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Biochemical and histological contributions to textural changes in watermelon fruit modulated by grafting

Georgios A. Soteriou [a], Anastasios S. Siomos [b], Dimitrios Gerasopoulos [b], Youssef Rouphael [c], Soteria Georgiadou [d], Marios C. Kyriacou [a,*]

[a] Postharvest Technology Laboratory, Agricultural Research Institute, Nicosia, Cyprus
[b] Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[c] Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy
[d] National Reference Laboratory for Animal Health, Histopathology Section, Veterinary Services, Nicosia, Cyprus

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ABSTRACT

Increased watermelon fruit flesh firmness is systematically incurred with grafting on *Cucurbita* hybrid rootstocks (heterografting). Possible differences in mesocarp cell wall constitution and histology between heterografted, homeografted (self-grafted) and non-grafted watermelon were examined, as well as their contributions to fruit texture. Firmness correlated positively ($r = 0.78$, $p < 0.001$) with cell density (cells mm^{-2}) which was higher in heterografts (5.83) than homeografts (4.64) and non-grafted controls (4.69). Mean cell size was smallest in heterografts and correlated negatively ($r = -0.75$, $p < 0.001$) with firmness. Cell wall material, particularly the water-insoluble pectin fractions associated with firmness, were highest in heterografts. No associations with firmness were found for cell wall neutral sugars and membrane permeability. Higher parenchymatic cell density with higher content of alcohol insoluble residue and more abundant water-insoluble pectin fractions underscore enhanced firmness in heterografts. Possible implication of osmolytes in rootstock-mediated cell pressure regulation warrants further investigation.

1. Introduction

Grafting watermelon [*Citrullus lanatus* (Thunb) Matsum & Nakai] cultivars onto disease resistant rootstocks has become a prerequisite for watermelon production, especially in areas where intensive cultivation is practiced and soil pathogen population has built up. Further to conferring resistance against soil borne pathogens, versatile interspecific hybrid rootstocks employed for heterografting watermelon may enhance root performance and boost crop yield by promoting scion growth through regulated hormone transfusion from the heterogeneous root (San Bautista et al., 2011). Superior yield performance of grafted plants has also been associated with enhanced nutrient uptake and improved water use efficiency (Rouphael, Cardarelli, Colla, & Rea, 2008). Resilient rootstocks have been found to support high watermelon yields under stressful abiotic conditions owing to traits such as increased tolerance to salinity, to adverse temperature regimes, to adverse soil pH, and heavy metal soil contamination (Davis et al., 2008).

Numerous reports of rootstock-mediated effects on watermelon crop performance and quality attributes are available, especially with reference to inter-specific (*Cucurbita maxima* × *C. moschata*) hybrid rootstocks. Provided the absence of incompatibility, grafting onto *Cucurbita* rootstocks may impart a positive effect on marketable yield, mean fruit weight and/ or mean number of fruits per plant, notwithstanding actual yield variation across seasons and edafoclimatic conditions (Huitron, Diaz, Dianez, & Camacho, 2007; Soteriou & Kyriacou, 2015). Moreover, commercially established types of interspecific hybrid rootstocks generally do not to seem to deteriorate key sensory quality traits of watermelon fruit,

* Corresponding author.
E-mail address: m.kyriacou@ari.gov.cy (M.C. Kyriacou).

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such as the soluble solids content (SSC) or soluble sugars (glucose, fructose, sucrose) content of the flesh (Proietti et al., 2008; Soteriou, Kyriacou, Siomos, & Gerasopoulos, 2014).

Besides sugar content, and pulp color determined by lipophilic carotenoid content, the textural characteristics of the fruit flesh constitute another important sensory quality trait of watermelon partly responsible for guiding consumer preferences in respect to this commodity. Despite evidence of wide genotypic variation influencing watermelon flesh firmness, heterografting confers nevertheless significant effects on firmness mediated by the type of rootstock employed (Bruton, Fish, Roberts, & Popham, 2009). Appraisals of inter-specific *Cucurbita* rootstocks in particular have demonstrated a consistent trend for increased fruit firmness when used for heterografting diploid and triploid watermelon scion cultivars (Bruton et al., 2009; Soteriou et al., 2014; Yamasaki, Yamashita, & Furuya, 1994). However, no previous studies have attempted to interpret the exact nature of the rootstock-mediated effect on watermelon fruit texture by unravelling potentially underlying biochemical and histological aspects of it. Such an effect could be associated with rootstock mediation in the differentiation of fruit cell morphology, cell turgor and cell wall structural properties (Harker, Redgwell, Hallett, Murray, & Carter, 1997). No studies are currently available that elucidate possible histological differences in the fruit mesocarp of grafted and non-grafted watermelon. Moreover, no model of primary cell wall structure currently explains all of the measured physical properties pertaining to fruit tissues, although certain cell wall components have been prominently associated with fruit firmness. Cellulose microfibrils embedded in a varied matrix of pectin and glycan polysaccharides constitute the main components structuring cell wall (Brummell, 2006). In certain fruit species, the evolution of fruit firmness during ripening is linked with events in cell wall metabolism which configure at full maturity what is termed "melting texture"; however, watermelon fruit is not a member of this group since its maturation profile is not accompanied by major cell wall modifications related to pectin solubilization and depolymerisation events (Redgwell et al., 1997). Consequently, watermelon textural differences resulting from grafting are not expected to originate from differential metabolism of cell wall components during fruit ripening.

