HortScience 52(7):936–940. 2017. doi: 10.21273/HORTSCI11255-16

Heritability and Genetic Variance Components Associated with Citrulline, Arginine, and Lycopene Content in Diverse Watermelon Cultigens

Todd C. Wehner[1]
Department of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Rachel P. Naegele
USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 S. Riverbend Avenue, Parlier, CA 93648-9757

Penelope Perkins-Veazie
Plants for Human Health Institute, NC Research Campus NCSU, 600 Laureate Way, Kannapolis, NC 28081

Additional index words. cucurbit, human health, *Citrullus lanatus*, pH, Brix

***Abstract*. Citrulline, arginine, and lycopene are naturally occurring compounds found in watermelon, *Citrullus lanatus* (Thumb) Matsum & Nakai, with beneficial effects on plant growth and human health. This study evaluated seven commercial cultivars and one breeding line for citrulline, arginine, and lycopene content in mature fruit grown at two locations in North Carolina. Correlations among these compounds and fruit quality traits (percent soluble solids and flesh pH) were evaluated. Watermelon cultigens evaluated were chosen for their fruit trait diversity. 'Yellow Doll' and NC-517 possessed the highest citrulline and combined concentration of citrulline and arginine of all cultigens evaluated. Lycopene content was highest in 'Dixielee', followed by 'Sugar Baby', and 'Allsweet', each of which have different shades of red flesh color. Location and its interaction with genotype had no significant effect on arginine or lycopene concentration. Broad-sense heritability was estimated for each trait. Arginine content (89%) and lycopene content (99%) had very high heritability. Citrulline content (41%), percent soluble solids (46%), and flesh pH (61%) had moderate heritability. Lycopene was positively correlated with flesh pH ($r = 0.517$) and negatively correlated with percent soluble solids ($r = -0.344$). Arginine content had a weak negative correlation with flesh pH ($r = -0.343$) and was not correlated with percent soluble solids.**

Watermelon [*Citrullus lanatus* (Thumb.) Matsum & Nakai] is an important crop grown worldwide. The United States is the sixth largest producer in the world, with an industry value of more than $430 million and an annual production of ≈2 million metric tons (FAO, 2012; NASS, 2014). Watermelons are grown throughout the United States, with production mainly in the southern states (Wehner, 2008). Recently, watermelon has gained national attention as a good source of antioxidants (lycopene), arginine, and the arginine precursor, citrulline (Hong et al., 2015; Kaore and Kaore, 2014; Wang et al., 2014).

Lycopene is a red-pigmented carotenoid with powerful antioxidant properties that serves as an intermediate for the biosynthesis of other carotenoids (DiMascio et al., 1989; Sandmann, 1994; Tomes et al., 1963). In red-fleshed watermelon, lycopene accounts for 70% to 90% of the total carotenoids; the remaining carotenoids include phytofluene, phytoene, β-carotene, lutein, nerosporene, and ζ-carotene (Gross, 1987; Tomes et al., 1963). In orange-fleshed watermelon, prolycopene, phytoene, and ζ-carotene are the major carotenoids, whereas in canary yellow and salmon yellow-fleshed watermelons, neoxanthin is the major carotenoid (Bang et al., 2010; Tadmor et al., 2004). Studies have shown that the content of lycopene and carotenoids increases rapidly and accumulates 10–12 d after pollination in diploid watermelons and continues to accumulate as the fruit mature (Lv et al., 2015). Lycopene concentration varied widely in the watermelon cultigens tested, ranging from 36 to 120 $mg \cdot kg^{-1}$ of fresh weight and can vary among production environments (Leskovar et al., 2004; Perkins-Veazie et al., 2001, 2006). The lycopene content has generally not been measured in most commercial cultivars.

In humans, lycopene scavenges singlet oxygen and peroxy radicals, and deactivates excited molecules or DNA chain breaking agents (Stahl et al., 1997). Several epidemiology studies found that lycopene reduced cancer cell growth and induced cell death in malignant leukemia, endometrial, mammary, lung, and prostate cancer cells (Amir et al., 1999; Collins et al., 2006; Kotake-Nara et al., 2001; Muller et al., 2002). Arab and Steck (2000) and Matos et al., (2000) reported that lycopene attached to low-density lipoproteins in blood plasma and protected against lipid peroxidation and foam cell production, both of which are implicated in the initiation of atherosclerosis. In other epidemiological studies, Steinmetz and Potter (1996) and Strandhagen et al. (2000) found that a diet consisting of fruits and vegetables rich in lycopene could protect against stroke and cardiovascular diseases, whereas Tarazona-Diaz et al. (2013) determined that watermelon juice containing lycopene and citrulline could improve athlete recovery and performance.

Besides lycopene, watermelons produce L-arginine (arginine) and its precursor L-citrulline (citrulline) in fruit and foliage (Akashi et al., 2001; Davis et al., 2011). In related *Citrullus* species, studies have shown that citrulline content increases in the foliage during drought stress and may improve plant tolerance to stress (Akashi et al., 2001; Wang et al., 2014). Similarly in melon, foliage citrulline content is an important indicator for drought stress (Kusvuran et al., 2013). In developing watermelon fruit, citrulline content is low, reaching peak levels just before maturity, and declining as fruit ages (Fish, 2014). In fruit, the value of citrulline accumulation as a stress tolerance is unknown.

