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QTLs associated with flesh quality traits in an elite × elite watermelon population

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Abstract Consumers prefer watermelon with sweet, red flesh, and the presence of lycopene, citrulline and arginine phytochemicals helpful for human health is an additional bonus. Breeders often select fruit with desirable flesh characteristics based on soluble solids content (Brix) and visual flesh color. Although marker assisted selection (MAS) of flesh traits would advance germplasm selection efficiency, the low heritability of Brix and lycopene content in red fleshed watermelon has hampered marker development. Here we describe the identification of QTLs associated with lycopene content, the amino acids citrulline and arginine, and the content of individual sugar (sucrose, glucose, fructose) in an elite × elite recombinant inbred line (RIL) population. Brix was most highly correlated with total sugars and glucose content, lycopene content was correlated with sucrose content, and citrulline and arginine content showed no correlation. A region on chromosome 5 was associated with sucrose, glucose, and fructose accumulation, while stable arginine content QTLs were identified on chromosomes 2 and 5.

Keywords *Citrullus lanatus* · Brix · Sugar · Fructose · Sucrose · Glucose · Lycopene · Citrulline · Arginine

Introduction

Watermelon [*Citrullus lanatus* (Thumb.) Matsum. and Nakai)] is enjoyed world-wide for its brightly colored, sweet and juicy flesh (Jeffrey 1978). U.S. watermelon production in 2015 was approximately 1.8 million metric tons and was valued at over $488 million dollars (USDA NASS). The U.S. watermelon industry depends on enticing consumers into regular consumption by producing fruit with appealing appearance, taste, and nutritional value. Increasing watermelon demand by producing higher quality fruit can be achieved by selecting for cultivars that have improved flavor and nutritional value (Kader 2008). The most important watermelon trait for consumers is sweet tasting, brightly colored flesh (Evans 2008; Maynard 2001).

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In watermelon breeding programs, selection for flesh sweetness is carried out in the field by measuring the soluble solids content (Brix) with a refractometer (Mitcham et al. 1996). Watermelon Brix is highly correlated with fruit sweetness, and has been used by breeders to develop cultivars with high quality fruit flesh (Pratt 1971). Several studies have attempted to map the Brix in watermelon to develop markers. A single QTL for Brix was identified in a cross between the cultivated Chinese inbred line 'garden female parent' and American inbred line 'LSW-177' on chromosome 2, although this QTL was not proven to be stable across different environments (Cheng et al. 2016). Sandlin et al. (2012) identified a Brix QTL on chromosome 2 in a cross between Strain II and Egusi, as well as three QTLs on chromosomes 1, 2, and 7 in the elite by elite 'Klondike Black Seeded' × 'New Hampshire Midget' population, with only the QTL on chromosome 1 showing stability across environments (R^2 = 7.3 and 8.4%). Stable QTLs for Brix were found in only two other studies, a QTL on chromosome 2 was stable across 2 years (R^2 = 29.3 and 31.4) (Ren et al. 2014), and two QTLs on linkage group 1 (R^2 = 19 and 17.6) were stable across three different environments (Xu et al. 2006). Both of these studies used an interspecific cross between a wild *C. amarus* (0.8 Brix) accession PI 296341-FR and an elite cultivar '97103' (11.0 Brix) that displayed extreme differences in Brix, resulting in high heritability (h^2 = 0.97) (Ren et al. 2014). Heritability in this interspecific cross differs from the low (H^2 = 0.20) (Sandlin et al. 2012) to moderate (H^2 = 0.46) (Wehner et al. 2017) heritability often seen in watermelon for this trait.

Low heritability of Brix in watermelon is well documented and explains the difficulty in identifying stable QTLs for this trait (Hashizume et al. 2003; Xu et al. 2006). Lack of stable Brix QTLs has made marker development for improved fruit quality challenging (Baldwin 2008), a problem that might be addressed by identifying QTLs associated with the underlying components of watermelon Brix. Soluble solids content primarily consists of the sugars, sucrose, glucose, and fructose, while other water-soluble components such as amino acids, organic acids, and water-soluble polysaccharides can also contribute to Brix. Mapping of the genes controlling the content of individual sugars (sucrose, glucose, fructose), and amino acids contributing to Brix and of lycopene content that controls red flesh color, may provide more stable QTLs for marker development and subsequent marker assisted selection of fruit quality in watermelon.

Sweetness of fruit is a key determining factor in consumer preference (Brueckner et al. 2007; Evans 2008). As watermelon contains very little organic acid, watermelon sweetness is determined by the amount of sugar accumulated in the flesh, as well as the composition of these sugars. The ratio of sucrose, glucose, and fructose content varies by cultivar and by developmental stage (Brown and Summers 1985; Yativ et al. 2010; Zhang and Ge 2016). Depending on the level of sucrose breakdown, cultivars may accumulate mostly fructose and glucose, or mostly sucrose (Ma et al. 2014; Pardo et al. 1997; Yativ et al. 2010).

Identification of QTLs controlling the content of specific sugars would be beneficial to breeders attempting to enhance fruit quality through selection of preferential sugar profiles. QTLs for sugar profile selection have been identified in other fruit crops, including apple (Guan et al. 2015) and peach (Dirlewanger et al. 1999). In watermelon, QTLs have been identified for sucrose (chromosomes 1, 2 and 5), glucose (chromosomes 1 and 6), and fructose (chromosomes 2, 3, 6, 8 and 10) content in the interspecific cross between PI 296341-FR and '97103' (Ren et al. 2014; Xu et al. 2006). QTLs for sucrose, glucose (chromosome 1), and fructose (chromosome 10) content were also identified by Cheng et al. (2016). However, none of these QTLs have been validated as being stable in different environments or in elite backgrounds, both of which are critical for marker assisted selection. Identification of stable QTLs controlling individual sugars in watermelon is needed to advance marker assisted selection of sugar content in watermelon.

