Citrulline and Arginine Are Moderately Heritable in Two Red-fleshed Watermelon Populations

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Abstract. Watermelon fruit [*Citrullus lanatus* (Thumb) Matsum & Nakai] is a natural source of phytonutrients, including lycopene, citrulline, and arginine. Two segregating, highly outcrossed North Carolina watermelon populations, NC High Yield (NCHYW) and NC Small Fruit (NCSFW), were evaluated for these traits and for indicators of ripeness (pH and soluble solids content). Parents tested in 2015 ($N_{SF} = 300$, $N_{HY} = 300$) were sampled for the above and offspring were tested in 2016 if the sampled fruit of the parents were of qualifying ripeness [soluble solids concentration (SSC) ≥ 8 , pH 5.5–6.5], resulting in 251 families ($N_{SF} = 72$, $N_{HY} = 175$). Narrow-sense heritability was estimated in each of the populations using the methods of 1) parent-offspring regression and 2) variance of half-sibling family means. Heritability for citrulline in NCHYW was moderate in both parent-offspring and half-sibling estimations (38% and 43%), as was arginine (40% and 44%) and lycopene (46% and 47%, respectively). Estimates for these traits in NCSFW were considerably different, with parent-offspring and half-sibling estimations for citrulline (65% and 22%), arginine (9% and 20%), and lycopene (44% and 68%). In NCHYW, moderate phenotypic correlations were found between SSC and citrulline (0.40), arginine (0.40), their combination (0.45), and lycopene (0.30) all of which were significant, except lycopene. Lycopene was significantly and weakly correlated to citrulline (0.22), but was not correlated to arginine (0.06). Similar correlations were found in NCSFW; SSC was significantly correlated to citrulline (0.24), arginine (0.18), and their combination (0.23), whereas lycopene was slightly correlated to citrulline (0.15) and not significantly correlated to arginine. Based on these heritabilities and phenotypic correlations, tandem selection for high lycopene and citrulline content may be accomplished efficiently using progeny rows with minimal replication using the NCSFW population, whereas replication with multiple years, rows, and locations may be necessary for creating stable lines using the NCHYW population.

The American Heart Association endorses watermelon as a heart-healthy food due to its low sodium and fat content (American Heart Association, 2018). Watermelon contains both lycopene and citrulline, bioactive compounds with implications in both plant and human health (Fish, 2012; Perkins-

Veazie et al., 2012; Tedesco et al., 1984). Lycopene, a red-pigmented carotenoid, serves as a strong antioxidant endogenously in plants and dietarily in humans. In plants, it is an intermediate in carotenoid biosynthesis, and in watermelons, it is found in greatest abundance in red-fleshed cultivars (70% to 90% of total carotenoids) (DiMascio et al., 1989; Tomes et al., 1963). Mutations in genes upstream and downstream in its synthesis generate other flesh colors, including orange, canary yellow, and salmon yellow watermelons (Kang et al., 2010; Tadmor et al., 2004). Lycopene and total carotenoid abundance increase rapidly at 10 to 12 d after pollination in diploid watermelons and continue to increase as the fruit matures (Lv et al., 2015).

Epidemiological studies have found that diets including high-lycopene fruit and vegetables (e.g., tomato, watermelon, grapefruit, and guava) may reduce risk of stroke and cardiovascular diseases (Steinmetz and Potter, 1996; Strandhagen et al., 2000). Lycopene is most known for its ability to reduce cancer cell growth and induce cell death in malignant leukemia, mammary, endometrial, lung, and prostate cancer cells (Amir et al., 1999; Muller et al., 2002; Kotake-Nara et al.,

2001). Lycopene also may affect cardiovascular health by slowing the development of atherosclerosis through attachment to low-density lipoproteins in blood plasma and protecting against lipid peroxidation and foam cell production (Arab and Steck, 2000; Gianetti et al., 2002; Matos et al., 2000). Mechanistically, lycopene scavenges radical oxygen species and quenches DNA chain breaking agents (Stahl et al., 1997).