In mammals, arginine plays an essential role in the nitric oxide pathway, contributing to improved immune processes and cardiovascular health. In rats, consumption of watermelon extracts with high citrulline resulted in improved lipid profiles, lower inflammation, and higher antioxidant capacity (Hong et al., 2015). In mice, hypothermia recovery and vascular endothelium function were improved in subjects administered a 1% dose of citrulline (Kobayashi et al., 2014). Free arginine, administered through supplements, can result in side effects such as nausea and gastrointestinal discomfort, prompting physicians to seek alternative forms of arginine (Collins et al., 2007; Hong et al., 2015). In humans, the arginine precursor, citrulline, is converted into arginine via argininosuccinate synthase (Collins et al., 2007).

In watermelon, citrulline has not been associated with fruit phenotypic traits such as flesh color (red, orange, salmon yellow, or white) (Davis et al., 2011). Previously, citrulline content was evaluated in 56 watermelon cultigens (cultivars, breeding lines, and PI accessions) at two locations in Texas and Oklahoma (Davis et al., 2011). Citrulline concentration varied greatly among cultigens. The highest citrulline concentrations were found in 'Tom Watson', PI 306364

Received for publication 26 Sept. 2016. Accepted for publication 23 Nov. 2016.
We would like to thank Tammy L. Ellington for assistance and support with field and laboratory experiments provided at North Carolina State University, Raleigh, NC.
[1]Corresponding author. E-mail: tcwehner@gmail.com.

(Northern Africa) and 'Jubilee', whereas the lowest citrulline concentrations were found in PI 164992 (Turkey), Low Sugar 177, and Low Sugar 194. These data suggest that citrulline has likely not been indirectly selected by breeders during cultivar development. Both unadapted lines and commercial cultigens may have useful variation in citrulline content. The variation in arginine content has not been directly evaluated in watermelon to date.

Watermelon breeding has resulted in a diversity of fruit sizes, rind color, rind patterns, and flesh color. Most cultivars have soluble solids content (°Brix) between 8% and 15% and acidity (flesh pH) of 5.18–5.60 (Corey and Schlimme, 1988). Studies have shown that variation in citrulline and lycopene exist within cultivated watermelons, but wild relatives and heirloom cultivars can also serve as good sources of trait variation for genetic improvement (Wehner, 2008). Many of these wild and weedy relatives have undesirable characteristics such as high or low flesh pH or low sugar content. Estimates of heritability and correlations among traits can help in the selection of breeding strategies and selection of parent material. Few studies to date have looked at the heritability and correlation of health compounds such as lycopene, arginine, and citrulline with fruit quality traits such as percent soluble solids and flesh acidity. The objectives of this study were to a) evaluate a diverse set of watermelon cultigens for lycopene, citrulline, and arginine content; b) determine the heritability of those traits; and c) measure the correlation of health compound concentrations with fruit acidity and soluble solids content.

Materials and Methods

Germplasm and field design. Eight diploid watermelon cultigens were evaluated for lycopene, citrulline, arginine, flesh acidity, and percent soluble solids content using field-grown fruit (Table 1). Cultigens were chosen to represent a range of sizes, shapes, rind patterns, and flesh colors (including canary yellow, salmon yellow, coral red, and scarlet red) (Table 1). Seeds for each genotype were sown in 72-cell polyethylene flats filled with an artificial soilless growing medium (4P *Fafard* soilless mix; Conrad Fafard Inc., Agawam, MA) in a greenhouse at the Horticultural Field Laboratory at North Carolina State University, Raleigh, NC. The flats were moistened to capacity after seeding, and held in the greenhouse at 25–30 °C until full seedling emergence. The transplants were moved to cold frames for 1 week of acclimation before transplanting into the field at the two-true-leaf stage. The plants were transplanted into raised beds covered with black polyethylene mulch and grown according to the recommendations in the North Carolina Extension Service and Southeastern US Vegetable Crops handbook (Holmes and Kemble, 2009; Sanders, 2004).

The treatment unit for each plot was a 3.7 m-long plot on rows with 3.1 m from center to center. Soils were a sandy clay loam at the Cunningham station near Kinston and sandy loam at the Horticultural Crops Research Station near Clinton, NC. Missing or damaged transplants were replaced 1 week after the initial transplanting. The experiment was a randomized complete block design with three replications, two locations, and eight cultigens. Fruit from each plot were harvested at maturity according to the number of days to maturity, as well as the indicators of maturity; i.e., a brown and dry tendril at the node bearing the fruit, a dull waxy fruit surface, a light-colored groundspot on the fruit, and a dull sound of the fruit when struck (Maynard, 2001).

