J. Amer. Soc. Hort. Sci. 139(4):433–441. 2014.

Quantitative Trait Loci and Candidate Genes Associated with Fatty Acid Content of Watermelon Seed

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Additional index words. egusi, linoleic acid, oleic acid, palmitic acid, SOP, stearic acid

Abstract. Seed oil percentage (SOP) and fatty acid composition of watermelon (*Citrullus lanatus*) seeds are important traits in Africa, the Middle East, and Asia where the seeds provide a significant source of nutrition and income. Oil yield from watermelon seed exceeds 50% (w/w) and is high in unsaturated fatty acids, a profile comparable to that of sunflower (*Helianthus annuus*) and soybean (*Glycine max*) oil. As a result of novel non-food uses of plant-derived oils, there is an increasing need for more sources of vegetable oil. To improve the nutritive value of watermelon seed and position watermelon as a potential oil crop, it is critical to understand the genetic factors associated with SOP and fatty acid composition. Although the fatty acid composition of watermelon seed is well documented, the underlying genetic basis has not yet been studied. Therefore, the current study aimed to elucidate the quality of watermelon seed oil and identify genomic regions and candidate genes associated with fatty acid composition. Seed from an F_2 population developed from a cross between an egusi type (PI 560023), known for its high SOP, and Strain II (PI 279261) was phenotyped for palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2). Significant ($P < 0.05$) correlations were found between palmitic and oleic acid (0.24), palmitic and linoleic acid (–0.37), stearic and linoleic acid (–0.21), and oleic and linoleic acid (–0.92). A total of eight quantitative trait loci (QTL) were associated with fatty acid composition with a QTL for oleic and linoleic acid colocalizing on chromosome (Chr) 6. Eighty genes involved in fatty biosynthesis including those modulating the ratio of saturated and unsaturated fatty acids were identified from the functionally annotated genes on the watermelon draft genome. Several fatty acid biosynthesis genes were found within and in close proximity to the QTL identified in this study. A gene (*Cla013264*) homolog to fatty acid elongase (FAE) was found within the 1.5-likelihood-odds (LOD) interval of the QTL for palmitic acid ($R^2 = 7.6\%$) on Chr 2, whereas *Cla008157*, a homolog to omega-3-fatty acid desaturase and *Cla008263*, a homolog to FAE, were identified within the 1.5-LOD interval of the QTL for palmitic acid ($R^2 = 24.7\%$) on Chr 3. In addition, the QTL for palmitic acid on Chr 3 was located ≈0.60 Mbp from *Cla002633*, a gene homolog to fatty acyl- [acyl carrier protein (ACP)] thioesterase B. A gene (*Cla009335*) homolog to ACP was found within the flanking markers of the QTL for oleic acid ($R^2 = 17.9\%$) and linoleic acid ($R^2 = 21.5\%$) on Chr 6, whereas *Cla010780,* a gene homolog to acyl-ACP desaturase was located within the QTL for stearic acid ($R^2 = 10.2\%$) on Chr 7. On Chr 8, another gene (*Cla013862*) homolog to acyl-ACP desaturase was found within the 1.5-LOD interval of the QTL for oleic acid ($R^2 = 13.5\%$). The genes identified in this study are possible candidates for the development of functional markers for application in marker-assisted selection for fatty acid composition in watermelon seed. To the best of our knowledge, this is the first study that aimed to elucidate genetic control of the fatty acid composition of watermelon seed.

Watermelon is an economically important crop of the Cucurbitaceae family, popular for its sweet edible flesh (Robinson and Decker-Walters, 1997). However, the seeds of watermelon provide a significant source of nutrition and income in other parts of the world including China (Zhang, 1996), Israel (Edelstein and Nerson, 2002), Iran (Baboli and Kordi, 2010), and Africa (Achigan-dako et al., 2008; Al-Khalifa, 1996; El-Adawy and Taha, 2001).

Watermelon seeds are rich in oils [greater than 50% (Baboli and Kordi, 2010)] and proteins [greater than 27% (Al-Khalifa, 1996; Baboli and Kordi, 2010; Loukou et al., 2007)], thus playing a crucial role in supplementing the nutrients of the staple carbohydrate foods of the poor, who cannot afford animal-derived protein foods (Achu et al., 2005). The seed is manually dehulled to separate the kernels from the seedcoat and eaten raw, roasted, made into soup, or processed into cooking oil (Achigan-dako et al., 2008; Al-Khalifa, 1996). In addition, edible seeds of other cucurbits such as melon [*Cucumeropsis mannii* and *Cucumis melo* (Achigan-dako et al., 2008; Loukou et al., 2007)], squash [*Cucurbita pepo* (Idouraine et al., 1996)], pumpkin [*C. pepo* and *Cucurbita moschata* (Al-Khalifa, 1996)], and bottle gourd [*Lagenaria siceraria* (Achigan-dako et al., 2008; Achu et al., 2005)] are also nutritious and form a part of the diet in these communities.

The egusi watermelon (*Citrullus lanatus* ssp. *mucosospermus* var. *egusi*), which is easily identifiable by its unique fleshy, thick pericarp (Gusmini et al., 2004), is popularly cultivated for its edible seeds in Africa. The egusi seed is nutritious with a high SOP (Gusmini et al., 2004; Jarret and Levy, 2012; Prothro et al., 2012) and a high protein content (Gusmini et al., 2004). Whereas extensive research has been carried out toward improvement of the yield and quality of oil for the major oil crops such as soybean, sunflower, peanut (*Arachis hypogaea*), corn (*Zea mays*), and canola (*Brassica napus*), limited research has been done to improve these traits in cucurbit crops such as watermelon despite their agronomic and cultural importance (Loukou et al., 2007). The limited attention in research for cucurbits

Received for publication 6 Feb. 2014. Accepted for publication 28 Apr. 2014. This research was partially funded by the National Institute of Food and Agriculture (NIFA) Research and Education grant (GEO-2009-0418).
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relative to staple crops has led to their underuse and classification as orphan crops (Achigan-dako et al., 2008; Baboli and Kordi, 2010; International Plant Genetic Resources Institute, 2002). Of the more than 500,000 known plant species, only 12 are currently exploited commercially to produce vegetable oil despite the increasing world demand (Baboli and Kordi, 2010; Mabaleha et al., 2007). Most of this demand is attributed to novel non-food uses including biofuel, oleochemicals, lubricants, pharmaceuticals, and cosmetics (Jarret and Levy, 2012; Panthee et al., 2006).

