritability for yield traits than NCHYW1 by location and summed over both the locations. Though, both populations were developed from similar cultivars, but their method of development was different (Table 2). There was more recombination events in NCHYW2 as $F_1$ were allowed to open pollinate to obtain $S_0$ seeds. Thus both populations had different allele frequencies. Moreover, both the populations were tested in different years. Estimates of heritability are highly influenced by allele frequency and environments. Estimates of narrow-sense heritability were low (0.04 for NCHYW1, 0.12 for NCHYW2) for total fruit weight in both the populations of watermelon (Table 5). Heritability was not different from zero for NCHYW2 for total fruit weight. Similar estimates of fruit yield have been reported by Alliprandini et al. (2004) in soybean and Lippert et al. (1982) and Moon et al. (2004) in muskmelon. This indicated that small proportion of total fruit weight is controlled by additive gene action and multiple genes are involved. Limited gain can be achieved from selection in such cases. Relatively higher gain can be expected in NCHYW2 because of higher

heritability. Similarly, low estimates of heritability were also observed for marketable fruit weight, 0.06 in NCHYW1 and 0.15 in NCHYW2. Estimates of narrow-sense heritability of total fruit number were 0.04 in NCHYW1 and 0.16 in NCHYW2, respectively. Negligible gains can be made in NCHYW1 for marketable fruit weight and fruit number because narrow-sense heritability is close to zero. However, slow gains are possible in NCHYW2 by multi-environment trialing and use of replicated progeny rows. Fruit size (0.18 in NCHYW1, 0.19 in NCHYW2) had slightly higher heritability than fruit yield. Gusmini and Wehner (2007) recorded low to intermediate levels of heritability for fruit weight in their study using 6 related generations. Similar results were also reported in muskmelon (Moon et al., 2004). Percent culls had low estimates of heritability in NCHYW1 and would be treated as non-heritable. Thus, breeders should not select against cull fruit as they are purely environmental. This way time and labor for recording cull fruit can be saved. Heritability estimates may be higher due to a slight bias that results because both parents and progeny were grown at same location (Clinton), though in different years (Smalley et al., 2004). The possible bias due to environmental correlations for parent and offspring at Clinton might have been reduced because parents and progeny were tested in different fields.

Broad-sense heritability (per-plot basis) was also estimated using $S_{0:1}$ progeny data (Table 6). Estimates of broad-sense heritability were higher than narrow-sense heritability. Estimates were higher for NCHYW2 than NCHYW1. Estimates varied 0.13 to 0.21 for total fruit weight, 0.11 to 0.16 for total fruit number, and 0.15 to 0.22 for marketable fruit weight, 0.31 to 0.32 for fruit size, and 0.06 to 0.26 for percent culls for NCHYW1 and NCHYW2,

respectively. However, estimates of heritability per-plot basis were inflated due to confounding of G x E component of variance in genetic variance. Heritability on per-plot basis was higher for fruit size compared to other traits. Estimates of realized heritability were close approximation of narrow-sense heritability. Slope of regression equation estimates narrow-sense and realized heritability, so there values tend to be closer.

Predicted gain from selection was calculated by using both narrow-sense and realized heritability (Table 6). Predicted gain by direct selection at 10% selection intensity (k=1.76) was calculated for all traits over locations using narrow-sense heritability. Overall, genetic gains were higher for NCHYW2 than NCHYW1 because of higher heritability estimates for the former population. Total fruit weight can be increased by 1.94 Mg $ha^{-1}$ in NCHYW1 to 3.66 Mg $ha^{-1}$ in NCHYW2 per cycle. The progress in gain in yield, hence increase in the population mean would be 2% to 5% per selection cycle. It might take several generations to accumulate favorable genes. Similarly, genetic gains for fruit number and marketable fruit weight were lower use due to lower heritability estimates. It is potentially possible to change fruit size by 0.79 kg in NCHYW1 and 0.98 kg in NCHYW2 in one cycle of selection. Gusmini and Wehner (2007) suggested recurrent selection as effective way to improve populations for fruit weight in their study. Selection to reduce the percentage of cull fruit should not be practiced in field because they are not genetically controlled (due to negligible estimates of heritability). Realized gains from selection were similar to predicted gain (Table 6).

Predicted response for trait Y based on trait X is presented in Table 7. Marketable fruit weight and fruit size as a selection criteria produced best predicted response in total fruit weight in NCHYW1 (2.31 Mg $ha^{-1}$ and 2.72 Mg $ha^{-1}$, respectively compared to 1.94 Mg $ha^{-1}$ by direct selection). High genotypic correlation of total fruit weight with marketable fruit weight and fruit size is the reason behind higher predicted response (Table 3). However, in NCHYW2, only marketable fruit weight as a selection criterion produced higher total fruit weight yield (4.10 Mg $ha^{-1}$ vs. 3.66 Mg $ha^{-1}$). Predicted responses by indirect selection did not produce better gains over direct selection using other traits as selection criteria for yield. This is because of low to intermediate level of genetic correlation between total fruit weight and other traits. Simulated response to indirect selection was also calculated by selecting 10% superior parental individuals and evaluated in offspring (Table 8). Values were higher than based on predicted response for indirect selection which was calculated from correlated response equation. In NCHYW1, total fruit weight as selection criterion produced higher marketable weight whereas in NCHYW2, total fruit number as a selection criterion yielded more total and marketable weight.

## Conclusions

Genotypic and phenotypic correlations among traits estimated in this study give an indication of characters that may be useful in selection (Johnson et al., 1955). This also provides valuable information for reducing the number of traits to be evaluated in a watermelon breeding program. Genotypic correlations among the traits for which selection is practiced may have important implications in breeding procedures. Total fruit weight and

marketable fruit weight are highly positively correlated, confirming the results of Gusmini and Wehner (2005). Thus the efficiency of breeding programs can be increased by measuring total fruit weight, since it will reliably predict marketable fruit weight. Total fruit weight was highly and positively correlated with fruit size. Thus fruit size could be used as an indirect selection criterion for yield. Fruit number was negatively correlated with fruit size indicating that selection for more fruit would result in smaller fruit. Total fruit weight was negatively correlated with percent culls that were advantageous to breeders because he would not have to spend more time for counting culls. Simulated response to indirect selection produced higher total and marketable weight when total fruit number was used as selection criterion in NCHYW2.

Heritability estimates provide guidelines to plan an effective breeding scheme for the trait. Parent-offspring regression has been used extensively in animal and plant breeding, based on the fact that traits are heritable from parent to offspring (Smalley et al., 2004). Environment, genetic variation, and precision in measurement all affect the heritability estimates. The results of this study confirm that watermelon yield is a low heritability trait. This indicated that additive gene action played minor role in controlling the expression of measured traits. These results are in agreement with the findings of Lippert et al. (1982), Moon et al. (2004), Munshi and Verma (1998), in melon, Doijode and Sulladmath (1985), and Yavasani (1997) in pumpkin. Watermelon belongs to same family as melon, gourd, and pumpkin and so it is interesting that the results were similar. Based on parent-offspring regression estimates for yield, selection among parents would not be effective in NCHYW1,

and gains from selection would be low in NCHYW2 population. A breeding scheme that would allow greater recombination would be more efficient. Recurrent selection for high yield would be more appropriate, though it would take more generations to produce useful lines. Fruit size had higher heritability than other traits and was consistent across populations. It should be possible to change the fruit size by recurrent selection (Gusmini and Wehner, 2007). Low to intermediate level of heritability and masking of genotypic value by environment would require multi-environment trials and use of replicated progeny rows. At the same time, selection for qualitative traits (TSS, flesh color, fruit shape) should also be practiced. These heritability estimates are based on single plant basis and are usually lower than based on plot basis (Smalley et al., 2007). Heritability estimates using per-plot basis were higher than narrow-sense heritability. Realized heritability estimate were close approximation of narrow-sense heritability. Other questions that will need to be answered in future studies are the importance of genotype x environment interaction for yield in watermelon.

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Table 1. Cultivars used to develop NCHYW1 and NCHYW2 populations[z].

| Cultivar | Source | Year of release | Trait |
|---|---|---|---|
| [y]*Set 1* | | | |
| Calhoun Gray | Louisiana State Univ. | 1965 | Disease resistant |
| Dixielee | Univ. of Florida | 1979 | Red flesh, high sugar |
| Mountain Hoosier | Seminis | 1930[x] | High yield |
| Big Crimson | Seminis | -[v] | High yield |
| Starbrite | Seminis | 1990 | High yield |
| Legacy | Syngenta | - | Fruit type |
| *Set 2* | | | |
| Red-N-Sweet | Louisiana State Univ. | 1987 | Red flesh, high sugar |
| Big Crimson | Seminis | - | High yield |
| Sangria ($F_1$) | Syngenta | 1985 | Fruit type |
| Early Arizona | Seminis | - | High yield |
| Charleston Gray[u] | USDA | 1954 | Disease resistant |
| Star-N-Stripes[u] | Seminis | - | High yield |

[z] Cultivars in each set were crossed in half-diallel. [6(6-1)]/2 = 15 crosses each set.
[y] Cultivars were divided in to 2 sets to reduce the number of crosses to be made in the greenhouse.
[x] Cultivated since 1930. Year of release not documented.
[v] Not available.
[u] Cultivars not used to develop NCHYW1 but used in NCHYW2.

Table 2. Overview of population development and parent and offspring evaluation.

| Year | NCHYW1 | NCHYW2 |
|---|---|---|
| Summer'05 | GH[z]: Cultivars crossed to make $F_1$s | GH: Cultivars crossed to make $F_1$s |
| Summer'06 | GH: $F_1$ selfed or sibbed to get $F_2$ ($S_0$)[y] | --- |
| Summer'06 | 320 $S_0$ (parents) tested: Yield measured and $S_{0:1}$ seed harvested | Field: $F_1$ were let open pollinate to get $F_2$ ($S_0$) |
| Summer'07 | 240 $S_{0:1}$[x] progeny (offspring) planted and yield measured | 320 $F_2$ ($S_0$) tested: Yield measured and $S_{0:1}$ seed harvested |
| Summer'08 | ---- | 240 $S_{0:1}$ progeny (offspring) yield measured |

[z] GH= Greenhouse.
[y] $S_0$= Parental generation.
[x] $S_{0:1}$= Offspring generation.

Table 3. Genotypic (right side of diagonal) and phenotypic correlations (left side of diagonal) between paired traits for two populations. Genotypic correlations are based on genetic covariance of paired traits and genetic variance of each trait in offspring ($S_{0:1}$) generation. Phenotypic correlations are calculated directly from offspring generation

| *Correlations for NCHYW1* | | | | | |
|---|---|---|---|---|---|
| Trait | Total fruit wt. | Total fruit No. | Mark. Fruit wt. | Fruit size | Percent culls |
| Total fruit wt. | | -0.02$^{NS}$ | 0.97$^{***}$ | 0.66$^{***}$ | -0.38 |
| Total fruit No. | 0.52$^{***}$ | | 0.03$^{NS}$ | -0.74 | -0.21 |
| Mark. Fruit wt. | 0.91$^{***}$ | 0.40$^{***}$ | | 0.54$^{***}$ | -0.60$^{***}$ |
| Fruit size | 0.37$^{***}$ | -0.49 | 0.32$^{***}$ | | 0.14 |
| Percent culls | -0.13$^{**}$ | -0.08 | -0.49$^{***}$ | 0.10 | |
| *Correlations for NCHYW2* | | | | | |
| Total fruit wt. | | 0.31 | 1.00$^{***}$ | 0.53$^{*}$ | -1.34 |
| Total fruit No. | 0.64$^{***}$ | | 0.40$^{***}$ | -0.67$^{**}$ | -0.77$^{***}$ |
| Mark. Fruit wt. | 0.97$^{***}$ | 0.60$^{***}$ | | 0.44 | -1.30 |
| Fruit size | 0.31$^{***}$ | -0.46$^{***}$ | 0.30 | | 0.23 |
| Percent culls | -0.03 | 0.07$^{***}$ | -0.23$^{***}$ | 0.04 | |

$^{*}$, $^{**}$, $^{***}$ Significant at $P \leq 0.05$, 0.01, or 0.001, respectively
NS, not significant at $P \leq 0.05$

Table 4. Genotypic (right side of diagonal) and phenotypic correlations (left side of diagonal) between paired traits for two populations. Genotypic correlations are based on parent-offspring cross covariance for paired traits and parent- offspring covariance of for each trait. Phenotypic correlation s for paired traits was calculated directly from parental measurements.

| *Correlations for NCHYW1* | | | | | |
|---|---|---|---|---|---|
| Trait | Total fruit wt. | Total fruit No. | Mark. Fruit wt. | Fruit size | Percent culls |
| Total fruit wt. | | $-0.28^{NS}$ | $1.02^{***}$ | $0.72^{NS}$ | $-1.48^{*}$ |
| Total fruit No. | $0.75^{***}$ | | $0.02^{NS}$ | $-0.97^{***}$ | $-0.65^{NS}$ |
| Mark. Fruit wt. | $0.96^{***}$ | $0.68^{***}$ | | $0.49^{NS}$ | $-1.38^{***}$ |
| Fruit size | $0.31^{***}$ | $-0.23^{***}$ | $0.32^{***}$ | | $-0.07^{NS}$ |
| Percent culls | $0.01^{NS}$ | $0.27^{***}$ | $-0.21^{**}$ | $0.04^{NS}$ | |
| *Correlations for NCHYW2* | | | | | |
| Total fruit wt. | | 0.51* | $0.97^{***}$ | $0.11^{NS}$ | $-0.12^{NS}$ |
| Total fruit No. | $0.60^{***}$ | | $0.46^{*}$ | $-0.77^{***}$ | $0.11^{NS}$ |
| Mark. Fruit wt. | $0.92^{***}$ | $0.45^{***}$ | | $0.07^{NS}$ | $-0.36^{NS}$ |
| Fruit size | $0.31^{***}$ | $-0.48^{***}$ | 0.22** | | $0.08^{NS}$ |
| Percent culls | 0.15* | $0.42^{***}$ | $-0.16^{*}$ | $0.06^{NS}$ | |

$^{*}$, $^{**}$, $^{***}$ Significant at $P \leq 0.05$, 0.01, or 0.001, respectively
NS, not significant at $P \leq 0.05$

Table 5. The estimation of heritability of yield in two watermelon populations: NCHYW1and NCHYW2 using slope estimates in linear regression of offspring mean on one parent.

| Regression of Y on X | Sample size | Estimates of heritability | P-value |
|---|---|---|---|
| *Kinston* | | | |
| NCHYW1 | | | |
| Total fruit wt. | 225 | 0.01±0.03 | 0.7584 |
| Total fruit no. | 225 | 0.04±0.03 | 0.2803 |
| Mark. fruit wt | 225 | 0.04±0.03 | 0.2207 |
| Fruit size | 225 | 0.19±0.04 | <0.0001 |
| Percent culls | 225 | 0.06±0.05 | 0.2277 |
| NCHYW2 | | | |
| Total fruit wt. | 200 | 0.17±0.10 | 0.0995 |
| Total fruit no. | 200 | 0.19±0.06 | 0.0025 |
| Mark. fruit wt | 200 | 0.19±0.11 | 0.0780 |
| Fruit size | 200 | 0.25±0.06 | <0.0001 |
| Percent culls | 200 | 0.03±0.03 | 0.4163 |
| *Clinton* | | | |
| NCHYW1 | | | |
| Total fruit wt. | 225 | 0.06±0.03 | 0.0241 |
| Total fruit no. | 225 | 0.05±0.02 | 0.0348 |
| Mark. fruit wt | 225 | 0.09±0.03 | 0.0027 |
| Fruit size | 225 | 0.17±0.04 | 0.0002 |
| Percent culls | 225 | -0.01±0.05 | 0.8099 |
| NCHYW2 | | | |
| Total fruit wt. | 200 | 0.07±0.05 | 0.1226 |
| Total fruit no. | 200 | 0.13±0.05 | 0.0068 |
| Mark. fruit wt | 200 | 0.10±0.05 | 0.0428 |
| Fruit size | 200 | 0.13±0.03 | 0.0002 |
| Percent culls | 200 | 0.15±0.04 | 0.0004 |

Table 5 Continued

| Regression of Y on X | Sample size | Estimates of heritability | P-value |
|---|---|---|---|
| *Overall* | | | |
| NCHYW1 | | | |
| Total fruit wt. | 225 | 0.04±0.02 | 0.1024 |
| Total fruit no. | 225 | 0.04±0.02 | 0.0558 |
| Mark. fruit wt. | 225 | 0.06±0.02 | 0.0075 |
| Fruit size | 225 | 0.18±0.03 | 0.0001 |
| Percent culls | 225 | 0.02±0.04 | 0.5334 |
| NCHYW2 | | | |
| Total fruit wt. | 200 | 0.12±0.06 | 0.0453 |
| Total fruit no. | 200 | 0.16±0.04 | 0.0001 |
| Mark. fruit wt. | 200 | 0.15±0.06 | 0.0233 |
| Fruit size | 200 | 0.19±0.04 | 0.0001 |
| Percent culls | 200 | 0.09±0.03 | 0.0011 |

Table 6. Variances, heritability estimates, and genetic gain of watermelon population NCHYW1 and NCHYW2

| | $\mu^z$ | $\sigma^2_A{}^y$ | $\sigma^2_P{}^x$ | $h^2_n{}^w$ | $h^2_b{}^v$ | $h^2_r{}^u$ | $\Delta G_P{}^t$ | $\Delta G_r{}^u$ |
|---|---|---|---|---|---|---|---|---|
| ***NCHYW1*** | | | | | | | | |
| **Total fruit wt. (Mg/ha)** | | | | | | | | |
| Kinston | 49.53 | 7.61 | 761.28 | 0.01 | - | 0.08 | 0.48 | 3.89 |
| Clinton | 44.53 | 45.68 | 761.28 | 0.06 | - | 0.06 | 2.88 | 2.95 |
| Overall | 46.48 | 30.45 | 761.28 | 0.04 | 0.13 | 0.07 | 1.94 | 3.40 |
| **Total fruit no.** | | | | | | | | |
| Kinston | 9512.49 | 1000245.16 | 25006129.00 | 0.04 | - | 0.19 | 352.04 | 1672.20 |
| Clinton | 6846.90 | 1250306.45 | 25006129.00 | 0.05 | - | -0.08 | 440.05 | -704.09 |
| Overall | 8179.69 | 1125275.81 | 25006129.00 | 0.04 | 0.11 | 0.05 | 352.04 | 440.05 |
| **Marketable fruit wt. (Mg/ha)** | | | | | | | | |
| Kinston | 43.33 | 28.88 | 722.17 | 0.04 | - | 0.08 | 1.89 | 3.78 |
| Clinton | 40.73 | 65.00 | 722.17 | 0.09 | - | 0.05 | 4.26 | 2.36 |
| Overall | 42.03 | 43.33 | 722.17 | 0.06 | 0.15 | 0.07 | 2.84 | 3.31 |
| **Fruit size (kg)** | | | | | | | | |
| Kinston | 5.90 | 1.17 | 6.17 | 0.19 | - | -0.02 | 0.83 | -0.09 |
| Clinton | 7.07 | 1.05 | 6.17 | 0.17 | - | 0.21 | 0.74 | 0.92 |
| Overall | 6.48 | 1.11 | 6.17 | 0.18 | 0.31 | 0.10 | 0.79 | 0.44 |
| **Percent culls (% by weight)** | | | | | | | | |
| Kinston | 19.27 | 26.70 | 445.06 | 0.06 | - | -0.48 | 2.23 | -17.82 |
| Clinton | 13.15 | -4.55 | 445.06 | -0.01 | - | 0.42 | -0.37 | 15.60 |
| Overall | 16.21 | 8.90 | 445.06 | 0.02 | 0.06 | 0.97 | 0.74 | 36.01 |