Citrulline, a physiological amino acid with a high N:C ratio, has implications in both plant and animal health. Citrulline has been administered orally (citrulline malate) in both human and mouse studies to improve human health, including skeletal and muscle performance and for prevention of muscle loss (Pérez-Guisado and Jakeman 2010; Sadji et al., 2015), pharmacology (Levillain et al., 1997; Rougé et al., 2007; Thibault et al., 2011), immunology (Sureda et al., 2009), and neurology (Sase et al., 2013). Citrulline supplementation offers several pharmacokinetic advantages over arginine, including a more targeted role in human metabolism, increased bioavailability and absorption into the blood, and decreased side effects when administered orally (Bahri et al., 2013; Collins et al., 2007; Mandel et al., 2005; Oketch-Rabah et al., 2016; Tarazona-Diaz et al., 2013). Health areas of greatest interest include muscle recovery during exercise (Tarazona-Diaz et al., 2013) and vascular health. Vascular health benefits include increasing vasodilation, which is exhibited by decreased systolic and diastolic blood pressure (Figueroa et al., 2011), correcting erectile dysfunction (Drewes et al., 2003; Shiota et al., 2013), and decreasing incidence of vasospastic angina (Morita et al., 2013), a symptom of coronary heart disease.

The specific function and metabolism of citrulline are considerably less articulated in plants, compared with its characterization in humans, prokaryotes, and yeast. Several studies suggest that citrulline serves to abate abiotic stress, because citrulline accumulates dramatically in the foliage of watermelon and related species in response to drought, salinity, and high light. Citrulline may act as a compatible solute for osmotic adjustment, and as a reactive oxygen species scavenger during times of extreme oxidative stress (Akashi et al., 2001; Kawasaki et al., 2000; Smirnoff and Cumbes, 1989; Yokota et al., 2002). A coaccumulation of both citrulline and arginine under salt stress has been reported in higher plants (Ashraf and Harris, 2004; Mansour, 2000). This accumulation of citrulline is thought to occur catabolically due to upregulation of specific genes including deacetylases (Cla016179, Cla016181), further supporting these ideas (Joshi and Fernie, 2017; Winter et al., 2015). Guo et al. (2013, 2015) found that during fruit development, other genes involved in citrulline catabolism, like argininosuccinate lyase (Cla022154) and argininosuccinate synthase (Cla019267, Cla002609) were downregulated in the flesh, which is consistent with the greater abundance of citrulline found in

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the rind. Analysis of the same RNA sequencing profiles found that two biosynthetic enzymes, *N*-acetylornithine aminotransferase (Cla015337) and *N*-acetyl Glu synthase (Cla014036) were progressively upregulated during fruit development in only the rind.

Of the bioactive metabolites found in watermelon fruit, carotenoid content most directly correlates with the stage of fruit development and ripeness. Carotenogenesis increases during development in a sigmoid fashion, whereas reports on phenolic compounds, and vitamin C relative to fruit development are conflicting or inconclusive (Tili et al., 2011). Understanding carotenoid metabolism, the primary contributor to watermelon flesh color, is important to maintaining high quality in transport and storage, and may also relate to citrulline content.

Rimando and Perkins-Veazie (2005) studied variation in citrulline content in watermelon, considering ploidy level, cultivar, flesh color, and fresh weight (FW) vs. dry weight. Red watermelons had significantly less citrulline than orange and yellow-fleshed fruit on both a fresh and dry weight basis. The two yellow cultivars had similarly high citrulline, but there was significant variation within red and orange colors. Red flesh ranged from 0.70 to 3.5 g/kg FW citrulline (mean reported as 1.0), while orange flesh cultivars, 'Tendersweet Orange Flesh' and 'Orange Sunshine', had 0.50 and 3.0 g/kg FW citrulline, respectively (Rimando and Perkins-Veazie, 2005). However, 'Orange Sunshine' is seedless, whereas 'Tendersweet Orange Flesh' is seeded, grown using different cultural practices that may impact bioactive profiles. Both yellow-fleshed cultivars had high citrulline, with 3.6 g/kg for the seeded 'Summer Gold' and 3.5 g/kg for the seedless 'Solid Gold.' Still, carotenoids are considered a potential predictor of the amount of citrulline in watermelons. Another study found that six seeded cultivars yielded less citrulline than eight seedless cultivars, with 1.8 g/kg FW (seeded) and 2.4 g/kg FW (seedless) (Davis et al., 2013). To estimate effects of ploidy on citrulline content, six experimental lines (2x) and their autotetraploids (4x) and triploids (3x) were investigated for amount of citrulline produced. Of the six families, only one showed significant differences in citrulline content (3x and 4x > 2x), but when all families were averaged by ploidy, no significant differences among ploidy were observed. These data parallel results of Liu et al. (2010), using watermelon from nine triploid hybrids and their diploid and autotetraploid progenitors grown under greenhouse conditions. In contrast, 3x watermelons had higher citrulline content than 2x fruit in field-grown watermelons (Perkins-Veazie et al., 2006).