One work hypothesis is whether qualitative differences in fruit cell wall properties exist between grafted and non-grafted plants which might contribute to firmness differentiation in mature watermelon fruit. This work hypothesis is underpinned by previous studies which report higher flesh firmness in the fruit of heterografted compared to non-grafted plants throughout ripening (Soteriou et al., 2014).

Accordingly, the overall objective of the current work has been to investigate possible associations between watermelon fruit flesh cell wall properties and fruit firmness differentiation in response to grafting onto *Cucurbita* interspecific rootstock. Moreover, watermelon homeografts (self-grafted plants) were introduced in the current study in an attempt to investigate whether recorded textural differences are solely rootstock-mediated or derive partly from the grafting procedure itself.

2. Materials and methods

2.1. Plant material

Transplants of watermelon [*Citrullus lanatus* (Thunb) Matsum & Nakai] cv. Pegasus were heterografted on inter-specific (*Cucurbita maxima* Duchesne × *C. moschata* Duchesne) hybrid rootstock 'N101' or homeografted (self-grafted). Grafts were made after the appearance of the first true leaf on seedlings using the approach grafting method. The root system of the scion was removed about 15 days after grafting. Non-grafted plants were used as the control. All plants were produced by Solomou Nurseries (Nicosia, Cyprus).

2.2. Experimental conditions

The field experiment was carried out on alkaline (pH 7.5) clay-loam soil at the Zygi Experimental Station (34° 44′ 00″ N; 33° 20′ 15″ E) of the Agricultural Research Institute of Cyprus between April and July. The climate of the area is typical Mediterranean with most precipitation occurring between November and March; mean day-time temperature ranged from 29 to 40 °C during April-July. Base application of 350 kg ha^{-1} of compound fertilizer 14N-9.6P-7.5K was incorporated into the soil prior to planting. Plant spacing was 1.3 m on the row and 3.0 m between rows, resulting in a density of approximately 2560 plants·ha^{-1}. Standard irrigation, fertilization and pest control practices were applied as described previously by Soteriou and Kyriacou (2015). At the onset of anthesis (fruit setting) all flowers were inspected daily and tagged to secure uniformity in fruit harvest maturity (Soteriou et al., 2014).

2.3. Fruit quality assessment

Fruits were weighed and equatorial and meridian diameters were determined at harvest and used to calculate the fruit shape index. Yield and number of fruits per hectare were also determined. Assessment of quality was performed on the day of harvest. Fruit rind was measured at two representative points on each cross-sectioned fruit using an electronic calliper. Flesh firmness was measured using a TA.XT plus Texture Analyser (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell and a multiple puncture probe, consisting of a set of eight 4 mm-diameter probes in a circular 42 mm-diameter arrangement, operated at 2 mm·s^{-1} test speed. Flesh firmness was recorded as maximum resistance force to penetration to a depth of 50 mm around the heart of each cross-sectioned fruit.

Flesh color was measured at two loci in the heart region of each fruit using an 8 mm-aperture Minolta CR-400 Chroma Meter (Minolta, Osaka, Japan). Measurements were performed in the CIELAB color space and from the recorded parameters L^*, a^* and b^*, the chroma (C^*) and hue angle (h°) were deduced (Soteriou et al., 2014). Sensory analysis for overall organoleptic acceptability was performed by a trained panel based on 1–5 hedonic scale (1 = dislike; 3 = neither like nor dislike; 5 = like).

The heart of each fruit was excised and homogenized under low speed to prevent foaming. Part of the homogenate was filtered through double cheesecloth and the soluble solids content (SSC) at 20 °C of the filtered juice was measured on a temperature-compensating digital refractometer (RFM870; Bellingham-Stanley Ltd, Kent, UK). Part of the homogenate was transferred to 50 ml falcon tubes, instantly dip-frozen in liquid nitrogen, and then stored at −80 °C for further phytochemical analyses. Analysis of non-structural carbohydrates (glucose, fructose and sucrose) in the juice was performed by liquid chromatography on an Agilent HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with 1200 Series quaternary pump and a 1260 Series refractive index detector commanded by ChemStation software. Separation was performed on a 4.6 × 250 mm carbohydrate column at 35 °C (Waters, Milford, MA, USA) using an acetonitrile:water (75:25) mobile phase at a flow rate of 1.4 ml·min^{-1}. Quantification was performed against fructose, glucose and sucrose external standard calibrating curves with a coefficient of determination (R^2) > 0.9999. Recovery trials performed under the same operating conditions were in all cases on the order of 100%.