Data collection. For each location, three fruit of each genotype per replication were individually harvested for evaluation. Individual fruit were cut into half (stem to blossom) and watermelon flesh was scooped from the center of the fruit heart. For each replicate, the fruit were combined and stored in 1-quart plastic freezer bags. The bags were pressed to remove air before sealing. At least 20 g of heart tissue (without seeds) were harvested from each plot. The Ziploc® bags (SC Johnson & Son, Racine, WI) were firmly pressed to macerate the tissue sample. The samples were immediately placed on ice in an insulated cooler in the field and later stored at –80 °C (1 month) or for short periods at –20 °C (3–5 d) until lycopene analysis.

Phytonutrient content was analyzed at the Plants for Human Health Institute, NC Research Campus NCSU, Kannapolis, NC. Lycopene was measured using a Hunter Plus colorimeter (Hunter, NJ). Total lycopene content was determined as $mg \cdot kg^{-1}$ using the formula $(absorbance_{560nm} - absorbance_{700nm}) \times 37.8$, where 37.8 is the slope derived by plotting values from the colorimeter against the same samples analyzed with hexane extraction using a spectrophotometer and standardized with an external lycopene standard (Davis et al., 2003). Citrulline and arginine content were determined as $\mu g \cdot g^{-1}$ using high-performance liquid chromatography (Collins et al., 2007) and external standards of L-citrulline and arginine (Sigma-Aldrich, St. Louis, MO). Percent soluble solids and flesh pH were measured for bulked fruit samples using a digital refractometer (Atago PAL-1; Atago Inc., Bellevue, WA) and a digital pH meter (H260G; Hach, Loveland, CO) equipped with a stainless steel rounded electrode (PH77-SS; Hach), respectively. A flesh pH of 5.2–5.6 is ideal, a pH above 5.9 is considered over ripe (Corey and Schlimme, 1988).

Data analysis. Data were analyzed for genotype, environment, and genotype × environment interactions using the SAS (SAS Institute, Cary, NC) procedure for general linear models (PROC GLM). Locations, replications, and genotypes were analyzed as random effects. Analysis of variance was used to determine the size and significance of interactions for each trait of interest. Least squared means or adjusted trait means and least significant difference for each genotype were computed over the two environments for the traits of interest. Hereafter, "mean" is used to indicate least squared mean or adjusted trait mean. Pairwise comparisons were made if F ratio was significant (5% level). Variance components were estimated using PROC VARCOMP implemented within SAS.

Phenotypic variance was calculated without environmental variance according to Hallauer and Miranda (1981). Correlation among traits was calculated using Pearson's correlation coefficient (r) implemented in PROC CORR in SAS. Shapiro–Wilk test indicated normal distribution for all traits with the exception of lycopene. Transformation of lycopene using log10 or square root did not improve the distribution. Data were skewed toward the low tail because most cultigens had a low lycopene content. Broad-sense heritability (H) was calculated for each trait using the ratio of genotypic to phenotypic variance (from variance component estimation) as in Eq. [1], where VarG is equal to genetic variance, VarG × L is the variance due to genotype by location interactions, and the VarEr is the variance due to error (Eq. [1]).

$$H = \mathrm{VarG}/(\mathrm{VarG} + \mathrm{VarG} \times \mathrm{L}/2 + \mathrm{VarEr}/2 \times 3) \quad [1]$$

Results

Location was not significant in this study for any of the traits evaluated except pH, and data were combined for analyses. When the concentration of citrulline plus arginine was analyzed, cultigen ($P = 0.0425$) and the interaction between cultigen and location ($P = 0.0307$) were significant. The mean of the population was 3.82 ± 0.71 $\mu g \cdot g^{-1}$ with a minimum of 1.7 $\mu g \cdot g^{-1}$ and a maximum of 5.7 $\mu g \cdot g^{-1}$. Highest combined concentrations of citrulline and arginine were detected in cultigens NC-517 and Yellow Doll. The lowest concentrations were detected in cultivars Sugar Baby and Charleston Gray (Table 2).

When analyzed for citrulline content, replication was not significant. However,

Table 1. Characteristics of the eight cultigens of watermelon evaluated in this study.

Cultigen	Color flesh	Color gene	Days to maturity	Fruit size (kg)
Allsweet	Coral red	Y^{Crl}	90	9.1
Charleston Gray	Coral red	Y^{Crl}	85	8.2
Dixielee	Scarlet red	Y^{Scr}	90	9.3
NC-517	Canary yellow	*C*	85	6.4
Sugar Baby	Deep red	*Unknown*	85	4.5
Tendersweet OF	Orange	Y^{O}	90	8.6
Yellow Crimson	Salmon yellow	*y*	80	9.1
Yellow Doll	Canary yellow	*C*	65	3.0

Table 2. Mean values for citrulline, arginine, lycopene, and fruit quality traits of eight watermelon cultigens.