Flesh color of watermelon is a key factor in consumer preference, making selection for fruit with brightly colored flesh a priority for breeders (Evans 2008). The red carotenoid pigment lycopene accumulates in the flesh during fruit ripening and gives watermelon its characteristic red flesh (Di Mascio et al. 1989). There are many health benefits associated with lycopene, including high antioxidant capacity, improved cardiovascular health, and blood pressure regulation (Cheng et al. 2017). Breeders typically select for flesh color based on visual inspection due to the high correlation between lycopene content and red flesh color (r = 0.91) (Kang et al. 2010; Liu et al.

2015) as lycopene content quantification in watermelon flesh is tedious (Davis et al. 2003).

Considerable research has been done on the inheritance of flesh color and lycopene content in watermelon over the years (Henderson et al. 1998; Poole 1944), and a marker for selection between red and yellow flesh color has been developed (Bang et al. 2007). Although high heritability ($H^2 = 0.99$) of lycopene content has been observed in studies with diverse flesh color (yellow, orange, and red) (Wehner et al. 2017), red fleshed varieties also show large variability in lycopene content (Perkins-Veazie et al. 2001). While several studies have shown that lycopene content is highly affected by environment in tomato (Brandt et al. 2006; Kuti and Konuru 2005) and watermelon (Perkins-Veazie et al. 2005, 2001), mapping lycopene content in a population segregating between red flesh and pink flesh watermelon has not yet been done.

The presence of the amino acids citrulline and arginine offer additional phytonutrients in watermelon. Citrulline is a non-essential amino acid that derives its name from watermelon (*Citrullus lanatus*) and can act as a vasodilator, which helps combat muscle fatigue (Goubel et al. 1997) and promotes cardiovascular health (Akashi et al. 2001). Watermelon is the highest natural source of citrulline available (Rimando and Perkins-Veazie 2005). Citrulline is converted in the body to the conditionally essential amino acid arginine, which has various functions in human health. Improvements in symptoms of patients with sickle cell disease and obesity have been associated with arginine intake (Waugh et al. 2001). Increased arginine consumption has been linked to reduced obesity levels in humans due to increased insulin sensitivity and reduction of adiposity (McKnight et al. 2010). Dietary supplementation with arginine has been shown to improve cardiovascular, reproductive, and liver health (Wu et al. 2007). Arginine also plays critical roles in the body through cell signaling and can act as an antioxidant. It is the precursor in the synthesis of nitric oxide, a major cell signaling molecule and it plays a multi-organ role in the body (Wu et al. 2009). Environment is known to affect watermelon citrulline content (Davis et al. 2011), and moderate heritability of citrulline content ($H^2 = 0.41$) and high heritability of arginine content ($H^2 = 0.89$) in watermelon was reported by Wehner et al. (2017).

The current method of selection for fruit quality traits in watermelon (sugar content and flesh color) involves Brix measurement and visual fruit color assessment at fruit maturity. Marker assisted selection of fruit quality traits, which can be carried out on seedlings or seeds, would be very beneficial to watermelon breeders as it would be more efficient and cost effective by eliminating the need to grow fruit to maturity to make selections (Baldwin 2008). The aim of this study was to identify QTLs associated with fruit quality traits including sugar (sucrose, glucose, and fructose), lycopene, citrulline, and arginine content in cultivated watermelon to further the development of MAS in fruit quality breeding.

Materials and methods

Plant material and flesh sample collection

The watermelon RIL mapping population 'Klondike Black Seeded' (red fleshed) × 'New Hampshire Midget' (pink fleshed) (Sandlin et al. 2012) was planted in the field at the University of Georgia Durham Horticulture Farm (Watkinsville, GA) on May 27th, 2015 and May 18th, 2016. Plants were grown both years following University of Georgia Cooperative Extension Service recommendations. Five plants of 151 RILs, parents and F_1s were grown in a randomized complete block design with one plant per replication using between-row spacing of 1.83 m and in-row spacing of 0.9 m. Seedlings were transplanted onto white plastic with Cecil sandy loam soil and drip irrigation was used for water and fertilizer application. Mean air temperatures of 2015 for daily max, daily min and average were 32.9, 20.4, and 26 °C respectively and 32.5, 19.9, and 25.8 °C respectively for 2016. Total rainfall from planting to harvest was 19.6 cm in 2015 and 21.4 cm in 2016. Fruit were harvested at maturity on July 28th, July 29th, and Aug 5th, 2015 and July 25th and August 1st, 2016. Flesh samples were collected from the heart of the watermelon and held in a cooler with ice packs prior to freezing at − 20 °C. Flesh from between 1 and 6 fruit of each line was collected and pooled, irrespective of block, before shipping overnight on dry ice to NCSU (Kannapolis, NC) for further analysis.

Compositional analyses

Fruit composition assays of soluble solids content (Brix): BRX; pH; total soluble sugars: TSS; sucrose: SUC; glucose: GLU; lycopene: LYC; citrulline: CIT; and arginine: ARG, content were done using − 20 °C fruit (about 100 g) thawed to room temperature and pureed 30 s with a homogenizer (Polytron PT10-35, Brinkmann Kinematica, Bohemia, NY) equipped with a PT 10-35 GT generator. About 0.5 ml of puree was placed on a digital refractometer (Atago Palette, NY) to determine the Brix. The pH of each sample was measured using a stainless steel, round tip probe and an electronic pH meter (Hach, Loveland, CO). Total lycopene content measurement was made following the methods in Davis et al. (2003), with an UltraLab Color Scan Pro (Hunter Associates Laboratory, Inc., Reston, VA) with a wavelength range from 350 nm to 1050 nm. Citrulline and arginine content was determined following the method of Jayaprakasha et al. (2011) with modifications. About 0.2 g of puree was weighed into a 2 ml microcentrifuge tube. 1.2 mL 0.03 M H_3PO_4 was added and the centrifuge tube was vortexed for 1 min. Samples were sonicated for 30 min and then allowed to sit at room temperature for 10 min. Samples were centrifuged at 14,000 rpm at 4 °C for 20 min (Eppendorf microcentrifuge 5417R, Hauppauge, NY) and 1 mL of supernatant was filtered into HPLC vials with a 0.2 µm filter using a 17 mm nylon syringe filter, F2513-2 (Thermo Fisher Scientific, Waltham, MA).