Citrulline accumulation in watermelon fruit appears to be correlated to ripeness. Akashi et al. (2017) investigated spatial and temporal citrulline accumulation in watermelon fruits, noting that it peaks roughly with soluble solids content (SSC) (Akashi et al., 2017; Fish, 2014). Akashi et al. (2017) also described a bimodal accumulation pattern of

citrulline, with peel (4.4 ± 0.8 g/kg) having the highest content on a FW basis compared with heart (2.4 ± 0.99 g/kg FW) and rind (2.1 ± 0.94 g/kg FW). Citrulline content in the rind varied among cultivars, within cultivar, and with fruit stage. Despite reports of much variation among cultivars ($\pm 47.1\%$ cv in Fish, 2014), rind had consistently higher citrulline content than flesh in most studies that compared tissue types (Fish, 2014; Jayaprakasha et al., 2011; Rimando and Perkins-Veazie, 2005).

Davis et al. (2010–11) investigated response to environment using five watermelon cultivars produced in three locations: 'Cream of Saskatchewan', 'Red-N-Sweet', and 'Tendersweet Orange Flesh' in Clinton and Kinston, NC, and 'Black Diamond' and 'Dixielee' in Lane, OK. There was a wide range of values for citrulline within cultivar, but no significant differences among cultivars or across environments tested. Because location did not seem to affect within-cultivar variation, breeding for high citrulline content in watermelons across widely different environments may be possible. Other findings suggest that citrulline is significantly affected by environment; when grown in two locations (Oklahoma and Texas), citrulline content varied widely within the same cultivars (Fish and Bruton, 2010).

Breeding for plant metabolites proves difficult when environmental effects predominate. Citrulline concentrations change significantly with many factors, including cultural practices (grafting, planting densities, harvest date), environmental effects (growing season, location, year, drought, and salt stress), fruit ripeness, cultivar-level variation and correlations (genotype, ploidy, lycopene content, arginine content), and analytical methods (tissue type, tissue processing, sample storage, extraction method, analytical instrumentation). The objective of this study was to estimate narrow-sense heritability and phenotypic relationships among fruit metabolites in two red-fleshed watermelon populations.

Methods

Cultivation and field design

Summer 2015: Parents. In 2015, two North Carolina watermelon populations, NC Small Fruit (NCSFW) and NC High Yield (NCHYW), ($N_{SF} = 300$, $N_{HY} = 300$) were grown in Castle Hayne, NC. Plots were planted on raised, shaped beds with 3.1-m centers and single hills 1.2 m apart. Single fruit were harvested, and seeds were extracted from single-plant plots for planting in 2016 (Table 1).

Summer 2016: Offspring. Seeds from 2015 parents of qualifying ripeness ($SSC \geq 8$, pH 5.5–6.5) were planted in 2016 ($N_{SF} = 72$, $N_{HY} = 175$). Offspring were tested at two locations: the Horticultural Crops Research Station in Clinton, NC, and the Cunningham Research Station in Kinston, NC. The experiment was performed using randomized complete blocks with two

replications. Each population was planted in a randomized complete block design. Field layout was identical to the parent population, except that offspring were grown using six-plant plots 3.7 m long, instead of single-plant hills (Table 1).

Single-plant hills and six-plant plots were grown using horticultural practices recommended by the North Carolina Extension Service (Sanders, 2004). Irrigation was with drip tubes in beds covered with black polyethylene mulch. Soil type was a Norfolk fine sand at Castle Hayne, Orangeburg loamy sand at Clinton, and a Norfolk sandy loam at Kinston. Plants were manually trained each week in a spiral by turning all vines in a clockwise circle around the crown until the start of fruit set. Vine training may affect fruit set, but this allowed for efficient sampling of fruit from single plants later in the season, thus reducing experimental error.