Cultigen	Citrulline (μg·g^{-1})	Arginine (μg·g^{-1})	Cit + Arg[z]	Lycopene (kg·g^{-1})	Flesh acidity (pH)	Soluble solids (%)
Yellow Doll	3.55 a	0.64 d	4.18 a	14.51 e	5.55 bcd	11.60 a
NC-517	3.45 ab	0.90 c	4.35 a	12.99 e	5.61 b	10.95 abcd
Dixielee	3.04 abc	0.99 bc	4.03 ab	59.26 a	5.79 a	11.08 abc
Allsweet	2.82 bcd	1.15 b	3.97 ab	41.77 c	5.43 d	10.52 cd
Tendersweet Orange Flesh	2.59 cd	1.40 a	3.99 ab	20.33 d	5.56 bc	10.73 bcd
Sugar Baby	2.34 d	0.85 cd	3.19 c	53.37 b	5.82 a	10.30 d
Yellow Crimson	2.32 d	1.45 a	3.77 abc	14.86 e	5.44 cd	11.37 ab
Charleston Gray	2.21 d	0.86 c	3.07 c	39.84 c	5.65 b	10.42 cd

[z]Combined citrulline and arginine concentration in μg·g^{-1}.

the interaction between cultigen and location (P = 0.0176) and cultigen (P = 0.0008) were significant. The population mean for citrulline was 2.79 ± 0.56 μg·g^{-1} with a minimum of 1.20 μg·g^{-1} and a maximum of 4.76 μg·g^{-1}. Citrulline content was lowest in 'Yellow Crimson', 'Sugar Baby', and 'Charleston Gray'. Watermelon cultigens Yellow Doll and NC-517 had the highest citrulline content (Table 2).

Similarly, lycopene content was significantly affected by cultigen (P < 0.0001) but not replicate (P = 0.7708) or the interaction between cultigen and location (P = 0.3020). Lycopene mean for the population was 32.12 ± 3.65 mg·kg^{-1} with a minimum of 11.72 mg·kg^{-1} and a maximum of 62.46 mg·kg^{-1}. Highest lycopene was detected in red-fleshed watermelon cultigen, 'Dixielee', while yellow and orange-fleshed cultigens, 'Yellow Doll', 'Yellow Crimson', and NC-517 had the lowest lycopene (Table 2).

When analyzed for arginine content, replicate and the interaction between cultigen and location were not significant, while cultigen (P < 0.0001) was significant. For arginine, the population mean was 1.0 ± 0.2 μg·g^{-1} with a minimum of 0.5 μg·g^{-1} and a maximum of 1.8 μg·g^{-1}. Arginine content was highest in 'Yellow Crimson' and 'Tendersweet Orange Flesh' and lowest in 'Yellow Doll' (Table 2).

When analyzed for flesh acidity (pH), replicate (P = 0.0439), cultigen (P < 0.0001), and the interaction between cultigen and location (P = 0.0017) were significant. The population mean was 5.6 ± 0.1 with a minimum pH of 5.3 and a maximum of 6.04. Among cultigens, flesh pH was highest in 'Sugar Baby' and 'Dixielee', and lowest in 'Allsweet' (Table 2).

Percent soluble solids was significant for cultigen (P = 0.0136) but not replication or the interaction between cultigen and location. Percent soluble solids for the population was 10.9 ± 0.7 with a minimum of 9.3 and a maximum of 13.3. Percent soluble solids was highest in 'Yellow Doll' and 'Yellow Crimson' (Table 2). The lowest percent soluble solids was for 'Sugar Baby'.

In the analysis of variance, the cultigen mean square was the largest mean square for lycopene concentration, citrulline concentration, percent soluble solids, and flesh acidity (Supplemental Table 1). For arginine content and flesh pH, the mean squares for location and the location by cultigen interaction were also relatively large. When evaluating location effect for these three traits, most cultigens had a higher arginine content at Clinton compared with Kinston (Table 3). Watermelon cultigen Charleston Gray had the largest shift in arginine concentration between locations. Flesh pH was not consistently higher or lower at either location among the cultigens examined.

Table 3. Mean values for citrulline, arginine, and flesh pH of eight watermelon cultigens at two locations.

	Citrulline (μg·g^{-1})		Arginine (μg·g^{-1})		Flesh acidity (pH)	
Cultigens	CI[z]	KN[y]	CI	KN	CI	KN
Allsweet	3.04	2.61	1.22	1.08	5.4	5.4
Charleston Gray	2.95	1.46	1.12	0.61	5.5	5.8
Dixielee	2.80	3.28	0.95	1.02	5.7	5.9
NC-517	2.87	4.03	0.88	0.92	5.6	5.6
Sugar Baby	2.63	2.04	0.91	0.80	5.9	5.7
Tendersweet Orange Flesh	2.78	2.39	1.42	1.38	5.4	5.7
Yellow Crimson	2.32	—	1.45	—	5.4	—
Yellow Doll	3.86	3.23	0.63	0.64	5.5	5.6

[z]Horticultural crops research station, Clinton, NC.
[y]Cunningham research station, Kinston, NC.

Broad-sense heritability varied greatly among fruit traits evaluated. Arginine and lycopene were highly heritable, 89% and 99%, respectively. Broad-sense heritability was low to moderate for citrulline, flesh pH, and percent soluble solids (41%, 61%, and 46%, respectively) (Table 4). Variance component estimates indicated that cultigen was the primary contributor for variation within most traits. Location variance was small relative to cultigen and cultigen by location interactions for arginine, lycopene, and flesh pH, and near zero for other traits (citrulline and percent soluble solids) (Table 4).