Filtered samples (2 µL) were injected into a Hitachi LaChrom (Hitachi Ltd., Tokoyo), equipped with a UV-VS diode array detector (DAD), controlled temperature auto sampler (4 °C), and column compartment (25 °C). A Gemini 3u C18, 110 A, 250 × 4.6 mm 00G-4439-EO column and C18 4 × 2.0; AJO-4286 guard cartridge (Phenomenex, Torrance, CA) were used with a mobile phase of 0.015 M H_3PO_4 for detection of arginine and citrulline content. D-2000 software (Hitachi Ltd., Tokoyo) was used as the system run controller and for data processing.

For sugar analysis, about 0.1 g puree was weighed into a 2 ml microcentrifuge tube and 1.4 ml of distilled water added. Samples were vortexed for 1 min and then sonicated for 15 min and then placed on ice for 10 min. Samples were filtered as described above and a 5µL volume injected onto a Hitachi LaChrom (Hitachi Ltd., Tokoyo), equipped with a refractive index detector, controlled temperature auto sampler (4 °C), and column compartment (65 °C). A Rezez RCM-Monosaccharide Ca + 2 (8%), OOH-0130-KO column with Carbo-Ca 4X 3.0 mm ID, AJO-4493 guard cartridge (Phenomenex) was used with a flow rate of 0.6 mL min^{-1} with a distilled deionized water mobile phase (Phenomenex). D-2000 software (Hitachi Ltd., Tokoyo) was used as the system run controller and for data processing.

Compound concentrations were estimated using standard curves generated by injecting 10 µL of fructose, sucrose or glucose for sugars and citrulline and arginine (Sigma-Aldrich, St. Louis, MO). Compound identification was performed based on retention time compared to authentic standards. Each sample was run in triplicate and content reported as mg/g fresh weight (FW) (sugars) or g/100 g (amino acids). Total sugars is the sum of glucose, fructose and sucrose.

Data analysis

As ripe watermelon fruit have a flesh pH between 5 and 6 (Corey and Schlimme 1988; Rekha et al. 2012), only pooled samples that satisfied this criteria were used for further analysis. An analysis of variance ANOVA was performed using the model $Y_{ij} = \mu + G_i + Y_j + e_{ij}$, where Y_{ij} is the phenotypic value, μ is the general mean, G_i is the effect of the ith genotype, Y_j is the effect of the jth year and e_{ij} is the residual.

Broad sense heritability was calculated based on the methodologies of Holland et al. (2003) and Nyquist and Baker (1991) as described in Sandlin et al. (2012) where $H^2 = \left[\sigma^2_{RIL}/\left(\sigma^2_{RIL} + \sigma^2_{YEAR\times RIL}/r\right)\right]$, σ^2_{RIL} is the genetic variance among the genotypes, $\sigma^2_{RIL\times YEAR}$ is the variance of genotypes by year interaction, and r is equal to the number of environments.

JMP, Version 13.0.0 (SAS Institute Inc., Cary, NC) was used to calculate pairwise Pearson correlations (r) between all traits and the Shapiro and Wilk test for normality (Shapiro and Wilk 1965). Where data deviated from normality, arcsine square-root transformation were carried out. If transformation resulted in normal distributions, the transformed data was used for QTL mapping, otherwise the untransformed data were used. Non-parametric interval mapping (scanone; model="np") in R/QTL (Broman, et al. 2003)

was used to confirm QTL if the residual errors deviated from normality.

QTL and candidate gene identification

The genetic linkage map used in this study was based on 164 RILs and 378 SNP markers with an average distance between markers of 3.8 cM and a total length of 1438 cM making up 13 linkage groups (Sandlin et al. 2012). QTL identification and analysis was done using Windows QTL Cartographer 2.5 (Wang et al. 2011). The data for the two years were analyzed separately using composite interval mapping (CIM). Threshold values were determined from permutation tests for all traits analyzed (1000 permutations, $\alpha = 0.05$) (Churchill and Doerge 1994). The standard model 6 was selected using the following parameters: a walk speed of 1 cM, 5 control markers, forward and backward regression method, and a window size of 10 cM. This model improves QTL resolution by using control markers to control for genetic background. QTLs were considered minor, intermediate, and major based on R^2 values of $< 10\%$, between 10 and 25%, and over than 25% respectively.

Published research was used to select genes previously identified to function in key metabolic pathways for the traits of interest (Guo et al. 2013; Joshi and Fernie 2017; Zhu et al. 2017). The Cucurbit Genomics Database of the International Cucurbit Genomics Initiative (http://www.icugi.org) was then used to determine Mb location of candidate genes in the '97103' (Guo et al. 2013) genome to identify candidate genes that fell within our QTL regions.

Results

Phenotypic data

After removal of samples that fell outside of the desired pH range, the final number of lines analyzed was n = 98 in each year, with 76 lines common over both years. Continuous frequency distributions were observed for all traits (Fig. 1), suggesting that these traits are quantitative. Distributions were normal ($P < 0.05$) for all traits except BRX2016, SUC2015 and SUC2016. SUC2015 and SUC2016 had right-skewed bimodal distributions. SUC2015 and SUC2016 were arcsine square-root transformed before QTL analysis.

Transgressive segregation was observed for all traits and the average values for all traits except sucrose content were higher in 2015 than in 2016 (Fig. 1). Fructose was the dominant sugar, followed by glucose and sucrose.

As expected, there were several significant correlations among the different fruit traits (Table 1). BRX2015 was positively correlated with TSS2015, GLU2015, LYC2015, CIT2015, and ARG2015 ($P < 0.01$). BRX2016 was positively correlated with TSS2016, GLU2016, FRU2016, and ARG2016 ($P < 0.01$). There were also significant negative correlations between sucrose content and the reducing sugars content ($P < 0.01$), as well as a positive correlation between glucose content and fructose content in both years ($P < 0.01$). Significant positive correlations between lycopene content and sucrose content were observed in both years ($P < 0.01$ and $P < 0.05$) while significant negative correlations were found between LYC2016 and the reducing sugars GLU2016 and FRU2016. Arginine content was also significantly correlated with the reducing sugars content in 2015, and with fructose content in 2016. All traits except Brix and total soluble solids were significantly correlated across years.

