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Horticultural evaluation of exotic watermelon germplasm as potential rootstocks

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ABSTRACT

Watermelon grafting is on the rise worldwide as an agrotechnology aimed at preventing soilborne-pest damage. Grafting watermelon on *Cucurbita* rootstocks may negatively affect fruit size, shape and quality. However, grafting watermelon on watermelon rootstocks can prevent these negative effects. Twenty-one exotic watermelon accessions were evaluated as potential sources for watermelon rootstock breeding programs. Most of the watermelon accessions tested in the field as rootstocks for the mini-watermelon 'Extazy' gave yields similar to the nongrafted and self-grafted 'Extazy'. Four accessions: WAN, PI 457916, PI 307750 and PI 307609, produced significantly lower yields. The accessions BDA, CON, MAL, PI 296341 and PI 307609 were selected for detailed evaluation due to their previously found tolerance to soilborne pathogens. No difference was found in the total fruit quality index between nongrafted 'Extazy' fruit and 'Extazy' fruit from plants grafted on the different watermelon accessions. Fruit weight from plants grafted on *Cucurbita* rootstocks was higher than that from plants grafted on 'Extazy' or on the other watermelon accessions. No bitter flavor and no cucurbitacin were present in 'Extazy' fruits of plants grafted on bitter fruit watermelon accessions. Thus the examined exotic watermelon accessions did not adversely affect fruit quality and can be used as a basic germplasm for watermelon rootstock breeding. The most promising accession is PI 296341.

1. Introduction

The grafting of cucurbits is becoming a well-developed practice with many horticultural advantages. The primary motivation for grafting vegetable plants is to prevent damage caused by soilborne pests and pathogens (Oda, 2002). In the last few decades, however, grafting of vegetable plants has also been found to enhance tolerance to abiotic stresses, efficiency of water and nutrient use, and fruit yield and quality (Edelstein et al., 2011; Lee and Oda, 2003; Nisini et al., 2002; Oda, 2002; Rivero et al., 2003; Romero et al., 1997; Shimada and Moritani, 1977).

In Japan, *Lagenaria siceraria* is the most frequently used rootstock for watermelon grafting, accounting for 64% of the total number of grafts. It is followed by *Cucurbita* species (26%), *Benincasa hispida* (7%), and watermelon varieties used mainly to overcome Fusarium wilt (1%) (Kawaide, 1985). In Israel, however, most of the watermelons are grafted onto *Cucurbita* rootstocks. *Lagenaria* is less resistant to soilborne pathogens and does not reach the high yields of the *Cucurbita* stocks (R. Cohen, unpublished data).

Grafting can affect the plant's performance negatively or positively. There is consensus among researchers that grafting watermelons on *Cucurbita* or *Lagenaria* tends to increase vigor, fruit size and yield (Alexopoulos et al., 2007; Cushman and Huan, 2008; Davis and Perkins-Veazie, 2005–2006). On the other hand, there is some disagreement in the literature as to the effects of grafting on watermelon fruit quality. Characteristics that are often affected by grafting are fruit appearance (size, shape, color, and defects and decay), firmness, texture, flavor (sugar, acids and aroma volatiles) and health-related compounds (desired compounds such as minerals, vitamins, and carotenoids) (Rouphael et al., 2010).

Negative effects such as a thicker rind and lower sugar content [total soluble solids (TSS)] have been found in fruits of watermelon grafted onto *Cucurbita* and *Lagenaria* rootstocks, compared to nongrafted plants (Alexopoulos et al., 2007; Cushman and Huan, 2008; Davis and Perkins-Veazie, 2005–2006). The lower sugar content in grafted watermelons could be a result of lower activity of sucrose-metabolism enzymes in the late stage of fruit development (Liu et al., 2004). Bruton et al. (2009) reported that grafting increases fruit firmness by up to 25%. Davis et al. (2008) demonstrated that grafting watermelon increases lycopene and total carotenoids by 20%. It should be noted that the decrease in fruit quality does not represent a general phenomenon but depends on the specific

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scion–rootstock interaction and growing conditions (Yetisir and Sari, 2003).

Negative effects on fruit quality can be dramatically increased when mini-watermelon is grafted onto vigorous *Cucurbita* rootstocks. Indeed, in preliminary work, we found that grafting some mini-watermelon cultivars onto *Cucurbita* rootstocks results in fruit enlargement, shape deformation, appearance of white fibers in the heart of the fruit and overall severe negative effects on fruit quality. Grafting watermelon on watermelon rootstocks can eliminate fruit-quality issues caused by *Cucurbita* rootstocks.

Resistance to soilborne pathogens is the first requirement for effective rootstocks. In another study, we screened exotic watermelon accessions for resistance to various pathogens (Tyutyunik et al., 2012). As a second step, the effects of selected resistant accessions on horticultural traits need to be evaluated to find germplasm suitable for watermelon rootstock breeding programs, and that was the aim of the present study. The long-term objective is grafting watermelon on watermelon rootstocks that are resistant to various soilborne diseases with an emphasis on mini-watermelon, to avoid the negative effects on fruit quality induced by *Cucurbita* rootstocks.