Low, but significant correlations were detected among several pairs of traits (Table 5). The combined citrulline and arginine concentration was significantly correlated with percent soluble solids (r = 0.379). Arginine concentration and fruit flesh pH were weakly, but significantly, negatively correlated (r = –0.343). Citrulline and arginine concentrations were not correlated. Citrulline concentration was positively correlated with percent soluble solids (r = 0.391). Lycopene concentration was positively correlated with flesh pH (r = 0.517) and negatively correlated with percent soluble solids (r = –0.344).

Discussion

Watermelon is an economically important food crop with many potential health benefits from its natural concentrations of lycopene and citrulline. In mammals, a diet rich in lycopene and citrulline has been shown to result in improved blood circulation and lipid profiles, and to protect against cardiovascular disease and stroke (Hong et al., 2015; Steinmetz and Potter, 1996; Strandhagen et al., 2000). Historically, watermelon cultivars have not been bred for high lycopene or citrulline concentrations. Breeding for increased citrulline and lycopene could increase the marketability of watermelon as a health food, while contributing to improved plant stress tolerance (Akashi et al., 2001; Kusvuran et al., 2013; Wang et al., 2014).

In a previous study, watermelon cultigens were evaluated for citrulline concentration in field-grown fruit. Differences in citrulline concentration were evident among and within accessions (Davis et al., 2011). However, in this study, there were no effects of environment (location) on citrulline or lycopene concentrations. Citrulline concentration was stable among cultigens, although arginine varied greatly. Cultivars Allsweet, Charleston Gray, Yellow Crimson, Sugar Baby, and Tendersweet Orange Flesh were evaluated in two locations in both studies. When grown in Oklahoma, cultigens had consistently higher citrulline concentration than the same cultigens grown in Texas. When grown in Kinston, NC, most cultigens had a lower arginine and combined arginine and citrulline concentration than the same cultigen grown in Clinton, NC. Production practices were consistent between these two locations, although differences in soil type and weather may contribute to the variation. Breeding line NC-517 and cultivar Dixielee, of which neither had been previously evaluated, had higher concentrations in Kinston, NC, compared with Clinton, NC. In each study, 'Charleston Gray' had the highest variability between locations of the cultigens evaluated in either citrulline or arginine concentration. These data suggest that 'Charleston Gray' would not be a suitable cultivar for developing stable citrulline or arginine concentrations in watermelon.

Table 4. Variance component estimates for citrulline, arginine, lycopene, and fruit quality traits of eight watermelon cultigens.

Component	Cit + Arg[z]	Citrulline	Arginine	Lycopene	Flesh acidity (pH)	Soluble solids (%)
Variance component estimates						
Location	–0.0180	–0.0200	0.0013	0.1335	0.0011	–0.0061
Block	0.0391	0.0245	0.0006	–0.9148	0.0024	–0.0178
Cultigen	–0.0170	0.1102	0.0717	347.7468	0.0126	0.0999
Location × cultigen	0.2816	0.2113	0.0052	1.1860	0.0125	0.0943
Error	0.5100	0.3172	0.0373	13.3290	0.0106	0.4080
Quantitative genetic effects						
Phenotypic	0.2088	0.2688	0.0805	350.5612	0.0208	0.2151
Environmental	–0.0180	–0.0200	0.0013	0.1335	0.0011	–0.0061
Genotypic	–0.0170	0.1102	0.0717	347.7468	0.0128	0.0999
Broad-sense heritability (H)	–0.0812	0.4102	0.8905	0.9920	0.6138	0.4645

[z]Combined citrulline and arginine concentration in $\mu g \cdot g^{-1}$.

Table 5. Pearson's correlation coefficient of traits for eight watermelon cultigens.[z]

Trait	Cit + Arg[y]	Citrulline	Arginine	Lycopene	Flesh acidity (pH)
Citrulline	0.930*	—			
Arginine	0.460*	0.103	—		
Lycopene	–0.229	–0.191	–0.160	—	
Flesh pH	–0.215	–0.101	–0.343*	0.517*	—
Soluble solids (%)	0.379*	0.391*	0.080	–0.344*	–0.013

*Significant at $P = 0.05$.
[z]Data were normally distributed (Shapiro–Wilk test) for all traits except lycopene, which was skewed toward the low end of the curve.
[y]Combined citrulline and arginine concentration.