Germplasm

NC small fruit. The population was created in 2005 and included cultivars 'New Hampshire Midget', 'Minilee', and 'Allsweet', which contributed yield, earliness, quality, disease resistance, and different fruit size. This population was intercrossed nine times, while selecting for yield, earliness, quality, disease resistance, and small fruit size.

NC high yield. The population was created in 2005 from crosses of 'Calhoun Gray', 'Dixielee', 'Mountain Hoosier', 'Big Crimson', 'Starbrite F1', 'Legacy', 'Red-N-Sweet', 'Sangria F1', and 'Early Arizona'. The population was intercrossed five times while selecting for yield, earliness, quality, disease resistance, and large fruit size.

Sample collection

For parents, single fruit were harvested when ripe (indicated by a brown tendril near the peduncle, large fruit, filled seeds, red flesh) from each plot for quality analysis. Red flesh was chosen preferentially because some fruit in the population had mixes of red and yellow flesh. Watermelons were cut transversely between blossom- and stem-ends. Samples of 100-g size were taken from the center of the watermelon using an ice cream scoop and bagged individually in polyethylene bags of 4-mL thickness with a locking seal (Uline, Braselton, GA). NCSFW samples were mixed tissue samples (heart, locule, interlocule); NCHYW samples were from heart only. Samples were kept on ice for no longer than 6 h, after which they were frozen at -18°C until blended.

For the offspring, the same sampling procedure was applied using four fruit per plot, of which the three ripest were sampled for quality analysis using the ripeness indicators described previously. Samples were placed in plastic bags with a locking seal and later blended individually for ripeness qualification.

Blending and ripeness qualification

Samples in plastic bags were half-thawed in water, and seeds were removed before

Table 1. Count, mean, low value, and high value for soluble solids concentration (SSC) (°Brix), acidity (pH), lycopene, citrulline, and arginine for NC High Yield watermelon (NCHYW) and Small Fruit (NCSFW) populations across two locations and two replications.^z

Statistic	SSC (°Brix)	pH	Lycopene (mg·kg ⁻¹ FW)	Citrulline (g·kg ⁻¹ FW)	Arginine (g·kg ⁻¹ FW)	Cit+Arg ^y (g·kg ⁻¹ FW)
NCHYW parent population						
N	175	175	175	175	175	175
M	9.4 ± 0.9	5.49 ± 0.2	45.9 ± 9.0	1.60 ± 0.60	0.99 ± 0.21	260.0 ± 76.0
X _L – X _H	8–11.8	5.04–6.29	29.3–93.1	0.21–3.6	0.45–1.5	66.4–487.1
NCHYW offspring population						
N	645	646	653	642	642	642
M	10.3 ± 0.7	5.8 ± 0.1	42.8 ± 8.5	2.38 ± 0.57	1.2 ± 0.21	362
X _L – X _H	8.1–11.5	5.9–6.3	19.7–82.3	0.17–5.3	0.10–2.2	0.38–6.7
NCSFW parent population						
N	72	72	72	72	72	72
M	9.54 ± 1.0	5.56 ± 0.2	64.25 ± 14	1.27 ± 0.64	1.1 ± 0.27	2.3 ± 0.81
X _L – X _H	8.0–12.4	5.03–6.33	40.3–115.3	0.34–2.0	0.16–2.9	0.52–4.4
NCSFW offspring population						
N	263	258	271	263	263	263
M	10.0 ± 0.7	5.62 ± 0.2	52.3 ± 12.2	228.6 ± 90.3	142.6 ± 48.0	371.2 ± 129.6
X _L – X _H	8.1–11.7	5.00–6.46	27.6–84.5	0.27–5.6	0.14–3.0	0.15–8.4

^zParent values are of single watermelons planted in 2015; offspring values are averaged over two locations and two replications, with up to three fruit sampled per plot, planted in 2016.

^yCombined citrulline and arginine concentration in g/kg fresh weight (FW).

blending. Fruit was blended individually for 45 s or until homogenized into a consistent slurry using a blender (Model 7010S 1L and an MC-3 Mini container; Waring Laboratory, Torrington, CT).

Individual samples of parental watermelons were analyzed for SSC and pH. Fruit SSC and pH data were recorded for offspring, and purees of qualifying fruit (SSC ≥ 8, pH 5.0–6.5) pooled within plot by weight (within 0.1 g) and quality data were recorded, including SSC, pH, lycopene, citrulline, and arginine. SSC was measured using a digital refractometer (Atago PAL-1; Atago, Inc., Bellevue WA) and pH was measured using a digital meter (Model H260G; Hach, Loveland, CO) equipped with a stainless steel rounded electrode (PH77-SS; Hach).

