

L-Citrulline Levels in Watermelon Cultivars From Three Locations

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Additional index words. *Citrullus lanatus*, phytonutrients, amino acids, genetics by environment, quality

Producers of fresh fruits and vegetables face increasing production costs and more intense international market competition. Maximizing marketability by offering high quality produce that is also highly nutritious provides new market niches for crops such as watermelons [*Citrullus lanatus* (Thunb.) Matsum. and Nakai], but germplasm will have to be identified that has enhanced levels of nutrients. Surprisingly, there is little information on how genes can affect the nutritional quality of most fruits and vegetables. This preliminary study was undertaken to determine the importance of genetics versus environment effects in watermelon L-citrulline content, an amino acid that may help regulate blood pressure. Our results suggest that L-citrulline content can vary within a cultivar (one cultivar demonstrated a 0.4 to 4.9 mg/ml fresh sample deviation) even when grown and tested at one location. The data did not indicate a strong varietal difference on the average amount of L-citrulline accumulated (2.4 to 3.4 mg/ml fresh sample); more lines need to be screened to determine if breeding for high L-citrulline germplasm is possible. Location did not appear to significantly increase within-cultivar variation (one cultivar demonstrated a 1 to 4.9 mg/ml fresh sample deviation over two locations), this implies that it may be possible to develop lines with constantly high L-citrulline content across divergent growing environments.

Watermelon is the number two fresh vegetable crop in the world in terms of area harvested and total production (FAOSTAT data, 2007) and has recently been showcased as a healthy food since it is high in the anti-

oxidant, lycopene. However, the full nutrient potential of this crop is not known. While watermelon is the leading fruit and vegetable source of lycopene, it also contains a variety of antioxidants and amino acids which likely have additional health-promoting activities. Scientific literature and current medical results indicate that fruits and vegetables contain a host of compounds that appear to work synergistically. Boileau *et al.* (2003) indicated that lycopene alone is not responsible for reducing prostate cancer associated with increased tomato intake. Since tomatoes contain the glycoalkaloid tomatine, a demonstrated anticarcinogen, as well as several well-known phenolic compounds such as quercetin, this finding is not entirely unexpected and demonstrates the importance of maintaining the nutritional value of fruits and vegetables.

Amino acids have well established individual roles in disease prevention. Arginine, an essential amino acid, functions as one of the twenty building blocks of proteins and in free form as a physiologic amino acid. L-citrulline (hereafter referred to as citrulline) is a physiologic amino acid endogenous to most living systems. These amino acids are directly involved in clearing excess metabolic ammonia from the body and are indirectly involved in cardiovascular function, immunostimulation, and protein metabolism (Curtis *et al.*, 2005). Ingested arginine is cleared by hepatic cells, but citrulline is not and can serve as an arginine source in other parts of the body.

Watermelon is rich in citrulline (Tedesco *et al.*, 1984) but varietal differences have not been adequately stud-

ied, and effects of growing conditions have not been investigated at all. In an earlier report, fourteen watermelon cultivars were found to contain from 0.42 to 1.83 mg · g⁻¹ fresh weight of citrulline, with an average content of 1.5 mg · g⁻¹ (Rimando and Perkins-Veazie, 2005). It has been shown that humans can effectively absorb citrulline from watermelon, which also increased plasma arginine levels (Collins *et al.*, 2007; Mandel *et al.* 2005). Recently, subjects consuming watermelon or synthetic citrulline as a drink, combined with exercise, had reduced arterial blood pressure, compared to a placebo (Figureoa *et al.*, 2010). The current study represents a preliminary screen to determine the potential of breeding high citrulline producing watermelons with stable phenotype across multiple environmental locations.

Materials and Methods

Plant material: Five watermelon lines were tested ('Cream of Saskatchewan', 'Red-N-Sweet', 'Tender Sweet Orange Flesh', 'Black Diamond', and 'Dixielee'), three of which (Cream of Saskatchewan, Red-N-Sweet, Tender Sweet Orange Flesh) were grown during the summer of 2008 at two locations in North Carolina, Kinston and Clinton. Black Diamond and Dixielee were grown in 2008 at Lane, OK.

