COMPARISON OF FREE CITRULLINE AND ARGININE IN WATERMELON SEEDS AND FLESH

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ABSTRACT

Citrulline, a non essential amino acid found in all cucurbits but in highest amounts in watermelon, helps promote vasodilation in humans by stimulating the nitric oxide system. Arginine is present in all plants and is a primary amino acid in the nitric oxide system of mammals. Citrulline has been reported in both flesh and rind of watermelon.

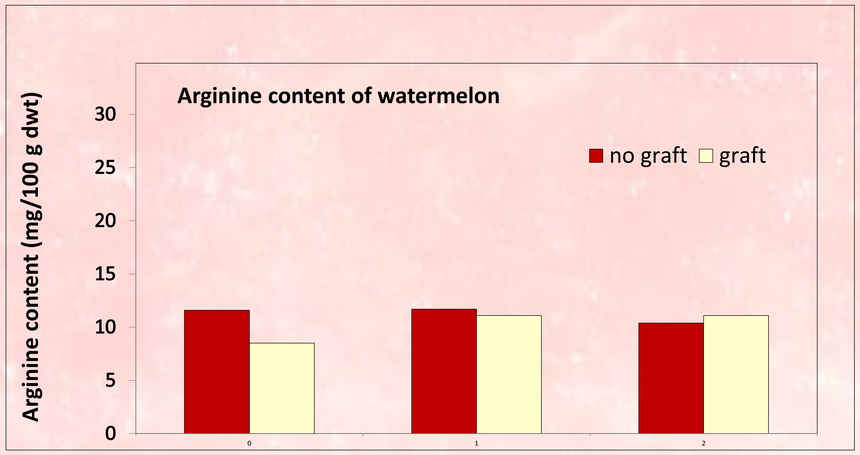
In this study, watermelon seeds (embryos) of various types and cultivars were analyzed for free citrulline and arginine and compared to amounts found in grafted and not grafted seedless watermelons. The citrulline content of watermelon flesh was much higher than that of seeds (about 2-2.5 vs 0.03 to 0.1 mg/g fresh weight), respectively. Arginine content was also higher (7-9 vs 0.3 to 1 mg/g fwt). The ratio of citrulline to arginine was very different in seeds (0.04 to 0.33) compared to flesh (2 to 4). The different ratios of citrulline and arginine between seeds and placental tissue most likely reflect the very different roles these tissues play in the reproduction system of watermelon. Citrulline can act as a storage mechanism for nitrogen while arginine is a nitrogen storage form in seeds.

INTRODUCTION

Citrulline is a little studied amino acid as it is considered non essential, and until recently, has not had a well defined role in human health or in plants. Named for watermelon (*Citrullus lanatus*), the cucurbit where it was first discovered and is found in highest quantities, citrulline was found to have vasodilator properties and to be an excellent alternative to arginine for treatment of some cardiovascular diseases (2). In watermelon plants, citrulline is thought to be a means of sequestering nitrogen in times of drought.

The content of citrulline in the rind and flesh of fruits, as well as in fruit from grafted or non grafted plants, has been reported (3). Arginine, a precursor of citrulline, and an important component of the nitric oxide system in animals, is present in much smaller amounts than citrulline in watermelon fruit.

The aim of these experiments was to establish relative amounts of citrulline and arginine content in watermelon seed embryos relative to the content in grafted or non grafted seedless watermelon.