To improve the nutritive value of watermelon seed and establish watermelon as a potential oil crop, it is critical to understand the genetic factors associated with SOP and fatty acid composition. Prothro et al. (2012) elucidated the genetic factors associated with SOP in watermelon and identified four QTL associated with the trait. Among these, the egusi locus, which is also associated with the egusi seed phenotype, explained 83% of the phenotypic variation observed in SOP. Further studies have revealed that the high SOP in the egusi seed is the result of a high kernel percentage [KP (Jarret and Levy, 2012; Meru and McGregor, 2013)], and that seed size plays a role in SOP in watermelon (Meru and McGregor, 2013).

The type and ratio of fatty acids in a given vegetable oil determine its nutrition, flavor, shelf life, and its potential application (Brown et al., 1975; Panthee et al., 2006; Wassom et al., 2008; XinYou et al., 2011; Yang et al., 2010). Edible vegetable oils and seeds with low saturated fat content are desirable because they produce lower levels of low-density lipoproteins that have been linked with arteriosclerosis and heart-related ailments (Wassom et al., 2008). Therefore, plant breeders aim to reduce the levels of saturated fatty acids while increasing the levels of unsaturated fatty acids in oil crops. On the contrary, increased levels of saturated fats are desirable for the margarine and similar industries for the development of solid or semisolid fats without harmful chemical processes such as hydrogenation or transesterification (Ascherio and Willett, 1997, Panthee et al., 2006).

The four primary fatty acids in watermelon seed oil are palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) with linoleic acid being the most abundant (Al-Khalifa, 1996; Baboli and Kordi, 2010; El-Adawy and Taha, 2001; Giwa et al., 2010; Jarret and Levy, 2012; Loukou et al., 2007; Oluba et al., 2008). A study of fatty acid composition in watermelon genebank accessions by Jarret and Levy (2012) documented the range of different fatty acids in watermelon seeds [linoleic acid (45.37% to 73%), oleic acid (7.89% to 33.95%), stearic acid (5.03% to 13.84%), palmitic acid (9.68% to 14.38%)]. The fatty acid composition in watermelon seed is similar to that found in other cucurbit crops. For instance, Al-Khalifa (1996) found similar levels of unsaturated fatty acids (oleic acid and linoleic acid) in watermelon (81.3%) and pumpkin [*C. pepo* (80.1%), *C. moschata* (79.9%)]. In comparison with the major oil crops, Baboli and Kordi (2010) found similar levels of palmitic acid in watermelon seed (11.36%) and soybean (11%) and similar levels of linoleic acid in watermelon seed (68.3%) and sunflower (68%). However, watermelon seed was lower in oleic acid [13.25% (sunflower = 18.6%, soybean = 23.4%)] but higher in stearic acid [7.04% (sunflower = 4.7%; soybean = 4%)].

Although the fatty acid composition of watermelon seed oil is well documented, knowledge about the underlying genetic factors is lacking. The recent sequencing and functional annotation of the watermelon genome presents an opportunity for further research into the molecular mechanisms underlying economically important traits including fatty acid composition (Guo et al., 2013). Similar tools have recently been made available for melon (Blanca et al., 2012) and cucumber [*Cucumis sativus* (Huang et al., 2009)] through the sequencing and functional annotation of their respective genomes. Previous studies aimed at understanding the regulation of stearoyl-acyl carrier protein desaturation led to the isolation of a full-length cDNA clone of stearoyl-ACP protein desaturase in cucumber (Shanklin et al., 1991; Shanklin and Somerville, 1991). However, no DNA markers have been developed for cucurbit crops for application in marker-assisted selection (MAS) for improved oil quality.

The association of DNA markers with genes regulating fatty acid composition has enabled the application of MAS to accelerate breeding for improved oil quality in canola (Hu et al., 2006), spring turnip rape [*Brassica rapa* (Tanhuanpaa et al., 1998)], sunflower (Hongtrakul et al., 1998; Perez-Vich et al., 2002), soybean (Cardinal et al., 2007; Fehr, 2007; Pham et al., 2010; Zhang et al., 2008), and peanut (Barkley et al., 2010, 2013; Chu et al., 2009). As a step toward developing such genomic tools for watermelon, the current study aimed to identify genetic loci and candidate genes associated with fatty acid composition in watermelon seed for future application in MAS for improved oil quality. To our knowledge, this is the first effort to identify DNA markers linked to genomic regions associated with fatty acid composition in cucurbit seeds.

Materials and Methods

Plant material and genotyping. The F_2 population (n = 142) from a cross between Strain II of the Japanese cultivar Yamato-cream [PI 279261 (normal seed type)] and an egusi type from Nigeria (PI 560023) used previously (Meru and McGregor, 2013; Prothro et al., 2012) to map loci controlling the egusi seed trait, SOP, and KP in watermelon was used in the present study. Single nucleotide polymorphism (SNP) assays were performed on the parents and progeny (F_2; n = 142) of the mapping population using an Illumina's GoldenGate SNP array and BeadStudio software (Illumina, San Diego, CA) as described in Sandlin et al. (2012). A genetic map was developed that included 357 SNP markers spanning 14 linkage groups (LGs) with an average gap of 4.2 cM between markers (Sandlin et al., 2012).