ANOVA showed significant effects for genotype for all traits, except TSS and for years for all traits, except SUC, CIT and ARG (Table S1). Estimated heritabilities for the traits were $H^2_{BRX} = 0.30, H^2_{TSS} = 0.28, H^2_{SUC} = 0.70$, $H^2_{GLU} = 0.51, H^2_{FRU} = 0.41, H^2_{LYC} = 0.60, H^2_{CIT} = 0.71, H^2_{ARG} = 0.73$.

QTL identification

Twenty-one significant QTLs were detected for the six traits over the two years (Table 2 and Fig. 2). All the sugar content QTLs were found on chromosomes 5 and 8 and QTLs for SUC2015 (R^2 = 23.3), SUC2016 (R^2 = 28.3), GLU2016 (R^2 = 9.6), and FRU2016 (R^2 = 18.3) co-localized on chromosome 5 (41.6–48.3 cM). The sucrose content QTL on chromosome 5 (*Qsur5-2*) was stable between the two years (39–45.4 cM, R^2 = 23.3; 39.6–48.3 cM, R^2 = 28.3). Two QTLs were identified for LYC2015 on chromosomes 3 (24–40.6 cM; R^2 = 18.5) and 11 (95.2–106.8 cM; R^2 = 9.7), but no QTLs for lycopene

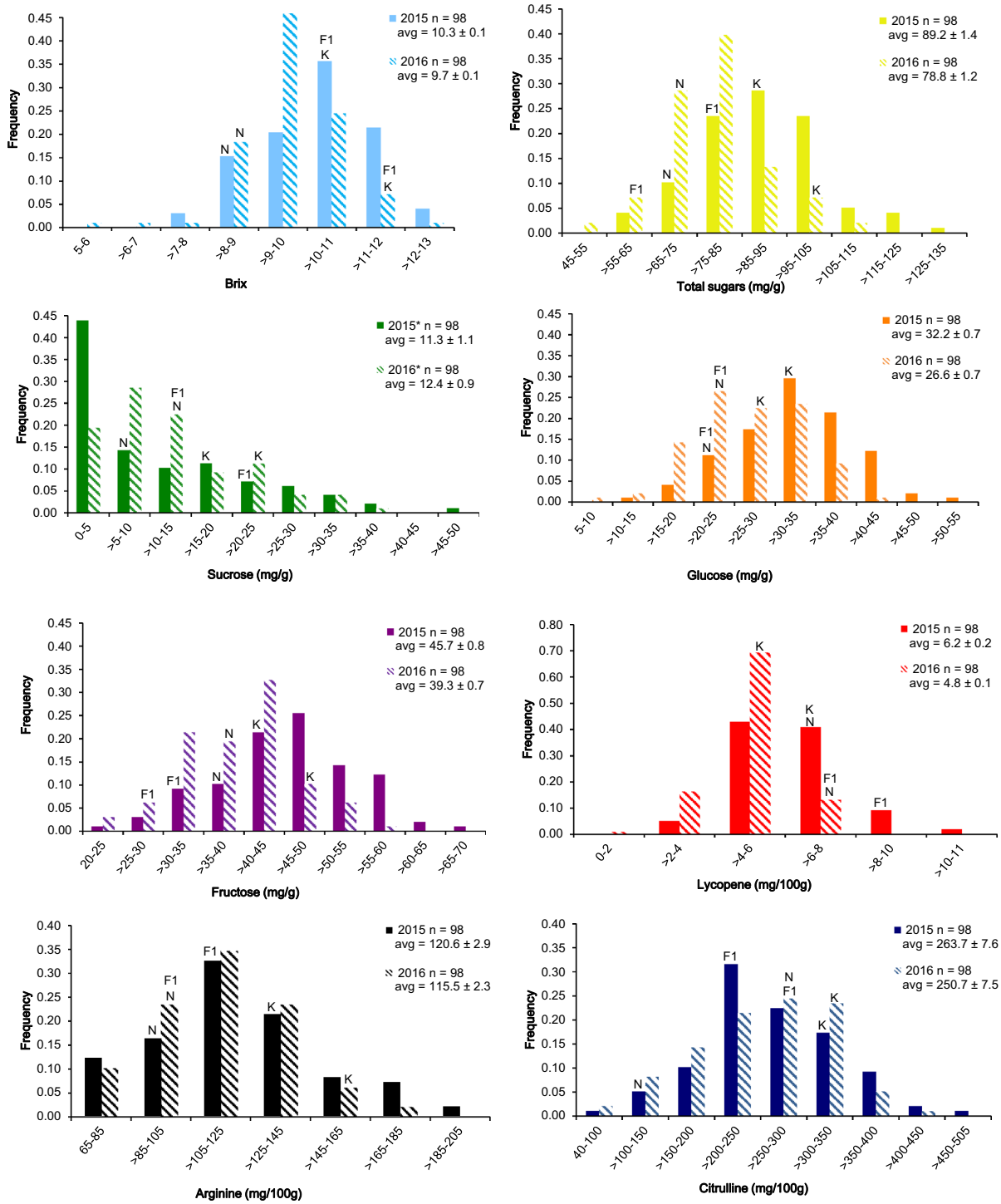

Fig. 1 Frequency distribution of fruit traits (soluble solids content (Brix), total soluble sugars, sucrose, glucose, fructose, citrulline, and arginine content) for 2015 (solid) and 2016 (striped) in 'Klondike Black Seeded' ("K") × 'New Hampshire Midget' ("N") watermelon RIL cross

Table 1 Pairwise correlations of fruit traits (soluble solids content (BRX), total sugar (TTS), sucrose (SUC), glucose (GLU), fructose (FRU), lycopene (LYC), citrulline (CIT), and arginine (ARG)) content in the 'Klondike Black Seeded' × 'New Hampshire Midget' watermelon RIL population for 2015 and 2016