Davis et al. (2011) also reported that differences in citrulline concentration were not associated with flesh color or pollination type based on citrulline concentrations of 56 cultigens with red, orange, salmon-yellow, or white flesh. Previously, Rimando and Perkins-Veazie (2005) determined that yellow-fleshed watermelons had higher citrulline concentrations compared with red- or orange-fleshed cultigens. No consistent differences were observed between red- and orange-fleshed cultigens. In our study, the highest citrulline concentration was detected in 'Yellow Doll' (canary yellow) and NC-517 (canary yellow). Cultivars Allsweet (red) and Dixielee (scarlet) had higher levels of citrulline than 'Tendersweet Orange Flesh' (orange) and 'Yellow Crimson' (yellow). Considering the differences among the eight cultigens tested in this study, it would be important to determine whether the other cultigens with canary yellow flesh color have higher citrulline compared with cultigens having red, orange, or salmon-yellow flesh. 'Charleston Gray' (coral red) and 'Sugar Baby' (deep red) had some of the highest levels of citrulline when grown in Lane, OK, while having moderate ('Sugar Baby') and low ('Charleston Gray') citrulline content in North Carolina. The differences in citrulline concentration among the same cultigens grown in different locations may be because of production practices or environmental (temperature, water, etc.) effects.

Lycopene content varies greatly across red, yellow, and orange-fleshed watermelons with red-fleshed cultigens having higher lycopene than yellow- or orange-fleshed cultigens. Perkins-Veazie et al. (2001) evaluated 13 watermelon cultigens for lycopene content, including 'Dixielee' and 'Crimson Sweet'. Lycopene ranged from 37.9 to 71.2 $\mu g \cdot g^{-1}$. Another study by Yoo et al. (2012) reported high variability in lycopene content (4.8–47.8 $\mu g \cdot g^{-1}$) among cultigens. These results have been mirrored by cultigen evaluations in India (Nagal et al., 2012). Similarly, in this study, lycopene ranged from 11.7 to 62.5 $\mu g \cdot g^{-1}$ and was different among fruit flesh colors. The scarlet red–fleshed watermelon cultigen ('Dixielee') had highest lycopene, followed by deep red ('Sugar Baby'), and the coral red ('Allsweet' and 'Charleston Gray') cultigens. The orange-fleshed cultigen ('Tendersweet Orange Flesh') had higher lycopene than the salmon-yellow ('Yellow Crimson'), and canary yellow-fleshed ('Yellow Doll' and NC-517) cultigens.

Breeding-improved lycopene, arginine, and citrulline concentration has been limited by the lack of information on the heritability of these traits. Previously, the general combining ability and heritability of lycopene content was found to be high in watermelon and suggested an additive effect for dominant genes (Zhang et al., 2010). In this study, lycopene content was highly heritable and demonstrated no cultivar × environment interaction. Similarly, arginine was also highly heritable with no cultivar × environment interaction. Despite being an arginine precursor, citrulline concentrations were highly environment-dependent and had low heritability. Based on variability and heritability data, breeding for improved arginine and lycopene content, but not citrulline, could result in effective gains using currently available cultigens. However, negative correlations with fruit quality traits should also be considered when breeding for improved arginine and lycopene.

Davis et al. (2013) reported that lycopene concentration was slightly positively correlated with percent soluble solids in six diploid watermelons, their induced autotetraploids, and their offspring. However, no correlations were detected between citrulline and percent soluble solids. In this study, minimal correlations were detected among citrulline, arginine, or lycopene concentrations and fruit quality. Arginine had a negative correlation with flesh pH, whereas citrulline only had a positive correlation with Brix. In contrast, lycopene had a negative correlation with Brix and a positive correlation with flesh pH. Further evaluation of more cultigens and germplasm will present clearer information on the potential correlations between citrulline or lycopene and fruit quality.

Arginine is affected by citrulline because citrulline is its precursor but is also affected by other physiological activities in the plant (Kawasaki et al., 2000). Thus, we expected some correlation between the two traits, especially because the cultigens in this study had moderate to high amounts of citrulline, yet the correlation measured was low (0.103) and not significant. The low (–0.344) but significant negative correlation between percent soluble solids and lycopene is the opposite of those previously reported. This is probably because the canary yellow cultigens had little lycopene, but were high in percent soluble solids. Thus, it is likely that both traits can be improved simultaneously by breeding.

Good flesh color, moderate flesh pH, and high soluble solids content are important quality traits in successful cultivars. As consumer preference for natural health foods increases, the demand for watermelons containing high citrulline and lycopene may increase. Breeding for high citrulline (heritability 0.41) and arginine (heritability 0.89) content should be possible because they are uncorrelated and have moderate to high heritability. Lycopene content had very high heritability (0.99) in this study as the trait could be measured accurately over locations and blocks. Orange and salmon yellow cultigens had consistently low lycopene, whereas scarlet red and coral red cultigens had consistently high lycopene content. Negative correlations of lycopene with percent soluble solids (–0.34) and arginine with flesh pH (–0.34) suggest that breeders will find it difficult to incorporate high lycopene, citrulline, and high fruit quality into a single inbred line.

Literature Cited

Akashi, K., C. Miyake, and A. Yokota. 2001. Citrulline, a novel compatible solute in drought-tolerant wild watermelons leaves, is an efficient hydroxyl radical scavenger. FEBS Lett. 508:438–442.

Amir, H., M. Karas, J. Giat, M. Danilenko, R. Levy, T. Yermiahu, J. Levi, and Y. Sharoni. 1999. Lycopene and 1, 25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. Nutr. Cancer 33:105–112.