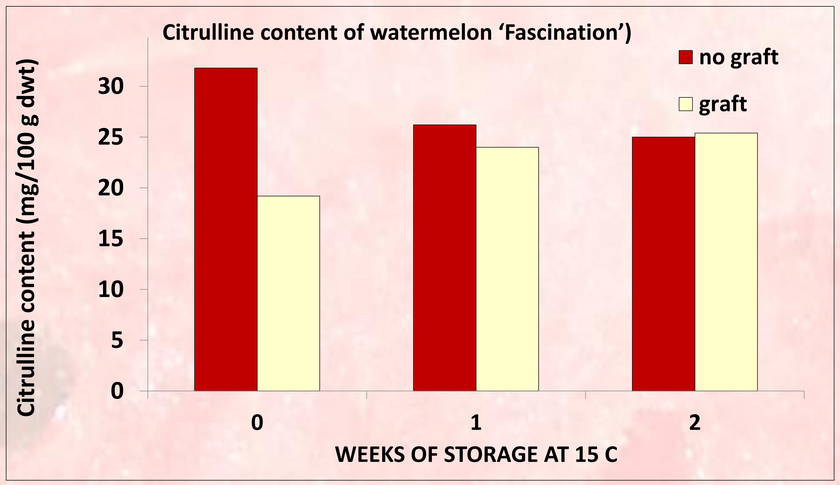


FIGURE 1. Citrulline and arginine content of watermelon flesh from grafted or not grafted plants

METHODS

Non-treated watermelon seeds of heirloom, hybrid seeded, or seedless types were purchased from seed companies. Twenty to 50 seeds coats were cut and embryos extracted (to make 2 g material) and frozen at -20 C. Grafted and not grafted seedless watermelons ('Fascination' with 'Carnivor' rootstock) were obtained from field plantings in SC. Heart (center) material (flesh) was cut from watermelons and immediately frozen at -20. All material was transferred to -80 C then freeze dried.

Freeze dried material was extracted with 0.03M phosphoric acid, centrifuged, and citrulline and arginine amino acids determined using a modified method of Jayaprakasha et al. (2011). A 5 µl filtered supernatant injected onto a Hitachi HPLC equipped with DAD (data collected at 207 nm) and a Gemini 3u C18 110A 250x4.6 mm column, mobile phase of 0.015M phosphoric acid at 0.5 ml/min at 25 C for 30 min.

A subset of samples were run using an amino analyzer to verify results. Free amino analysis was done using 0.5 ml supernatant diluted with 0.02N HCl to a final volume of 1 ml and analyzed using a Hitachi Model L-8900 Analyzer with 570 and 440 nm wavelengths. The analyzer was fitted with an analytical column (model 2622SC PF; 40-mm length, 6.0-mm ID), with a guard column. Separation of the amino acids was done by using a gradient of borate buffers and a temperature gradient of 30 to 70°C. Instrumental postcolumn derivatization was done with ninhydrin. An amino acid standard curves of 1 to 5 nM were prepared by making serial dilutions of an amino acid standard mixture containing 2.5 pmol of 18 individual amino acids in 0.02 N HCl.

RESULTS AND DISCUSSION

- Watermelon flesh was higher in citrulline content from not grafted plants at week 0. values became more similar with graft/no graft fruit with storage time. **FIGURE 1**
- Seed embryos for seedless watermelons were higher in ctirulline than embryos from seeds for seeded watermelons. **TABLE 1**
- The ratio of citrulline to arginine in watermelon flesh was 3:1 while in seeds, was 1:1 to 1:23.
- This difference in citrulline/arginine may reflect the use of different amino acids for nitrogen storage in the plant tissue vs seed tissue.

TABLE 1. Embryo content of citrulline and arginine in seeds for seedless or seeded watermelon.

Cultivar	Citrulline	Arginine	Ratio (C:A)
Heirloom seeded (diploid)	mg/100g fwt		
Charleston Gray	4.65	107.16	1:23
Carolina Cross	8.86	70.57	1:8
Georgia Rattlesnake	3.36	26.99	1:8
Hybrid seeded (diploid)			
Viking	4.50	46.2	1:10
Estrella	5.73	25.35	1:4
Lantha	3.67	17.45	1:4
Seedless (tetraploid)			
Citation	11.36	33.48	1:3
Fascination	64.35	88.41	1:1
Melody	13.54	45.52	1:3

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