	BRX2015	BRX2016	TTS2015	TTS2016	SUC2015	SUC2016	GLU2015	GLU2016
BRX2016	0.21							
TTS2015	0.46**	0.32**						
TTS2016	0.18	0.51**	0.24					
SUC2015	0.20	0.05	0.32**	0.00				
SUC2016	0.21	0.05	0.24	0.13	0.62**			
GLU2015	0.39**	0.32**	0.68**	0.25*	− 0.40**	− 0.24*		
GLU2016	0.01	0.45**	0.08	0.74**	− 0.36**	− 0.52**	0.41**	
FRU2015	0.18	0.19	0.68**	0.20	− 0.42**	− 0.21	0.80**	0.26*
FRU2016	0.05	0.34**	0.06	0.77**	− 0.37**	− 0.49**	0.31**	0.87**
LYC2015	0.50**	0.06	0.16	− 0.05	0.40**	0.36**	− 0.12	− 0.28*
LYC2016	0.27*	0.06	0.19	− 0.20	0.16	0.23*	0.06	− 0.31**
CIT2015	0.35**	− 0.17	− 0.02	− 0.11	0.14	− 0.02	− 0.07	− 0.15
CIT2016	0.12	0.06	− 0.09	0.09	− 0.15	− 0.22	0.09	0.17
ARG2015	0.30**	0.11	0.45**	0.14	0.04	0.00	0.32**	0.08
ARG2016	0.27*	0.29**	0.37**	0.21	− 0.04	− 0.04	0.36**	0.14

	FRU2015	FRU2016	LYC2015	LYC2016	CIT2015	CIT2016	ARG2015
BRX2016							
TTS2015							
TTS2016							
SUC2015							
SUC2016							
GLU2015							
GLU2016							
FRU2015							
FRU2016	0.32**						
LYC2015	− 0.15	− 0.22					
LYC2016	0.05	− 0.30**	0.53**				
CIT2015	− 0.15	− 0.01	0.23*	0.16			
CIT2016	− 0.03	0.24*	− 0.10	− 0.01	0.54**		
ARG2015	0.43**	0.15	0.15	0.01	0.06	− 0.03	
ARG2016	0.37**	0.26*	− 0.06	− 0.11	0.00	0.21	0.59**

Significance indicated by $P < 0.05$ (*) and $P < 0.01$ (**)

content were identified in 2016. Two QTLs were identified for CIT2015 on chromosomes 1 (60–68.7 cM; $R^2 = 11.5$) and 11 (112.1–133 cM; $R^2 = 12.9$) and one for CIT2016 on chromosome 8 (39.8–53.3 cM; $R^2 = 21.4$). ARG2015 and ARG2016 QTLs were stable across years and were found on chromosomes 2 (75.7–86.1 cM, $R^2 = 15.7$; 72.6–87.2 cM, $R^2 = 10.5$) and 5 (185.7–197.5 cM, $R^2 = 14.7$; 186.4–197.8 cM, $R^2 = 30.2$ cM).

Candidate gene identification

The total number of genes in the LOD-2 confidence interval for each QTL were: *Qbrx5-1:* 379; *Qbrx8-2:* 492; *Qsur5-2:* 206; *Qsur8-1:* 326; *Qglu5-1:* 265;

Table 2 Quantitative trait loci identified in the 'Klondike Black Seeded' × 'New Hampshire Midget' watermelon RIL population in 2015 and 2016 using composite interval mapping

Trait	Chr	QTL name	Year	Position (cM)	Maximum LOD	R^2	Additive effect[b]	LOD-1 support interval (cM)	LOD-1 support interval (cM)	LOD-2 support interval (cM)	LOD-2 support interval (cM)	LOD-2 flanking marker[a]	Marker location (Mb)[c]	LOD-2 flanking marker	Marker location (Mb)
Brix	5	*Qbrx5-1*	2016	189.6	3.6	12.4	0.374	181.8	200.3	177	205.6	NW0249733	26.7	NW0248728	30.0
Brix	8	*Qbrx8-2*	2016	0.0	4.0	13.4	− 0.397	0.0	2.5	0.0	4.5	NW0249245	20.9	NW0248412	25.3
Sucrose*	5	*Qsur5-2*	2015	44.8	8.0	23.3	0.024	41.6	45.2	39.0	45.4	NW0248917	2.5	NW0248590	3.9
Sucrose*	5	*Qsur5-2*	2016	44.8	9.0	28.3	0.021	42.1	47.7	39.6	48.3	NW0248917	2.5	NW0250541	4.2
Sucrose*	8	*Qsur8-1*	2015	50.6	3.4	8.8	− 0.015	41.1	54.4	34.7	56.7	NW0249239	20.2	NW0249249	23.4
Glucose	5	*Qglu5-1*	2015	17.9	4.1	12.8	− 2.670	13.7	24.6	8.0	26.5	NW0248569	0.4	NW0248917	2.5
Glucose	5	*Qglu5-2*	2016	45.4	3.1	9.6	− 2.162	44.8	48.5	44.8	48.5	NW0248917	2.5	NW0250541	4.2
Glucose	5	*Qglu5-3*	2015	66.6	4.1	11.9	− 2.595	59.5	71.8	54.3	73.1	NW0250541	4.2	NW0252146	7.1
Glucose	8	*Qglu8-1*	2016	49.6	5.2	17.4	3.005	48.4	50.9	46.4	51.6	NW0249873	20.6	NW0249249	23.4
Glucose	8	*Qglu8-2*	2015	135.5	4.7	14.5	− 2.858	132.3	137.5	130.7	137.5	NW0249735	3.3	NW0248954	14.9
Fructose	5	*Qfru5-1*	2015	16.9	4.9	18.3	− 3.630	13.9	20.9	13.6	21.3	NW0248569	0.4	NW0249824	2.2
Fructose	5	*Qfru5-2*	2016	45.4	4.8	18.3	− 3.106	39.3	48.5	32.6	48.5	NW0248917	2.5	NW0250541	4.2
Lycopene	3	*Qlyc3-1*	2015	31.8	5.2	18.5	0.683	26.6	37.5	24.0	40.6	NW0248648	20.7	NW0249140	23.3
Lycopene	11	*Qlyc11-1*	2015	105.2	3.1	9.7	− 0.504	98.0	106.8	95.2	106.8	NW0250927	2.7	NW0251300	10.3
Citrulline	1	*Qcit1-1*	2015	67.7	3.7	11.5	27.058	64.5	68.7	60.0	68.7	NW0248299	6.3	NW0250100	8.2
Citrulline	8	*Qcit8-1*	2016	48.6	7.0	21.4	36.008	44.8	51.5	39.8	53.3	NW0249239	20.2	NW0249249	23.4
Citrulline	11	*Qcit11-1*	2015	118.8	4.2	12.9	− 28.156	115.3	122.8	112.1	133	NW0248693	2.2	NW0250927	2.7
Arginine	2	*Qarg2-1*	2015	79.7	4.8	15.7	11.956	78.4	83.3	75.7	86.1	NW0249226	28.8	NW0251320	30.2
Arginine	2	*Qarg2-1*	2016	75.6	4.2	10.5	7.960	72.4	75.7	72.6	87.2	NW0249185	28.2	NW0251320	30.2
Arginine	5	*Qarg5-1*	2015	189.6	4.9	14.7	11.021	186.0	194.2	185.7	197.5	NW0247929	27.8	NW0248728	30.0
Arginine	5	*Qarg5-1*	2016	190.6	9.6	30.2	13.062	187.7	195.5	186.4	197.8	NW0247929	27.8	NW0248728	30.0

