

Fruit Quality Characteristics of 'Galia' F₁ Hybrid (*Cucumis melo* reticulatus group) Muskmelon Developed from a Transgenic Male Parent

J.M. Mitchell, D.J. Cantliffe, H.J. Klee
and S.A. Sargent
University of Florida-IFAS
Horticultural Sciences Dept.
Gainesville, Florida, 32611
USA

P.J. Stoffella
University of Florida-IFAS
Horticultural Sciences Dept.
Indian River Research and Education Center
Ft. Pierce, Florida, 34945
USA

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Abstract

Previous work has resulted in transformed 'Galia' male parental lines with an antisense ACC oxidase (CMACO-1) gene that inhibits the last step in ethylene biosynthesis. From this work, two transgenic male parental lines were developed. The transformed male lines tested PCR positive for the transgene, but were unselected for delayed ripening. Transgenic 'Galia' F₁ hybrids (TGMH-AS-1 and TGMH-AS-2) were developed from crossing the two transformed male lines to a wild-type female parental line through traditional breeding methods. During spring and fall 2004, transgenic 'Galia', wild-type 'Galia' and 'Gal-52' were grown in a passive-ventilated greenhouse in Citra, Florida. In spring 2004, no significant differences were observed between transgenic and wild-type 'Galia' fruit harvested fresh or after five days storage at 20°C. 'Galia' is highly susceptible to powdery mildew (*Sphaerotheca fuliginea*), which was unable to be controlled in spring 2004. Consequently, powdery mildew created significant stress on the plants and potentially negated any positive effect of the transgene. In fall, powdery mildew was controlled by fungicides. Fruit from the transgenic lines remained on the vine an average of five days longer than the wild-type. At full-slip stage, there were no differences in quality parameters (weight, length, width, color, soluble solids content and firmness) between the wild-type and transgenic 'Galia' fruits. Thus, the superior fruit quality, common of the 'Galia' F₁ hybrid is not negatively affected by the transgene.

INTRODUCTION

One of the major problems in the fresh produce industry is the difficulty of delivering quality products to the consumer with optimum flavor at reasonable costs. This is associated with the highly perishable nature of many fruits (McLaughlin, 2004). The Galia melon (*Cucumis melo* L. reticulatus group) is a highly desirable product in the U.S. because of its flavor, sweetness, and aroma, but a short postharvest life limits its continued acceptance by shippers. For example, attempts have been made to produce 'Galia' melons commercially in Puerto Rico, but these failed, principally because of the short postharvest shelf life of the fruits (D.J. Cantliffe, pers. commun.).

'Galia' melon is an F₁ hybrid grown primarily in Israel, Morocco, Turkey, and Spain, being exported principally to Europe where it is in high demand (Rodriguez et al., 2002). 'Galia'-type has become a trade name for other look-alike melon cultivars. Unfortunately, although these 'Galia'-type cultivars are firm, they lack the flavor, aroma, and high sugar content of the original 'Galia' hybrid. The 'Galia' cultivar is especially adapted to intensive irrigation and fertilization where yields of up to 50 tons/hectare of high quality fruits (13-15% Brix) have been recorded (Karchi, 2000). Fruits are round, medium netted, skin is yellow, and flesh is green and thick. In order to achieve peak flavor and sweetness 'Galia' melon must be picked at the full-ripe stage (Karchi, 1979) which thus limits shelf-life (Shaw et al., 2001).

Two transgenic lines (TGM-AS-1 and TGM-AS-2) of the male parent of 'Galia' muskmelon have been developed (Nuñez-Palenius et al., 2001, 2003). Fruit which developed on these lines exhibited delayed ripening. The approach used a gene encoding

the enzyme ACC oxidase (CMACO-1), which is involved in the last step of ethylene synthesis. Antisense technology shuts-off the gene, reducing the level of ethylene synthesis. The initial lines from tissue culture produced fruits that ripened either very slowly or essentially not at all and produced less ethylene as compared to wild-type fruits (Nuñez-Palenius, 2005). Other postharvest quality characteristics of the transgenic fruits were not significantly different from the wild-type fruits, denoting that the transgenic melons were comparable to the wild-type melons, even with the newly inserted gene. From the two transgenic male parental lines, transgenic F₁ 'Galia' hybrids (TGH-AS-1 and TGH-AS-2) were developed from crossing the two transformed male lines to a wild-type female parental line through traditional breeding methods. These were preliminary crosses, made without any selection criteria such as the delayed ripening characteristic.