Table 6 Continued

| | $\mu^z$ | $\sigma^2_A{}^y$ | $\sigma^2_P{}^x$ | $h^2_n{}^w$ | $h^2_b{}^v$ | $h^2_r{}^u$ | $\Delta G_P{}^t$ | $\Delta G_r{}^u$ |
|---|---|---|---|---|---|---|---|---|
| ***NCHYW2*** | | | | | | | | |
| **Total fruit wt. (Mg/ha)** | | | | | | | | |
| Kinston | 97.14 | 51.14 | 300.80 | 0.17 | - | 0.83 | 5.19 | 25.34 |
| Clinton | 59.07 | 21.06 | 300.80 | 0.07 | - | -0.62 | 2.14 | -18.92 |
| Overall | 78.11 | 36.01 | 300.80 | 0.12 | 0.21 | 0.12 | 3.66 | 3.66 |
| **Total fruit no.** | | | | | | | | |
| Kinston | 10086.00 | 1933526.74 | 10176646.00 | 0.19 | - | 0.23 | 1066.76 | 1291.35 |
| Clinton | 9364.67 | 1322963.98 | 10176646.00 | 0.13 | - | 0.11 | 729.89 | 729.89 |
| Overall | 9725.54 | 1628263.36 | 10176646.00 | 0.16 | 0.16 | 0.23 | 898.33 | 1526.14 |
| **Marketable fruit wt. (Mg/ha)** | | | | | | | | |
| Kinston | 94.99 | 53.10 | 279.52 | 0.19 | - | 0.86 | 5.59 | 25.31 |
| Clinton | 56.39 | 27.95 | 279.52 | 0.10 | - | -0.64 | 2.94 | -18.83 |
| Overall | 75.39 | 41.93 | 279.52 | 0.15 | 0.22 | 0.16 | 4.41 | 4.71 |
| **Fruit size (kg)** | | | | | | | | |
| Kinston | 10.09 | 1.77 | 7.09 | 0.25 | - | 0.62 | 1.17 | 2.90 |
| Clinton | 6.76 | 0.92 | 7.09 | 0.13 | - | -0.19 | 0.61 | -0.89 |
| Overall | 8.43 | 1.35 | 7.09 | 0.19 | 0.32 | 0.21 | 0.89 | 0.98 |
| **Percent culls (% by weight)** | | | | | | | | |
| Kinston | 4.19 | 9.32 | 310.65 | 0.03 | - | 0.30 | 0.93 | 9.31 |
| Clinton | 7.87 | 46.60 | 310.65 | 0.15 | - | -0.16 | 4.65 | -4.96 |
| Overall | 6.03 | 27.96 | 310.65 | 0.09 | 0.26 | 0.58 | 2.79 | 18.03 |

[z] $\mu$ = Population mean of parental ($S_0$) generation.
[y] $\sigma^2_A$ = Additive variance; It is overestimated by dominance and epistatic genetic variances.
[x] $\sigma^2_P$ = Phenotypic variance of parental ($S_{0:1}$) generation.
[w] $h^2_n$ = Narrow-sense heritability.
[v] $h^2_b$ = Broad-sense heritability.
[u] $h^2_r$ = Realized heritability.
[t] $\Delta G_P$ = Predicted gain from selection at 10% selection intensity (k=1.76) = $k\, h^2_n\, \sigma_P$.
[s] $\Delta G_r$ = Realized gain from selection at 10% selection intensity (k=1.76) = $k\, h^2_r\, \sigma_P$

Table 7. Comparison of direct selection and predicted response[z] for trait Y based on selection for trait X at 10% selection intensity. Values are absolute change predicted in offspring.

| | Correlated traits (Y) | | | | |
|---|---|---|---|---|---|
| Selected trait (X) | Total fruit wt. | Total fruit no. | Mark. Fruit wt. | Fruit size | Percent culls |
| *NCHYW1* | | | | | |
| Total fruit wt. | **1.94**[y] | -7.04 | 2.25 | 0.24 | -0.4 |
| Total fruit no. | -0.04 | **352.04** | 0.07 | -0.27 | -0.22 |
| Mark. Fruit wt. | 2.31 | 12.93 | **2.84** | 0.25 | -0.77 |
| Fruit size | 2.72 | -552.62 | 2.65 | **0.79** | 0.31 |
| Percent culls | -0.52 | -333.57 | -0.98 | 0.04 | **0.74** |
| *NCHYW2* | | | | | |
| Total fruit wt. | **3.66** | 241.17 | 3.95 | 0.38 | -4.32 |
| Total fruit no. | 1.31 | **898.33** | 1.82 | -0.55 | -2.87 |
| Mark. Fruit wt. | 4.1 | 347.92 | **4.41** | 0.35 | -4.69 |
| Fruit size | 2.44 | -655.88 | 2.19 | **0.98** | 0.93 |
| Percent culls | -4.25 | -518.78 | -4.44 | 0.14 | **2.79** |

[z] Predicted response ($CR_{y:x}$): $kr_g h_y h_x \sigma_y$

[y] Values in bold indicate direct selection response for trait Y.

Table 8. Simulated response[z] to indirect selection for trait Y based on selection for trait X at 10% selection intensity.

| | Correlated traits (Y) | | | | |
|---|---|---|---|---|---|
| Selectd trait (X) | Total fruit wt. | Total fruit no. | Mark. Fruit wt. | Fruit size | Percent culls |
| *NCHYW1* | | | | | |
| Total fruit wt. | **3.97**[y] | 284.26 | 5 | 0.16 | -3.14 |
| Total fruit no. | 0.45 | **498.65** | 2 | -0.46 | -5.3 |
| Mark. Fruit wt. | 3.12 | 459.69 | **3.73** | -0.03 | -1.87 |
| Fruit size | 2.16 | -300.48 | 1.85 | **0.5** | 1.03 |
| Percent culls | 5.13 | 30.87 | 9.11 | 0.07 | **-16.4** |
| *NCHYW2* | | | | | |
| Total fruit wt. | **3.64** | 91.96 | 4.54 | 0.18 | -2.01 |
| Total fruit no. | 9.09 | **1414.36** | 7.89 | -0.29 | 2.3 |
| Mark. Fruit wt. | 3.88 | -154.69 | **4.59** | 0.38 | -2.81 |
| Fruit size | -0.21 | -1118.49 | -0.27 | **1.14** | 0.01 |
| Percent culls | -4.96 | 965.55 | -2.55 | -1.62 | **-3.56** |

[z] Simulated response for indirect selection was based on selection of top 10% parent ($S_0$) from raw data for each trait. Response is realized in offspring generation.
[y]Values in bold indicate direct selection response for trait Y.

Figure 1. Field at Clinton showing $S_{0:1}$ progeny rows with 6 plants per plot.

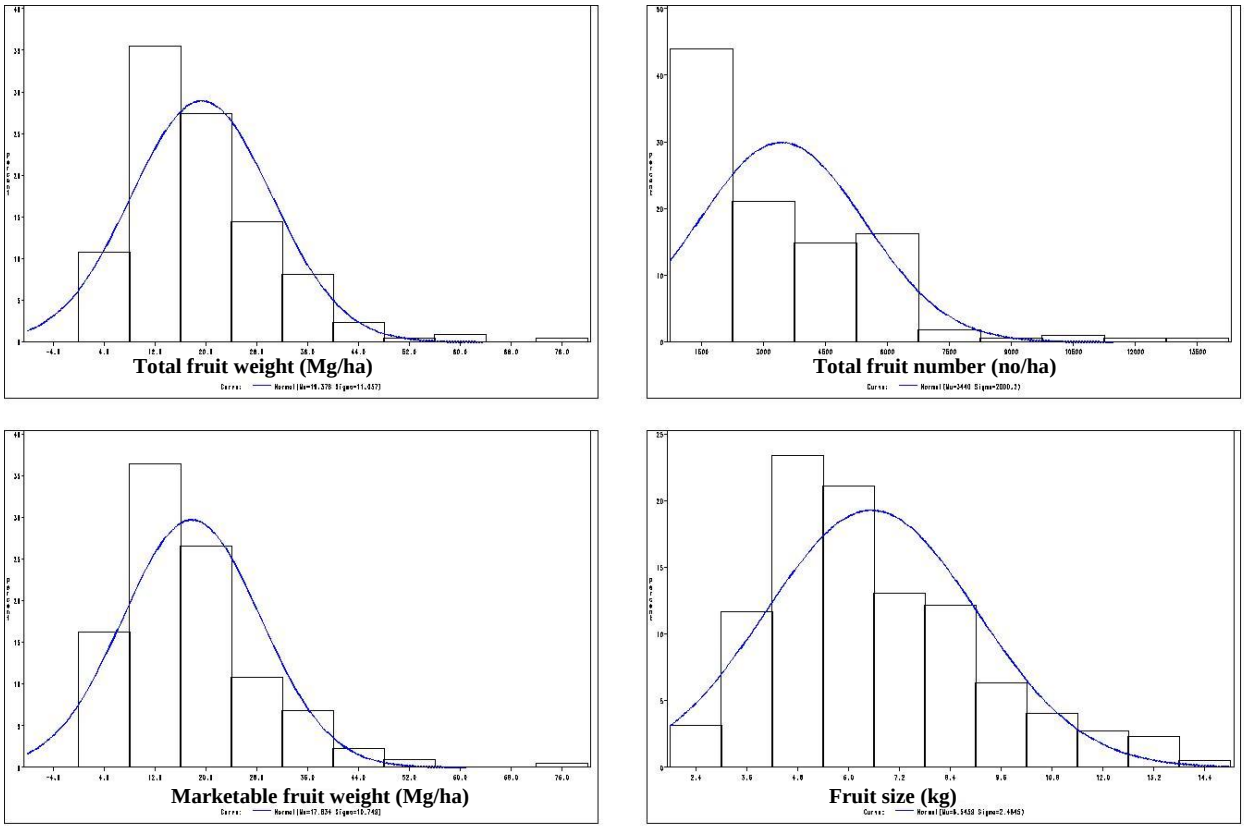


Figure 2. Distribution of frequency (with normal curve) of yield traits of $S_0$ generation in NCHYW1.

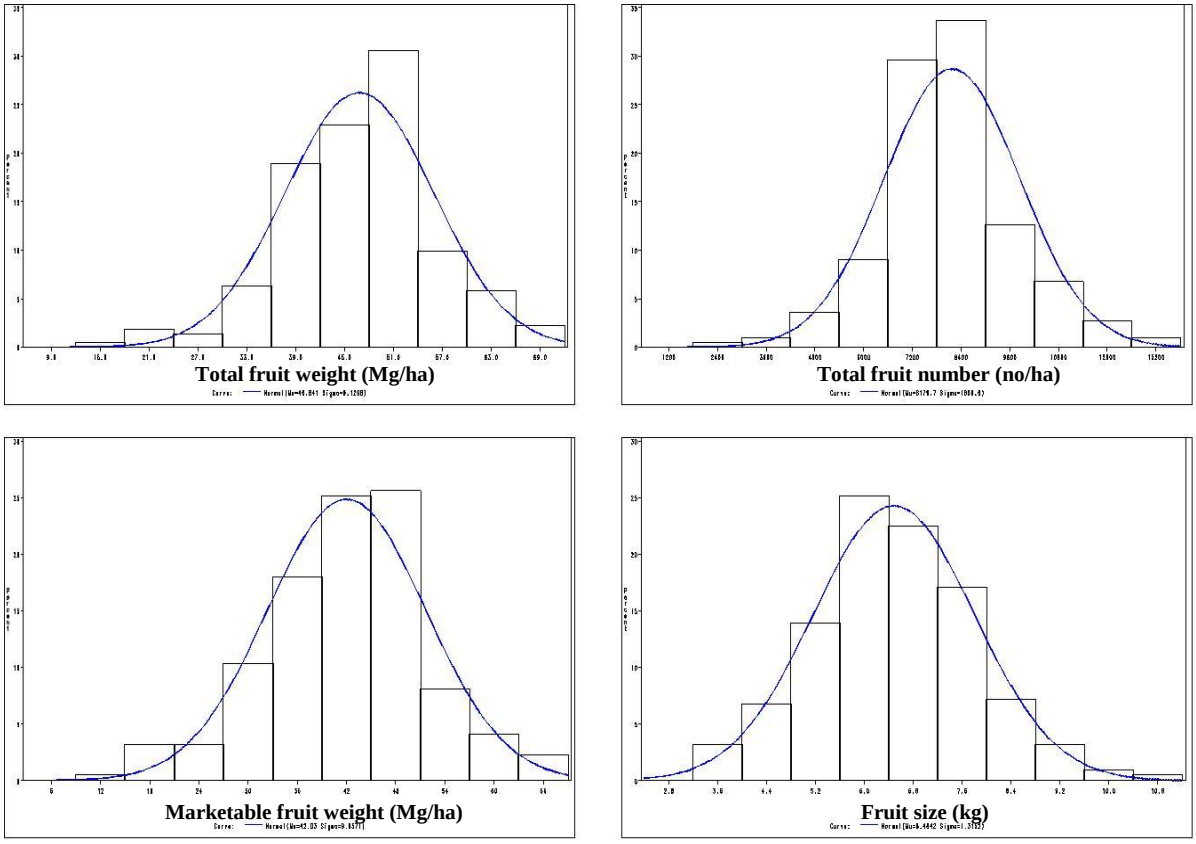


Figure 3. Distribution of frequency (with normal curve) of yield traits of $S_{0:1}$ generation in NCHYW1.

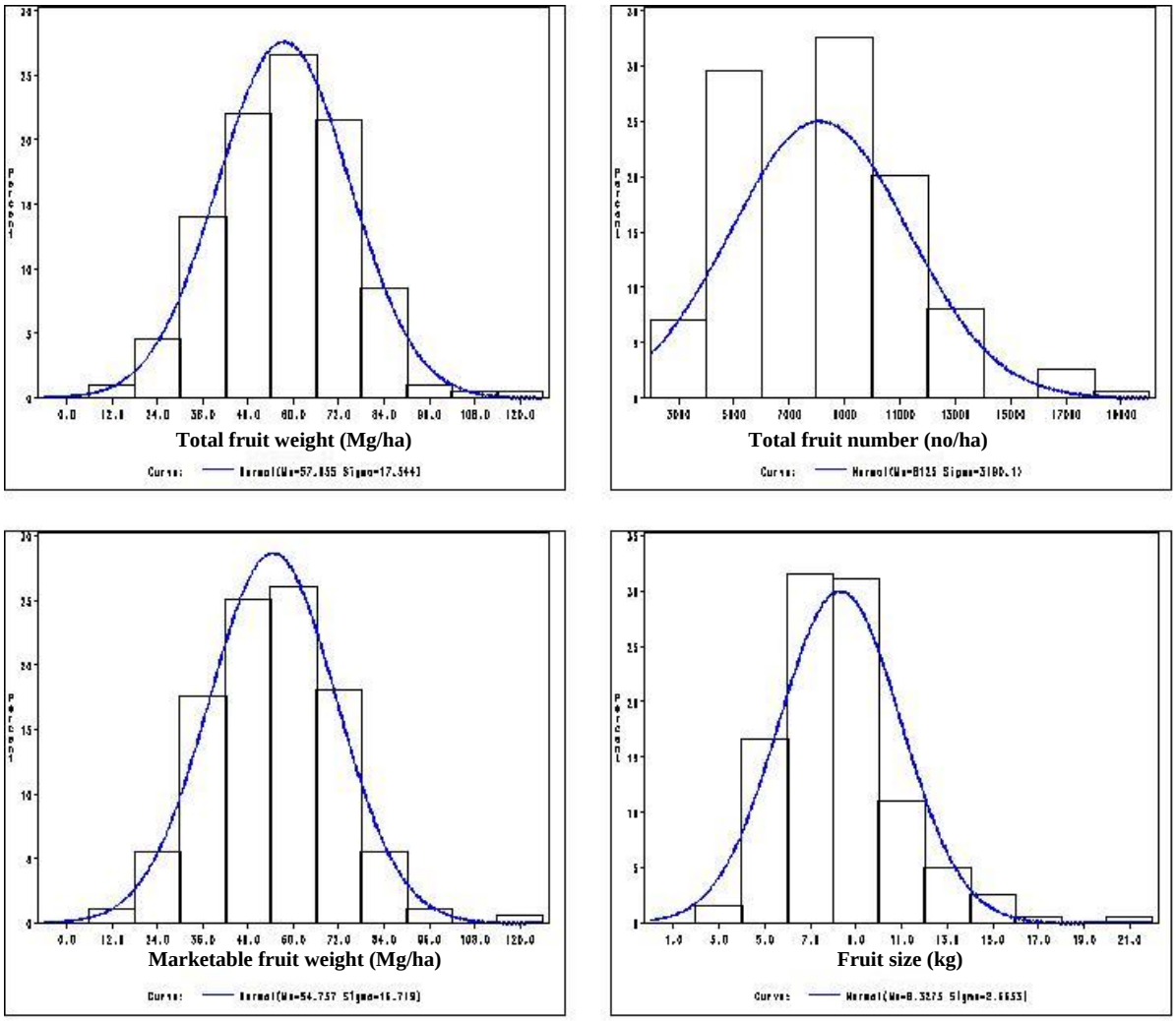


Figure.4. Distribution of frequency (with normal curve) of yield traits of $S_0$ generation in NCHYW2.

