Lycopene and Total Carotenoid Content as Descriptors for *Citrullus lanatus*: Limitations and Preliminary Trials

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Germplasm collections are valuable sources of genetic material for crop improvement. The watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) germplasm collection in Griffin, GA (5) is a good example of a collection that is contributing to the production of improved cultivars. This collection contains approximately 1600 genotypes of the cultivated taxa (*C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides*) and is routinely utilized by researchers for the identification of valuable traits such as disease resistance, improved yield, unique flesh characteristics, health-promoting compounds, etc. In order to make the *Citrullus* germplasm collection more user-friendly and valuable to the scientific community genotypes should be characterized to the fullest extent possible. Currently, about 15 characteristics (descriptors) such as flesh color, fruit weight and shape, rind thickness, brix, etc., are used to describe watermelon accessions in the Griffin genebank. However, as new uses are found and trends in watermelon consumption change, it is useful to add or modify descriptors. Two descriptors currently being evaluated are lycopene and total carotenoid content in fruit flesh.

Red fleshed watermelons contain high quantities of lycopene, a carotenoid that imparts the red color. This compound has powerful antioxidant properties and has been shown to lower the risk of myocardial infarction (6) and some cancers. Few red fruits and vegetables contain detectable quantities of lycopene and the USDA-NCC Carotenoid Database officially considers watermelon, on average, to contain higher levels of lycopene than other fresh fruits and vegetables (4). Because of the potential health benefits of lycopene, there is interest in increasing its content in commercial cultivars.

Red and pink fleshed watermelon also contain carotenoids related to lycopene, such as β-carotene and pro-lycopene. The quantities of non-lycopene carotenoids in watermelon have been reported as high as 40% (8) but is more typically between 10-20% of the total carotenoids present in commercial varieties (Perkins-Veazie, unpublished results). Orange watermelons contain predominately pro-lycopene but the other carotenoids present have been reported to comprise as much as 50% of the total carotenoid content (9). Watermelon with white and yellow flesh contain, as of yet, unidentified carotenoids.

The germplasm descriptor list for *Citrullus* does not currently contain a descriptor for fruit pigment content. Addition of descriptors that report fruit lycopene and total carotenoid content will make this information available to the industry in an easy to search format. The following potential descriptors were evaluated: maximum lycopene content, range of lycopene content in red fruit, mean lycopene content of red fruit, percent of red fruit, maximum total carotenoid content, range of total carotenoid content in pigmented fruit, mean total carotenoid content of pigmented fruit, and percent of pigmented fruit. Plant Introduction (PI) lines were evaluated in 2000-2003. One to thirteen pigmented fruits from each of eight PI lines was collected for lycopene and six for total carotenoid analysis.

Results: Lycopene content in fruit flesh ranged from 10 to 81 $\mu g \cdot g^{-1}$ fresh weight (FW). The largest range of lycopene content within a line was 57 $\mu g \cdot g^{-1}$ (PI 385964). PI 288232 had the highest lycopene content detected (81 $\mu g \cdot g^{-1}$) in one fruit and PI 319212 had the highest overall lycopene content with a mean of 64 $\mu g \cdot g^{-1}$ (Table 1). Among the yellow and orange watermelon the total carotenoid content ranged from 3-13 $\mu g \cdot g^{-1}$. The largest range of total carotenoid content within the eight lines was 3-13 $\mu g \cdot g^{-1}$ (PI 629111). This PI line also had the highest total carotenoid content detected (13 $\mu g \cdot g^{-1}$) in one fruit and PI 629111 and NSL 29605 had the highest overall total carotenoid content 8 $\mu g \cdot g^{-1}$ (Table 2).

Using this data, we developed potential descriptors and descriptor codes for the *Citrullus lanatus* PI collection. The four descriptors and their codes are listed in Tables 3 and 4.