Plants grown in OK were arranged in a randomized complete block design and fertility and care provided as outlined in Motes and Cuperus (1995). All NC samples were nonrandomized and had ten plants per cultivar at each of the two locations. Flesh samples representing each replicate (OK only) and each location were collected from ripe fruit only. Maturity was assessed by external and internal characteristics (i.e., waxyness, tendril death, brix, firmness, seed maturity). A digital refractometer was used to determine Brix. Samples were collected from heart tissue, pureed, and stored at -80°C.

Citrulline quantification: Citrulline was analyzed using a TLC plate methodology which is a slight modification of a Brenner and Niederwieser (1960) method. Citrulline was quantified on the basis of a citrulline standard (Sigma-Aldrich, St. Louis, MO). Briefly, 40 g samples were pureed using a Brinkmann Polytron Homogenizer (Brinkmann Instruments, Inc., Westbury, New York) with a 20 mm O.D. blade. One ml of the liquid puree was centrifuged at 15,800g for 10 min to remove debris. Supernatants were diluted to make a 10% and a 20% solution in deionized water. Ten ul of the diluents were loaded on a 20 x 20 cm silica gel matrix (200 um layer thickness, 5-17 um particle size) TLC plate (Sigma). The spots were air dried, then amino acids were resolved using a solvent (2:1:1 n-butanol: acetic acid: deionized

water). Plates were developed with 0.2% ninhydrin in ethanol by baking at 95°C for 5 to 10 min. Densitometric scans of the citrulline spots were visualized and calculated against standards using a Kodak Image station (model 440CF, Eastman Kodak, Rochester, NY). Data in both figures is given as mg/g fresh weight, since 1 ml of watermelon puree is very nearly and consistently 1 g.

Results and Discussion

Though amino acid analysis of protein hydrolysates is generally considered a routine procedure, where as quantification of physiologic citrulline is complicated by presence of glutamine. The method used here estimates citrulline and glutamine together. Since ripe watermelon contains from 3 to 10 times more citrulline than glutamine (Fish and Bruton, 2010), the amount of glutamine in the samples should not adversely affect the reliability of comparisons of citrulline levels among samples.

The five cultivars analyzed had average citrulline content of 2.4 to 3.4 mg/ml fresh sample. These values correlated well with a previous study (overall average 2.4 mg/g) of 14 varieties (Rimando and Perkins-Veazie, 2005). Two cultivars in our study, Cream of Saskatchewan and Tender Sweet Orange Flesh, were also tested in the Rimando and Perkins-Veazie study (2005). The earlier report showed slightly lower citrulline values than we determined (1.0 and 0.5 mg/g, compared to 3.1 and 2.6 mg/g). Ours data showed, on average, higher citrulline values. The difference between the two studies was likely due to the different methods used. This underlies the importance of maintaining one method for comparison between lines. However, we can not rule out that environmental conditions caused differences in the samples between the two studies.

There was no statistical difference between lines in our study. Thus, we were not able to determine if genotype affects average citrulline values. More varieties need to be tested to determine if breeding for high citrulline germplasm is possible.

Our results indicated that within cultivar differences are quite high in some cultivars (one cultivar demonstrated a 0.4 to 4.9 mg/ml fresh sample deviation) even when grown and tested from one location. It might be possible to breed for lines with more stable citrulline expression. Since location did not appear to significantly increase within-cultivar variation (one cultivar demonstrated a 1 to 4.9 mg/ml fresh sample deviation over two locations), it may be possible to breed lines with consistently high citrulline content across widely different growing environments.

Acknowledgements

The authors would like to thank Amy Helms and Cody Sheffield for technical help. This project was partially funded by the National Watermelon Promotion Board and the National Watermelon Association.

Disclaimer

The use of trade names in this publication does not imply endorsement by the USDA of the products named or criticism of similar ones not mentioned.