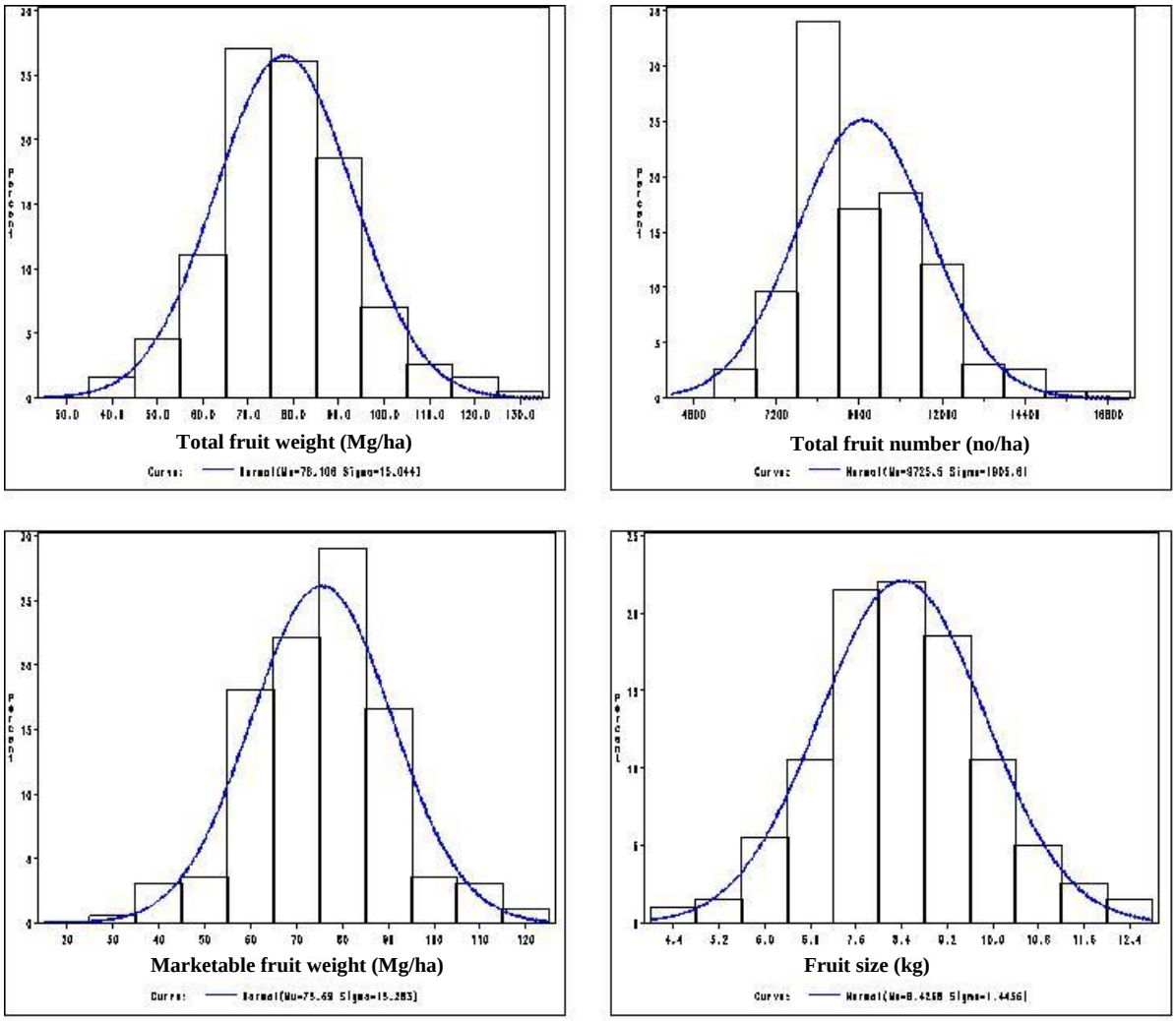


Figure 5. Distribution of frequency (with normal curve) of yield traits of $S_{0:1}$ generation in NCHYW2.

# CHAPTER TWO

# INHERITANCE OF FRUIT YIELD, RIND PATTERN AND FRUIT SHAPE USING SIX RELATED GENERATIONS IN WATERMELON

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**Inheritance of fruit yield, rind pattern and fruit shape using six related generations in watermelon**


## Abstract

The watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] has high variability for fruit size, shape, rind pattern, and flesh color. This study was designed to measure the quantitative inheritance of total fruit yield, total fruit number and fruit size and qualitative inheritance of rind phenotypes (solid dark green vs. wide stripe) and fruit shape (elongate vs. round). For each of the three families, 'Mountain Hoosier x Calsweet', 'Mountain Hoosier' x 'Minilee', and 'Early Arizona' x 'Minilee', six generations ($P_aS_1$, $P_bS_1$, $F_1$, $F_2$, $BC_1P_a$, $BC_1P_b$) were developed. Each family was tested in summer 2008 in three environments in North Carolina. Phenotypic data were analyzed with the $\chi 2$ method to test the segregation of Mendelian genes. Deviations from the expected segregation ratios based on hypothesized single incompletely dominant gene for elongate vs. round fruit shape and single dominant gene for solid dark green rind vs. wide stripe, and solid dark green vs. gray rind pattern were recorded, raising questions on the inheritance of these traits. However, inheritance of solid dark green rind vs. light (gray) rind showed duplicate dominant epistasis. The *g-1* and *g-2* genes were identified to control gray rind when in homozygous recessive form. For quantitative traits, the mean and variance were calculated. Total fruit weight, total fruit number, and fruit size showed deviation from normal distribution. $F_2$ variances were not homogeneous except for fruit number, so data were presented by location and families. Mean fruit weights were higher at Kinston than Clinton (M) and Clinton (P). Environmental

variance was larger than genetic variance for total fruit weight, fruit number, and fruit size. Broad-sense heritability was estimated to be low to intermediate in effect for total fruit weight (0.28, 0.31, and 0.57), total fruit number (0.74, 0.37, and 0.94), and fruit size (0.61, 0.29, and 0.44) at Kinston, Clinton (M), and Clinton (P), respectively. Mean estimates of narrow-sense heritability were 0.59, 0.68, and 0.43 for total fruit weight, total fruit number, and fruit size, respectively. Calculations to estimate the number of effective factors mediating fruit yield, fruit number, and fruit size suggested that these traits were multigenic. Based on these results, it is recommended to use quantitative approaches to make gain in fruit yield, fruit number, and fruit size. Recurrent selection using replicated progeny rows would be most useful for traits with low heritability and high environmental influence in watermelon.

## Introduction

The watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*] has been bred to improve yield, quality, and disease resistance, to diversify fruit and plant type (i.e., seeded vs. seedless fruit, and large vs. dwarf vines), and to adapt useful cultivars to different production areas around the world. Watermelon breeders have contributed to the development of new cultivars and to the understanding of the genetics of useful traits in this crop. In the United States, many cultivars were released in the late 1800s and early 1900s with adaptation to the western or eastern production areas: e.g., 'Angeleno', 'Chilean', and 'Kleckley Sweet' were popular in California, while 'Florida Favorite' and 'Georgia Rattlesnake' were popular in the southeastern United States (Whitaker and Jagger, 1937). The first reported genetic studies on watermelon were from the late 1930s and early 1940s and

involved the adapted inbred cultivars developed in the previous few decades of watermelon breeding. The emphasis of these investigations was on major traits, such as rind, flesh, and seed-coat colors, fruit shape and weight, and sex expression (Poole, 1944; Poole and Grimball, 1945; Poole et al., 1941; Porter, 1933, 1937; Weetman, 1937).

Yield varies among watermelon accessions, old cultivars, and modern elite cultivars (Wehner, 2008). Growers want high weight of marketable fruit per acre and to harvest one load (51 Mg) per hectare (Gusmini and Wehner, 2007; Maynard, 2001; Wehner, 2008). Many studies in watermelon are on qualitative genes and many gene lists have been published (Cucurbit Gene List Committee, 1979, 1982, and 1987; Guner and Wehner, 2004; Henderson, 1991, 1992; Rhodes and Dane, 1999; Rhodes and Zhang, 1995). However, very few quantitative trait loci studies have been published for watermelon yield and size. Gusmini and Wehner (2005a) screened a diverse set of watermelon cultivars for fruit yield, number, and fruit size. They recorded wide variation in yield and found that sources for high yield are available. Studies in the 1970s also showed varietal differences for yield and yield components in several other countries (Chhonkar, 1977; Sidhu and Brar, 1978; Thakur and Nandpuri, 1974). The next step will be to develop populations using sources of higher yield and incorporate them into a breeding program. To improve any quantitative trait, estimating variances and heritability are helpful. Previous studies, it has been reported that non-additive gene action played a role for fruit yield in watermelon (Brar and Nandpuri, 1974; Prosvirnin, 1978; Sachan and Nath, 1976). Low to intermediate levels of broad- and narrow- sense

heritability for fruit yield and fruit weight have been recorded (Brar and Nandpuri, 1974; Gusmini and Wehner, 2005a; Prasad et al., 1988; Vashishtha et al., 1983).

Watermelon breeders are interested in developing elite cultivars using novel phenotypes including different fruit shapes and rind patterns. The rind (skin) colors and patterns of watermelon fruit have been one of the objectives of breeding. Watermelon has a green rind, ranging from light to dark, from solid to striped , and intermittent to spotted (Guner and Wehner, 2003), and the inheritance of these rind types has been studied. Researchers have proposed various models of inheritance of rind pattern in watermelon cultivars. In 1937, Weetman proposed that three alleles at a single locus determine the inheritance of striped and solid green (dark and light rind). The *D* allele for dark green is dominant to the *d* allele for light green rind, and the $d^s$ allele, which produces stripes, is dominant to *d* and recessive to *D* (Weetman, 1937). This allelic series was renamed to *G*, $g^s$, and *g* by Poole in 1944 and this hypothesis has been reported in all the gene lists for watermelon (Cucurbit Gene List Committee, 1979; Cucurbit Gene List Committee, 1982; Cucurbit Gene List Committee, 1987; Guner and Wehner, 2003; Henderson, 1991; Henderson, 1992; Rhodes and Dane, 1999; Rhodes and Zhang, 1995; Wehner and Guner 2004), using the notation adopted above. Weetman (1937) also hypothesized that two loci (S, dominant for striping, and D, dominant for dark green rind) could be controlling the background color and foreground stripe pattern. However, Porter (1937) reported that dark green rind was completely dominant to light green in the two crosses involving two different dark green cultivars (‘Angeleno’ and ‘California Klondike’). He reported incomplete dominance of dark green rind in the cross ‘California

Klondike' x 'Thurmond Gray', the latter cultivar being described as gray (yellowish green). There are reports of dominance of solid dark green over gray rind (Wehner, 2008). Gusmini and Wehner (2006) also studied the inheritance of spotted (*Sp*), yellow belly (*Yb*), and intermittent rind pattern (*ins*) that can be used to develop specialty cultivars.

To date, there is no strong evidence for either of the two hypotheses proposed by Weetman for the inheritance of different shades of solid green rind and striped rind in watermelon. Nevertheless, dark green (*D*, renamed *G*) is completely dominant to light green (*d*, renamed *g*) in crosses with a light green parent. On the other hand, in crosses of dark green cultivars with gray cultivars (light green background), genes for rind color behave as incomplete dominant and produce the medium green type that is also commonly observed in watermelon. Possibly, the multi-allelic series at the *g* locus needs to include an allele for the background of the gray watermelons that is different from the *g* allele for light green rind. The inheritance of gray rind pattern has never been studied directly. Future research should be conducted to study the effect of the G allele for solid dark green rind against gray cultivars.

Watermelon fruit can be round, oval, blocky, or elongate in shape (Maynard, 2001). The genetics of fruit shape have not been widely studied, but the round, oval, and elongate phenotypes are determined by the incomplete dominance of the *O* gene. The homozygous dominant plants have elongated fruit (*OO*), the homozygous recessive fruit is round (spherical) (*oo*), and the heterozygous fruit is oval (*Oo*) (Guner and Wehner, 2003; Guner and Wehner, 2004; Poole and Grimball, 1945; Weetman, 1937; Wehner and Guner 2004). In

addition, the shape of the fruit can be predicted by the shape of the ovary shape at anthesis, thus making ovary shape a useful marker for things such as hybrid seed production (Warid and Abd el Hafez, 1976).'Mountain Hoosier' and 'Early Arizona' have dark green solid rinds with round fruit shape. These cultivars are hypothesized to have genotypes '*oo*' for round fruit shape and '*GG*' for solid dark green rind. 'Calsweet' has wide striped ($g^s g^s$) rind with elongate fruit shape (*OO*) whereas 'Minilee' has gray rind pattern (*gg*) with round shape (*oo*).

Various methods are available to study the quantitative and qualitative inheritance of traits. Variances and heritability can be estimated using parent-offspring regression (Holland et al. 2003; Nyquist, 1991), North Carolina Design I, North Carolina Design II (Comstock and Robinson, 1948), and North Carolina Design III (Comstock and Robinson, 1952). Each method has unique advantages and disadvantages. Among other methods, a design based on the measure of variance from six generations ($P_aS_1$, $P_bS_1$, $F_1$, $F_2$, $BC_1P_a$, and $BC_1P_b$) can be used to estimate environmental, additive, dominance, and phenotypic variances (Gusmini and Wehner, 2007; Tetteh, 2008). Using this strategy, $F_2$ variance estimates the total phenotypic variance. Non-segregating generations ($P_a$, $P_b$, and $F_1$) give an estimate of environmental variance (Wright, 1968).The additive variance is estimated by subtracting the sum of backcross variances from twice the phenotypic variance as an extension of single locus model under the hypothesis of absence of linkage and genetic-by-environment interactions (Warner, 1952). The estimates of broad- and narrow-sense heritability can be derived from

estimates of genotypic, additive and phenotypic variance. These generations can be used test the segregation ratios for single gene (fruit shape and rind pattern).

The objectives of these experiments were to study the inheritance and genetic variance of fruit yield, fruit shape, and rind pattern from families of 'Mountain Hoosier x Calsweet', 'Mountain Hoosier x Minilee', and 'Early Arizona' x 'Minilee' based on six related generations ($P_aS_1$, $P_bS_1$, $F_1$, $F_2$, $BC_1P_a$, and $BC_1P_b$). This study was conducted to confirm already reported genes. Heritability estimates from this study were also compared with those calculated by parent-offspring regression in first chapter.

## Materials and Methods

*Traits and crosses.* Three families were developed from 3 crosses of watermelon inbred cultivars or lines to estimate the heritability of fruit yield, rind pattern, and fruit shape (Table 1). Each of the two high yielding cultivars 'Mountain Hoosier' and 'Early Arizona' was crossed with the low yielding cultivars 'Calsweet' or 'Minilee' (Fig 1.). In this way three families were developed; 'Mountain Hoosier x Calsweet', 'Mountain Hoosier x Minilee', and 'Early Arizona' x 'Minilee'. Fourth family, 'Early Arizona x Calsweet', was not planted as it did not have enough seeds. Six generations were developed ($P_aS_1$, $P_bS_1$, $F_1$, $F_2$, $BC_1P_a$, $BC_1P_b$) for each family which were grown in the greenhouses at Horticultural Field Laboratories, North Carolina State University in Raleigh, North Carolina. Parents were self-pollinated to obtain enough seeds for making future crosses, so they were noted as $P_aS_1$ and $P_bS_1$. These parents also differed for other horticulturally important traits (Table 1). 'Mountain Hoosier' x 'Calsweet' was studied for inheritance of fruit shape and rind pattern.

‘Mountain Hoosier’ is round fruited with solid dark green rind whereas ‘Calsweet’ is elongate and has gray rind. ‘Mountain Hoosier’ x ‘Minilee’ and ‘Early Arizona’ x ‘Minilee’ were studied to determine the inheritance of solid dark green rind from ‘Mountain Hoosier’ and ‘Early Arizona’ against gray rind from ‘Minilee’.

*Cultural practices.* Seeds of the six generations for each family were sown in 72-cell polyethylene flats in the greenhouses at North Carolina State University. The artificial soilless growing medium 4P *Fafard* soilless *mix* (Conrad Fafard Incorporated, Massachusetts), was used. The medium was moistened to capacity after seeding and held in the greenhouse at constant temperature (25-30 ºC) until full emergence. The transplants were moved to an open cold frame at the field site for acclimation two weeks prior to transplanting. The seedlings were transplanted by hand at the two-true-leaf stage. Missing or damaged transplants were replaced a week after transplanting. In the field, raised beds were made up with drip irrigation tubes and covered with black polyethylene mulch. The experiment was conducted using horticultural practices recommended by the North Carolina Extension Service (Sanders, 2004). The soil types were Orangeburg loamy sand at Clinton, and a Norfolk sandy loam at Kinston. In order to keep families, generations, and plants separate for data collection, each plant was manually trained each week into a spiral shape by turning all the vines in a clockwise circle around the crown until about 70% of the plants in the field set fruit (Fig. 2). The vine training allowed easy tracing of the fruit to the plant that produced it, resulting in high accuracy.

*Fruit shape and rind pattern.* The field test was run in the summer of 2008 at two research stations: Horticultural Crops Research Station in Clinton, North Carolina, and Cunningham Research Station in Kinston, North Carolina. In this experiment, we identified locations as Kinston, Clinton (M), and Clinton (P) where M and P stands for two site names at Clinton research station. Though this was a study of Mendelian traits, and replication was not necessary over locations, families were divided into three sets (one set per location) as a precautionary measure in case of adverse environmental conditions or unpredicted disease epidemics occurs at one location. All six generations of each family were planted at each location as one set without replication. Transplants were placed in rows in the following order and number: $P_aS_1$, (10), $P_bS_1$ (10), $BC_1P_a$, (30) $BC_1P_b$ (30), $F_1$ (20), $F_2$ (100) at Clinton (M) and Clinton (P) locations and $P_aS_1$ (10), $P_bS_1$ (10), $F_1$ (20), $BC_1P_a$ (30), $BC_1P_b$ (30), $F_2$ (100) at Kinston. At Clinton, each field was 0.4 ha with eight rows 60 m long, and each family occupied four rows. At Kinston each field was 0.4 ha with six rows 85 m long and each family occupied three rows. The fields had raised shaped beds (rows) on 3.1 m centers with single hills 1.2 m apart.

We analyzed the data by family and then pooled the data over families (wherever applicable) after testing for homogeneity of variances using the O'Brien F-test (Ostle and Malone, 1988; Steel et al., 1997). O'Brien F-test was used because it is insensitive to deviation of distributions from normal. We performed segregation analysis and goodness-of-fit tests (Ramsey and Schafer, 1997) using the SAS-STAT statistical package (SAS Institute, Cary, North Carolina) and the SASGene 1.2 program (Liu et al., 1997), based on $\chi 2$ testing

of the expected segregation ratios for a single gene. All $\chi 2$ tests were performed with a 95% confidence level ($\alpha$=0.05). Names and symbols for new genes proposed herein are in conformance with gene nomenclature rules for the *Cucurbitaceae* family (Cucurbit Gene List Committee, 1982).

*Total fruit yield, Total fruit number, and fruit weight.* Distributions of the $F_2$ populations were tested for normality using Shapiro-Wilk's statistic (Shapiro and Wilk, 1965) in PROC UNIVARIATE procedure of SAS-STAT. We tested the $F_2$ data for homogeneity of variances using O'Brien F-test (Ostle and Malone, 1988; Steel et al., 1997). Since the variances were heterogeneous, we analyzed data by family and location. The variance components, phenotypic (P), environmental (E), genotypic (G) and additive (A) variances in each generation were estimated using Warner (1952) and Wright's (1968) formula:

$$\sigma^2 (P) = \sigma^2 (F_2)$$

$$\sigma^2 (G) = \sigma^2 (P) - \sigma^2 (E)$$

$$\sigma^2 (E) = \frac{\sigma^2 (P_a) + \sigma^2 (P_b) + [2\ \sigma^2 (F_1)]}{4}$$

$$\sigma^2 (A) = [2\ \sigma^2 (F_2)] - [\sigma^2 (BC_1P_a) + \sigma^2 (BC_1P_b)]$$

Narrow sense heritability estimates were calculated using the ratio of additive variance to phenotypic variance. Negative estimates for genetic variances are possible with the experimental design adopted. Negative estimates should be considered equal to zero (Robinson et al., 1955), but should be reported "in order to contribute to accumulation of knowledge, which may, in the future, be properly interpreted" (Dudley and Moll, 1969;

Gusmini and Wehner, 2007). In this experiment, we considered negative estimates equal to zero for calculation of the mean estimates over families and locations. When a negative estimate was derived from another negative estimate (e.g. narrow-sense heritability and genetic gain derived from additive variance), it was considered close to zero or omitted (Gusmini and Wehner, 2007).

A quantitative estimate for the minimum number of effective factors (Mendelian gene or quantitative trait loci ) controlling total fruit weight, total fruit number, and fruit size can be determined by using the methods of Lande (1981), Mather and Jinks (1982) and Wright (1968). However, in the present experiment we used methods of Wright (1968) and Mather and Jinks (1982).