Individual purees (parents) or pooled homogenates (offspring) were further processed using a homogenizer for 15 s (Polytron PT 10-35 GT; Kinematica, Bohemia, NY), aliquoted into 1.5-mL tubes, and frozen at –18 °C. Aliquots were transported on ice to Kannapolis, NC, where they were stored at –80 °C until extraction.

Lycopene quantification

Lycopene concentration was measured using 5-mL thawed aliquots diluted in 15-mL deionized water. Sample absorbance at 560 and 700 nm was measured using an UltraScan PRO colorimeter (Hunter Laboratory, Reston, VA). Total lycopene content was calculated using the formula (Abs560 – Abs700) × DF (wt/volume) slope (31.8). The slope was calculated by plotting values from the colorimeter vs. the same sample when analyzed with hexane extraction using a spectrophotometer and standardized with an external lycopene standard (Davis et al., 2003). Units were expressed as mg·kg⁻¹ FW.

Citrulline and arginine extraction and quantification

Extraction. Frozen watermelon purees stored at –18 °C were thawed at room temperature, 0.03M H₃PO₄ (1.2 mL) added

to 0.2 g ± 0.01 g aliquots of puree, and vortexed for 1 min. Purees were then sonicated (30 min), left at room temperature to rest (10 min), and then centrifuged (23,447 g_n, 4 °C, 20 min; centrifuge 5417 R; Eppendorf, Hamburg, Germany). Supernatants (1 mL) were filtered into amber high-performance liquid chromatography (HPLC) vials (17-mm nylon syringe filter, F2513-2; Thermo Scientific, Waltham, MA) and frozen at –80 °C until HPLC analysis.

Quantification. Citrulline and arginine concentrations were determined using a modified method of Jayaprakasha et al. (2011). HPLC was performed using an Elite LaChrom, Hitachi (Tokyo, Japan) system equipped with an autosampler and photodiode array detector. A 5 µL volume of filtered supernatant was injected onto a Gemini 3u C18, 110 Å, 250 × 4.6 mm, 00G-4439-EO column and C18 4 × 2.0; AJO-4286 SecurityGuard cartridges (Phenomenex, Torrance, CA) and amino acids separated using a mobile phase of 0.015 M H₃PO₄ at 0.5 mL/min at room temperature (25 °C) with a runtime of 30 min.

Data analysis

Data were analyzed with general linear models (PROC GLM) in SAS v9.1 (SAS, 2006). Location, replication, and genotype were considered random effects. Analysis of variance was used to determine significance of each variance component and their interactions for each of the six traits. Variance components were estimated using PROC VARCOMP in SAS.

Narrow-sense heritability (h²) was calculated for each trait using two methods. First, a parent-offspring regression was calculated using the SAS mixed procedure (PROC MIXED), which included parent data (one location) and offspring data (averaged over two replications each at two locations). Second, h² was calculated using the variance of half-sibling family means, using PROC VARCOMP. Variance components were estimated for additive and environmental, additive × environment, and other components

of interest. The equation used here was specific to heritability calculations for half-siblings, where σ²_F is family (cultigen-wise variance), σ²_{P_HS} is phenotypic variance of half-sibling values, σ²_{LF} is location by family interaction variance, σ²_e is error variance, s = number of locations, b = number of blocks, and n = number of samples per plot (Isik et al., 2017):

$$\frac{\sigma_F^2}{\sigma_{P_HS}^2} = \frac{\sigma_F^2}{\sigma_F^2 + \frac{\sigma_{LF}^2}{s} + \frac{\sigma_e^2}{sb}}$$

Correlations between quality traits were calculated using the SAS procedure PROC CORR.

Results

Analysis of variance showed that location had the largest variance for citrulline, arginine, lycopene, pH, and SSC in NCHYW, and replication within location was the largest variance for citrulline plus arginine. Within the NCSFW population, the largest variance for pH and lycopene was location, whereas variance was highest for replication within location for citrulline, arginine, citrulline plus arginine, and SSC (Table 2).