Fatty acid analysis. Phenotyping was carried out on seed derived from 142 open-pollinated F_2 individuals (Prothro et al., 2012), the parental lines, and F_1. The 15 seeds from each F_2 plant and parental lines that were manually dehulled with a steel blade for the Meru and McGregor (2013) study were used for fatty acid analysis. The seed kernels from each sample were crushed with a mortar and pestle and 200 mg of the powder was weighed and transferred into 2.2-mL 96-well plates (Fisher Scientific, Pittsburgh, PA). Fatty acid methyl esters (FAMEs) were prepared using the standard method for analyses of fatty acid composition in fats and oils recommended by the American Oil Chemist's Society [Ce 1-62 (American Oil Chemist's Society, 2009)]. To extract oil from the samples, 2 mL of hexane (Fisher Scientific) was added to each well followed by incubation at room temperature (25 °C) for 15 min. The supernatant (0.5 mL) from each sample was transferred to a new 96-well plate and evaporated to dryness with a stream of

N_2 at 50 °C on a 96-well evaporator. To each well, 0.2 mL of ethyl ether (BDH, Poole, U.K.) was added to solubilize the lipids followed by the addition of 0.2 mL 0.1 M KOH and incubation at 50 °C for 10 min to convert the lipids into FAMEs. The methylation reactions were stopped by adding 0.2 mL of 0.15 M HCl. The samples were incubated at room temperature for 15 min, and an aliquot (0.5 mL) of the organic layer containing FAMEs was transferred to 2-mL autosampler vials (Fisher Scientific) for gas chromatography (GC) analysis. The FAMEs were separated by injecting 1 μL of sample onto a GC column (DB-23) in a gas chromatograph (6890 Series; Agilent, Wilmington, DE) equipped with an autosampler carousel. A standard (15A; Nu-Control Prep, Elysian, MN) with known concentrations for palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids was used to identify peaks. The profile for the fatty acids was estimated from chromatograms using ChemStation Software (Agilent). Fatty acid extraction was carried out twice from different portions of the original ground sample and the average value for each line used in the data analysis.

Correlations, mean separation, and QTL detection. Pearson correlations between the values of the phenotypic traits were calculated using JMP (Version 11; SAS Institute, Cary, NC). The values for SOP for the population were previously determined by Prothro et al. (2012), whereas those of seed size (seed weight) were determined by Meru and McGregor (2013). Student's *t* test was performed in JMP to identify differences in the means of the various fatty acids in normal (n = 100) and egusi (n = 42) seed subpopulations.

The fatty acid values were arcsine square root-transformed before QTL analysis because they are expressed as a proportion of total fatty acids in the oil (Wills et al., 2010). Composite interval mapping (CIM) was used to detect QTL with a 5-cM window in WinQTL Cartographer Version 2.5 (Wang et al., 2011a). The standard model (Model 6) with a walk speed of 1 cM was used for CIM analysis and the population type was designated as "RF3" (Wang et al., 2011a). Statistical significance of a QTL was determined by likelihood-odds thresholds set by 1000 permutations [$\alpha = 0.05$ (Churchill and Doerge, 1994)]. QTL detected on the same LG were not considered different unless they were separated by at least 20 cM (Ravi et al., 2011). All LGs and QTL were visualized using MapChart 2.2 (Voorrips, 2002).

Fatty acid biosynthesis genes in watermelon. The genes involved in fatty acid biosynthesis in watermelon were determined by comparing annotated genes in the watermelon draft genome (Guo et al., 2013; International Cucurbits Genomics Initiative, 2012) with genes reported to be involved in fatty acid biosynthesis for other crops (Byfield et al., 2006; Cahoon et al., 1994; Cardinal et al., 2007; Kachroo et al., 2008; Li et al., 2010).

Candidate genes. Markers flanking the 1.5-LOD [≈95% confidence interval (Silva et al., 2012)] interval for all the QTL were identified on the linkage map and their corresponding positions on the watermelon physical map (Guo et al., 2013) determined. The regions between the flanking markers were then inspected for genes in the fatty acid biosynthesis pathway.

Results and Discussion

Phenotypic analysis of traits. Linoleic acid was the predominant fatty acid detected. The levels of palmitic acid and linoleic acid in the Strain II parent (9.36% and 70.72%, respectively) were higher than in the egusi parent (8.50% and 61.15%, respectively), whereas those of stearic acid and oleic acid in the egusi parent (7.92% and 22.42%, respectively) were higher than in Strain II (5.59% and 14.33%, respectively) (Table 1). These results are similar to those reported by Jarret and Levy (2012) for egusi (palmitic = 9.68% to 12.82%, stearic = 8.63% to 13.84%, oleic = 8.14% to 17.26%, linoleic = 58.95% to 71.10%) and for the seeds of edible watermelon (palmitic = 9.68% to 14.38%, stearic = 5.03% to 11.52%, oleic = 7.89% to 25.67%, linoleic = 48.7% to 73%). In comparison with the major oil crops, the degree of unsaturation (oleic acid and linoleic acid) found in this study for watermelon (Strain II = 85.1%, egusi = 83.6%) was similar to that of soybean (84.4%) and sunflower (88.6%) (Baboli and Kordi, 2010).

Extraction of crude oil from watermelon seeds for subsistence use is common in West Africa (Achigan-dako et al., 2008; Jarret and Levy, 2012; Oluba et al., 2008). However, this oil may have a low shelf life and less stability at high cooking temperature as a result of high linoleic acid content, which is highly oxidative. High levels of linoleic acid content (57%) in soybean has led to breeding efforts to decrease the content of this fatty acid in favor of oleic acid, which is more stable at high cooking temperature (Baboli and Kordi, 2010; Lee et al., 2007). Linoleic acid is more oxidative as a result of the presence of two double bonds as opposed to oleic acid, which has a single double bond (Lee et al., 2007). For watermelon seed to be exploited commercially for the production of cooking oil, it is necessary to breed for reduced levels of linoleic acid and increased levels of oleic acid to improve its stability and shelf life. From the study on watermelon genebank accessions by Jarret and Levy (2012), it is clear that natural variation exists in oleic acid (7.89% to 33.95%) and linoleic acid (45.37% to 73%) and can be exploited in breeding to produce cultivars of high oleic acid and lower linoleic acid. Adoption of watermelon as a minor oil crop would improve farmers' earnings while concurrently reducing the amount of solid waste resulting from the disposal of watermelon seeds (Baboli and Kordi, 2010; El-Adawy and Taha, 2001).

Table 1. Watermelon seed oil fatty acid composition of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) in Strain II (PI 279261), egusi (PI 560023), F_1, and F_2 population (n = 142) derived from a cross between Strain II and egusi.