The objective of this experiment was to compare the transgenic F₁ 'Galia' hybrids (TGH-AS-1 and TGH-AS-2) to commercial wild-type 'Galia' and a 'Galia'-type F₁ cultivar ('Gal-52') for yield, fruit quality, and shipping ability under simulated commercial conditions.

MATERIALS AND METHODS

Two trials were conducted in spring and fall 2004 to compare production, yield and postharvest quality of the transgenic F₁ 'Galia' hybrids (TGH-AS-1 and TGH-AS-2) to commercial wild-type 'Galia' and 'Gal-52' ('Galia'-type cultivar) muskmelons.

Production Methods

For each experiment, transplants were grown from seed and sown in polystyrene trays (Speedling, Bushnell, FL, USA) with cell sizes of 3.8-cm Sq x 6.4-cm H and 128 cells per flat. The growing medium used was a professional fine grade mixture (Premier ProMix FPX, Quakertown, PA, USA). Seedlings were grown at the University of Florida, Gainesville, FL campus in a growth chamber (Controlled Env. Ltd., Winnipeg, Manitoba, Canada) at temperatures of 28°C (day) and 22°C (night) with 12-hour daily artificial lighting. When cotyledons were fully expanded, seedlings were fertilized once per week with a solution of 100 ppm each of 20N: 20P: 20K (Peters Professional All Purpose Plant Food, Spectrum Group, St. Louis, MO, USA). In spring 2004, seeds were sown on 9 Jan. and in fall, 2004 on 6 July, 2004.

A polymerase chain reaction (PCR) analysis was used to identify seedlings with the transgene. When seedlings had two fully expanded true leaves, a 1.5 cm sample was cut from the youngest leaf tissue of each seedling. DNA was extracted using a modified Doyle and Doyle (1987) procedure, named DNA microprep for tomato/CTAB. The PCR reaction was conducted in a DNA Thermal Cycler 480 (Applied Biosystems, Foster City, CA, USA). PCR analysis was completed on every putative transgenic 'Galia' seedling.

After transgenic seedlings were identified, all transplants were moved to the greenhouse at Citra, FL for production. In spring 2004 plants were transplanted on 21 Feb. and in fall 2004 on 18 Aug. Both trials were conducted in a passive-ventilated, high-roof, saw-tooth greenhouse (TOP greenhouses, Ltd., Barkan, Israel). Commercial greenhouse production techniques for producing 'Galia' melons hydroponically were used according to guidelines from Shaw et al. (2001).

Plant spacing was 30 cm between plants and 90 cm between rows. Plants were grown in 11 L nursery pots (Lerio Co., Kissimmee, FL, USA) with pine bark media and fertigated through a 1-inch polyhose and WPCJ pressure-compensating drip emitters (Netafim USA, Fresno, CA, USA). A programmable timer, Sterling 12 (Superior Controls, Co., Inc., Valencia, CA, USA) was used for irrigation. Scheduling was based on plant need to achieve 10-20% leachate volume. Nutrients were delivered with each irrigation event using a Dosatron (Dosatron Int., Clearwater, FL, USA) injection system.

Plants were grown vertically, pruned to a single stem and twisted around individual twine for support. Intense pruning began one week after planting. All laterals were removed up to the 8th node and each subsequent lateral was pruned at the second node after fruit set. Plants were pollinated by bumble bees (*Bombus impatiens*, Natupol,

Koppert Biological Systems, Inc., Romulus, MI, USA).

Preventive fungicides were used weekly for powdery mildew (*Sphaerotheca fuliginea*) and gummy stem blight (*Didymella bryoniae*). *Fusarium oxysporum* developed in the fall trial as a result of hurricane damage. The *Fusarium* was suppressed through application of the fungicide Thiophanate-methyl (Topsin, Cerexagri, Inc., Phila., PA, USA) at a rate of 1.68 g per 3.78 L three to four times per week. Powdery mildew (*Sphaerotheca fuliginea*) also developed, but was controlled by spraying applications of potassium bicarbonate (Milstop, BioWorks, Inc., Fairport, NY, USA) at a rate of 5 g per L every five days.