Variance component estimates suggest that error was the greatest source of variation for all traits of interest in the NCHYW population, followed by cultigen. Error and cultigen were primary sources of variation in the NCSFW population for citrulline plus arginine, SSC, and pH. Sources of variation for citrulline were error followed by location × cultigen and replication within location. Variance for lycopene came primarily from location, followed by error and cultigen (Table 3).

Narrow-sense heritability varied greatly among the traits evaluated. In NCHYW, heritability from parent-offspring regression was moderate in lycopene (46%), citrulline (38%), and arginine (40%), and moderate-to-low for arginine plus citrulline (29%), pH (30%), and SSC (17%). Heritability using

Table 2. Analysis of variance (df and mean squares) for soluble solids concentration (SSC) ($^{\circ}$ Brix), acidity (pH), lycopene, citrulline, and arginine for NC High Yield watermelon (NCHYW) and Small Fruit (NCSFW) populations across two locations and two replications.

Source	df ^z	SSC	pH	Lycopene	Citrulline	Arginine	Cit+Arg ^y
NCHYW offspring population							
Location	1	17.24*	3,435.28*	3,715.56*	5,508.69*	63,008.89*	24,783.76*
Rep(Loc)	2	2.72*	215.23	403.27*	416.33	26,687.82*	31,308.43*
Cultigen	175	0.89*	136.30*	108.37*	622.09*	4,584.36*	6,340.70*
Loc $\times$ CIt ^x	171	0.38	82.98	61.97*	383.43	2,660.71	4,324.16
Error	289	0.35	73.71	41.91	351.66	671,776.84	3,678.39
NCSFW offspring population							
Location	1	0.92	8,126.31*	14,166.71*	29,056.01*	928.76	40,374.38
Rep(Loc)	2	2.37*	215.65	93.93	71,659.27	19,333.52*	165,400.09
Cultigen	71	0.72*	164.092	187.65*	9,301.12*	2,620.94*	18,140.47
Loc $\times$ CIt ^x	71	0.33	140.15	73.56	7,507.17	1,356.36	16,074.33*
Error	262	0.26	136.76	56.7	5,947.17	1,859.21	12,561.77

^zDegrees of freedom.

^yCombined citrulline and arginine concentrations.

^xLocation and cultigen interaction.

*Significant at 0.05 *P*.

Table 3. Variance components for soluble solids concentration (SSC) ($^{\circ}$ Brix), acidity (pH), lycopene, citrulline, and arginine for NC High Yield watermelon (NCHYW) and Small Fruit (NCSFW) populations across two locations and two replications using the variance of half-sibling family means.

Statistic	SSC	pH	Lycopene	Citrulline	Arginine	Cit+Arg ^z
NCHYW statistical variance component estimates						
Location	0.045	10.96	10.36	115.97	11.74	11.19
Rep (Loc)	0.016	1.01	2.33	147.39	-0.547	172.79
Cultigen	0.14	14.49	13.14	513.96	66.82	533.93
Loc $\times$ CIt ^y	0.013	5.91	8.6	114.39	-13.15	161.83
Error	0.35	73.07	43.08	2,426.4	374.92	3,847.8
NCHYW quantitative genetic estimates						
Additive	0.57	57.94	52.55	2,055.85	267.3	2,135.71
Phenotypic	0.67	79.17	67.62	2,719.64	354.45	3,178.58
<i>h</i> ²	0.6	0.41	0.47	0.43	0.44	0.34
NCSFW statistical variance component estimates						
Location	-0.0195	61.25	106.88	238.89	-119.42	11.19
Rep (Loc)	0.0463	-1.4	0.165	1,168.3	256.01	172.79
Cultigen	0.12	6.55	34.73	565.58	139	533.93
Loc $\times$ CIt ^y	0.0256	5.78	3.155	1,379.9	252.26	161.83
Error	0.27	135.81	57.96	5,457	1,786.6	3,847.8
NCSFW quantitative genetic estimates						
Additive	0.47	26.2	138.92	2,262.3	556.01	2,715.31
Phenotypic	0.55	63.04	154.98	4,316.52	1,128.78	7,077.02
<i>h</i> ²	0.6	0.15	0.68	0.22	0.2	0.14

^zCombined citrulline and arginine concentrations.

^yLocation and cultigen interaction.