				F_2 population				
	Strain II	Egusi	F_1	Mean	SD	Range	Minimum	Maximum
Fatty acid	Fatty acid composition (% by wt)							
Palmitic	9.36	8.50	10.95	10.57	0.58	3.01	9.07	12.08
Stearic	5.59	7.92	8.08	8.37	1.22	7.61	4.33	11.94
Oleic	14.33	22.42	18.50	18.8	3.03	16.81	12.46	29.28
Linoleic	70.72	61.15	62.48	62.12	3.25	19.63	50.65	70.28

Within the population, palmitic acid ranged from 9.07% to 12.08%, stearic acid from 4.33% to 11.94%, oleic acid from 12.46% to 29.28%, and linoleic acid from 50.65% to 70.28% (Table 1). Transgressive segregation was observed in one direction for palmitic acid and linoleic acid and in both directions for stearic acid and oleic acid whereby the progeny had trait values outside the range of the parents. Transgressive segregation is primarily associated with antagonistic additive effects (Rieseberg et al., 1999). Linoleic acid was found to be significantly ($P < 0.05$) negatively correlated to palmitic (–0.37), stearic (–0.21), and oleic (–0.92) fatty acids. However, palmitic acid was significantly positively correlated (0.24) to oleic acid (Table 2). Oyenuga and Fetuga (1975) found a significant positive correlation between palmitic acid and oleic acid but a negative correlation between palmitic acid and linoleic acid in watermelon. On the contrary, Jarret and Levy (2012) did not find significant correlations between these fatty acids in watermelon. In wheat (*Triticum aestivum*), Wang et al. (2011b) found linoleic acid to be significantly negatively correlated to palmitic, stearic, oleic, and linolenic acids. Palmitic acid was the only trait significantly correlated with SOP (–0.22) (Table 2). This relationship may explain the observation in egusi watermelon whose seeds have high SOP (Gusmini et al., 2004; Jarret and Levy, 2012; Meru and McGregor, 2013; Prothro et al., 2012) but lower levels of palmitic acid relative to other watermelon types (Jarret and Levy, 2012). In the current study, significant differences ($P < 0.05$) were found between palmitic acid levels in normal (low SOP) and egusi (high SOP) seeds further supporting this relationship. However, no unique QTL for palmitic acid were identified when mapping normal and egusi seed separately (data not shown). In canola, Zhao et al. (2008) found a negative correlation between oil content and palmitic acid (–0.34), whereas in soybean, a negative correlation (–0.53) between linoleic acid and oil content has been documented (Li et al., 2011). No significant differences were found between egusi and normal seeds in the population for the levels of stearic acid, oleic acid, and linoleic acid (data not shown). Seed weight was significantly positively correlated with oleic acid (0.27) but significantly negatively correlated with linoleic acid (–0.24). These results are similar to those obtained in soybean by Liu et al. (1995) who found a positive correlation between seed size and oleic acid but a negative correlation between seed size and linoleic acid.

Detection of QTL. Genetic analysis detected a total of eight QTL on Chr 2, 3, 5, 6, 7, and 8, three each for palmitic acid and oleic acid and one each for stearic acid and linoleic acid (Fig. 1; Table 3). The QTL for oleic acid (R^2 = 17.9%) colocalized with a QTL for linoleic acid (R^2 = 21.5%) on Chr 6. Colocalization of the two traits can be expected because of the high negative correlation between the traits (–0.92) (Table 2), which is consistent with the fact that the two fatty acids share a common pathway where desaturation of oleic acid by omega-6 fatty acid desaturase-2 (FAD2) yields linoleic acid in plants (Bachlava et al., 2009; Liu et al., 2011; Sharma et al., 2002; Yu et al., 2008). Colocalization of QTL for oleic acid and linoleic acid is common in crops including corn (Wassom et al., 2008; Yang et al., 2010), wheat (Wang et al., 2011b), and soybean (Panthee et al., 2006).

Table 2. Pearson correlations for fatty acids, seed oil percentage [SOP (Prothro et al., 2012)], and seed weight (Meru and McGregor, 2013) in the Strain II (PI 279261) × egusi (PI 560023) F_2 watermelon population (n = 142).

Trait	SOP	Seed wt	Palmitic acid	Stearic acid	Oleic acid
Palmitic acid	–0.22*	0.01			
Stearic acid	0.11	0.02	–0.01		
Oleic acid	0.12	0.27*	0.24*	–0.15	
Linoleic acid	–0.12	–0.24*	–0.37*	–0.21*	–0.92*

*Significant at $P < 0.05$.

Colocalization was also observed between the QTL for oleic acid and linoleic acid on Chr 6 and QTL for seed size (seed weight) in normal seed identified by Meru and McGregor (2013). These results might explain the significant correlation observed between seed size and the two fatty acids in the current study (Table 2). QTL for oleic acid were also detected on Chr 2 (R^2 = 10.7%) and Chr 8 (R^2 = 13.5%). QTL for palmitic acid were identified on Chr 2 (R^2 = 7.6%), Chr 3 (R^2 = 24.7%), and Chr 5 (R^2 = 12.7%), whereas a single QTL for stearic acid was identified on Chr 7 (R^2 = 10.2%) (Fig. 1; Table 3).

Fatty acid biosynthesis genes in watermelon. An examination of the functionally annotated genes in the watermelon draft genome (Guo et al., 2013) revealed 80 genes involved in fatty acid biosynthesis (Supplemental Table 1). Of particular interest among the identified genes are those central in regulating the ratio of saturated (palmitic acid and stearic acid) and unsaturated (oleic acid and linoleic acid) fatty acids. This ratio is important because it determines the potential application of vegetable oils (Brown et al., 1975; Panthee et al., 2006; Wassom et al., 2008; XinYou et al., 2011; Yang et al., 2010). The fatty acyl-ACP thioesterase-B (FATB) regulates the amount of saturated fatty acids by hydrolyzing the acyl group from saturated-ACP (16:0-ACP and 18:0-ACP) to release free saturated fatty acids (Barker et al., 2007; Bonaventure et al., 2003). In watermelon, five genes potentially encoding this enzyme were identified on several chromosomes. Similarly, five genes are predicted to encode FATB genes in peanut (Yin et al., 2013) and one each in arabidopsis [*Arabidopsis thaliana* (Barker et al., 2007; Bonaventure et al., 2003)] and jatropha [*Jatropha curcas* (Natarajan and Parani, 2011)]. In soybean, several low-palmitic acid cultivars have been developed by mutagenesis (ethyl methanesulfonate and N-nitroso-N-methyl urea and X-radiation) and molecular analysis of these cultivars has revealed mutation in various FATB genes (Cardinal et al., 2007).