Mather`s method: $$\frac{\left[\mu(P_b)-\mu(P_a)\right]^2}{\left[2\times\sigma^2(F_2)\right]-\left[\sigma^2(BC_1P_b)+\sigma^2(BC_1P_a)\right]}$$

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

The general assumptions for the estimation of number of effective factors are that no linkage exists between the loci involved, the effects of all loci are equal, and all alleles for increasing the value of a trait are in a single parent, there are no dominance and no epistasis.

The predicted gain from selection per cycle was predicted for selection intensities of 5%, 10% and 20% using the formula: $h_n^2 \times \sqrt{\sigma^2(P)}$ multiplied by *k*, the selection differential in

standard deviation units (Hallauer and Miranda, 1988). Statistical analysis for quantitative traits was carried out using SASQuant statistical package (Gusmini et al., 2007).

## Results

*Elongate vs. round fruit shape.* 'Mountain Hoosier' was crossed with 'Calsweet' to determine the inheritance of fruit shape in watermelon (Table 2). Fruit shape is reported to be controlled by a single incompletely dominant gene, resulting in fruit that are elongate (*OO*), oval (*Oo*), or round (*oo*) (Guner and Wehner, 2003, 2004; Poole and Grimball, 1945; Weetman, 1937). This study was conducted to validate the already reported gene by crossing 'Mountain Hoosier' (round fruit) and 'Calsweet' (elongate fruit). Our hypothesis was that elongate, oval, and round fruit shape should segregate in 1:2:1 ratio in $F_2$, oval and round should segregate in 1:1 ratio when $F_1$ were backcrossed to 'Mountain Hoosier' ($BC_1P_a$), and in 1:1 ratio (oval: elongate) when $F_1$ were backcrossed to 'Calsweet' ($BC_aP_b$). In the $F_1$ generation, fruit were mixture of oval and round. Segregation in $F_1$ indicated that one of the parents was heterozygous. According to reported gene, all $F_1$ should be oval (*Oo*). The $F_2$ individuals segregated 22:116:133 (Elongate: oval: round), and the $\chi^2$ was 96.54 (P-value=0.0001) which rejected our hypothesis and showed that data were not consistent with expected segregation ratio of 1:2:1. The fruit in $BC_1P_a$ generation segregated in 25:55 (Oval: round) with $\chi^2$ of 11.25 (P-value= 0.0008). This again confronts the expected segregation ratio of 1:1. No $\chi^2$ was reported for $BC_1P_b$ (backcross to 'Calsweet') as round fruit were observed in this generation which was not expected. Results again indicated that 'Calsweet' might be heterozygous for *O* gene. Results of this study do not support the theory of single

incompletely dominant gene controlling the fruit shape in 'Mountain Hoosier' x 'Calsweet' family. Results should be verified by testing more families for this trait. It was observed that oval fruit varied in their shape. There might be more categories that are still undefined e.g. oblong, globular and blocky.

*Solid dark green vs. wide stripe rind pattern.* A cross was made between 'Mountain Hoosier' and 'Calsweet' to determine inheritance of solid dark green rind against wide striped rind (Table 3). Solid dark green rind pattern is hypothesized to be controlled by the *G* allele which is dominant to the $g^s$ allele that produces wide striped rind. The hypothesis of this part of study was that fruit rind in $F_2$ generation should segregate in 3 (solid dark green):1(wide stripe). The ratio should be 1 (solid dark green):1(wide stripe) when $F_1$ were backcrossed to 'Calsweet'. $F_1$ generation fruit were all solid dark green (54:0). This indicated that solid dark rind pattern is inherited as single dominant gene. $F_2$ and $BC_1P_b$ ratios were observed to verify results from $F_1$ generation. However, segregation ratios from these generations did not confirm the single gene hypothesis for solid dark green rind (Guner and Wehner, 2004; Gusmini and Wehner, 2005b). The $F_2$ generation segregated in a 221:45 ratio (Solid dark green: wide stripes) with a $\chi^2$ value of 9.27 (P-value= 0.000) indicating that solid dark green rind pattern did not segregate in expected ratio of 3:1 against wide striped rind. The backcross to 'Calsweet' resulted in a 52:32 ratio between solid dark green vs. wide stripe. The $\chi^2$ value 4.76 (P-value= 0.029) did not support this 1:1 segregation ratio either. These results cannot be explained by a single dominant gene controlling the solid dark rind against wide stripe. However, these results do not corroborate the previous findings. The $g^s$

allele was proposed for all striped pattern, although there are narrow, medium, wide striped fruit occur that might not be explained in qualitative manner (Guner and Wehner, 2004). Stripes patterns (narrow, medium, and wide) might interact with solid dark green rind differently and thus an area for study.

*Solid dark green vs. gray rind pattern.* The *G* allele for solid dark green rind is dominant to *g* allele which controls gray rind pattern. The $F_2$ generation should segregate in 3:1 ratio phenotypically and backcross to recessive parent should segregate in 1:1 ratio. In 'Mountain Hoosier' x 'Minilee' and 'Early Arizona' x 'Minilee' families, $F_1$ indicated control of single dominant gene for solid dark green rind over gray rind pattern as all fruit were solid dark green (Appendix Table 2). $F_2$ plants segregated 229:20 ($\chi^2$ =38.23, P-value= 0.00) in 'Mountain Hoosier' x 'Minilee' and 239:18 ($\chi^2$ =44.39, P-value= 0.0) in 'Early Arizona' x 'Minilee'. Plants in the $BC_1P_b$ generation (backcross to 'Minilee') segregated 59:23 (Solid dark green: Gray) with $\chi^2$ of 15.80 (P-value=0.00) in 'Mountain Hoosier' x 'Minilee' and 62:24 (Solid dark green: Gray) with 16.79 (P-value=0.00) in 'Early Arizona' x 'Minilee'. Both the families showed significant distortion from expected ratio in both $F_2$ and backcross generations. The hypothesis of single gene controlling solid dark rind against gray rind was disapproved based on this study. Segregation ratios still indicated that solid dark rind is under control of some dominant genes. Different genes might be interacting epistatically to distort the 3:1 segregation ratio. The segregation ratios in the $F_2$ and backcross generations were found to be closely associated with dominant duplicate epitasis gene action (15:1). Both the families were tested for duplicate dominant epistasis using $\chi^2$ test. If that is true the $F_2$

generation should segregate as 15:1 (Solid dark green: gray), $BC_1P_b$ should segregate 3:1, and $BC_1P_a$ and $F_1$ should be all solid dark green rind. All the fruit in the $F_1$ and $BC_1P_a$ (backcross to parent with solid green rind) were solid dark green in both families, which supported our initial hypothesis (Table 4). $F_2$ segregated in 229:20 ($\chi^2$ = 1.06, P-value = 0.30) in 'Mountain Hoosier' x 'Minilee' and 239:18 ($\chi^2$ = 0.27, P-value = 0.61) in 'Early Arizona' x 'Minilee' and were in conformity with the expected segregation ratio of 15:1. Similar results were also obtained when the data was pooled over families for $F_2$ ($\chi^2$ = 1.20, P-value = 0.27). Backcrossing to recessive parent 'Minilee' resulted in expected segregation ratio of 3:1 (Solid dark green: Gray) with $\chi^2$ of 0.26 (P-value= 0.61), 0.57(P-value=0.45), and 0.79(P-value= 0.37) in 'Mountain Hoosier' x 'Minilee', 'Early Arizona' x 'Minilee', and when the data was pooled over families, respectively.

Our hypothesis testing confirmed that solid dark green rind vs. gray rind is under control of duplicate dominant epistasis gene action. We propose that two dominant genes *g-1* and *g-2* are controlling the gray rind. Either of allele of two genes can give solid dark green rind when present together or alone in dominant form (*G-1 or G-2*). When these genes are present in homozygous recessive form, they produce gray rind. *G-1_ g-2g-2_, g-1g-1G-2_,* and *G-1-G-1_G-2_* will produce solid dark green rind where as *g-1g-1g-2g-2* will produce gray rind. We propose naming new recessive genes for gray rind as *g-1* and *g-2*.

*Total fruit weight, total fruit number, and fruit size.* The data were analyzed using both Mendelian and quantitative approaches. In our experiments, total fruit weight, total fruit number, and fruit size were quantitative traits. Discrete classes were not observed within the

$F_2$ segregating population of any of the family. A test of normality (Shapiro-Wilk's test) revealed that normal distribution did not occur for total fruit weight, total fruit number, and fruit size in the $F_2$ population of any of the family. The results of the test for fruit weight are, for 'Mountain Hoosier' x 'Calsweet': W =0.94 and Pr < W of <0.0001; for 'Mountain Hoosier' x 'Minilee': W =0.95 and Pr < W of <0.0001; and for 'Early Arizona' x 'Minilee': W =0.91 and Pr < W of <0.0001. The results for total fruit number were, 'Mountain Hoosier' x 'Calsweet': W =0.86 and Pr < W of <0.0001; for 'Mountain Hoosier' x 'Minilee': W =0.82 and Pr < W of <0.0001; and for 'Early Arizona' x 'Minilee': W =0.77 and Pr < W of <0.0001. Shapiro-Wilk's test values (W) for fruit size were, 0.94 (Pr<W of <0.0001), 0.95 (Pr<W of <0.0001), and 0.93(Pr<W of <0.0001). The distributions of $F_2$ generations for these traits are presented by location and family in Fig 3, 4, and 5 in the form of box plots. This quantitative analysis involved calculation of genetic variance estimates, heritability, number of effective factors (genes controlling the trait), and predicted gain from selection.

Overall mean weights for all the generations were higher at Kinston than at Clinton (M) and Clinton (P) (data not shown). Total fruit weight of parental inbred lines at Kinston was closer to reported expected weights (Gusmini and Wehner, 2005a). Mean yield of first parent was consistently higher than second parent indicating that parental inbreds differed across families for fruit yield. However, parental inbreds showed differences for total fruit number in family 'Mountain Hoosier' x 'Calsweet' and 'Mountain Hoosier' x 'Minilee' only. Fruit size of 'Mountain Hoosier' was comparable to 'Calsweet'. Parental inbreds in other two

families (‘Mountain Hoosier’ x ‘Minilee’ and ‘Early Arizona’ x ‘Minilee’) differed for fruit size.

The estimated variances were not homogenous across locations based on O’Brien F-test (P-value= 0.0001) (Ostle and Malone, 1988; Steel et al., 1997) for total fruit weight. Families were found to be homogeneous (P-value=0.8074). Measured $F_2$ variances were homogenous for total fruit number by location (P-value=0.8758) but heterogeneous by family (P-value=0.0068). Fruit size was highly heterogeneous by both location (P-value=0.0050) and family (P-value=0.0001). Due to heterogeneity of variances, the data were analyzed by location and family. Finally, the data were also pooled by locations.

In many cases, parental variance was larger than the $F_2$ variance for total fruit weight, total fruit number, and fruit size that should not happen as parents are inbred and more uniform (Table 5). This anomaly might be due to environmental factors. Overall, the mean parental variances of the high yielding cultivars (‘Mountain Hoosier’ and Early Arizona) was higher than low yielding cultivars (‘Calsweet’ and ‘Minilee’) (210.39 vs. 128.56, respectively) for total fruit weight (Table 5). However, there were not large difference in parental variance for total fruit number and fruit size. $F_2$ variance for total fruit weight was consistent among families within locations except for Clinton (P). However, there were large differences in $F_2$ variance within families. $F_2$ variance for total fruit number was consistent for locations (mean) but variable within locations and families. For fruit size, higher mean $F_2$ variances were observed for Kinston followed by Clinton (P) and then Clinton (M). Large $F_2$ variance was indicative of a large amount of phenotypic variability in the experiment. There

was large difference in backcross variance within and among families, and within and across locations for total fruit weight (Table 5). This might lead to different estimates of additive variance and narrow-sense heritability for different families and locations. Mean backcross variance was almost consistent for total fruit number and fruit size across locations. However, there were differences in backcross variance for total fruit number within family and location. $BC_1P_a$ variance varied from 2.43 in 'Mountain Hoosier' x 'Minilee' at Clinton (M)) to 9.97 in 'Early Arizona' x 'Minilee' at Clinton (M)) and $BC_1P_b$ variance was 3.62 in 'Mountain Hoosier' x 'Calsweet' at Clinton (P)) to 28.20 in 'Mountain Hoosier' x 'Minilee' at Clinton (M)). Fruit size had heterogeneous variances. Backcross variance with first parent ($BC_aP_1$) varied 0.80 to 19.12 for 'Mountain Hoosier' x 'Minilee', 'Early Arizona' x 'Minilee' at Clinton(P), respectively and backcross variance with second parent ranged from 0.72 to 7.56 for 'Mountain Hoosier' x 'Minilee', 'Early Arizona' x 'Minilee' at Clinton(P), respectively. These differences in backcross variance account for differences in narrow-sense heritability (Gusmini and Wehner, 2007).

Overall, environmental variance was larger than genetic variance (151.82 vs. 58.50) for total fruit weight (Table 6). However, genetic variances were larger than environment across within Clinton (P) for 'Mountain Hoosier' x 'Calsweet' and 'Mountain Hoosier' x 'Minilee' families. Some of genetic variances had negative estimates. Negative estimates of variance are possible with the design adopted in this experiment (Gusmini and Wehner, 2007). Observations were recorded on single plant basis which is not a good measure to estimate variances of complex traits like yield. These traits are usually evaluated in replicated progeny

rows. Robinson et al. (1955) reported that negative estimates of variance should be considered equal to zero. However, they are reported here for accumulation of knowledge and to avoid the bias in the future reviews (Dudley and Moll, 1969, Gusmini and Wehner, 2007). Negative estimates of variances have been reported but estimates of heritability and genetic gain were omitted in this study (Gusmini and Wehner, 2007). Environmental variances were also larger than genetic variances for total fruit number and fruit size across locations and families. These data suggest a larger blurring effect of environment on genotype for total fruit yield, total fruit number, and fruit size.

Negative estimates of additive variance were recorded for total fruit weight for Kinston and Clinton (M) when pooled over families and overall mean pooled over locations (Table 6). Overall means values were deflated by extreme negative values of additive variance in each location (e.g., -397.30 at Kinston, -340.80 at Clinton (M)). Many families recorded positive estimates of additive variance especially, all families at Clinton (P). Overall mean additive variance was larger than genetic variance for total fruit number and fruit size.

Broad-sense heritability had low to intermediate level of estimates for total fruit weight at Kinston, Clinton(M), and Clinton(P) (0.28, 0.31, and 0.57, respectively) (Table 6). It ranged 0.08 to 0.71 with an overall mean of 0.39. It can be inferred that for every unit difference in total fruit weight, 39% of the difference is due to genetic variation. The remaining 61% is due to blurring of genotype by environment and experimental errors. Comparatively higher level of estimates for broad-sense heritability were recorded for total fruit number at Kinston, Clinton (M), and Clinton (P) (0.74, 0.37 and 0.94, respectively) with

an overall mean of 0.68. This indicated that major portion of variation in fruit number was due to genotype. Fruit size also showed low to intermediate level of broad-sense heritability when pooled over families at each location (0.61, 0.29, and 0.44, respectively). Similar estimates for broad-sense heritability for yield, fruit number, and fruit size have been reported in previous studies (Chhonkar, 1977; Sidhu and Brar, 1978; Vashistha et al. (1983). Narrow-sense heritability estimates were larger than broad-sense heritability for fruit yield and fruit number. The data indicates that additive components play a major role in the improvement of these traits. Estimates of heritability were much higher than estimates based on parent-offspring regression. These estimates may be overestimated as they are derived from $F_2$ generation and backcrosses which were measured on single plant basis. Measurements on single plant basis are not effective in complex traits like yield. Moreover, there may be linkage disequilibrium existing in the $F_2$ and backcross generations which might bias the estimates. Overall mean narrow-sense heritability was 0.59, 0.68, and 0.43 for total fruit weight, total fruit number, and fruit size, respectively. Gusmini and Wehner (2007) found 0.59 estimate of narrow-sense heritability for fruit size in their study.

In this study, two methods (Mather and Jinks, 1982 and Wright, 1968) were used to find the number of effective factors (Mendelian genes) that controls the total fruit weight, total fruit number, and fruit size (Table 7). Our data shows that the mean number of effective factors controlling total fruit weight was found to be 3.2. Mean value of effective factors ranged from -4.9 to 22.7. These effective factors are calculated from variance estimates and may not be very precise. A maximum number of 32 genes control yield in ‘Mountain

Hoosier' x 'Minilee' family at Clinton (M). Overall mean effective factors that controlled fruit number and fruit size were 0.5 and 2.5, respectively which were comparatively lower number. Effective factors as high as 23.6 were recorded for fruit size in family 'Mountain Hoosier' x 'Minilee' at Clinton (M). Such large variations are possible in estimates of effective factors with the experimental design adopted, still provide us data showing that yield is a quantitative trait and controlled by many genes.

Estimated gain from selection depends on family and location. They will even differ for families having common parent. These numbers may be overestimated due to inflated estimates of narrow-sense heritability. Gains were higher at lower selection intensity (Table 7), but consistent for fruit size across locations and families. Gains with negative estimated have been omitted (Gusmini and Wehner, 2007). Mean gain varied with selection intensity (16.5 Mg $ha^{-1}$, 14.2 Mg $ha^{-1}$, and 11.1 Mg $ha^{-1}$ at 5%, 10%, and 20% selection intensity, respectively). Mean gain for total fruit number was 4973, 4248, and 3380 at 5%, 10%, and 20% selection intensity, respectively which were very high. Mean gains were 2.0 kg, 1.7 kg, and 1.4 kg for fruit size at 5%, 10%, and 20% selection intensity.

## Discussion

Segregation ratios of 'Mountain Hoosier' (round) x 'Calsweet' (elongate) family did not support the hypothesis of single incompletely dominant gene controlling fruit shape. This is possible because earlier reports might be based on crosses made of different parents. 'Calsweet' fruit are more oblong than elongate. Shapes may vary small elongate to large elongate, small round to large round, blocky, oblong, and globular. It is worthwhile to study

fruit shape quantitatively by taking length: diameter ratio. This would give an opportunity to properly interpret fruit shape. Results should be verified over multiple families. 'Mountain Hoosier' x 'Calsweet' was also studied to validate the previous hypothesis of complete dominance of solid dark green rind over wide striped rind (Cucurbit gene list committee, 1982, 1987; Guner and Wehner, 2003, 2004). However, segregation ratios did not support the hypothesis. The previous studies reported that the *G* allele is for dark green and is dominant to the $g^s$ allele, which produces stripes. The *G* allele is for dark green and the $g^s$ represent all stripe patterns. Future studies might be concentrated on making crosses between solid dark green fruit with fruit of different stripe width.

Two families, 'Mountain Hoosier' x 'Minilee' and 'Early Arizona' x 'Minilee' were made to confirm the complete dominance of solid dark green rind over gray rind. This study disapproves the single gene hypothesis. However, this study demonstrates that solid dark green rind is inherited in duplicate dominant epistasis fashion against gray rind. The *g-1* and *g-2* genes were named to control gray rind in homozygous recessive form. The genotype of gray rind fruited watermelon would be *g-1g-1g-2g-2*.