Insect pests were monitored with daily scouting. Several beneficial insects were released to help augment pest management. A parasitic wasp of the green peach aphid (*Myzus persicae* var. *persicae*), *Aphidius colemani* (Koppert Biological Systems, Inc., Romulus, MI, USA) was released. *Neoseiulus californicus* (Biotactics Inc., Perris, CA) predatory mites were used to control the two-spotted spider mite (*Tetranychus urticae*); and *Encarsia formosa* and *Erotmocerus eremicus* (ENERMIX, Koppert Biological Systems, Inc., Romulus, MI, USA) were released to control whitefly (*Trialeurodes vaporariorum*).

Postharvest Analyses

Fruits were harvested when the external ground color was golden-yellow and the fruits were at full-slip stage. After harvest, postharvest data on fruit number, weight, length and width were measured.

In spring 2004, fruits were divided into two groups, one tested on the day of harvest and the other stored for five days at 20°C and 85% relative humidity. A 2.5 cm slice was taken from the equatorial region of each fruit after the preliminary postharvest data (weight, length and width) were obtained. Once sliced, flesh color, flesh thickness, firmness and soluble solids content were measured. Internal color was measured by reflectance with the Chromameter (Minolta-CR-200, Japan) and a caliper (Digimatic Mycal, Mitutoyo, Japan) was used to measure flesh thickness from peel to cavity. A firmness reading was taken at two equidistant points on the equatorial region of each fruit slice using an Instron Universal Testing Instrument (Model 4411-C8009, Canton, MA, USA). The Instron was fitted with a 50-kg load cell and an 11-mm convex probe. Firmness data were expressed as the maximum force (Newton) attained during deformation. Soluble solids content was measured with a temperature-compensating, hand-held refractometer (Model 10430, Reichert Scientific Instrument, Buffalo, NY, USA) from two samples taken from the equatorial region of the fruit.

Stored melons were placed in a 20°C storage room after preliminary postharvest data were recorded. Respiration and ethylene evolution were measured daily for five consecutive days beginning 24 hours after harvest. To determine ethylene and respiration (CO₂), each melon was sealed in an airtight Tupperware container for one hour allowing ethylene and CO₂ to accumulate. Two samples were taken from the headspace using a hypodermic syringe through a rubber septa: 0.5-ml for CO₂ was injected into in a gas chromatograph (GC) (Gow-Mac Instruments, Series 580, Bridgewater, NJ, USA) equipped with a thermal conductivity detector, and a 1.0-ml sample for ethylene measured in a GC (Tracor 540, Tremeetrics Analytical Division, Austin, TX) equipped with a photoionization detector and an Alumina F1 column with a mesh size of 80/100. After determining ethylene and CO₂ on the 5th day, the same postharvest parameters were measured as described above.

In fall 2004, fruits were not stored due to low yields. All postharvest parameters (weight, length, width, color, flesh thickness, firmness, and soluble solids content) were measured on the day of harvest.

Data Analysis

Both experiments were conducted in a randomized complete block design (RCBD). The spring trial had three replications and the fall trial had four replications. Data were analyzed using the GLM procedure (SAS Institute, Version 9, Cary, NC, USA). All data presented were subject to Duncan's Multiple Range Test.

RESULTS

Spring 2004

Midway through the season a severe powdery mildew (*Sphaerotheca fuliginea*) infestation occurred throughout the greenhouse. Fungicides (Myclobutanyl (Nova, Dow AgroSciences, Canada), Azoxystrobin (Quadris, Zeneca Ag Products Inc., USA) and hydrogen dioxide (Oxidate, BioSafe Systems, Glastonbury, CT, USA)) were used on the crop to control the disease.

Each plant yielded two to four fruits from 5 May until 10 June. There was no variation observed in disease tolerance or plant hardiness among the treatments. There were no significant differences in yield or in quality measurements between any treatments for all melons harvested (Table 1).

For melons sliced on the day of harvest, soluble solids content (SSC) was significantly lower for the transgenic melons in the TGH-AS-1 line compared to the other treatments (Table 2). The exact reason for the low SSC content is not known, however, the high powdery mildew levels may have stressed the plants, resulting in accelerated fruit ripening and abscission. Severe powdery mildew affects fruit quality, causing reduced fruit yields, incomplete ripening and poor flavors (Zitter et al., 1996). Exterior color of 'Gal-52' was significantly different compared to the other cultivars at harvest, however all interior flesh color was similar (data not shown).