The number of genes potentially encoding acyl-ACP desaturases in watermelon (12 genes) was higher than that in peanut [eight genes (Yin et al., 2013)] and jatropha [four genes (Natarajan and Parani, 2011)]. Acyl-ACP desaturases are soluble enzymes that introduce double bonds into acyl-ACPs in oxygen-dependent reactions to form monounsaturated fatty acids and hence modulate the ratio of saturated and unsaturated fatty acids (Guy et al., 2011). For example, the stearoyl-ACP protein desaturase (SADP) introduces a double bond at C_9 in stearic acid (18:0) to form oleic acid (18:1). In soybean, mutations in the SADP have been sought to increase the levels of stearic acid (and reduce the levels of oleic acid) in soybean oil to decrease the cost of hydrogenation in the food processing industry (Byfield et al., 2006). Although stearic acid is a saturated fatty acid, it does not increase cholesterol levels in humans and thus does not pose a health risk in contrast to palmitic acid (Ruddle et al., 2013). Most of the high stearic acid

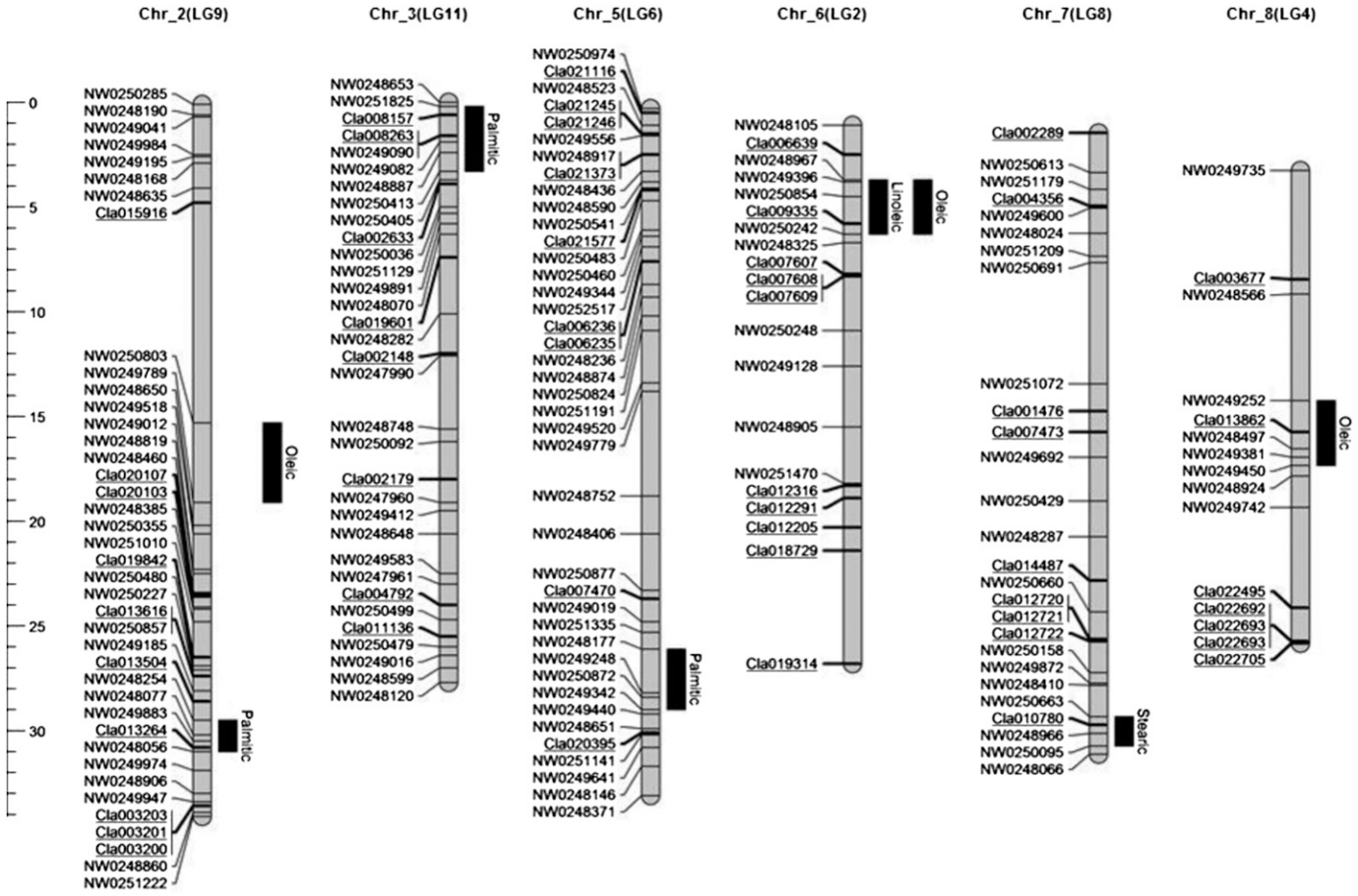

Fig. 1. Quantitative trait loci [length of the bar represents the region between the markers flanking the 1.5-likelihood-odds (LOD) support interval] identified by composite interval mapping for the content of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) in watermelon seed in the Strain II (PI 279261) × egusi (PI 560023) watermelon population. The genes involved in the fatty acid biosynthesis pathway are underlined. Figure generated using MapChart Version 2.2 (Voorrips, 2002).

soybean germplasm lines have been developed using mutagenesis targeting SADP (Ruddle et al., 2013), although a natural mutation in the gene has also been reported in one cultivar (Byfield et al., 2006; Ruddle et al., 2013).