Arab, L. and S. Steck. 2000. Lycopene and cardiovascular disease. Amer. J. Clin. Nutr. 71:1691S–1695S.

Bang, H., A.R. Davis, S. Kim, D.I. Leskovar, and S.R. King. 2010. Flesh color inheritance and gene interactions among canary yellow, pale yellow and red watermelon. J. Amer. Hort. Sci. 135:362–368.

Collins, J.K., P. Perkins-Veazie, and W. Roberts. 2006. Lycopene: From plants to humans. HortScience 41:1135–1144.

Collins, J.K., G. Wu, P. Perkins-Veazie, K. Spears, L. Claypool, R.A. Baker, and B.A. Clevidence. 2007. Watermelon consumption increases plasma arginine concentrations in adults. Nutrition 23:261–266.

Corey, K.A. and D.V. Schlimme. 1988. Relationship of rind gloss and groundspot color to flesh quality of watermelon fruits during maturation. Sci. Hort. 34:211–218.

Davis, A.R., W. Fish, and P. Perkins-Veazie. 2003. A rapid hexane-free method for analyzing lycopene content in watermelon. J. Food Sci. 68:328–332.

Davis, A.R., C.L. Webber, W.W. Fish, T.C. Wehner, S. King, and P. Perkins-Veazie. 2011. L-citrulline levels in watermelon cultigens tested in two environments. HortScience 46:1572–1575.

Davis, A.R., C.L. Webber, W.G. Liu, P. Perkins-Veazie, A. Levi, and S. King. 2013. Watermelon quality traits as affected by ploidy. HortScience 48:1113–1118.

DiMascio, P., S.P. Kaiser, and H. Sies. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. Arch. Biochem. Biophys. 274:532–538.

Fish, W.W. 2014. The expression of citrulline and other members of the arginine metabolic family in developing watermelon fruit. Internat. J. Innovations Res. 2:665–672.

Food and Agricultural Organization of the United Nations (FAO). 2012. <www.fao.org/faostat/en/>.

Gross, J. 1987. Pigments in fruits. Academics Press, New York, NY.

Hallauer, A.R. and J.B. Miranda Fo. 1981. Quantitative genetics in maize breeding, 2nd ed. Iowa St. Press, Ames, IA.

Holmes, G.J. and J.M. Kemble. 2009. Southeastern U.S. 2009 vegetable crops handbook [Online]. Accessed 30 Oct. 2009; verified 24 Oct. 2011. <http://www.lsuagcenter.com/NR/rdonlyres/55456C33-CBB9-45E1-BA34-859CEFA09724/54862/2009_SEVG1.pdf>.

Hong, M.Y., N. Hartig, K. Kaufman, S. Hooshmand, A. Figueroa, and M. Kern. 2015. Watermelon consumption improves inflammation and antioxidant capacity in rats fed an antherogenic diet. Nutr. Res. 35(3):251–258.

Kaore, S.N. and N.M. Kaore. 2014. Citrulline: Pharmacological perspectives and role as a biomarker in disease and toxicities, p. 883–905. In: R.C. Gupta (ed.). Biomarkers in toxicology. Academic Press, Elsevier Inc., Waltham, MA.

Kawasaki, S., C. Miyake, T. Kohchi, S. Fujii, M. Uchida, and A. Yokota. 2000. Responses of wild watermelon to drought stress: Accumulation of an ArgE homologue and citruline in leaves during water deficits. Plant Cell Physiol. 41 (7):864–873.

Kobayashi, Y., K. Narita, K. Chiba, H. Takemoto, M. Morita, and K. Morishita. 2014. Effects of L-citrulline diet on stress-induced cold hypersensitivity in mice. Pharmacogn. Res. 6:297–302.

Kotake-Nara, E., M. Kushiro, H. Zhang, T. Sugawara, K. Miyashita, and A. Nagao. 2001. Carotenoids affect proliferation of human prostate cancer cells. J. Nutr. 131:3303–3306.

Kusvuran, S., H.Y. Dasagan, and K. Abak. 2013. Citrulline is an important biochemical indicator in tolerance to saline and drought stresses in melon. Sci. World J. 2013:253414.

Leskovar, D.I., H. Bang, K.M. Crosby, N. Maness, J.A. Franco, and P. Perkins-Veazie. 2004. Lycopene, carbohydrates, ascorbic acid and yield components of diploid and triploid watermelon cultivars are affected by deficit irrigation. J. Hort. Sci. Biotechnol. 79:75–81.

Lv, P., N. Li, H. Liu, H. Gu, and W. Zhao. 2015. Changes in carotenoid profiles and the expression pattern of the genes in carotenoid metabolisms during fruit development and ripening in four watermelon cultigens. Food Chem. 174:52–59.

Matos, H.R., P. Di Moscio, and M.H. Medeiros. 2000. Protective effect of lycopene on lipid peroxidation and oxidative DNA damage in cell culture. Arch. Biochem. Biophys. 383: 56–59.