Gusmini and Wehner (2005a) showed that genetic variation exists in watermelon cultivars for yield, both for fruit weight and number. It is possible to improve yield by plant breeding. Based on the results of our experiment, it should be possible to increase watermelon fruit yield and number and change the fruit size. Low to intermediate levels of narrow-sense heritability were reported in this study indicating that yield traits has low level of additive gene action and environment has a blurring effect on the genotype. Recurrent

selection is usually used for population improvement to accumulate the effective factors in watermelon. Use of large intercrossing blocks would be required due to large number of plants. Large environmental variation and low to intermediate level of heritability may require progeny testing using self-pollination of half-sib families and trialing in multiple locations using replicated progeny rows.

Estimates of heritability in this study were appreciably higher than estimates of heritability based on parent-offspring relationship in chapter 1 of this dissertation. Estimates of narrow-sense heritability were more reliable based on parent-offspring regression and heritability based on per-plot basis obtained from offspring generations as observed in chapter 1. We do not expect heritability of complex traits like yield to be as high as 0.91 which was observed in this study. Potential bias is possible because estimates of variance and heritability were based on single-plant basis of six-related generations in this study. The parent, $F_2$, and backcross generations were used in this study which was essentially based on single plant measurements. Gusmini and Wehner (2007) also observed inflated estimates of heritability for fruit size based on single plant in their study conducted by using six related generations. Complex traits like yield are evaluated using replicated yield trials as these are highly influenced by environment (Casler, 1982; Nyquist, 1991). The estimates based on single plant basis are very prone to environmental bias and human error, thus can give biased estimates. The estimates of heritability in this chapter 1 were based on breeding value of parental generation that was determined using family rows. Thus results were more reliable and free from bias due to environment and human error while recording data. It is

recommended that estimates of heritability and variance should be conducted based on progeny testing. Another reason that might have contributed to bias in estimates of heritability is that vines were trained in spiral. There is possibility that plants might have lost female flowers in that process which are source of yield. Results of natural outcrossing study (chapter 3) were not applicable to this study since controlled crosses were made in the greenhouse.

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Table 1. Crosses and traits analyzed for qualitative inheritance of phenotypic traits in watermelon fruit during summer 2008 in Clinton and Kinston, North Carolina. [z]

| | Trait of interest | |
|---|---|---|
| Cross (or Family) | Phenotype | Gene |
| 'Mountain Hoosier' x 'Calsweet' | Elongate fruit shape | *O* |
| | Solid dark green rind | *G* |
| 'Mountain Hoosier' x 'Minilee' | Gray rind | *g* |
| 'Early Arizona' x 'Minilee' | Gray rind | *g* |

[z] Six generations ($P_aS_1$, $P_bS_1$, $F_1$, $F_2$, $BC_1P_a$, $BC_1P_b$) for each family were developed using the greenhouses at North Carolina State University in Raleigh, North Carolina. All crosses were also analyzed for yield.

Table 2. Single locus goodness-of-fit test for fruit shape in watermelon.[z]

| Generation | Total | Elongate[y] | Oval[x] | Round[w] | Expected[v] | $\chi^2$ | df | P-value |
|---|---|---|---|---|---|---|---|---|
| 'Mountain Hoosier' x 'Calsweet' | | | | | | | | |
| $P_aS_1$[u] | 29 | 0 | 0 | 29 | | | | |
| $P_bS_1$[t] | 12 | 12 | 0 | 0 | | | | |
| $F_1$ | 53 | 0 | 37 | 16 | | | | |
| $F_2$ | 271 | 22 | 116 | 133 | 1(E):2(B):1(R) | 96.54 | 2 | 0.0001 |
| $BC_1P_a$ | 80 | 0 | 25 | 55 | 1(B):1(R) | 11.25 | 1 | 0.0008 |
| $BC_1P_b$ | 83 | 20 | 45 | 18 | 1(E):1(B) | -[s] | - | - |

[z] Data are ratings from family 'Mountain Hoosier' x 'Calsweet' of *Citrullus lanatus var. lanatus* from three locations viz. Kinston, Clinton (M), and Clinton (P).
[y] Elongate shape is supposed to be controlled by single dominant gene *O*.
[x] Oval is supposed to be heterozygous (*Oo*)
[w] Round is supposed to be homozygous for recessive allele (*oo*)
[v] Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation
[u] $P_a$ was carrier of recessive allele (Round fruit)
[t] $P_b$ was carrier of dominant allele (Elongate fruit)
[s] No $\chi^2$ value is reported as round fruit has no expected ratio

Table 3. Single locus goodness-of-fit test for rind pattern in watermelon.[z]

| Generation | Total | Solid dark[y] | Wide stripe[x] | Expected[w] | $\chi^2$ | df | P-value |
|---|---|---|---|---|---|---|---|
| 'Mountain Hoosier' x 'Calsweet' | | | | | | | |
| $P_aS_1$[v] | 29 | 29 | 0 | | | | |
| $P_bS_1$[u] | 12 | 0 | 12 | | | | |
| $F_1$ | 54 | 54 | 0 | | | | |
| $F_2$ | 266 | 221 | 45 | 3:1 | 9.27 | 1 | 0.000 |
| $BC_1P_a$ | 81 | 81 | 0 | | | | |
| $BC_1P_b$ | 84 | 52 | 32 | 1:1 | 4.76 | 1 | 0.029 |

[z] Data are ratings from family 'Mountain Hoosier' x 'Calsweet' of *Citrullus lanatus var. lanatus* from three locations viz. Kinston, Clinton (M), and Clinton (P)
[y] Solid dark was the standard rind pattern
[x] Wide stripe was the mutant rind pattern
[w] Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation
[v] $P_a$ was carrier of dominant allele (solid dark green)
[u] $P_b$ was carrier of dominant allele (wide stripe)

Table 4. Goodness-of-fit test for duplicate dominant epistasis for rind pattern in watermelon.[z]

| Generation | Total | Solid dark[y] | Gray[x] | Expected[w] | $\chi^2$ | df | P-value |
|---|---|---|---|---|---|---|---|
| 'Mountain Hoosier' x 'Minilee' | | | | | | | |
| $P_aS_1$[v] | 27 | 27 | 0 | | | | |
| $P_bS_1$[u] | 26 | 0 | 26 | | | | |
| $F_1$ | 58 | 58 | 0 | | | | |
| $F_2$ | 249 | 229 | 20 | 15:1 | 1.06 | 1 | 0.30 |
| $BC_1P_a$ | 84 | 84 | 0 | 1:0 | | | |
| $BC_1P_b$ | 82 | 59 | 23 | 3:1 | 0.26 | 1 | 0.61 |
| 'Early Arizona' x 'Minilee' | | | | | | | |
| $P_aS_1$[v] | 26 | 26 | 0 | | | | |
| $P_bS_1$[u] | 28 | 0 | 28 | | | | |
| $F_1$ | 56 | 56 | 0 | | | | |
| $F_2$ | 257 | 239 | 18 | 15:1 | 0.27 | 1 | 0.61 |
| $BC_1P_a$ | 83 | 83 | 0 | 1:0 | | | |
| $BC_1P_b$ | 86 | 62 | 24 | 3:1 | 0.57 | | 0.45 |
| Pooled over families | | | | | | | |
| $P_aS_1$[v] | 53 | 53 | 0 | | | | |
| $P_bS_1$[u] | 54 | 0 | 54 | | | | |
| $F_1$ | 114 | 114 | 0 | | | | |
| $F_2$ | 506 | 468 | 38 | 15:1 | 1.20 | 1 | 0.27 |
| $BC_1P_a$ | 167 | 167 | 0 | 1:0 | | | |
| $BC_1P_b$ | 168 | 121 | 47 | 3:1 | 0.79 | | 0.37 |

[z] Data are ratings from family 'Mountain Hoosier' x 'Minilee' and 'Early Arizona' x 'Minilee' of *Citrullus lanatus var. lanatus* from three locations viz. Kinston, Clinton (M), and Clinton (P)
[y] Solid dark was the standard rind pattern
[x] gray was the mutant rind pattern
[w] Expected was the hypothesized segregation ratio for duplicate dominant epistasis inheritance for each segregating generation
[v] $P_a$ was carrier of dominant allele (solid dark green )
[u] $P_b$ was carrier of dominant allele (gray)

Table 5. Phenotypic variances by generation for the watermelon families tested for yield traits in 2008 at Kinston and Clinton, NC. [z]

| Pedigree | $\sigma^2(P_a)$ | $\sigma^2(P_b)$ | $\sigma^2(F_1)$ | $\sigma^2(F_2)$ | $\sigma^2(BC_1P_a)$ | $\sigma^2(BC_1P_b)$ |
|---|---|---|---|---|---|---|
| *Total fruit weight* | | | | | | |
| Kinston | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 277.25 | 215.96 | 398.76 | 304.49 | 592.93 | 413.38 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 560.07 | 11.75 | 238.72 | 285.66 | 374.68 | 242.72 |
| ‘Early Arizona’ x ‘Minilee’ | 213.01 | 94.32 | 158.07 | 302.11 | 183.66 | 149.49 |
| Mean | 350.11 | 107.34 | 265.18 | 297.42 | 383.76 | 268.53 |
| Clinton(M) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 118.23 | 532.42 | 15.22 | 103.42 | 220.03 | 327.63 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 426.10 | 16.61 | 45.97 | 143.76 | 56.25 | 217.12 |
| ‘Early Arizona’ x ‘Minilee’ | 35.04 | 39.52 | 59.88 | 85.92 | 88.01 | 42.99 |
| Mean | 193.12 | 196.18 | 40.36 | 111.03 | 121.42 | 195.91 |
| Clinton(P) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 56.46 | 69.00 | 45.01 | 186.60 | 194.51 | 79.00 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 181.58 | 120.81 | 149.98 | 369.27 | 422.12 | 192.11 |
| ‘Early Arizona’ x ‘Minilee’ | 25.81 | 56.71 | 95.88 | 60.27 | 37.95 | 60.89 |
| Mean | 87.95 | 82.17 | 96.96 | 205.38 | 218.19 | 110.66 |
| Overall mean | 210.39 | 128.56 | 134.17 | 204.58 | 241.12 | 191.70 |
| *Total fruit number (‘000,000)* | | | | | | |
| Kinston | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 7.72 | 0 | 6.01 | 5.94 | 5.08 | 8.54 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 9.04 | 5.43 | 6.45 | 8.68 | 4.10 | 6.35 |
| ‘Early Arizona’ x ‘Minilee’ | 9.04 | 11.7 | 10.7 | 20.3 | 11.8 | 15.0 |
| Mean | 8.60 | 5.71 | 7.72 | 11.64 | 6.99 | 9.96 |
| Clinton(M) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 3.22 | 6.63 | 1.92 | 5.14 | 2.52 | 8.87 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 18.1 | 3.22 | 4.87 | 15.2 | 2.43 | 28.20 |
| ‘Early Arizona’ x ‘Minilee’ | 9.04 | 14.5 | 5.54 | 9.36 | 9.97 | 5.28 |
| Mean | 10.12 | 8.12 | 4.11 | 9.90 | 4.97 | 14.12 |
| Clinton(P) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 9.73 | 6.99 | 3.40 | 6.30 | 9.72 | 3.62 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 8.04 | 15.4 | 4.02 | 13.0 | 5.39 | 8.42 |
| ‘Early Arizona’ x ‘Minilee’ | 8.14 | 10.5 | 17.1 | 7.81 | 6.77 | 22.10 |
| Mean | 8.63 | 10.96 | 8.17 | 9.04 | 7.29 | 11.38 |
| Overall mean | 9.11 | 8.26 | 6.67 | 10.19 | 6.42 | 11.82 |

Table 5 Continued

| Pedigree | $\sigma^2(P_a)$ | $\sigma^2(P_b)$ | $\sigma^2(F_1)$ | $\sigma^2(F_2)$ | $\sigma^2(BC_1P_a)$ | $\sigma^2(BC_1P_b)$ |
|---|---|---|---|---|---|---|
| *Fruit size* | | | | | | |
| Kinston | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 14.93 | 29.85 | 12.97 | 9.91 | 10.93 | 6.27 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 18.79 | 1.76 | 7.22 | 8.21 | 8.73 | 3.64 |
| ‘Early Arizona’ x ‘Minilee’ | 1.73 | 1.27 | 1.08 | 3.27 | 2.42 | 2.82 |
| Mean | 11.82 | 10.96 | 7.09 | 7.13 | 7.36 | 4.24 |
| Clinton(M) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 6.82 | 7.38 | 5.87 | 5.15 | 12.13 | 4.74 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 3.52 | 0.53 | 10.48 | 6.40 | 7.33 | 4.06 |
| ‘Early Arizona’ x ‘Minilee’ | 0.73 | 1.09 | 1.79 | 3.03 | 3.72 | 1.44 |
| Mean | 3.69 | 3.00 | 6.05 | 4.86 | 7.73 | 3.41 |
| Clinton(P) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 1.49 | 2.97 | 6.37 | 6.69 | 7.24 | 3.78 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 13.76 | 0.11 | 9.86 | 15.63 | 19.12 | 7.56 |
| ‘Early Arizona’ x ‘Minilee’ | 1.57 | 1.17 | 1.11 | 2.27 | 0.80 | 0.72 |
| Mean | 5.61 | 1.42 | 5.78 | 8.20 | 9.05 | 4.03 |
| Overall mean | 7.04 | 5.13 | 6.31 | 6.73 | 8.05 | 3.89 |

[z] Data are from three families of high-by low yielding cultivars of Citrullus lanatus var. lanatus from three locations viz. Kinston, Clinton (M), and Clinton (P). M and P represent planting sites at Clinton.

Table 6. Variance[z] and heritability estimates for the watermelon families tested for yield traits in 2008 at Kinston and Clinton, NC.[y]

| Pedigree | $\sigma^2(P)$[x] | $\sigma^2(E)$[w] | $\sigma^2(G)$[v] | $\sigma^2(A)$[u] | $h^2_b$[t] | $h^2_n$[s] |
|---|---|---|---|---|---|---|
| *Total fruit weight* | | | | | | |
| Kinston | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 304.49 | 322.68 | -18.19 | -397.3 | -[r] | - |
| ‘Mountain Hoosier’ x ‘Minilee’ | 285.66 | 262.32 | 23.34 | -46.08 | 0.08 | - |
| ‘Early Arizona’ x ‘Minilee’ | 302.11 | 155.87 | 146.24 | 274.06 | 0.48 | 0.91 |
| Mean | 297.42 | 246.97 | 50.46 | -56.44 | 0.28 | 0.91 |
| Clinton(M) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 103.42 | 170.27 | -66.85 | -340.80 | - | - |
| ‘Mountain Hoosier’ x ‘Minilee’ | 165.41 | 133.66 | 31.75 | 57.45 | 0.19 | 0.35 |
| ‘Early Arizona’ x ‘Minilee’ | 85.92 | 48.58 | 37.34 | 40.83 | 0.43 | 0.48 |
| Mean | 118.25 | 117.50 | 2.24 | -80.84 | 0.31 | 0.42 |
| Clinton(P) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 186.60 | 53.87 | 132.73 | 99.68 | 0.71 | 0.53 |
| ‘Mountain Hoosier’ x ‘Minilee’ | 394.60 | 150.59 | 244.01 | 174.96 | 0.62 | 0.44 |
| ‘Early Arizona’ x ‘Minilee’ | 60.27 | 68.57 | -8.30 | 21.71 | - | 0.36 |
| Mean | 213.82 | 91.01 | 122.81 | 98.56 | 0.57 | 0.44 |
| Overall mean | 209.83 | 151.82 | 58.50 | -12.90 | 0.39 | 0.59 |
| *Total fruit number(‘000,000)* | | | | | | |
| Kinston | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 5.94 | 4.93 | 1.01 | -1.73 | 0.17 | - |
| ‘Mountain Hoosier’ x ‘Minilee’ | 8.68 | 6.84 | 1.84 | 6.91 | 0.21 | 0.80 |
| ‘Early Arizona’ x ‘Minilee’ | 20.30 | 10.50 | 9.78 | 13.90 | 0.48 | 0.68 |
| Mean | 11.64 | 7.42 | 4.21 | 6.36 | 0.29 | 0.74 |
| Clinton(M) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 5.14 | 3.42 | 1.72 | -11.20 | 0.33 | - |
| ‘Mountain Hoosier’ x ‘Minilee’ | 15.20 | 7.76 | 7.46 | -0.18 | 0.49 | - |
| ‘Early Arizona’ x ‘Minilee’ | 9.36 | 8.65 | 0.71 | 3.46 | 0.08 | 0.37 |
| Mean | 9.90 | 6.61 | 3.30 | -2.64 | 0.30 | 0.37 |
| Clinton(P) | | | | | | |
| ‘Mountain Hoosier’ x ‘Calsweet’ | 6.30 | 5.88 | 0.42 | -7.34 | 0.07 | - |
| ‘Mountain Hoosier’ x ‘Minilee’ | 13.00 | 7.86 | 5.18 | 12.30 | 0.40 | 0.94 |
| ‘Early Arizona’ x ‘Minilee’ | 7.81 | 13.20 | -5.39 | 13.30 | - | - |
| Mean | 9.04 | 8.98 | 0.07 | 6.08 | 0.24 | 0.94 |
| Overall mean | 10.19 | 7.67 | 2.52 | 3.27 | 0.28 | 0.68 |

Table 6 Continued

| Pedigree | $\sigma^2(P)^x$ | $\sigma^2(E)^w$ | $\sigma^2(G)^v$ | $\sigma^2(A)^u$ | $h^2_b{}^t$ | $h^2_n{}^s$ |
|---|---|---|---|---|---|---|
| *Fruit size* | | | | | | |
| Kinston | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 9.91 | 17.68 | -7.77 | 2.62 | - | 0.26 |
| 'Mountain Hoosier' x 'Minilee' | 8.21 | 8.75 | -0.54 | 4.05 | - | 0.49 |
| 'Early Arizona' x 'Minilee' | 3.27 | 1.29 | 1.99 | 1.30 | 0.61 | 0.40 |
| Mean | 7.13 | 9.24 | -2.10 | 2.66 | 0.61 | 0.38 |
| Clinton(M) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 5.15 | 6.48 | -1.33 | -6.56 | - | - |
| 'Mountain Hoosier' x 'Minilee' | 6.40 | 6.25 | 0.15 | 1.41 | 0.02 | 0.22 |
| 'Early Arizona' x 'Minilee' | 3.03 | 1.35 | 1.68 | 0.89 | 0.55 | 0.30 |
| Mean | 4.86 | 4.69 | 0.17 | -1.42 | 0.29 | 0.26 |
| Clinton(P) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 6.69 | 4.30 | 2.39 | 2.36 | 0.36 | 0.35 |
| 'Mountain Hoosier' x 'Minilee' | 15.63 | 8.40 | 7.24 | 4.58 | 0.46 | 0.29 |
| 'Early Arizona' x 'Minilee' | 2.27 | 1.24 | 1.03 | 3.02 | 0.45 | 1.33 |
| Mean | 8.20 | 4.65 | 3.55 | 3.32 | 0.42 | 0.66 |
| Overall mean | 6.73 | 6.19 | 0.07 | 1.21 | 0.44 | 0.43 |

[y] Data are from three families of high-by low yielding cultivars of *Citrullus lanatus var. lanatus* from three locations viz. Kinston, Clinton(M), and Clinton(P). M and P represent planting sites at Clinton.