2. Materials and methods

2.1. Characterization of mini- and midi-watermelon fruit quality following grafting on Cucurbita rootstocks

The mini-watermelon cv. Extazy and the midi-watermelon cv. Leopard (Hazera Genetics, Israel) were grafted onto 'TZ-148' (*Cucurbita maxima* × *C. moschata*) rootstock (Tezier, France) and planted in fields with no history of soilborne disease at Newe Ya'ar Research Center. The same mini- and midi-watermelon cultivars were also planted as nongrafted plants. Each treatment consisted of 40 plants (10 plants per plot × 4 replicates). Eight randomly selected fruits from each plot were taken at harvest (fully mature) for quality measurements. Another eight fruits were evaluated after 6 and 14 days of storage at 20 °C, 70% relative humidity. The fruits were tested for the following quality parameters: rind thickness (mm); TSS (%Brix); flesh firmness (N) and weight (kg). The TSS content was tested by squeezing the juice of the fruit flesh onto a digital refractometer (Atago, Japan) and the results were expressed as %Brix. The firmness of the rind was tested with a penetrometer (8-mm diameter plunger) (Silverado, USA). Fruit-quality index was determined by a panel of three tasters (experts), based on flesh color, fibers, seeds, flesh status and taste. The index was rated on a scale of 1–5, with 1 being very bad and 5, excellent.

2.2. Plant material and growth conditions

The tested watermelon accessions included commercial cultivars which are not commonly grown in Israel and wild watermelon accessions (identified by their PI numbers). Hereafter we term these accessions "exotic watermelon accessions". Their names and origins are presented in Table 1.

Two field experiments were conducted to evaluate the horticultural performance of watermelons grafted onto the exotic accessions serving as rootstocks in northern Israel at the Newe Ya'ar Research Center in the spring–summer seasons of 2011 and 2012. Both experiments were planted in fields with no history of soilborne disease in the first week of April, on raised beds spaced 2 m from center to center. Each bed had one row, with 0.5-m spacing between plants. Standard cultural practices including drip fertigation were employed, as in local commercial cultivation. The climate is a Mediterranean type, and lacks summer rainfall. The mini-watermelon 'Extazy' was grafted (splice grafting) on the watermelon accessions, and nongrafted and self-grafted 'Extazy' were used as controls. Grafting was made at "Hishtil Nurseries" (Ashkelon, Israel) with over 90% success. Each plot consisted of ten plants, and watermelon cv. Crimson Sweet was used as the pollenizer, two plants per plot. The experiments were designed as randomized blocks with four replicates, and each subplot was 10 m^2. Standard cultural practices, including drip fertigation, were applied. Fruits were harvested when fully mature in July 2011 and 2012.

Table 1
Full names, abbreviations and origins of the evaluated watermelon accessions.

Accession name (abbreviation)	Country of origin
Black diamond (BDA)	USA
Congo (CON)	Congo
Cream of Saskatchewan (COS)[a]	Russia
Early moonbeam (EMO)	USA
Hungarian (HUN)	Hungary
King and queen (KAQ)	USA
Malali (MAL)	Israel
Nwau-858770 (NWAU)	China
Red-seeded citron (RSC)	Africa
Wanli (WAN)	Philippines
WIS	No data
PI 270549	Ghana
PI 273481	Ethiopia
PI 296341	Cape Province, South Africa
PI 307609	Nigeria
PI 307750	Philippines
PI 326515	Ghana
PI 441722	Brazil
PI 457916	Liberia
PI 459075	Botswana
PI 482260	Zimbabwe
Extazy – commercial mini	"HaZera Genetics", Israel, used as scion
Leopard – commercial midi	"HaZera Genetics", Israel, used as scion

[a] Imported to Canada from Russia by an immigrant.

2.3. Microscopic evaluation of fruit flesh from grafted and nongrafted plants

Fruits of mini-watermelon 'Extazy' grafted onto the *Cucurbita* rootstock 'TZ-148' and nongrafted watermelon were tested for differences in flesh cells (size and shape). The fruits were cut in the center and 0.5-cm samples were taken. The samples were fixed in FAA (formalin, acetic acid, alcohol) solution (50% ethanol, 10% formaldehyde, 5% acetic acid, v/v) for 24 h followed by a dehydration series in which they were consecutively dipped into the following ethanol:tert-butanol (Finkelman Chemicals Ltd., Israel) concentrations (%): 75:25, 55:45, 35:50, 20:50, 10:40. After drying, the samples were fixed in melted paraffin at 60 °C (Paraplast plus, Oxford Labware, USA). The samples were cut by microtome (Leica RM2245, Wetzlar, Germany), and colored with safranin and fast-green (Sigma–Aldrich, USA). Chitin, suberin, and lignin were colored red, and cytoplasm and cellulose were colored green. Other sections were colored with toluidine blue-O (TBO) (Sigma–Aldrich) which is used for differential coloring of biological cell walls. All photographs were taken under a light microscope (DMLB 100S, Germany).

2.4. Cucurbitacin analysis

Plant extraction for HPLC analysis was performed using fleshy tissue from the center of the fruit ground with liquid nitrogen to a fine powder. About 0.5 g weighed frozen tissue was extracted with hexane/water (5 ml/5 ml) and then with chloroform (5 ml). The chloroform solution was evaporated to dryness in a Savant (Holbrook, USA) SpeedVac apparatus and resuspended in 500 μl MeOH. Samples were then filtered through a 0.45-mm Acrodisc syringe