Increased levels of oleic acid and concomitant reduction in levels of linoleic acid have been achieved in oil crops by targeting FAD2 that converts oleic acid into linoleic acid by inserting a double bond at C_{12} (Heppard et al., 1996; Yu et al., 2008). High oleic acid peanut cultivars have been obtained both through radiation-induced mutagenesis and exploitation of natural mutations in FAD2 (Yu et al., 2008). Four genes potentially encoding FAD2 were identified in watermelon on Chr 2, 6, and 11, a number lower than that of FAD2 in peanut (13) (Yin et al., 2013).

Candidate genes. The genes *Cla013264* (E-value: 2E-200) and *Cla008263* (E-value: 2E-195) homologs to 3-ketoacyl-CoA synthase in *Populus trichocarpa* (Supplemental Table 1) were found within the 1.5-LOD interval of the QTL for palmitic acid on Chr 2 (R^2 = 7.6%) and Chr 3 (R^2 = 24.7%), respectively. 3-ketoacyl-CoA synthase is a subunit of fatty acid elongase (FAE) complex that condenses acyl-CoA with malonyl-CoA as the first step in the synthesis of very long fatty acids (Bach et al., 2008; Bach and Faure, 2010; Barret et al., 1998). The association of this QTL with variation in palmitic acid may be explained by the use of palmitic acid to form palmitoyl-CoA, which is used as a substrate by 3-ketoacyl-CoA synthase (Bach et al., 2008; Bach and Faure, 2010). In canola, FAE is associated with variation in erucic acid (22:1) and is targeted in breeding for low erucic acid (cooking oil) and high erucic acid (industrial applications) canola cultivars (Barret et al., 1998; Fourmann et al., 1998).

In addition, a gene (*Cla008157*) homolog (E-value: 1E-136) to omega-3 desaturase (FAD3) in *Betula pendula* (Supplemental Table 1) was found within the flanking markers of the QTL for palmitic acid on Chr 3. FAD3 catalyzes the insertion of a double bond into linoleic acid to yield linolenic acid and is a central target for breeders in manipulating the levels of linolenic acid, which is undesirable in soybean oil (Anai et al., 2005). Interestingly, *Cla002633,* a homolog (E-value: 4E-61) to the FATB gene in *Ricinus communis* (Supplemental Table 1), is just outside (≈0.60 Mbp from NW0250413) the 1.5-LOD interval of the palmitic acid QTL on Chr 3. Fatty acid thioesterase B hydrolyzes the acyl group from palmitoyl-ACP to release free palmitic acid and ACP (Bonaventure et al., 2003; Cardinal et al., 2007). A natural deletion in the FATB gene of soybean was shown to result in reduced palmitic acid levels (Cardinal et al., 2007). Similarly, reduction in the levels of palmitic acid has been reported in chemical and induced mutants that resulted in perturbation of FATB isoforms in soybean (Cardinal et al., 2007).

Table 3. Chromosomal (Chr) positions and the corresponding 1.5-likelihood-odds (LOD) support interval associated with fatty acid composition in the Strain II (PI 279261) × egusi (PI 560023) F_2 watermelon population.

Fatty acid	LG[z]	Position (cM)	Chr[y]	LOD[x]	R^2 (%)[w]	Additive effect[v]	Dominance effect[v]	LOD-1 0.5 support interval (cM)	Flanking marker[u]	Position on Chr (Mbp)[y]	LOD-1 0.5 support interval (cM)	Flanking marker[u]	Position on Chr (Mbp)[y]
Palmitic	9B	106.74	2	3.67	7.55	0.20	–0.44	98.08	NW0248254	29.63	112.74	NW0248056	31.13
Palmitic	11B	44.96	3	10.70	24.73	0.67	0.35	38.53	NW0250413	3.36	59.39	NW0251825	0.33
Palmitic	6	188.02	5	4.94	12.67	–0.45	–0.12	173.02	NW0248177	26.20	199.21	NW0249342	29.07
Stearic	8	7.99	7	3.85	10.17	–0.85	–0.82	2.68	NW0250663	29.41	12.45	NW0250095	30.82
Oleic	9B	3.01	2	5.13	10.67	–1.65	0.04	0.00	NW0250803	15.40	5.27	NW0249789	19.15
Oleic	2	36.59	6	6.43	17.86	–1.69	–2.06	23.89	NW0248967	3.77	44.59	NW0250242	6.44
Oleic	4	17.57	8	6.46	13.48	1.78	–0.63	12.30	NW0249450	17.39	35.37	NW0249252	14.33
Linoleic	2	36.59	6	5.88	21.46	1.59	2.09	13.89	NW0248967	3.77	43.59	NW0250242	6.44

[z]Linkage group in Sandlin et al. (2012).
[y]Chromosome and position in the draft watermelon genome sequence (Guo et al., 2013; Ren et al., 2012).
[x]Log_{10} likelihood ratio.
[w]Phenotypic variation explained.
[v]Negative values indicate that the effect is contributed by the allele from the egusi parent (PI 560023).
[u]Marker sequence information available in Sandlin et al. (2012).

The gene (*Cla009335*) homolog (E-value: 1E-26) to ACP in *Fragaria vesca* (Supplemental Table 1) was found within the flanking markers of the QTL for oleic acid and linoleic acid on Chr 6. ACPs are acidic proteins involved in de novo fatty acid synthesis, acyl chain modification, and chain-length termination during fatty acid biosynthesis (Li et al., 2010).

A gene (*Cla010780*), homolog (E-value: 6E-46) to the SADP gene in *Picea glauca,* and *Cla013862,* a homolog (E-value: 3E-173) to the SADP gene in *R. communis,* were located within the flanking markers of QTL for stearic acid on Chr 7 and oleic acid on Chr 8, respectively. The SADP gene catalyzes the conversion of stearic acid to oleic acid (Barker et al., 2007; Byfield et al., 2006) and may explain why the gene is associated with both the QTL for stearic acid and oleic acid in watermelon seed. In soybean, silencing of SADP resulted in a 3.6-fold reduction in oleic acid levels and a 6-fold increase in stearic acid levels when compared with the wild type (Kachroo et al., 2008), whereas in *Thunbergia alata,* overexpression of the gene led to accumulation of oleic acid (Cahoon et al., 1994). Physiologically, alteration in the function of SADP in plants results in changes in membrane integrity and fluidity, which in turn affect a plant's ability to acclimatize to cold or activate the salicylic-dependent pathogen defense pathway (Kachroo et al., 2008).