[x] $\sigma^2(P) = \sigma^2(F_2)$ = phenotypic variance

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

[v] $\sigma^2(G) = \sigma^2(P) - \sigma^2(E)$ = genetic variance

[u] $\sigma^2(A) = \left[2\sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)\right]$ = additive variance

[t] $h^2_b$ = broad-sense heritability

[s] $h^2_n$ = narrow-sense heritability

[r] Negative estimate from negative estimate of additive variance

Table 7. Estimates of number of effective factors and predicted gain from selection under different selection intensities for the watermelon families tested for yield traits in 2008 at Kinston and Clinton, NC. [z]

| Pedigree | Effective factors | | | Gain from selection[y] | | |
|---|---|---|---|---|---|---|
| | M[x] | W[w] | Mean | 5% | 10% | 20% |
| *Total fruit weight* | | | | | | |
| Kinston | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | -0.5 | -9.3 | -4.9 | -[v] | - | - |
| 'Mountain Hoosier' x 'Minilee' | -5.3 | 2.7 | -1.3 | - | - | - |
| 'Early Arizona' x 'Minilee' | 0.7 | 0.8 | 0.8 | 32.5 | 27.8 | 22.1 |
| Mean | -1.7 | -2.5 | -2.1 | 32.5 | 27.8 | 22.1 |
| Clinton(M) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | -0.2 | -0.5 | -0.35 | - | - | - |
| 'Mountain Hoosier' x 'Minilee' | 32.4 | 13.0 | 22.7 | 2.4 | 2.1 | 1.7 |
| 'Early Arizona' x 'Minilee' | 0.3 | 0.2 | 0.3 | 9.1 | 7.8 | 6.2 |
| Mean | 10.8 | 6.6 | 8.7 | 5.8 | 5.0 | 4.0 |
| Clinton(P) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 0.0 | 0.0 | 0.0 | 15.0 | 12.8 | 10.2 |
| 'Mountain Hoosier' x 'Minilee' | 0.2 | 0.1 | 0.15 | 13.3 | 11.4 | 9.1 |
| 'Early Arizona' x 'Minilee' | 0.0 | -0.3 | -0.15 | 5.8 | 4.9 | 3.9 |
| Mean | 0.1 | -0.1 | 0.0 | 11.4 | 9.7 | 7.3 |
| Overall mean | 4.1 | 2.3 | 3.2 | 16.6 | 14.2 | 11.1 |
| *Total fruit number* | | | | | | |
| Kinston | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | -1.3 | 1.1 | -0.1 | - | - | - |
| 'Mountain Hoosier' x 'Minilee' | 0.9 | 1.4 | 1.2 | 4833 | 4129 | 3285 |
| 'Early Arizona' x 'Minilee' | 0.0 | 0.1 | 0.1 | 6336 | 5413 | 4306 |
| Mean | -0.4 | 1.0 | 0.3 | 5585 | 4771 | 3796 |
| Clinton(M) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 0.0 | 0.1 | 0.1 | - | - | - |
| 'Mountain Hoosier' x 'Minilee' | -1.0 | 0.4 | -0.3 | - | - | - |
| 'Early Arizona' x 'Minilee' | 0.1 | 3.0 | 1.6 | 2332 | 1992 | 1585 |
| Mean | 0.0 | 1.2 | 0.6 | 2332 | 1992 | 1585 |
| Clinton(P) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | -1.4 | 3.1 | 0.9 | - | - | - |
| 'Mountain Hoosier' x 'Minilee' | 1.2 | 1.0 | 1.1 | 7002 | 5982 | 4759 |
| 'Early Arizona' x 'Minilee' | 0.0 | -0.1 | -0.1 | - | - | -6654 |
| Mean | -0.1 | 1.0 | 0.0 | 7002 | 5982 | 4759 |
| Overall mean | -0.2 | 1.1 | 0.5 | 4973 | 4248 | 3380 |

Table 7 Continued

| Pedigree | Effective factors | | | Gain from selection[y] | | |
|---|---|---|---|---|---|---|
| | M[x] | W[w] | Mean | 5% | 10% | 20% |
| *Fruit size* | | | | | | |
| Kinston | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 0.5 | -0.2 | 0.2 | 1.7 | 1.5 | 1.2 |
| 'Mountain Hoosier' x 'Minilee' | 6.5 | -13.4 | -3.5 | 2.9 | 2.5 | 2.0 |
| 'Early Arizona' x 'Minilee' | 1.2 | 0.3 | 0.8 | 1.5 | 1.3 | 1.0 |
| Mean | 2.7 | -3.5 | -0.4 | 2.0 | 1.8 | 1.4 |
| Clinton(M) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | -0.1 | -0.3 | -0.2 | - | - | - |
| 'Mountain Hoosier' x 'Minilee' | 6.3 | 23.6 | 15.0 | 1.1 | 1.0 | 0.8 |
| 'Early Arizona' x 'Minilee' | 0.6 | 0.2 | 0.4 | 1.1 | 0.9 | 0.7 |
| Mean | 2.3 | 7.8 | 5.1 | 1.1 | 1.0 | 0.8 |
| Clinton(P) | | | | | | |
| 'Mountain Hoosier' x 'Calsweet' | 0.3 | 0.7 | 0.5 | 1.9 | 1.6 | 1.3 |
| 'Mountain Hoosier' x 'Minilee' | 3.6 | 0.8 | 2.2 | 2.4 | 2.0 | 1.6 |
| 'Early Arizona' x 'Minilee' | 0.0 | 0.2 | 0.1 | 4.1 | 3.5 | 2.8 |
| Mean | 1.3 | 0.6 | 1.0 | 2.8 | 2.4 | 1.9 |
| Overall mean | 2.1 | 2.9 | 2.5 | 2.0 | 1.7 | 1.4 |

[y] Data are from three families of high-by low yielding cultivars of *Citrullus lanatus var. lanatus* from three locations viz. Kinston, Clinton(M), and Clinton(P). M and P are planting sites at Clinton.

[y]Gain from selection = k x $h_n^2 \times \sqrt{\sigma^2(P)}$ .

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

[x] M = Mather's method: $$\frac{\left[\mu(P_b)-\mu(P_a)\right]^2}{2}$$
$$\overline{\left[2\times\sigma^2(F_2)\right]-\left[\sigma^2(BC_1P_b)+\sigma^2(BC_1P_a)\right]}$$

[v] Negative estimate from a negative estimate of additive variance.

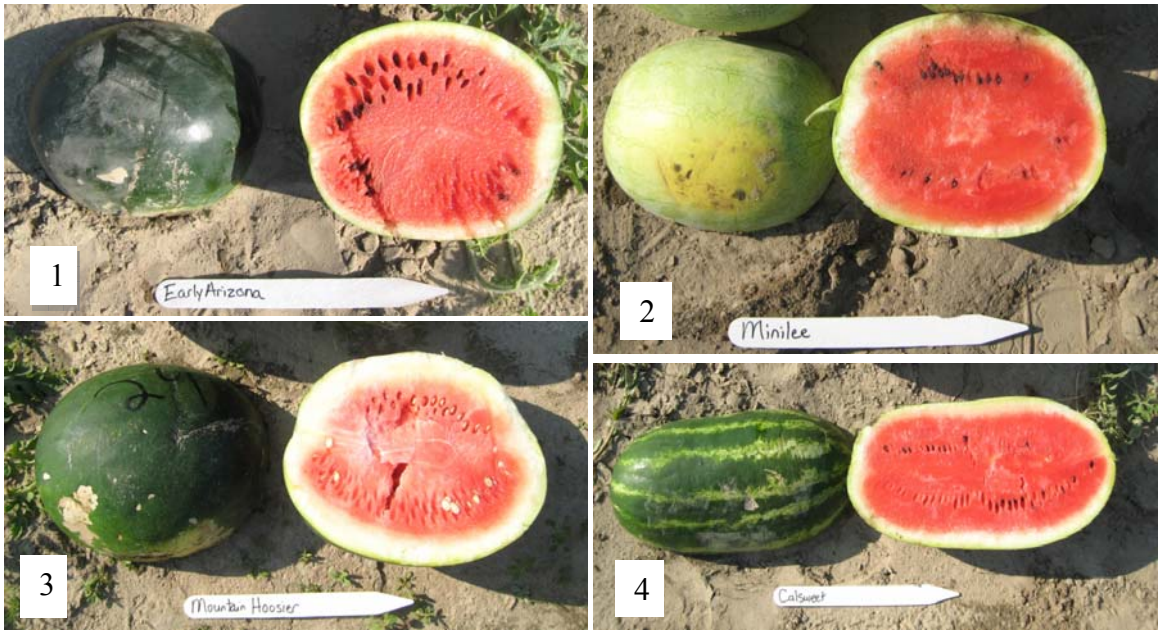


Figure 1. Cultivars used to develop families. 1. ‘Early Arizona’ with round fruit shape and solid dark green rind; 2.‘Minilee’ showing round fruit shape and gray rind; 3. ‘Mountain Hoosier’ with round fruit shape and solid dark green rind; 4. ‘Calsweet’ with elongate fruit shape with wide stripe rind.

Figure 2. Families being trained in spiral to help in identifying individual plants at Clinton (M).

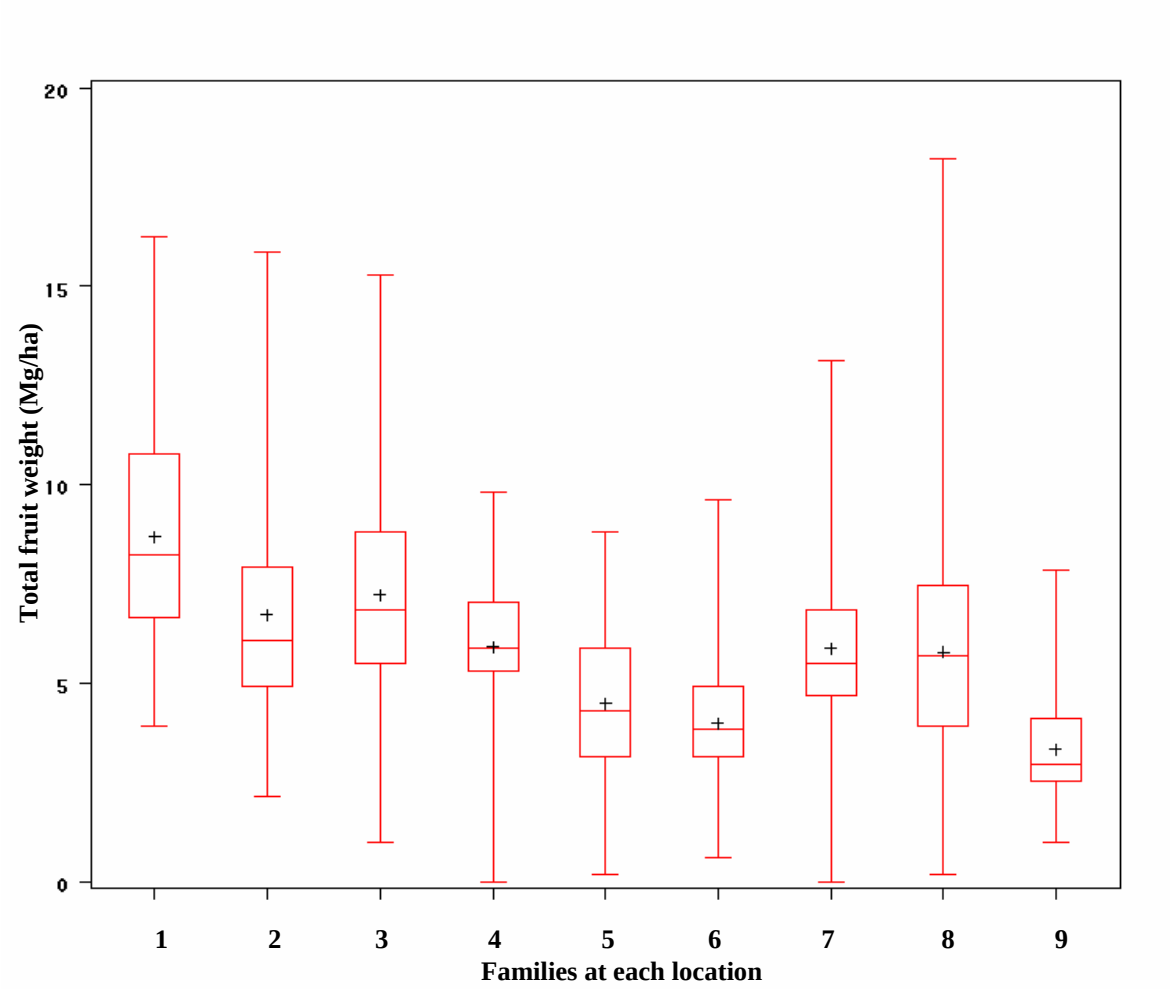


Figure 3. Box plots showing the distribution of $F_2$ data for the three families at three locations for total fruit weight. 1, 4, 7= 'Mountain Hoosier' x 'Calsweet' at Kinston, Clinton (M), and Clinton (P); 2, 5, 8= 'Mountain Hoosier' x 'Minilee' at Kinston, Clinton (M), and Clinton (P); 3, 6, 9= 'Early Arizona' x 'Minilee' at Kinston, Clinton (M), and Clinton (P), respectively.

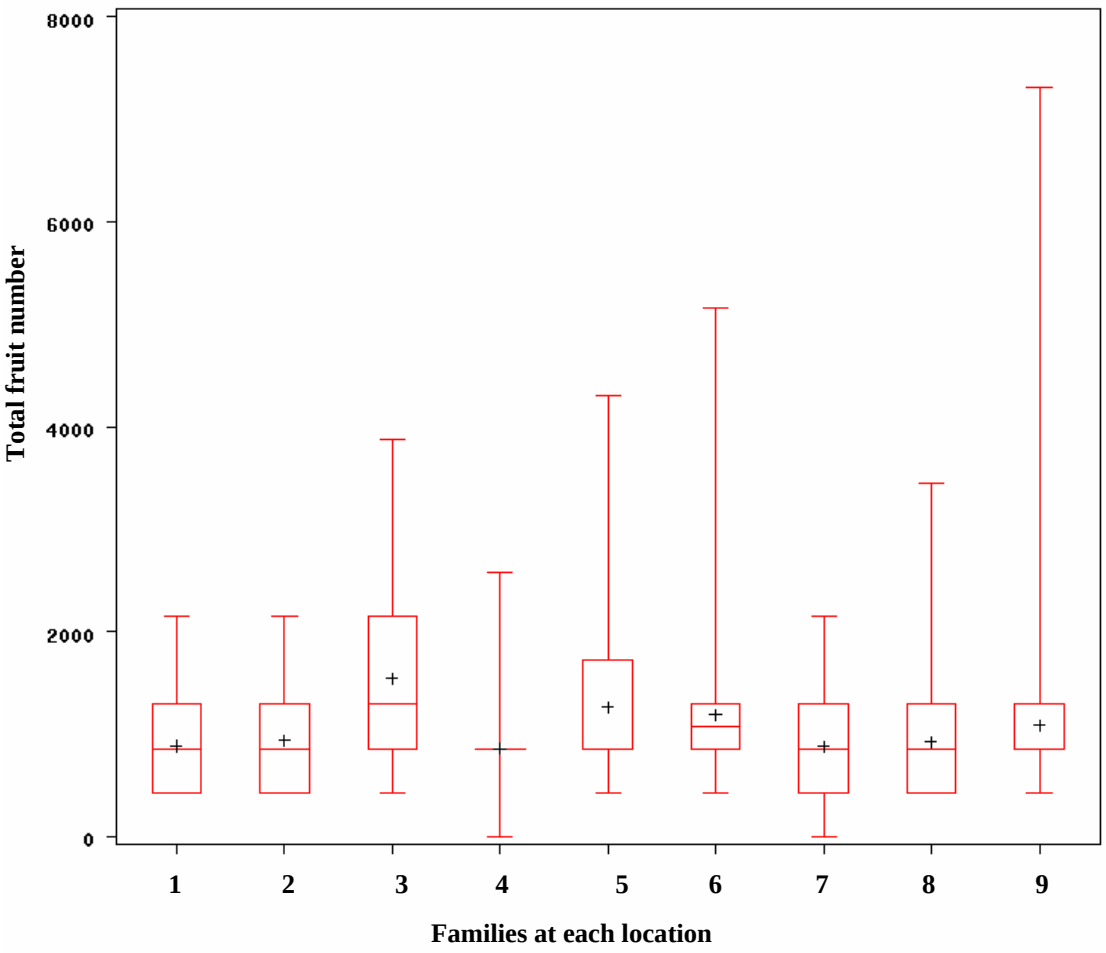


Figure 4. Box plots showing the distribution of $F_2$ data for the three families at three locations for total fruit number. 1, 4, 7= 'Mountain Hoosier' x 'Calsweet' at Kinston, Clinton (M), and Clinton (P); 2, 5, 8= 'Mountain Hoosier' x 'Minilee' at Kinston, Clinton (M), and Clinton (P); 3, 6, 9= 'Early Arizona' x 'Minilee' at Kinston, Clinton (M), and Clinton (P), respectively.

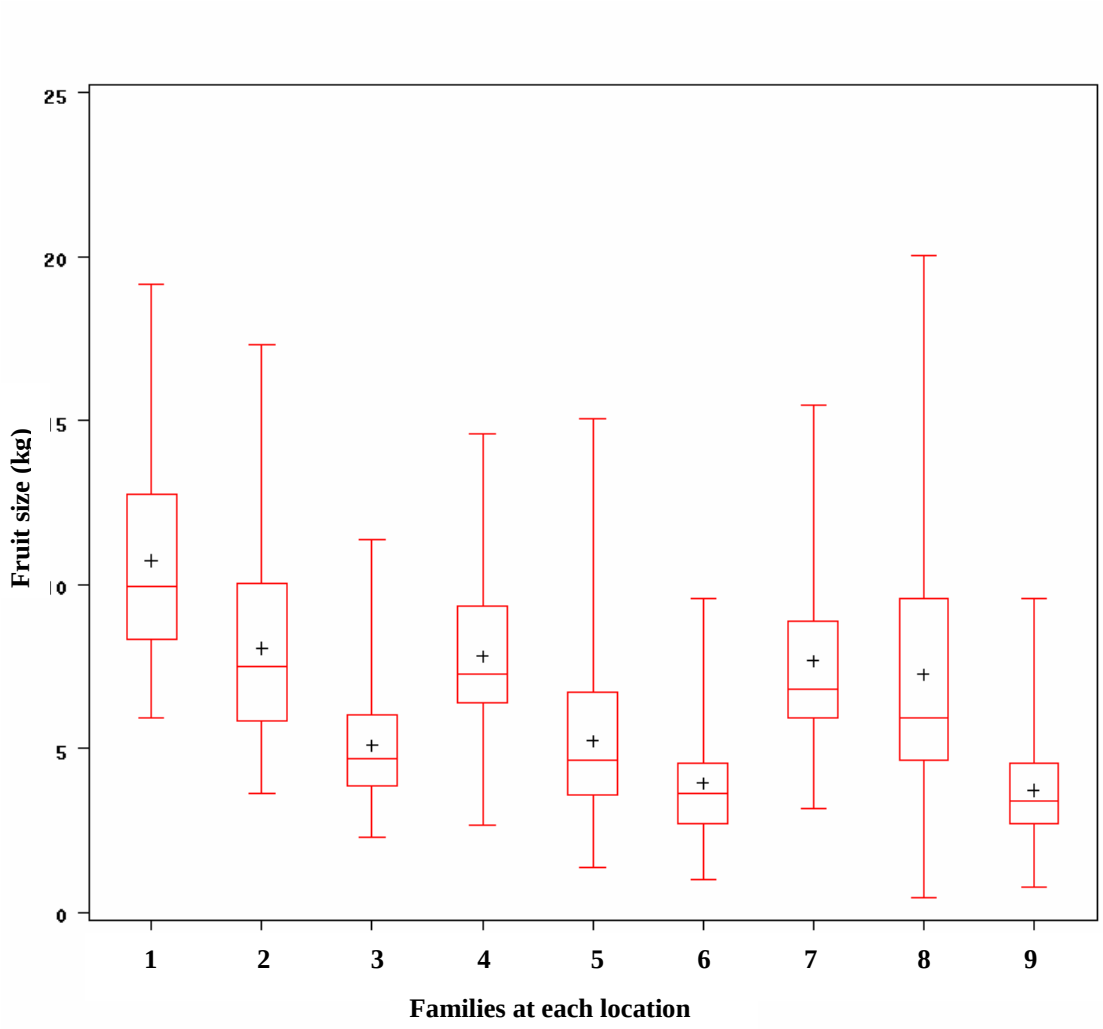


Figure 5. Box plots showing the distribution of $F_2$ data for the three families at three locations for fruit size. 1, 4, 7= 'Mountain Hoosier' x 'Calsweet' at Kinston, Clinton (M), and Clinton (P); 2, 5, 8= 'Mountain Hoosier' x 'Minilee' at Kinston, Clinton (M), and Clinton (P); 3, 6, 9= 'Early Arizona' x 'Minilee' at Kinston, Clinton (M), and Clinton (P), respectively.