*Arcsine square-root transformed for normality

[a]Markers previously published in Sandlin et al. (2012)

[b]Negative values indicate an effect contributed by an allele from the male parent ('New Hampshire Midget')

[c]Mb location of markers in '97103' genome Guo et al. (2013)

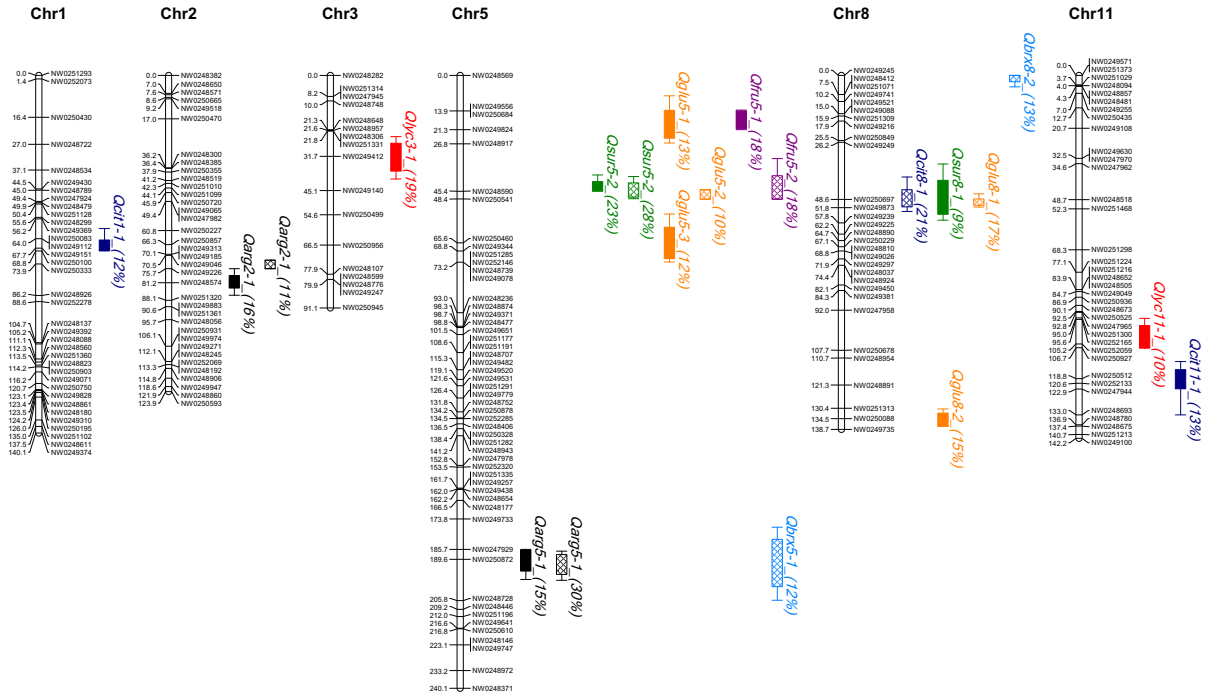

Fig. 2 Genetic map (cM) of the 'Klondike Black Seeded' × 'New Hampshire Midget' RIL population indicating the quantitative trait loci (QTL) identified for soluble solids content (brx, light blue), sucrose content (suc, green), glucose content (glu, orange), fructose content (fru, purple), lycopene content (lyc, red), citrulline content (cit, dark blue), arginine content (arg, black) in 2015 (solid) and 2016 (cross-hatch). Bars and whiskers indicate LOD-1 and LOD-2 support intervals, respectively. The number in parenthesis after the QTL name is the phenotypic variation explained by the QTL

Qglu5-2: 206; *Qglu5-3:* 305; *Qglu8-1:* 287; *Qglu8-2*: 411; *Qfru5-1:* 231; *Qfru5-2:* 206; *Qlyc3-1:* 102; *Qlyc11-1:* 308; *Qcit1-1:* 162; *Qcit8-1:* 326; *Qcit11-1:* 31; *Qarg2-1:* 202 and *Qarg5-1:* 259. Several candidate genes were identified in the QTL regions on chromosome 5 associated with the sugar traits. These included *Cla021522* (phosphate permease), *Cla021325* (pectinesterase), *Cla021640*, *Cla021693* (membrane transporters), *Cla021639*, *Cla021641, Cla021642, Cla021643* (sugar/inositol transporters), *Cla021807* (galactokinase), and *Cla021809* (neutral invertase). Candidate genes for citrulline included *Cla022154* (Argininosuccinate lyase), *Cla022273* (N-acetylglutamate Kinase), and *Cla022392* (Ornithine decarboxylase) in the QTL region on chromosome 8, and *Cla006553* (Pyrroline-5-carboxylate synthase) which was located near the QTL region on chromosome 11 (1,262,283–1,270,007 bp). Two arginine candidate genes controlling plasma membrane transporters were found in the QTL region on chromosome 5, *Cla020511* and *Cla020512*, and one candidate gene was located near the same QTL region, *Cla020781* (Ornithine carbamoyltransferase) (26,837,924–26,841,318 bp).