*h*² = narrow-sense heritability.

half-siblings found similar heritabilities for citrulline (43%), arginine (44%), citrulline plus arginine (29%), lycopene (47%), and pH (40%). Heritability from half-siblings was higher for SSC (60%, Table 4). In NCSFW, heritabilities from parent-offspring regression were high for citrulline (65%) and SSC (60%), moderate for lycopene (44%) and citrulline plus arginine (33%), and low for arginine, pH, and SSC (9.4%, 9.8%, and $\approx$ 0%, respectively). Estimation using half-siblings revealed significantly different values. Citrulline had low heritability (22%) compared with its parent-offspring estimate (65%). Arginine was low (14%), along with pH (15%). Lycopene was more heritable in NCSFW considering half-siblings (68%) compared with parent-offspring (44%, Table 4).

Both populations had weak, positive correlations between lycopene and citrulline ($r_{HY} = 0.22$; $r_{SF} = 0.15$) and lycopene and citrulline plus arginine ($r_{HY} = -0.20$; $r_{SF} = 0.13$). Citrulline and arginine were positively

correlated in both populations, with NCSFW having a much stronger correlation ($r_{HY} = 0.43$; $r_{SF} = 0.73$). In both populations, SSC was correlated significantly with citrulline ($r_{HY} = 0.40$; $r_{SF} = 0.24$), arginine ($r_{HY} = 0.40$; $r_{SF} = 0.18$), and citrulline plus arginine ($r_{HY} = 0.45$; $r_{SF} = 0.23$). Although pH was negatively correlated with all other traits and was significant for each trait in at least one population, significance was not consistent between populations (significant for citrulline plus arginine and citrulline in NCHYW, and significant for arginine and lycopene in NCSFW) (Table 5).

Discussion

Watermelon is an economically significant crop globally and has important nutritive and bioactive profiles and properties with high concentrations of lycopene and citrulline in addition to potassium and vitamins C and A. In mammals, these phytonutrients can improve vasodilation, cardiovascular health,

and reduce risks for stroke and several cancers (Collins et al., 2006; Perkins-Veazie et al., 2012). Breeding to increase lycopene and citrulline in watermelon may also help increase stress tolerance in the plant (Akashi et al., 2001; Kusvuran et al., 2013; Wang et al., 2014).

Genetic variance was present for all six quality traits in both the parents and the offspring of the two populations studied (Table 1). For citrulline, there are several studies that report variation among and within cultigen in different environments, including that of Davis et al. (2011) and Wehner et al. (2017), which prompted this study.

Lycopene abundance varies greatly in red, orange, and yellow-fleshed watermelon, with red-fleshed cultigens containing lycopene as the major carotenoid, followed by orange-fleshed and yellow-fleshed cultigens. Perkins-Veazie et al. (2016) found great variation in lycopene among 50 watermelon cultivars, ranging from 33 to 100 mg/kg. Yoo et al. (2012) and Nagal et al. (2012) both reported high variation in lycopene among cultigens.

Heritability estimates are used to plan efficient breeding strategies to improve trait value and are now available to improve citrulline and arginine. Zhang et al. (2010) reported the general combining ability and heritability of lycopene, which were relatively high, suggesting a potential additive effect due to dominant genes. A study considering broad-sense heritability of fruit quality traits in a variety of red, orange, and yellow flesh found that arginine and lycopene were highly heritable (89% and 99%, respectively) (Wehner et al., 2017). Broad-sense heritability for citrulline, pH, and SSC was low to moderate (41%, 61%, and 46%, respectively) (Wehner et al., 2017). However, there have been no studies reporting narrow-sense heritability of lycopene, citrulline, or arginine in watermelon.

In our study, environment was a significant contributor in all traits for NCHYW and had the largest mean squares, although error and cultigen had the largest variance components, except for lycopene, where location was the largest. Despite that, heritability for lycopene was moderate (47%). In NCHYW, heritability was essentially the same for citrulline and arginine, considering both parent-offspring regression (38% and 40%, respectively) and half-siblings (43% and 44%, respectively). In NCSFW, heritabilities were considerably different and there were fewer significant variance components, possibly due to insufficient sample size. Variance components for all traits were more consistently distributed in the NCHYW population, with error predominating, followed by cultigen. This difference may be from the larger sample size, compared with NCSFW ($N_{HY} \approx 650$; $N_{SF} \approx 230$), failure to capture three ripe fruits from 5% of plots, different harvest dates for second collections when three rip fruits were not found during the first harvest ($\approx$ 10% of plots), and the differing pedigrees of the two watermelon populations.