# CHAPTER THREE

# THE RATE OF NATURAL OUTCROSSING IN WATERMELON

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## The Rate of Natural Outcrossing in Watermelon


### Abstract

Watermelon (*Citrullus lanatus*) is a cross-pollinated crop. Estimation of the rate of natural outcrossing is important to plant breeders to determine the minimum isolation distance required for seed increase and to calculate precise estimates of genetic variance, covariance with in family, and heritability that in turn helps design a suitable breeding strategy for crop improvement. There is little inbreeding depression in watermelon, indicating a lack of dominance variance, and possibly a high rate of self-pollination. Hence, the objective of this study was to determine the rate of natural outcrossing in watermelon to estimate the distance that pollen can be transferred by honeybee (*Apis mellifera* L.). The experiment was a split plot in a randomized complete block design with 7 replications and conducted at 2 locations: Kinston and Clinton, NC. Whole plots were the 8 in-row spacing treatments: 0.6, 1.2, 1.8, 2.4, 3.0, 3.7, 4.3, and 4.9 m. Sub plots were 2 cultivars: 'Allsweet' and 'Mickylee'. The cultivar, 'Moon and Stars' was used as a pollen donor since it has a useful spotted (*Sp*) marker gene. 'Moon and Stars' has bright yellow spots on leaves, fruit and cotyledons due to the *Sp* gene that is dominant to uniform color. Plants were grown in rows 3.1 m apart. Each plant was trained in a spiral pattern to keep each plant separate, and to make it easy to identify the fruit belonging to each plant. Analysis of variance showed significant differences in the rate of natural outcrossing due to in-row spacing. Linear regression of the rate of natural outcrossing on in-row spacing was significant. Regression coefficient of -2.98 indicated that the rate of natural outcrossing decreased with increased in-

row spacing. However, location, cultivar, and the interaction effects were not significant. Close in-row spacing had a significantly higher rate of natural outcrossing: 11%, 17% and 11% at 0.6, 1.2 and 1.8 m, respectively. On the other hand, the rate of natural outcrossing rate was significantly lower (1.8%) where a wider in-row spacing (≥ 4.3 m) was used. This indicated that a high rate of self-pollination can be achieved in watermelon plants trained in a spiral and spaced more than 5 m apart. Thus, watermelon breeders can make use of breeding methods suited to self-pollinated crops provided that plants are spaced more than 5 m apart. The rate of outcrossing must be taken into account to estimate heritability and variance in watermelon populations.

## Introduction

Plant populations are classified as autogamous, allogamous, or mixed mating types. All the species included in *Cucurbitaceae* are classified as allogamous (cross-pollinated) including watermelon, which has monoecious and andromonoecious flowering habits. The *a* locus determines sex expression in watermelon, producing monoecious (*AA*) or andromonoecious (*aa*) sex expression (Guner and Wehner, 2004; Rhodes and Dane, 1999; Rhodes and Zhang, 1995; Rosa, 1928). Cross-pollination in watermelon is mediated by honeybees (*Apis mellifera* L.) and bumblebees (*Bombus impatiens* Cresson) that visit the flower to collect pollen and nectar (Delaplane and Mayer, 2000; Free, 1993; McGregor, 1976). Although >85% of watermelon pollinators are honey bees, bumble bees have been reported to be a better pollinator than honey bees in watermelon (Stanghellini et al., 1998). Most of the pollen is removed in 2 hours after anthesis in watermelon (Stanghellini et al.,

2002) by pollinators. Stephen (1970) and Lord (1985) reported in cucumber that at least 8-12 visits are required for fruit set. However, Gingras et al. (1999) suggested that a single visit is enough to induce fruiting. In addition to insect pollinators, the outcrossing rate is also reported to be influenced by staminate flower and pollen production as affected by the genotype and environment. Stanghellini and Schultheis (2005) reported variability in pollen grain production in 27 watermelon cultivars.

The movement of insect pollinators in a field is strongly directional, with pollinators moving to the nearest neighboring flowers within the same row (Cresswell et al., 1995; Handel, 19982; Walters and Schultheis, 2009; Zimmerman, 1979). Pollen movement was restricted to 3 m from the donor plant in muskmelon (Handel, 1982) and 2 to 3 m in cucumber (Handel, 1983). So, the rate of natural outcrossing is influenced by plant spacing. In watermelon, the rate of natural outcrossing (measured between-row only) was near zero for rows separated by 6 m or more (Rhodes et al., 1987) and averaged 0.8% for rows 3 to 6 m apart. Walters and Schultheis (2009) recorded an outcrossing rate near to zero in plants spaced more than 10 m apart. Ferreira et al. (2002) reported an outcrossing rate of 65% and inbreeding coefficient as high as 0.41 in andromonoecious families of watermelon. When averaged over monoecious and andromonoecious families, the outcrossing rate was 77% (Ferreira et al., 2000, 2002). However, these authors did not report the plant spacing adopted in the experiment. The rate of natural outcrossing has been measured for cucumber families planted in isolation blocks. Wehner and Jenkins (1985) reported that natural outcrossing rate (mean and range over replications) was 36% (29-43%) cross-, 17% (0-42%) sib-, and 47%

(23-77%) self-pollination. Thus, 64% of pollinations were self- or sib-, but not cross-pollination among families. Jenkins (1942) in another study recorded 30 to 35% natural self pollination. Watermelon was expected to have a mixed mating system since it is similar to cucumber in plant growth and sex expression. Moreover, there was no significant inbreeding depression in watermelon (Wehner, 2008), indicating a high rate of self-pollination in the species. Self-pollination can occur in both monoecious and andromonoecious populations (Ferreira et al., 2000). Allard (1960) suggested that cucurbits evolved as small populations in nature, thus having high levels of inbreeding.

Estimation of natural outcrossing rate is useful for plant breeders especially when experiments are run to estimate components of genetic variance. Usually in cross-pollinated crops, it is assumed that individuals produced from a single parent are half-sib families and variances are calculated based on the assumption of half-sibs. However, these estimates of genetic variance might be overestimated if there is inbreeding (self pollination). Crop improvement methods for self-pollinated crops are different from cross-pollinated crops. Common methods for crop improvement employed in watermelon are: pedigree breeding and recurrent selection (Fehr, 1987; Wehner, 2008). If the natural outcrossing rate is found to be high, watermelon populations can be improved by intercrossing selected families in isolation blocks by recurrent selection. Intercrossing can play an important role in genetic gain. Wehner and Cramer (1996) reported genetic gain using recurrent selection in cucumber populations. The same results could be expected for watermelon. Another major application for these results is for plant breeders interested in self-pollinating plants after crossing two or

more lines in a breeding program (e.g. Pedigree breeding or bulk breeding) without the use of controlled pollination in greenhouses or insect-proof (screen) cages.

The objective of this study was to determine the rate of natural outcrossing in watermelon cultivars as affected by environment, cultivar, and in-row spacing, and to determine the distance pollen can be transferred by pollinators such as honeybee.

## Material and Methods

The experiment was conducted at the Horticultural Crops Research Station, Clinton and Cunningham Research Station, Kinston, North Carolina during summer 2008. The soil type at Clinton was a Norfolk (fine-loamy, siliceous, thermic, Typic Kandiudults), and a Norfolk sandy loam at Kinston. Standard horticultural practices were used as recommended by the North Carolina Extension Service (Sanders, 2004).

*Treatment plots*. The experiment was a split plot in a randomized complete block design with 7 replications at each location (Fig. 1). Whole plots were the 8 in-row spacing: 0.6, 1.2, 1.8, 2.4, 3.0, 3.7, 4.3, and 4.9 m). The in-row spacing is defined as the distance between cultivar used to track the rate of natural outcrossing and pollen donor cultivar with marker gene. The sub plots were 2 cultivars (‘Allsweet’ and ‘Mickylee’). The cultivars, ‘Allsweet’ and ‘Mickylee’ were used in each treatment plot to measure the natural outcrossing rate from their progeny. Cultivars were selected from different geographical locations in U.S. ‘Allsweet’ is cultivated in midwestern U.S. whereas ‘Mickylee’ is a southeastern cultivar. ‘Allsweet’ has long cylindrical shape with striped rind, whereas ‘Mickylee’ has small round fruit with gray rind (Wehner, 2002). All fruit that were set on 'Allsweet' and 'Mickylee' were

the result of a combination of self- and cross-pollination. The cultivar, ‘Moon and Stars’ was planted next to plants of 'Allsweet' and 'Mickylee' as a pollen donor with a spotted (*Sp*) marker gene. ‘Moon and Stars’ has large, elongate fruit, dark green rind with yellow spots, and firm, sweet flesh with dotted seeds (Fig. 2). The bright yellow spots on the rind and leaves are dominant to uniform green rind and foliage color, and are due to a single dominant gene, *Sp* (Poole, 1944; Rhodes, 1986; Guner and Wehner, 2004). The *Sp* gene was used as a marker to track the natural outcrossing rate in the progeny of ‘Allsweet’ and ‘Mickylee’. Additional border plants were planted for closer in- row spacing (0.6 m, 1.2 m, and 1.8 m) to avoid outcrossing from pollen donor plant of the next plot, since they were physically closer to each other than the other treatments (Fig. 1). Transplants were grown in 72-cell polyethylene flats in the greenhouse at North Carolina State University in Raleigh, NC. A soilless growing medium was used (Fafard 4P consisting of Canadian sphagnum peatmoss, perlite, vermiculite, processed pine bark, Conrad Fafard Inc., Anderson, South Carolina). The transplants were moved to cold frames when they were 4 weeks old, and transplanted to the field after one week of acclimation. Transplants were planted on 0.5 m wide raised beds with black plastic mulch and drip irrigation. Rows were 3.1 m apart (center to center). Plants were trained in a spiral arrangement each week starting when the vines reached the edge of the raised bed, and ending at the time of fruit set (Gusmini and Wehner, 2007). No disease problems were observed. Honeybees were placed in the field at the stage of first flowers opening using the recommended rate of 2 active hives/ha.

*Progeny evaluation plots*. One ripe fruit was harvested from each plant of 'Allsweet' and 'Mickylee' from each treatment plot. All seeds from each fruit were assumed to be half-sib families. Plots for progeny evaluation were 1.5 x 5.2 m with 1 m alleys between them. Progeny were evaluated at the 4 true-leaf stage using 100 plants per plot to calculate rate of natural outcrossing. Progeny were planted on 28 July, 2008. The first evaluation was done on 21 August, 2008 and the second evaluation was done on 2 September, 2008, to confirm the results.

Progeny with bright yellow spots on their leaves were progeny resulting from pollination by carrying the *Sp* allele from 'Moon and Stars'. The rate of natural outcrossing was measured as the percentage of spotted plants out of the total. The data were analyzed to study the affect of in-row spacing on rate of natural outcrossing using the MEANS, REG and GLM procedures of SAS (SAS Institute, Inc., Cary, NC). The locations, in-row spacing, and cultivars were considered as fixed effects in this experiment.

## Results

Location and cultivar did not influence the rate of natural outcrossing significantly (Table 1). In-row spacing had a highly significant effect on the rate of natural outcrossing in watermelon. Interaction of location and in-row spacing, location and cultivar, and in-row spacing and cultivar were also not significant. Although interaction effects are not significant, the data are presented for each location and cultivar to show the range of effects of those treatments. Finally, the data for the rate of natural outcrossing is summarized by pooling data over location and cultivar.

The rate of natural outcrossing decreases as the distance between plants increased in the rows at both locations (Table 2). A similar trend is also observed for each cultivar. The rate of natural outcrossing was pooled over location and cultivar since interactions are not significant. Linear regression analysis with in-row spacing as dependent variable for the rate of natural outcrossing was significant. General linear model explained 74% variation ($R^2$) which was high (Fig 3.). Regression equation estimated was:

The rate of natural outcrossing = 15.64 – 2.98 (in-row spacing).

The slope of regression equation (-2.98) indicated that the rate of natural outcrossing decreased with increasing in-row spacing. Even if plants are intermingled (zero in-row spacing), outcrossing would not be more than 15.64%. The rate of natural outcrossing is low (1.8%) at the wider in-row spacing lengths of 4.3 m and 4.9 m. Outcrossing should occur at a low frequency at a spacing greater than 5 m,. On the other hand, closer in-row spacing such as 0.6 and 1.2 m has significantly higher rate of natural outcrossing (11% and 16.9%, respectively). The closest in-row spacing (0.6 m) had the maximum rate of natural outcrossing (59.2 %), and it gradually decreases with increasing in-row spacing under maximum column (Table 2). Wider in-row spacing (4.9 m) is the least outcrossed (9.2%).

## Discussion

The rate of natural outcrossing relative to location, cultivar, and in-row spacing is measured in this experiment. Locations do not show significant effect, indicating that Kinston and Clinton are similar in environmental conditions in affecting flowering and pollination. These locations may be grouped under one mega-environment. This is important

since sex expression in watermelon is highly dependent on nutrition, air temperature, light, and humidity (Wehner, 2008). The two cultivars also do not influence the rate of natural outcrossing; indicating that they are similar in attracting pollination vectors such as bees, and have similar sex expression, which might include trait such as staminate: pistillate flower ratio. These cultivars are adapted to different environments across U.S. (Wehner, personal communication). A large variability exists in watermelon cultivars for staminate: pistillate flower ratio and pollen production that might affect outcrossing rate (Stanghellini and Schultheis, 2005).

Walters and Schultheis (2009) detected strong directionality in the pollen flow, with most occurring within rows. They also observed pollen flow across rows up to 10 %. Rhodes et al. (1987) recorded 4% mean pollen flow across rows. Thus the rate of natural outcrossing might be underestimated slightly in this experiment, since pollen from border rows (across rows) might have been transferred to treatment plots. However, in our experiment, we trained plants in the tight spiral to avoid spread of vines which these authors did not do in their study. Thus pollen flow across rows in our study should be minimal. The results show the significant effect of in-row spacing on the rate of natural outcrossing in watermelon. Most of the pollen grains are deposited on the nearest available flower (Cresswell et al., 1995) and pollen availability is diluted as distance from the pollen donor is increased (Fig. 3). Our study indicates that a high degree of self-pollination can be achieved in watermelon plants trained in a spiral and spaced >5 m apart in the rows (Table 2, Fig 3). Results of this study are in agreement with other research findings (Handel, 1982, 1983; Handel and Mishkin, 1984;

Hokanson et al., 1997a, 1997b; Walters and Schultheis, 2009) in that pollen is generally carried short distances (0.5 to 2 m) in cucurbits. Sometimes, pollen may be transferred a long distance (5 to 10 m) at a low frequency (1% to 2%) which we recorded in our experiment. In a breeding program where isolation space is valuable, these results are useful. Pollen transfer between plants can also be reduced by spiral training. Spiral training reduces the spread of vines and reduces the exposure of flowers to bees from other vines. Breeders can advance the generations by inbreeding if they use the proper in-row spacing, thus saving space and labor costs for self-pollination in the field or greenhouse. On the other hand, close in-row spacing (<0.6 m) may not be useful for intercrossing families in a recurrent selection program unless hand pollination is used. The rate of natural outcrossing is not high enough to match the effectiveness of intercrossing by hand. Treating the vines with growth regulators to increase staminate flower production has not been effective so far (Wehner, personal communication).

Watermelon breeders often calculate estimates of genetic variance and covariance among family members (e.g. half-sibs) in their populations. Estimates are often miscalculated if the mating system is not well studied, especially in watermelon where the outcrossing rate is dependent on in-row spacing. The coancestry of individuals is higher when parents are spaced apart, due to the increase in self-pollination. The results of this study support that concept. Genetic variance is: $\sigma^2_G = (1+F)\ \sigma^2_A + (1-F)\ \sigma^2_G + 4FD_1 + 4FD_2 + F\ (1-F)\ H$, where F is the inbreeding coefficient (Weir and Cockerham, 1976). We recommend that watermelon breeders take into account the inbreeding rate and/or outcrossing rate of populations at the in-row spacing that they are using in their breeding plots (Ferreira et al., 2000, 2002). The first

chapter of this dissertation did make use of findings of this study (Kumar, 2009). In our NCHYW1 and NCYYW2 populations, plants in parental generations were spaced at 3.05 m apart which has outcrossing rate of 4 % has based on this study. Self-pollinated crops can have an outcrossing rate of 1-3% (Fujita et al., 1997; Lesley, 1924). Thus offspring were treated as the result of self-pollination. Estimates of variance and heritability were calculated by the procedure recommended for self-pollinated crops. However, results of this study were not applicable to the study conducted by sex related generations (Chapter 2), since controlled crosses were made in the greenhouse.

In conclusion, watermelon appears to act more like a self-pollinated crop when plants are spaced >5 m apart. A spacing of 10 m may be needed to be safe. For increasing the seed of elite inbreds, 100 m isolation is recommended since it requires zero outcrossing (Rhodes et al., 1987). Components of genetic variances should be estimated by taking the rate of natural outcrossing into account. Future studies are designed to reduce the bias due to border row and in-row spacing.