The genes identified in this study are possible candidates for the development of functional markers for application in MAS for improved oil quality in watermelon seed. The main QTL for palmitic acid on Chr 3 (R^2 = 24.7%) and that for oleic acid (R^2 = 17.9%) and linoleic acid (R^2 = 21.5%) on Chr 6 present possible targets in MAS for manipulating the levels of saturated and unsaturated fatty acids in watermelon seed. In the major oil crops, the key genes in the fatty acid biosynthesis pathway have been cloned and well characterized (Barkley et al., 2013; Byfield et al., 2006; Cardinal et al., 2007). Availability of these genomic tools in these crops has led to the development of functional markers and identification of markers tightly linked to key fatty acid biosynthesis genes. Allele specific primers for the FAD2 gene have been developed for canola (Hu et al., 2006), spring turnip rape (Tanhuanpaa et al., 1998), sunflower (Hongtrakul et al., 1998; Perez-Vich et al., 2002), soybean (Pham et al., 2010), and peanut (Barkley et al., 2010, 2013; Chu et al., 2009). Similarly, candidate genes for SADP (Pham et al., 2010; Zhang et al., 2008) and FATB (Cardinal et al., 2007; Fehr, 2007) have been tagged with molecular markers in soybean.

In breeding for oil quality in watermelon seed, targeting genes encoding FATB, SADP, and FAD2 would be logical because these enzymes are central in determining the fatty acid composition in oil crops. Given the variation in fatty acid composition reported for watermelon genebank accessions by Jarret and Levy (2012) [linoleic acid (45.37% to 73%), oleic acid (7.89% to 33.95%), stearic acid (5.03% to 13.84%), palmitic acid (9.68% to 14.38%)], conventional breeding can be used to develop watermelon cultivars of superior oil quality by selection of appropriate combinations of the naturally occurring alleles in the gene pool.

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Supplemental Table 1. Chromosomal (Chr) placement of genes encoding enzymes involved in fatty acid biosynthesis in watermelon and their homology (E-value) to corresponding genes in other plant species (Guo et al., 2013; International Cucurbits Genomics Initiative, 2012).

				Homology[x]		
Unigene[z]	Chr[y]	Symbol	Enzyme	Accession no.	E-value	Species
Cla015916	2	FAE	3-ketoacyl-CoA synthase	NP_179223	5E-57	*Arabidopsis thaliana*
Cla013264	2	FAE	3-ketoacyl-CoA synthase	XP_002313455	2E-200	*Populus trichocarpa*
Cla020107	2	FAE	3-ketoacyl-CoA synthase	XP_002315256	1E-155	*P. trichocarpa*
Cla020103	2	FAE	3-ketoacyl-CoA synthase	XP_002327205	5E-167	*P. trichocarpa*
Cla008263	3	FAE	3-ketoacyl-CoA synthase	XP_002313455	2E-195	*P. trichocarpa*
Cla007470	5	FAE	3-ketoacyl-CoA synthase	XP_002309451	4E-168	*P. trichocarpa*
Cla020395	5	FAE	3-ketoacyl-CoA synthase	ABA01490	9E-267	*Gossypium hirsutum*
Cla021116	5	FAE	3-ketoacyl-CoA synthase	ABX10440	1E-210	*G. hirsutum*
Cla021577	5	FAE	3-ketoacyl-CoA synthase	XP_002312562	5E-226	*P. trichocarpa*
Cla006236	5	FAE	3-ketoacyl-CoA synthase	BT004205	1E-118	*A. thaliana*
Cla006235	5	FAE	3-ketoacyl-CoA synthase	BT004205	4E-123	*A. thaliana*
Cla012291	6	FAE	3-ketoacyl-CoA synthase	ACY78677	3E-257	*Pistacia chinensis*
Cla002289	7	FAE	3-ketoacyl-CoA synthase	XP_002309451	2E-82	*P. trichocarpa*
Cla022495	8	FAE	3-ketoacyl-CoA synthase	ACQ41892	3E-256	*Camellia oleifera*
Cla017158	10	FAE	3-ketoacyl-CoA synthase	XP_002298171	2E-173	*P. trichocarpa*
Cla012095	4	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	Q9X248	1E-18	*Thermotoga maritima*
Cla022705	8	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	P28643	3E-116	*Cuphea lanceolata*
Cla003677	8	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	XM_003608029	7E-07	*Medicago truncatula*
Cla017175	10	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	Q93X67	1E-13	*Brassica napus*
Cla017174	10	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	P51831	4E-15	*Bacillus subtilis*
Cla016982	10	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	Q9X248	1E-18	*T. maritima*
Cla001918	11	fabG	3-oxoacyl-(Acyl-carrier-protein) reductase	AF324985	1E-12	*A. thaliana*
Cla013616	2	ACCase	Acetyl-CoA carboxylase	ACN85391	3E-55	*Suaeda salsa*
Cla002148	3	ACCase	Acetyl-CoA carboxylase	AAA75528	0.0	*Glycine max*
Cla001454	4	ACCase	Acetyl-CoA carboxylase	AY142630	5E-88	*A. thaliana*
Cla001455	4	ACCase	Acetyl-CoA carboxylase	ACR61637	9E-163	*Jatropha curcas*
Cla018129	4	ACCase	Acetyl-CoA carboxylase	FJ441419	3E-246	*A. thaliana*
Cla021246	5	ACCase	Acetyl-CoA carboxylase	FJ441419	4E-145	*A. thaliana*
Cla021245	5	ACCase	Acetyl-CoA carboxylase	FJ441419	3E-26	*A. thaliana*
Cla009059	1	ACP	Acyl carrier protein	P53665	2E-16	*A. thaliana*
Cla019842	2	ACP	Acyl carrier protein	CAA04768	4E-31	*Fragaria vesca*
Cla019842	2	ACP	Acyl carrier protein	CAA04768	4E-31	*F. vesca*
Cla019842	2	ACP	Acyl carrier protein	CAA04768	4E-31	*F. vesca*
Cla002179	3	ACP	Acyl carrier protein	ABP38063	6E-46	*J. curcas*
Cla002179	3	ACP	Acyl carrier protein	ABP38063	6E-46	*J. curcas*
Cla021373	5	ACP	Acyl carrier protein	ACJ07135	2E-39	*Arachis hypogaea*
Cla009335	6	ACP	Acyl carrier protein	CAA04768	1E-26	*F. vesca*
Cla019314	6	ACP	Acyl carrier protein	P93092	5E-29	*Casuarina glauca*
Cla015524	9	ACP	Acyl carrier protein	P93092	1E-19	*C. glauca*
Cla012205	6	DESA1	Acyl-[ACP] desaturase	XP_002517641	3E-61	*Ricinus communis*
Cla010780	7	DESA1	Acyl-[ACP] desaturase	AAM12238	6E-46	*Picea glauca*
Cla012720	7	DESA1	Acyl-[ACP] desaturase	ADC80920	2E-157	*Vernicia fordii*
Cla012721	7	DESA1	Acyl-[ACP] desaturase	D3YLK2	9E-61	*V. fordii*
Cla012722	7	DESA1	Acyl-[ACP] desaturase	ADC80920	2E-50	*V. fordii*
Cla022693	8	DESA1	Acyl-[ACP] desaturase	XP_002531889	1E-189	*R. communis*
Cla013862	8	DESA1	Acyl-[ACP] desaturase	XP_002526163	3E-173	*R. communis*
Cla022692	8	DESA1	Acyl-[ACP] desaturase	XP_002531889	6e-172	*R. communis*
Cla022693	8	DESA1	Acyl-[ACP] desaturase	XP_002531889	1E-189	*R. communis*
Cla014825	9	DESA1	Acyl-[ACP] desaturase	ADC80920	1E-59	*V. fordii*
Cla000627	11	DESA1	Acyl-[ACP] desaturase	ACG59946	6E-193	*R. communis*
Cla016938	11	DESA1	Acyl-[ACP] desaturase	ADC80920	3E-14	*V. fordii*
Cla013504	2	fabF	Beta 3-keto-acyl-ACP synthase II	NP_178533	9E-179	*A. thaliana*
Cla011136	3	fabF	Beta 3-keto-acyl-ACP synthase II	ADK23940	8E-207	*G. hirsutum*
Cla019601	3	fabF	Beta 3-keto-acyl-ACP synthase II	ACJ07141	2E-212	*A. hypogaea*
Cla011561	1	fabH	Beta 3-keto-acyl-ACP synthase III	XP_002529789	4E-172	*R. communis*
Cla007607	6	CPA-FAS	Cyclopropane fatty acid synthase	ABG37642	2E-141	*P. trichocarpa*