Discussion: These preliminary experiments tested the feasibility of using lycopene and total carotenoid content as descriptors for *Citrullus lanatus* PI lines and were used to formulate descriptor codes for documenting various carotenoid contents. Although the proposed descriptors are less informative than the actual data values (Table 5 vs. 1 and 2), the codes are consistent with the GRIN database and are in a format that is easy to use with a search engine. Since PI lines are often heterogeneous in their expression and accumulation of carotenoids, just reporting the average carotenoid content would not be very meaningful. Therefore, we suggest reporting the carotenoid content in eight ways: maximum lycopene content, range of lycopene content in pigmented fruit, mean lycopene content of pigmented fruit, percent of red pigmented fruit, maximum total carotenoid content, range of total carotenoid content in pigmented fruit, mean total carotenoid content of pigmented fruit, and percent of pigmented fruit. These eight descriptors supply information on the number of fruit that are pigmented, how many of the pigmented fruit are red versus yellow or orange, amount of lycopene and or total carotenoids in the fruit, and the range of these compounds present in the PI lines. Non-pigmented fruit are not included because they are typically low in carotenoids (unpublished data) and thus would skew the data making it less informative. In this report, total carotenoid content is documented for yellow and orange fruit only, but it is informative to list this trait for red watermelon as well. Since carotenoid profiles are not well characterized for yellow and orange watermelon total carotenoid content is suggested as a descriptor and not descriptors for individual compounds, such as pro-lycopene.

Obviously, analyzing more fruit will provide more accurate descriptor data. This is especially true since the production environment can affect lycopene levels by 10-20% (7) and PI lines are often grown in the greenhouse which can reduce carotenoid accumulation. For optimal results we recommend testing at least 10 plants from homogeneous lines and more from lines that demonstrate heterogeneity for fruit color. We are currently analyzing heterogeneous lines to determine an optimal number of fruit for reporting on these descriptors. Only reporting on ripe fruit is imperative, since under-ripe fruit can have 40% less lycopene than ripe fruit of the same variety (Perkins-Veazie, unpublished data).

Methods: *Watermelon Fruit*: *Citrullus lanatus* PI lines from the USDA's *Citrullus lanatus* germplasm collection in Griffin, GA were transplanted in a 0.5 ha field of Bernow fine sandy loam soil at the South Central Agricultural Research Laboratory in Lane, OK. Seedlings were transplanted in April at the three-leaf stage, on black plastic mulch with drip tape. Plants were 0.9 m apart in a single row 9.1 m in length, 3.6 m between reps, and with a 3.1 m alley separating rows. The cultural methods were performed according to Oklahoma State University Extension guidelines (Bulletin No. F-6236). PI 270546 was grown in the greenhouse in winter 2001 until fruit was ripe.

Watermelons were harvested when external appearance suggested ripeness. Ripeness was determined by total soluble solid content, texture, and seed development. Heart tissue from ripe, pigmented watermelons were collected and stored in –80^{o}C until they were processed.

Sample Preparation: All steps from the time watermelons were cut lengthwise were performed in subdued lighting at room temperature. The excised tissue was cut into approximately 2.6 mm^3 cubes or smaller. Samples were pureed either fresh or after storage at -80^{o}C. Tissue (25-500 g) was homogenized in a Waring blender until chunks were less then 4 mm^3 then pureed using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, N.Y.) with a 20 mm O.D. blade to produce a uniform slurry with particles smaller then 2 mm^3. The samples were not allowed to heat or froth.

Lycopene Detection Method: This method was performed as in Fish et al.(3). Samples were kept on ice unless otherwise stated. Briefly, approximately 0.6 g (determined to the nearest 0.01 g) duplicate samples were weighed from each puree into two 40 ml amber screw-top vials (Fisher, #03-391-8F) that contained 5 ml of 0.05% (w/v) BHT in acetone, 5 ml of 95% ethanol, and 10 ml of hexane. Purees were stirred on a magnetic stirring plate during sampling. To extract lycopene from the samples, they were placed on ice on an orbital shaker at 180 rpm for 15 min. Then, 3 ml of deionized water were added to each vial and the samples were shaken for an additional 5 minutes on ice. The vials were then left at room temperature for 5 min. to allow for phase separation. Absorbance of the upper, hexane layer was measured in a 1 cm path length quartz cuvette at 503 nm after blanking with hexane. The lycopene content of watermelon was then estimated using the absorbance at 503 nm and the sample weight (3, 1).

Total Carotenoid Detection Method: Frozen samples were weighed and homogenized with enough acetone to cover the sample then transferred to a filter paper lined funnel and washed with acetone until all the color was removed. Then 50 ml of hexane was added to the acetone/carotenoid mixture. Water was added to facilitate separation of the layers. The hexane layer was removed, transferred to a 1 cm quartz cuvette and read at 485 nm to estimate total carotenoids (2).

Acknowledgments: Special thanks are given to Dr. Julie Collins, Dr. Penelope Perkins-Veazie, and Dr. Wenhua Lu for their thorough reviews. Thanks to Anthony Dillard, Amy Helms, and Ashly Gammon for technical assistance. This work was partially funded by the Cucurbit Crop Germplasm Committee.