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Table 1. Analysis of variance to show the significance and nonsignificance of main effects and interactions for the rate of natural outcrossing in watermelon.

| Source of variation | DF | Mean Square | P-values |
|---|---|---|---|
| Location | 1 | 199.94 | 0.1987 |
| Replication (Location) | 12 | 108.02 | 0.4625 |
| Spacing | 7 | 614.56 | 0.0003 |
| Spacing x Location | 7 | 68.64 | 0.8232 |
| Replication (Location*Spacing) | 76 | 134.18 | 0.1823 |
| Cultivar | 1 | 13.15 | 0.7288 |
| Spacing x Cultivar | 7 | 110.37 | 0.4270 |
| Cultivar x Location | 1 | 260.84 | 0.1255 |
| Residual error | 73 | 108.59 | -- |

Table 2. The rate of natural outcrossing in watermelon due to in-row spacing.[z]

| In-row Spacing (m) | Mean[y] | | Mean[x] | | | | Overall mean |
|---|---|---|---|---|---|---|---|
| | Clinton | Kinston | Allsweet | Mickylee | Min. | Max. | |
| 0.6 | 10.7 | 11.9 | 12.5 | 9.7 | 0 | 59.2 | 11.0 |
| 1.2 | 16.4 | 17.4 | 21.9 | 11.9 | 0 | 52.5 | 16.9 |
| 1.8 | 13.7 | 9.2 | 7.9 | 13.2 | 0 | 56.8 | 11.0 |
| 2.4 | 10.5 | 2.4 | 5.8 | 7.3 | 0 | 33.3 | 6.6 |
| 3.0 | 5.4 | 2.5 | 5.0 | 3.1 | 0 | 20.4 | 4.0 |
| 3.7 | 6.2 | 6.6 | 5.7 | 7.7 | 0 | 50.8 | 6.7 |
| 4.3 | 3.7 | 0.9 | 1.5 | 2.2 | 0 | 16.9 | 1.8 |
| 4.9 | 2.7 | 1.2 | 2.4 | 1.2 | 0 | 9.2 | 1.8 |
| LSD[w] | 11.1 | | 7.9 | | | | 5.6 |

[z]Data are means of 7 replications of 1 plant per hill, trained in spiral
[y]Locations were insignificant at α= 0.05
[x]Cultivars were insignificant at α= 0.05
[w]Least significant difference at α= 0.05

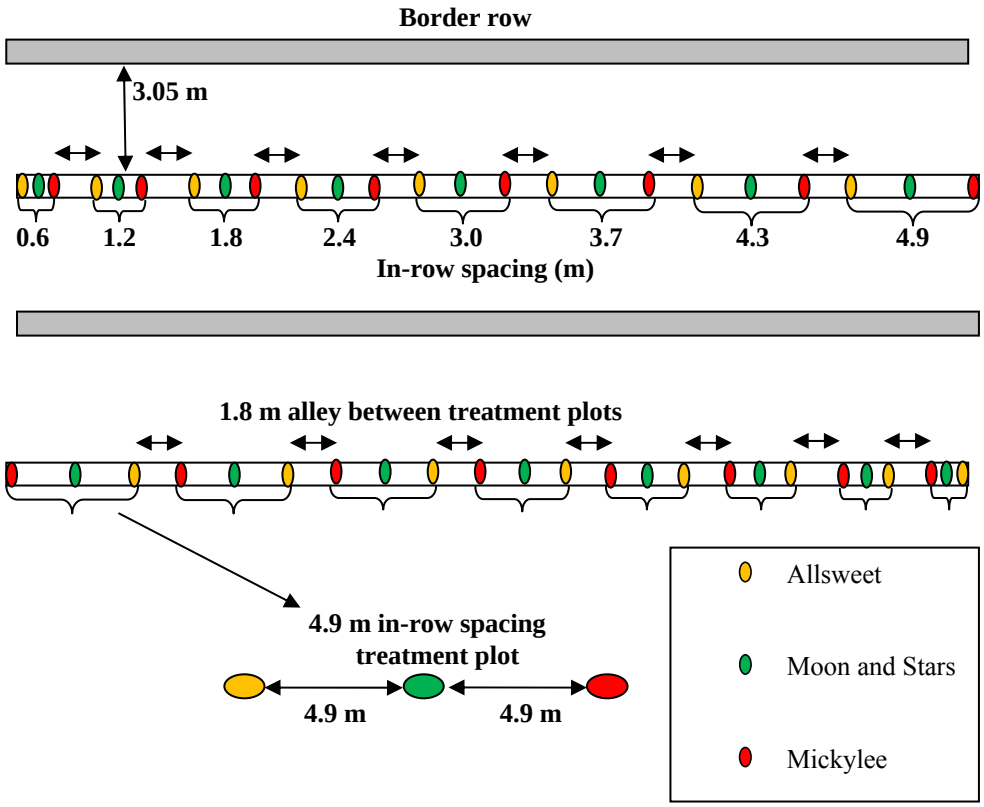


Figure 1. Field design for natural outcrossing study conducted at Clinton and Kinston, NC (only two replications are shown). Treatment plot consisted of 3 cultivars: 'Allsweet', and 'Mickylee' on the outside and 'Moon and Stars' in the center. 'Moon and Stars' was a source of pollen donor of marker gene to 2 other cultivars in the same plot. Additional border plant was planted at each end of 0.6 m, 1.2 m, and 1.8 m in-row spacing treatment plots (not shown). Design was split plot in randomized complete block with 8 in-row spacing as whole plot and 2 cultivars ('Allsweet' and 'Mickylee') as sub-plot. Border rows were planted with 'Allsweet'.

Figure 2. ‘Moon and Stars’ fruit with bright yellow spots due to *Sp* gene.

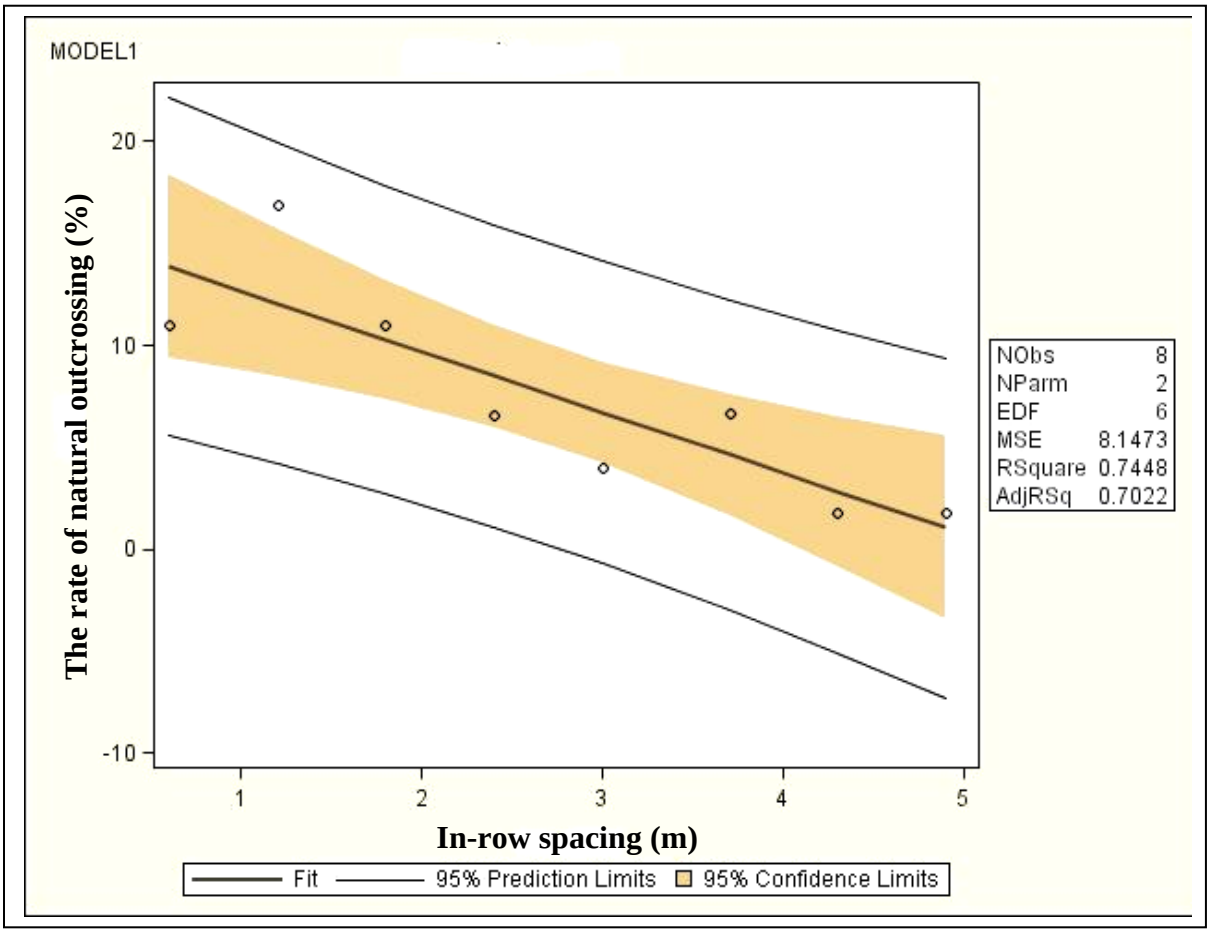


Figure 3. The rate of natural outcrossing in watermelon as a function of in-row spacing. The data were pooled over 2 locations, 2 cultivars, and 7 replications. Regression equation: The rate of natural outcrossing = 15.64 – 2.98 (In-row spacing).

# GENERAL CONCLUSIONS

## Summary of Research Findings

The research findings presented herein have improved the ability of watermelon breeders to design an efficient breeding program for improvement of watermelon yield, develop cultivars with novel phenotypes (fruit shape and rind pattern), and understand the mating system of watermelon.

The results of parent-offspring regression indicate that total fruit weight, total fruit number, marketable fruit weight, fruit size (weight per fruit), and percent culls have low heritability. The heritability estimates are comparatively higher for North Carolina High Yielding Watermelon 2 (NCHYW2) than North Carolina High Yielding Watermelon 1 (NCHYW1) population. This might due to different procedures adapted to develop these populations and it might have resulted in difference in allele frequencies. Moreover, both the populations were tested in different years. Estimates of variance and heritability are dependent on allele frequency, environments, and years in which populations are tested. Estimates of narrow-sense heritability vary from 0.04 to 0.12 for total fruit weight, 0.04 to 0.16 for total fruit number, and 0.06 to 0.15 for marketable fruit weight, 0.18 to 0.19 for fruit size, and 0.02 to 0.09 for percent culls, between NCHYW1 and NCHYW2, respectively. Results indicated that selection would not be effective for yield in NCHYW1, whereas selection for yield in NCHYW2 would be slow and take time to make useful gain. In such cases, recurrent selection or other long term breeding procedure is recommended for population improvement to accumulate favorable genes for yield. Breeders should not waste

time and resources selecting for low percent culls, since heritability is close to zero, indicating that culls are controlled entirely by environment in these populations. Estimates of broad-sense heritability (per-plot basis) were higher than narrow-sense heritability. Estimates of realized heritability were similar to narrow-sense heritability in both the populations.

Genotypic and phenotypic correlations from this study will help watermelon breeders reduce the number of traits to be evaluated and make gains by indirect selection. Total fruit weight and marketable fruit weight are strongly positively correlated indicating that evaluating for either one is sufficient. Total fruit weight is also positively correlated with fruit size. Total fruit number and fruit size are negatively correlated. Therefore, selection for higher fruit number would result in small fruit size. Predicted response in total fruit weight was higher than direct selection using marketable fruit weight and fruit size as the selection criteria.

In our study, fruit shape was not controlled by single incompletely dominant gene (*O*), as reported in previous studies. The family 'Mountain Hoosier' (round) x 'Calsweet' (elongate) was used to study the inheritance of elongate (*OO*), oval (*Oo*), and round (*oo*) shapes. It is important to define fruit shape more precisely. In future studies, fruit shape should be studied quantitatively by measuring length-diameter (LD) ratio. It will then be possible to estimate the fraction of genetic and environmental effects on fruit shape in watermelon. Moreover, results should be verified using several families of elongate fruit cultivars crossed with round fruit cultivars. Families, 'Mountain Hoosier' x 'Calsweet', 'Mountain Hoosier' x 'Minilee', and 'Early Arizona' x 'Minilee', were used to study the

inheritance of solid dark green rind pattern (‘Mountain Hoosier’ and ‘Early Arizona’) against wide stripe (‘Calsweet’) and gray rind pattern in watermelon (‘Minilee’). Like fruit shape, the results to study the inheritance of rind pattern do not conform to previous published reports that solid dark green rind color is controlled by single dominant gene (*G)* against wide striped ($g^s$) and gray rind (*g*). However, segregation ratios of $F_2$ and backcross confirmed that solid dark green rind vs. gray rind is inherited under duplicate dominant gene action. Two genes *g-2* and *g-1* in homozygous recessive form (*g-1g-1 g-2g-2*) produce gray rind, and all other allele combinations (genotypes) would produce solid dark green rind.

High yield and desired fruit size are important breeding goals in many crops. Low to intermediate level of heritability were reported for total fruit weight, total fruit number, and fruit size in a study involving three families (‘Mountain Hoosier’ x ‘Calsweet’, ‘Mountain Hoosier’ x ‘Minilee’, and ‘Early Arizona’ x ‘Minilee’) consisting of six related generations. This indicates a low to moderate level of additive gene action. However, it should be possible to make improvement in these traits by recurrent selection. Low heritability would be a limiting factor in making rapid gains in selection. Breeders should use multiple environment trialing and replicated progeny rows for effective selection.

Estimates of heritability in chapter 2 (based on single plant basis of six-related generations) were appreciably higher than estimates of heritability based on parent-offspring relationship in chapter 1 of this dissertation. Estimates of narrow-sense heritability were more reliable based on parent-offspring regression and broad-sense heritability ( per-plot basis)  obtained from offspring generations as observed in chapter 1. We do not expect

heritability of complex traits like yield to be as high as 0.91 which was observed in chapter 2. Potential bias is possible because estimates of variance and heritability were based on single-plant measurements of six-related generations. The parent, $F_2$, and backcross generations were used in chapter which was essentially based on single plant. Complex traits like yield are evaluated using replicated yield trials as these are highly influenced by environment. The estimates based on single plant basis are very prone to environmental bias and human error, thus provide biased estimates as observed in chapter 2. The estimates of heritability in chapter 1 were based on breeding value of parental generation that was determined using replicated progeny rows. Thus results were more reliable and relatively free from bias due to environment and human error while recording data. It is recommended that estimates of heritability and variance should be conducted by design that involves progeny testing like North Carolina design I, North Carolina Design II, and North Carolina Design III. Another reason that might have contributed to bias in estimating heritability is that vines were trained in spiral and that the plants might have lost female flowers in that process which are source of yield.

The determination of the rate of natural outcrossing is important to plant breeders. Watermelon has a mixed mating system, so determination of outcrossing in this crop is important for designing a suitable breeding strategy. We find that the rate of natural outcrossing in watermelon is dependent on in-row spacing. A high degree of self-pollination occurs when plants are spaced >5 m apart in the row. Thus, breeding techniques used for self-pollinated crops may be used in that case. However, closer in-row spacing does not

produce much cross pollination to intercross families by open-pollination. Thus intercrossing should be done using controlled pollinations in greenhouse. Results might be underestimated due to pollination from border rows. Further studies should be conducted to reduce the potential bias due to transfer of pollens from the border rows, and plant to plant distance at end of plot.

Watermelon breeders often calculate estimates of genetic variances in their populations. Estimates are often miscalculated if the mating system is not well studied, especially in watermelon where the outcrossing rate is dependent on in-row spacing. The coancestry of individuals is higher when parents are spaced apart, due to the increase in self-pollination. In NCHYW1 and NCYYW2 populations, plants in parental generations were spaced at 3.05 m apart. Outcrossing rate is 4 % when plants are spaced 3.05 m apart based on outcrossing study in chapter 3. Self-pollinated crops can have an outcrossing rate of 1-3%. Thus we treated offspring as the result of self-pollination. Estimates of variance and heritability were calculated by the procedure followed for self-pollinated crops. However, results of this study were not applicable to the study conducted by six related generations (Chapter 2), since controlled crosses were made in the greenhouse.

**Implications for watermelon breeders**

Watermelon production in the U.S. is valued at $435 million annually, just below vegetable crops such as tomato and pepper. Traits of interest in watermelon breeding are yield, biotic and abiotic stress resistance, fruit shape and size, rind pattern, and nutrition (sugar, lycopene, and citrulline). The research literature available in watermelon breeding in

these traits is scanty or contradictory. The main problem is that germplasm used to conduct previous studies is not available to conduct future studies. However, the USDA-ARS watermelon germplasm collection in Griffin, Georgia, has over 1,500 accessions that need to be screened for specific gene of interest. Genetic variances for quantitative traits should be estimated among different genetic backgrounds. Based on these variances, populations can be improved for traits of interest.

The research information should be updated in GRIN database (http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl) of National Plant Germplasm system of USDA-ARS. The genetic base of cultivated watermelon is narrow. Watermelon breeders should collaborate with international institutes to introduce new germplasm and broaden the existing genetic base. Wild germplasm should also be incorporated to introduce biotic and abiotic stress resistance. Researchers should send seeds of germplasm used to report new genes to watermelon gene curators, Drs. Todd C. Wehner and Stephen R. King, for future maintenance.

# APPENDIX

Table 1. Test of normality for different traits in NCHYW1 and NCHYW2 populations based on Shapiro- Wilk's test.

| Trait | p-value < W | | | |
|---|---|---|---|---|
| | NCHYW1 | | NCHYW2 | |
| | $S_0$ [x] | $S_{0:1}$ [y] | $S_0$ | $S_{0:1}$ |
| Total fruit weight | 0.194 | 0.0370 | 0.0001 | 0.0430 |
| Total fruit number | 0.0001 | 0.0001 | 0.0001 | 0.0170 |
| Marketable fruit weight | 0.1100 | 0.0460 | 0.0001 | 0.0540 |
| Fruit size | 0.0001 | 0.8510 | 0.0001 | 0.7170 |
| Percent culls | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

[x] $S_0$= Parental generation
[y] $S_{0:1}$= Offspring generation

Table 2. Single locus goodness-of-fit test for rind pattern in watermelon.[z]

| Generation | Total | Solid dark[y] | Gray[x] | Expected[w] | $\chi^2$ | df | P-value |
|---|---|---|---|---|---|---|---|
| 'Mountain Hoosier' x 'Minilee' | | | | | | | |
| $P_aS_1$[v] | 27 | 27 | 0 | | | | |
| $P_bS_1$[u] | 26 | 0 | 26 | | | | |
| $F_1$ | 58 | 58 | 0 | | | | |
| $F_2$ | 249 | 229 | 20 | 3:1 | 38.23 | 1 | 0.000 |
| $BC_1P_a$ | 84 | 84 | 0 | 1:0 | | | |
| $BC_1P_b$ | 82 | 59 | 23 | 1:1 | 15.80 | 1 | 0.000 |
| 'Early Arizona' x 'Minilee' | | | | | | | |
| $P_aS_1$[v] | 26 | 26 | 0 | | | | |
| $P_bS_1$[u] | 28 | 0 | 28 | | | | |
| $F_1$ | 56 | 56 | 0 | | | | |
| $F_2$ | 257 | 239 | 18 | 15:1 | 44.39 | 1 | 0.000 |
| $BC_1P_a$ | 83 | 83 | 0 | 1:0 | | | |
| $BC_1P_b$ | 86 | 62 | 24 | 1:1 | 16.79 | | 0.000 |

[z] Data are ratings from family 'Mountain Hoosier' x 'Minilee' and 'Early Arizona' x 'Minilee' of *Citrullus lanatus var. lanatus* from three locations viz. Kinston, Clinton(M), and Clinton (P)

[y] Solid dark was the standard rind pattern

[x] gray was the mutant rind pattern

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

[v] $P_a$ was carrier of dominant allele (solid dark green )

[u] $P_b$ was carrier of dominant allele (gray)