Discussion

QTLs associated with fruit quality traits were identified in an elite by elite cross and candidate genes within these regions were noted. Trait correlations with Brix identified in this study indicate that selection for Brix may lead to the inadvertent selection of specific sugars or bioactive compounds, which has significant implications given the regular use of Brix measurements in the selection process. Significant correlations were found between Brix, GLU2015 and GLU2016, as well as Brix and FRU2016. High correlations for these traits were also observed by Ren et al. (2014) in a cross between elite '97103' and wild PI 296341-FR, where strong correlations between Brix and the content of the reducing sugars glucose (r = 0.88) and fructose (r = 0.92), along with

moderate correlation for sucrose content (r = 0.65) were observed.

Selection using Brix measurements may also influence the amino acid content of cultivars, as significant correlations between Brix and arginine content were identified in both years. These correlations differed from results reported by Wehner et al. (2017) who showed no correlation for these two traits, although they found a significant correlation between Brix and citrulline content (r = 0.39). For lycopene, positive correlations (r = 0.37) with Brix have been reported with red fleshed watermelon (Perkins-Veazie et al. 2001) and as expected, negative correlations were seen for studies using yellow fleshed watermelons (r = − 0.34) (Wehner et al. 2017). Significant correlations between lycopene content and Brix were only found in 2015 in this study (r = 0.50).

The detection of QTLs for Brix in only one year (2016) is likely due to the low heritability of this trait in watermelon. In the current study heritability ($H^2_{BRX} = 0.30$) was slightly higher than reported previously for the same population but measured across two different environments (H^2 = 0.20) (Sandlin et al. 2012). Moderate heritability of Brix (H^2 = 0.46) has been reported in watermelon cultivars, with high heritability (h^2 = 0.97) reported only for inter-specific crosses between *C. amarus* and *C. lanatus* (PI 296341-FR × '97103'). The difference between parental Brix for these two species is very large (0.8 vs 11) and resulted in high heritability and stable QTL detection on chromosome 2 (Ren et al. 2014). These parental differences were much greater than those observed for the populations in this study, where average Brix for the parents was similar to that reported previously for 'New Hampshire Midget' and 'Klondike Black Seeded' (Sandlin et al. 2012). Brix QTLs for the PI 296341-FR × '97103' interspecific cross were also identified by Xu et al. (2006). Unfortunately, these QTLs were reported before the watermelon genome was published and linkage groups cannot be associated with chromosomes, making it impossible to compare the location of these QTLs. One QTL for BRX2016 on chromosome 8 was found to overlap with Brix and fructose content QTLs as reported by Ren et al. (2014). However, none of the individual sugar content QTLs identified in this study overlapped with the BRX2016 QTL.

The observed significant correlations between the content of different sugars were expected as sucrose can be converted into glucose and fructose in watermelon fruit (Brown and Summers 1985). Heritability of sucrose content ($H^2_{SUC} = 0.70$) was higher in the present study than reported by Ren et al. (2014) in the *C. amarus* × *C. lanatus* population (H^2 = 0.65), while heritability for glucose and fructose content was lower in the present study ($H^2_{GLU} = 0.51$ vs 0.86; $H^2_{FRU} = 0.41$ vs 0.89). These differences are probably at least in part due to the different genetic backgrounds used in the two studies. The majority of the phenotypic variance for sugar content was explained by major QTLs associated with sucrose content (*Qsur5-2*) and intermediate QTLs associated with glucose content (*Qglu5-1*, *Qglu5-2*, *Qglu5-3*) and fructose content (*Qfru5-1*, *Qfru5-2*) detected on chromosome 5. The region on chromosome 5 where QTLs for content of all three sugars co-localized in 2016 may provide a candidate region for genetic control of sugar accumulation. Although the locations of all chromosome 5 sugar content QTLs did not overlap in both years, it is possible that they represent a single locus. The power to detect a QTL as well as the accuracy of its location are dependent on the population size used and the heritability of a given trait (Bogdan and Doerge 2005). The exact QTL locations become increasingly variable as population size decreases (Li et al. 2006, 2010) and the small population size (n = 98) used in the current study may explain the inconsistent location of the sugar content QTLs in this region of chromosome 5. Results of QTL studies realized with populations of small sizes are more affected by environmental factors. QTLs were also identified on chromosome 8 for sucrose content (*Qsur8-1*) and glucose content (*Qglu8-1*, *Qglu8-2*), with *Qsur8-1* and *Qglu8-1* co-localizing. QTLs for sucrose and glucose content were previously identified on chromosomes 5 and 8 (Cheng et al. 2016; Ren et al. 2014), though at different positions.

Co-localization of sugar content QTLs in watermelon is not surprising, given that accumulation of one sugar is dependent on the loss of another. In watermelon fruit ripening, both fructose and glucose content increase at 24–36 days after anthesis (DAA), followed by a continuous decrease, especially of glucose, through ripening at 42 days (Elmstrom and Davis 1981). In contrast, sucrose content remains

relatively low until 36 DAA after which it increases linearly to 42 DAA. Glucose and fructose are the primary photosynthetic products transported in watermelon, with subsequent synthesis of sucrose (Guo et al. 2013). This sugar relationship indicates that there is overlap for the genetic control of accumulation of individual sugars. Co-localization of sugar content QTLs has also been described previously in other crops, including tomato (Fulton et al. 2002) and peach (Dirlewanger et al. 1999).