Maynard, D.N. (ed.). 2001. Watermelons. Characteristics, production, and marketing.ASHS Press, Alexandria, VA. p. 1–227.

Muller, K., K.L. Carpenter, I.R. Challis, J.N. Skepper, and M.J. Arends. 2002. Carotenoids induce apoptosis in the T-lymphoblast cell line Jurkat E6.1. Free Radic. Res. 36:791–802.

Nagal, S., C. Kaur, H. Choudhary, J. Singh, B.B. Singh, and K.N. Singh. 2012. Lycopene content, antioxidant capacity and color attributes of selected watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) cultigens grown in India. Intl. J. Food Sci. Nutr. 63:996–1000.

NASS. 2014. Agricultural Statistics. Washington DC. <https://www.nass.usda.gov/Publications/Ag_Statistics/2014/Ag%20Stats%202014_Complete%20Publication.pdf>.

Perkins-Veazie, P., J.K. Collins, A.R. Davis, and W. Roberts. 2006. Carotenoids content of 50 watermelon cultivars. J. Agr. Food Chem. 54:2593–2597.

Perkins-Veazie, P., J.K. Collins, S. Pair, and W. Robert. 2001. Lycopene content differs among red fleshed watermelon cultivars. J. Agr. Food Chem. 81:983–987.

Rimando, A.M. and P.M. Perkins-Veazie. 2005. Determination of citrulline in watermelon rind. J. Chromatogr. 1078:196–200.

Sanders, D.C. (ed.). 2004. Vegetable crop guidelines for the Southeastern U.S. 2004–2005. North Carolina Veg. Growers Assn, Raleigh, NC.

Sandmann, G. 1994. Carotenoid biosynthesis in microorganism and plants. Eur. J. Biochem. 223:7–24.

Stahl, W., S. Nicolai, K. Briviba, M. Hanusch, G. Broszeit, M. Peters, H.D. Martin, and H. Sies. 1997. Biological activities of natural and synthetic carotenoids: Induction of gap junctional communication and singlet oxygen quenching. Carcinogenic 18:89–92.

Steinmetz, K.A. and J.D. Potter. 1996. Vegetables, fruits, and cancer prevention: A review. J. Amer. Diet. Assoc. 96:1027–1039.

Strandhagen, E., P.O. Hansson, I. Bosaeus, B. Isaksson, and H. Eriksson. 2000. High fruit intake may reduce mortality among middle-aged and elderly men. The study of men born in 1913. Eur. J. Clin. Nutr. 54:337–341.

Tadmor, Y., S. King, A. Levi, A. Davis, A. Meir, B. Wasserman, J. Hirschberg, and E. Lewinsohn. 2004. Comparative fruit colouration in watermelon and tomato. Food Res. Intl. 38:837–841.

Tarazona-Diaz, M.P., F. Alacid, M. Carrasco, I. Martinez, and E. Aguayo. 2013. Watermelon juice: Potential functional drink for sore muscle relief in athletes. J. Agr. Food Chem. 61:7522–7528.

Tomes, M.L., K.W. Johnson, and M. Hess. 1963. The carotene pigment contain of certain red fleshed watermelons. Proc. Amer. Soc. Hort. Sci. 82:460–464.

Wang, Z.Y., H.T. Hu, L.R. Goertzen, J.S. McElroy, and F. Dane. 2014. Analysis of the *Citrullus colocynthis* transcriptome during water deficit stress. PLoS One 9(8):E104657.

Wehner, T.C. 2008. Watermelon. p. 381–418. In: J. Prohens and F. Nuez (eds.). Handbook of plant breeding; vegetables I: Asteraceae, Brassicaceae, Chenopodiaceae, and Cucurbitaceae. Springer Science+Business LLC, New York, NY.

Yoo, K.S., H. Bang, E.J. Lee, K. Crosby, and B.S. Patil. 2012. Variation of carotenoid, sugar, and ascorbic acid concentrations in watermelon genotypes and genetic analysis. Hort. Environ. Biotechnol. 53:552–560.

Zhang, F., H. He, and Y. Xu. 2010. Watermelon lycopene content correlation with flesh colour and genetic research. ISHS Acta Horticulturae 856: International Symposium on Vegetable Safety and Human Health. [Abstract].

Supplemental Table 1. Analysis of variance (df and means squares) for six fruit quality traits across eight watermelon cultigens, two locations, and four replications.

Source	df[z]	Citrulline + Arginine[y]	Citrulline	Arginine	Lycopene	Flesh acidity (pH)	Soluble solids (%)
Location	1	1.2352	0.6697	0.0875	12.7720	0.0945	0.4033
Rep (Loc)	4	0.8228	0.5167	0.0418	6.0102	0.0298	0.2658
Cultigen	7	1.2530	1.6126	0.4831	2103.3674	0.1246	1.2903
Loc × cultigen	7	1.3547	0.9511	0.0528	16.8868	0.0481	0.6909
Error	28	0.5099	0.3172	0.0372	13.3289	0.0105	0.4079

[z]Degrees of freedom.
[y]Combined citrulline and arginine.