Continued next page

Supplemental Table 1. Continued.

Unigene[z]	Chr[y]	Symbol	Enzyme	Homology[x]		
				Accession no.	E-value	Species
Cla007608	6	CPA-FAS	Cyclopropane fatty acid synthase	AAT74602	4E-20	*G. hirsutum*
Cla007609	6	CPA-FAS	Cyclopropane fatty acid synthase	NM_113256	0.0	*A. thaliana*
Cla007473	7	CPA-FAS	Cyclopropane fatty acid synthase	Q3L7F1	2E-57	*G. hirsutum*
Cla001981	9	CPA-FAS	Cyclopropane fatty acid synthase	AAT74602	3E-16	*G. hirsutum*
Cla001982	9	CPA-FAS	Cyclopropane fatty acid synthase	ABG37642	6E-12	*P. trichocarpa*
Cla003203	2	fabI	Enoyl-[ACP] reductase	ABB83365	3E-89	*Malus domestica*
Cla004792	3	fabI	Enoyl-[ACP] reductase	ABB83365	1E-92	*M. domestica*
Cla014934	9	FATA	Fatty acyl-ACP thioesterase A	AAB51523	2E-144	*Garcinia mangostana*
Cla003904	1	FATB	Fatty acyl-ACP thioesterase A	XP_002526311	5E-150	*R. communis*
Cla002633	3	FATB	Fatty acyl-ACP thioesterase A	XP_002511148	4E-61	*R. communis*
Cla012316	6	FATB	Fatty acyl-ACP thioesterase A	XP_002515564	1E-183	*R. communis*
Cla015415	9	FATB	Fatty acyl-ACP thioesterase A	ABI20760	6E-21	*G. max*
Cla016747	11	FATB	Fatty acyl-ACP thioesterase A	ABC47311	6E-171	*Populus tomentosa*
Cla014487	7	fabZ	Hydroxymyristoyl ACP dehydratase	BT098415	1E-22	*G. max*
Cla008157	3	FAD3	Omega 3 fatty acid desaturase	AAN17504	1E-136	*Betula pendula*
Cla006639	6	FAD3	Omega 3 fatty acid desaturase	XP_002511936	5E-218	*R. communis*
Cla004356	7	FAD3	Omega 3 fatty acid desaturase	ACE80931	1E-250	*Cucumis sativus*
Cla003201	2	FAD2	Omega-6 fatty acid desaturase	AAO37752	5E-194	*Trichosanthes kirilowii*
Cla003200	2	FAD2	Omega-6 fatty acid desaturase	AAT72296	8E-146	*Nicotiana tabacum*
Cla018729	6	FAD2	Omega-6 fatty acid desaturase	ABU96742	6E-206	*J. curcas*
Cla017539	10	FAD2	Omega-6 fatty acid desaturase	AAS19533	4E-213	*Cucurbita pepo*
Cla003659	1	PPT	Palmitoyl-protein thioesterase	AK317387	1E-82	*A. thaliana*
Cla003660	1	PPT	Palmitoyl-protein thioesterase	ACG35638	7E-115	*Zea mays*
Cla005617	10	PPT	Palmitoyl-protein thioesterase	BT020483	6E-81	*A. thaliana*

[z]Unigene assigned in the draft watermelon genome (Guo et al., 2013; Ren et al., 2012).
[y]Chromosome in the draft watermelon genome sequence (Guo et al., 2013; Ren et al., 2012).
[x]Homology to sequences in Genbank, Swiss-Prot, TrEMBL, and TAIR (International Cucurbits Genomics Initiative, 2012).