The importance of key sugar transporter genes in sugar accumulation indicates the need to identify their locations in the genome for marker development. Several different enzymes and sugar transporters have been shown to affect the process of sugar accumulation during fruit ripening, along with many transcription factors (Guo et al. 2013). Invertases, sucrose synthase (SuSy), and sucrose phosphate synthase (SPS) are the three main active enzyme families in the sugar metabolism of fruits, with insoluble invertases playing a critical role in sucrose accumulation in watermelon (Yativ et al. 2010). Several candidate genes in the region on chromosome 5 were identified, including *Cla021522* (phosphate permease), *Cla021325* (pectinesterase), *Cla021640*, *Cla021693* (membrane transporter), *Cla021639*, *Cla021641, Cla021642, Cla021643* (sugar/inositol transporters), *Cla021807* (galactokinase), and *Cla021809* (neutral invertase) (Guo et al. 2013; Zhu et al. 2017). Further research will be needed to determine the role of these candidate genes in sugar accumulation in watermelon.

Heritability of lycopene content in the current study ($H^2_{LYC} = 0.70$) was lower than described in Wehner et al. (2017) where broad sense heritability was estimated to be 0.99. The latter study includes cultivars with yellow and orange flesh and a much larger range of lycopene contents. High variability of lycopene content among red fleshed watermelon has been described previously (Perkins-Veazie et al. 2001, 2005) and may explain the lack of stable QTLs for lycopene content in this study. A major QTL ($R^2 = 83.5$) for lycopene content was mapped previously on chromosome 4 in a F_2 population from a cross between parents with pale yellow and red flesh (Liu et al., 2015). However, mapping lycopene content in a population segregating for red vs pink flesh color is proving more challenging.

An interesting result for lycopene content was its positive correlation with sucrose content in both years. This trend is also found in other fruits, such as sweet orange red fleshed mutants (Liu et al. 2007; Xu et al. 2009) and tomato, where lycopene accumulation was shown to directly depend on the sucrose concentration present (Telef et al. 2006). Visual selection of fruit based on red flesh color (high lycopene) may therefore result in the inadvertent selection of high sucrose content. Sucrose, glucose and fructose sugars have been shown to be perceived differently by panelists in other fruits, meaning that this sugar selection may affect consumer preference. There is not currently enough research on watermelon taste perception to know whether this selection would result in positive or negative taste perception (Pangborn 1963).

Heritability for citrulline content ($H^2_{CIT} = 0.71$) was higher than observed by Wehner et al. (2017) ($H^2 = 0.41$), while heritability for arginine content was lower in the present study ($H^2_{ARG} = 0.73$ vs 0.89). Although citrulline is the precursor of arginine, correlations between these two traits were not significant (Table 1). Wehner et al. (2017) also failed to detect a significant correlation between citrulline and arginine content when studying eight different watermelon cultigens. Accumulation of citrulline content in watermelon increases as the fruit matures and reaches its peak just prior to full maturity, before declining over time (Akashi et al. 2017; Fish 2014; Guo et al. 2013). Citrulline content values for both years were within the range reported by Davis et al. (2011) and Wehner et al. (2017).

Guo et al. (2013) found downregulation of argininosuccinase and two argininosuccinate synthase genes during watermelon flesh development, indicating that citrulline accumulation in the fruit flesh is likely due to decreased citrulline degradation. Current research suggests that this accumulation is due to both an increase in transport of citrulline into the fruit and a simultaneous decrease in the catabolism of citrulline (Guo et al. 2013; Joshi and Fernie 2017). Candidate citrulline genes coding for Argininosuccinate lyase, *Cla022154*, N-acetylglutamate kinase, *Cla022273*, and ornithine decarboxylase, *Cla022392*, were found in the citrulline LOD-2 QTL region of chromosome 8. Additionally, *Cla006553*, a candidate gene coding for pyrroline-5-carboxylate synthase was found close (1,262,283–1,270,007 bp) to the QTL region on

chromosome 11 (Joshi and Fernie 2017). The lack of correlation between citrulline and arginine content is intriguing and may indicate that arginine content is primarily dependent on transport into the fruit, with citrulline catabolism providing only a minimal amount of arginine.

Two stable QTLs for arginine content (*Qarg2-1* and *Qarg5-1*) were identified in the current study. To our knowledge, this is the first identification of QTLs associated with arginine content in watermelon flesh. Arginine accumulation candidate genes *Cla020511* and *Cla020512*, both of which function in plasma membrane transport, were identified within the LOD-2 QTL region on chromosome 5. A candidate gene coding for the enzyme ornithine carbamoyl transferase, *Cla020781* (Joshi and Fernie 2017), was also close (26,837,924–26,841,318 bp) to the same arginine QTL region on chromosome 5. Arginine is formed from the enzymatic breakdown of citrulline and thus genetic control of these two amino acids was expected to coincide (Joshi and Fernie 2017). However, as expected from the lack of correlation between citrulline and arginine content, there was no overlap in QTLs associated with these two traits. Unlike arginine, no stable QTL was identified for citrulline content.

The need to select fruit with sweet, bright red flesh that is high in nutritional compounds is important to watermelon breeders due to its high demand from the consumer (Evans 2008; Hashizume et al. 2003). Development of markers for selection of these fruit quality traits would enhance efficient selection of new cultivars with more bioactive compounds (Baldwin 2008; Kader 2008). In the current study we used a biparental RIL population from a cross between two cultivars to identify QTL associated with fruit quality traits. The use of biparental populations for QTL detection is still the norm in watermelon and other cucurbits where the development of multi-parental populations is cost prohibitive. The Klondike Black Seeded cultivar used as one of the parents is one of the Klondike-type cultivars developed in California in the early to mid-1900s (Parris, 1949). Klondike cultivars are an integral part of the pedigrees of many subsequent watermelon cultivars, and therefore the QTL identified here should be more broadly relevant. We identified stable QTLs for fruit quality traits (sucrose and arginine content) that provide potential targets for marker assisted selection of sucrose and amino acid content. Additionally, QTL co-localization of sugar content traits identified on chromosome 5 may indicate the identification of a major QTL controlling accumulation of all three sugars (sucrose, glucose, and fructose) in watermelon.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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