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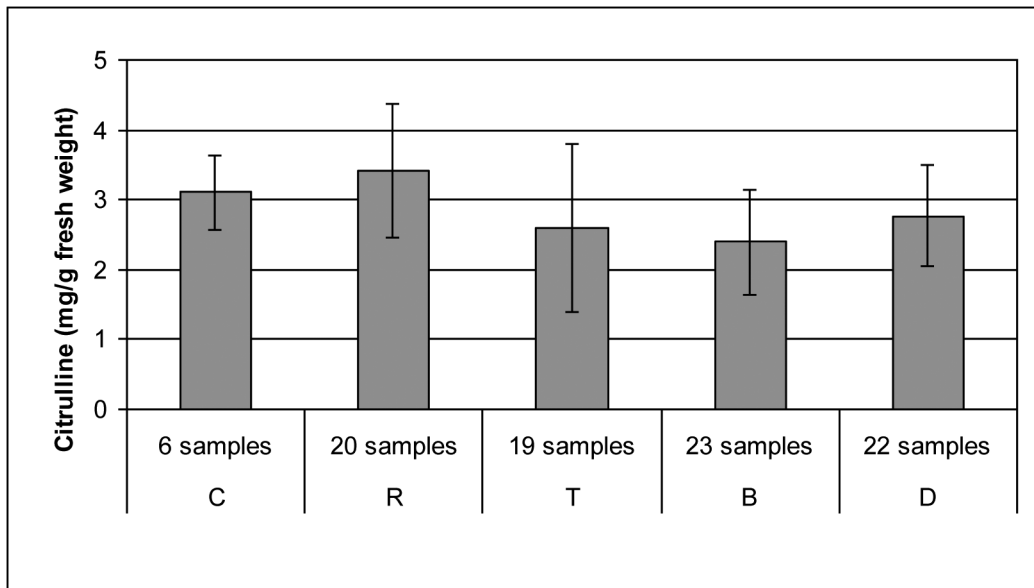


Figure 1. Comparison of citrulline quantities and deviation in five cultivars. The watermelon lines are C, Cream of Saskatchewan; R, Red-N-Sweet; T, Tender Sweet Orange Flesh, B, Black Diamond, D, Dixielee. The number of each cultivar tested is listed. Standard deviation is shown with error bars.

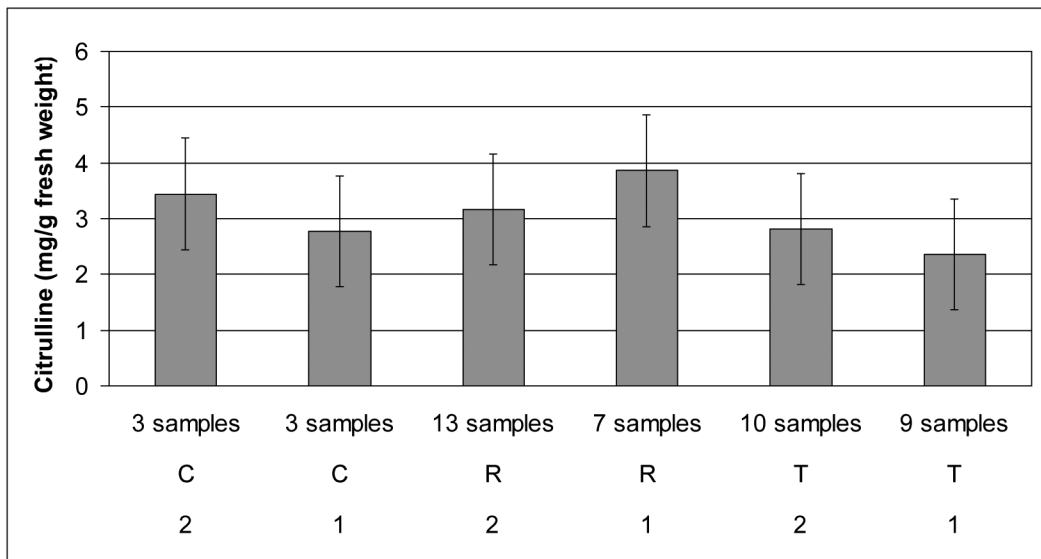


Figure 2. Effect of location on three watermelon lines. The number of each cultivar per location is listed. The watermelon lines are C, Cream of Saskatchewan; R, Red-N-Sweet; T, Tender Sweet Orange Flesh. Location is denoted by a 1, or a 2. Standard deviation is shown with error bars.