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Table 1: Lycopene content analysis for 8 PI lines.

PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Lycopene Content µg/g[y]	Lycopene Content Range µg/g[x]	Mean Lycopene Content µg/g[x]	% Plants with Pigmented Fruit[w]
270546	1	35	NA	NA	13
288232	4	81	33-81	56	100
319212	2	76	52-76	64	55
385964	13	70	13-70	44	57
525085	2	62	59-62	61	75
525096	2	44	27-44	36	78
560000	2	70	40-70	55	8
482291	5	32	10-32	23	95

[z]Each fruit was harvested from a different plant.
[y]Maximum lycopene content among the fruit analyzed within this PI line.
[x]Range of lycopene content within this PI line.
[w]At least 10 plants were analyzed for this data.

Table 2: Total Carotenoid content analysis for 6 yellow or orange PI lines.

PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Total Carotenoid Content µg/g[y]	Total Carotenoid Content Range µg/g[x]	Mean Total Carotenoid Content µg/g[x]	% Plants with Pigmented Fruit[w]
219887	3	11	5-11	7	100
229749	1	7	NA	NA	NA
601228	3	6	4-6	4	100
629111	2	13	3-13	8	100
NSL29605	3	9	7-9	8	100
NSL68237	3	12	5-12	10	100

[z]Each fruit was harvested from a different plant.
[y]Maximum carotenoid content among the fruit analyzed within this PI line.
[x]Range of carotenoid content within this PI line.
[w]At least 2 plants were analyzed for this data if reported.

Table 3: List of lycopene descriptors and their codes and definitions for red watermelon fruit.

Codes	Maximum Lycopene Content	Mean Lycopene Content of Red Fruit	Range of Lycopene Content in Red Fruit	Percent of Red Fruit
	Definitions			
1	≤10 μg/g	≤10 μg/g	≥125 μg/g	0 %
2	11-25 μg/g	11-25 μg/g	100-125 μg/g	1-10 %
3	25-50 μg/g	25-50 μg/g	75-100 μg/g	11-25 %
4	50-75 μg/g	50-75 μg/g	50-75 μg/g	26-50 %
5	75-100 μg/g	75-100 μg/g	25-50 μg/g	51-75 %
6	100-125 μg/g	100-125 μg/g	11-25 μg/g	76-90 %
7	≥125 μg/g	≥125 μg/g	≤10 μg/g	91-100 %

Table 4: List of total carotenoid descriptors and their codes and definitions for watermelon fruit.

Codes	Maximum Total Carotenoid Content	Mean Total Carotenoid Content of Pigmented Fruit	Range of Total Carotenoid Content in Pigmented Fruit	Percent of Pigmented Fruit
	Definitions			
1	≤10 μg/g	≤10 μg/g	≥125 μg/g	0 %
2	11-25 μg/g	11-25 μg/g	100-125 μg/g	1-10 %
3	25-50 μg/g	25-50 μg/g	75-100 μg/g	11-25 %
4	50-75 μg/g	50-75 μg/g	50-75 μg/g	26-50 %
5	75-100 μg/g	75-100 μg/g	25-50 μg/g	51-75 %
6	100-125 μg/g	100-125 μg/g	11-25 μg/g	76-90 %
7	≥125 μg/g	≥125 μg/g	≤10 μg/g	91-100 %

Table 5: Data from table 1 and 2 listed in descriptor format.

PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Lycopene Content	Range of Lycopene in Red Fruit	Mean Lycopene Content of Red Fruit	Percent of Red Fruit
270546	1	3	NA	NA	3
288232	4	5	5	4	7
319212	2	5	6	4	5
385964	13	4	4	3	5
525085	2	4	7	4	5
525096	2	3	6	3	6
560000	2	4	5	4	2
482291	5	3	6	2	7
PI Number	Number of Fruits/Plants Analyzed[z]	Maximum Total Carotenoid Content	Range of Total Carotenoid in Pigmented Fruit	Mean Total Carotenoid Content of Pigmented Fruit	Percent of Pigmented Fruit
219887	3	2	7	1	7
229749	1	1	NA	NA	NA
601228	3	1	7	1	7
629111	2	2	7	1	7
NSL29605	3	1	7	1	7
NSL68237	3	2	7	1	7

[z]Each fruit was harvested from a different plant.