Davis et al. (2013) determined that SSC and lycopene content were slightly and

Table 4. Estimates of narrow-sense heritability for soluble solids concentration (SSC) (°Brix), acidity (pH), lycopene, citrulline, and arginine for NC High Yield watermelon (NCHYW) and Small Fruit (NCSFW) populations across two locations and two replications using parent-offspring regression and the variance of half-sibling family means.

Estimation/trait	SSC	pH	Lycopene	Citrulline	Arginine	Cit+Arg ^z
NCHYW						
h ² by parent-offspring	0.17	0.31	0.46	0.38	0.40	0.29
h ² by half-siblings	0.60	0.40	0.47	0.43	0.44	0.34
NCSFW						
h ² by parent-offspring	-0.34 ^y	0.10	0.44	0.65	0.09	0.33
h ² by half-siblings	0.60	0.15	0.68	0.22	0.20	0.14

^zCombined citrulline and arginine concentrations.

^yNegative heritability values indicate a heritability close to zero.

h² = narrow-sense heritability.

Table 5. Pearson correlation coefficient soluble solids concentration (SSC) (°Brix), acidity (pH), lycopene, citrulline, and arginine for NC High Yield watermelon (NCHYW, left) and Small Fruit (NCSFW, right) populations across two locations and two replications.

	SSC	pH	Lycopene	Citrulline	Arginine	^z NCSFW Cit+Arg ^y
SSC	-	0.40*	0.31*	0.24*	0.18*	0.23*
pH	0.38	-	0.49*	0.08	0.14*	0.11
Lycopene	0.3	0.29	-	0.15*	0.06	0.13*
Citrulline	0.40*	0.19*	0.22*	-	0.73*	0.97*
Arginine	0.40*	0.16	0.07	0.43*	-	0.88*
Cit+Arg ^z	0.45*	0.20*	0.20*	0.96*	0.66*	-
NCHYW						

^zCombined citrulline and arginine concentrations.

*Indicates significant at 0.05 *P*.

“-” indicates division between the two data sets.

positively correlated, using a study of six diploid cultivars, their autotetraploids, and their triploid progeny. No correlation was found between citrulline and SSC. In contrast, this study found moderate correlations between SSC and citrulline (0.40), arginine (0.40), citrulline plus arginine (0.45), and lycopene (0.30), which included only diploids. Lycopene was only weakly correlated with citrulline (0.22) and not correlated with arginine (0.06). Similarly, directional and significant correlations were found in NCSFW, although correlations were generally weaker.

Plant breeders interested in developing cultivars that have high citrulline (or arginine) or high lycopene content should be able to make good progress because the heritabilities are generally in the moderate range (25% to 50%). Selection should be based on progeny rows, perhaps with only a single replication. Single-plant selection would not be advised unless heritability was above 50% to 100% (the range for high heritability).

In this study, heritability estimates were calculated using both parent-offspring regression and the variance of half-sibling family means. In both estimations for the NCHYW population, heritability of citrulline was moderate (40%), as was arginine (≈40%) and lycopene (≈50%). Heritabilities of these traits in the NCSFW population were highly varied, likely due to the smaller population size and cultivars chosen for the genesis of each population. Overall, the narrow-sense heritability estimates in this study were much lower compared with the broad-sense heritabilities of our 2017 study, suggesting there may have been additional alleles coding for fruit quality traits in the genotypes chosen for the 2017 study. In

addition, the NCHYW was intercrossed more times than the NCSFW population, although the selection intensity was the same for both populations. This may account for the higher degree of segregation and variance in NCSFW, in addition to the different pedigrees of the population progenitors. The wide variation within cultivar in the 2017 study might have caused shifts in the means, hence the significant differences among cultivars and across environments. Significant correlations were identified in the NCHYW population, with significant, positive correlations of SSC to citrulline (0.40), arginine (0.40), and their combination (0.45). In NCHYW, lycopene was weakly but significantly correlated to citrulline (0.22), although not correlated to arginine (0.06). Similar correlations were identified in NCSFW, although weaker. Based on these heritabilities and correlations, tandem selection for high lycopene and citrulline content may be accomplished without excessive plot replication.

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