For melons stored at 20°C for five days there were no differences in quality measurements, ethylene evolution and respiration between transgenic and wild-type 'Galia'. However, 'Gal-52' was significantly firmer after storage (Table 3) but produced more ethylene and CO₂ than the other melons (Fig. 1). No significant differences in external or internal color were observed (data not shown).

Fall 2004

The experiment was repeated in fall 2004, using the same cultivars and production methods. However, two hurricanes damaged the greenhouse and the crop three and five weeks after planting respectively. Twenty-five percent of the plants developed severe *Fusarium oxysporum*. There were no differences observed for plant susceptibility to *Fusarium* or powdery mildew (data not shown). One to two fruits were harvested per plant at full-slip from 18 Oct. to 17 Nov. 2004.

'Gal-52' yielded slightly more fruit (0.2 per plant) than wild-type and transgenic 'Galia' (Table 4). Significant differences were also observed in days to harvest. Transgenic 'Galia' remained on the vine an average of five days longer than wild-type 'Galia' and seven days longer than 'Gal-52' (Table 4). Significant differences were found in external color between treatments, however all internal flesh color was similar (data not shown).

DISCUSSION

The transgenic 'Galia' hybrid used in this research resulted from a cross of an unselected transgenic male parent and the wild-type female parent. Since the male line was not selected, other than by PCR analysis for the transgene, the level of expression was unknown.

In the spring trial delayed ripening was not evident. Severe powdery mildew infestation stressed the plants, potentially inducing high levels of ethylene. Ethylene production is often seen in stressed plants (Srivastava, 2001; Morgan and Drew, 1997). Only ethylene production was reduced in the transgenic fruit, not ethylene perception. As a result, if ethylene is present, the fruit is still able to perceive ethylene and ripen.

The transgenic fruits that remained on the vine longer than the wild-type demonstrated that the antisense (CMACO-1) gene was expressed. Since no significant differences in fruit quality factors were found at full-slip, potentially a longer shelf-life, high quality 'Galia' melon can be developed, especially after the male transgenic lines are selected for delayed ripening and stabilized through selfing for four or more generations.

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Tables

Table 1. Mean fruit characteristics for transgenic 'Galia' (TGH-AS-1 and TGH-AS-2), wild-type 'Galia' and 'Gal-52' melons, spring 2004.

Treatment	Fruit no./ plant	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Days to harvest
TGH- AS-1	2.0 ^z	759	124	109	48
TGH-AS-2	2.6	799	128	109	54
Galia	2.6	722	123	108	52
Gal-52	2.5	735	126	109	46
Significance	NS	NS	NS	NS	NS

^z Means separated within each column by Duncan's multiple range test (P=0.05)

^{NS} Non significant

Table 2. Mean fruit quality characteristics for transgenic 'Galia' (TGH-AS-1 and TGH-AS-2), wild-type 'Galia' and 'Gal-52' melons evaluated on the day of harvest, spring 2004.

Treatment	SSC (°Brix)	Flesh thickness (mm)	Flesh firmness (N)
TGH-AS-1	4.8b ^z	24.1	26.5
TGH-AS-2	9.3a	24.8	23.2
Galia	10.3a	23.7	23.7
Gal-52	9.4a	25.3	29.7
Significance	*	NS	NS

^z Means separated within each column by Duncan's multiple range test (P=0.05)

* and ^{NS} Significant and non significant

Table 3. Mean fruit quality characteristics for transgenic 'Galia' (TGH-AS-1 and TGH-AS-2), wild-type 'Galia' and 'Gal-52' melons stored for 5 days at 20°C, spring 2004.

Treatment	SSC (°Brix)	Flesh thickness (mm)	Flesh firmness (N)
TGH-AS-1	5.7b ^z	25.7	3.83b
TGH-AS-2	8.1a	26.2	3.09b
Galia	9.4a	25.6	3.82b
Gal-52	8.9a	25.3	5.75a
Significance	NS	NS	*

^z Means separated within each column by Duncan's multiple range test (P=0.05)

* and ^{NS} Significant and non significant respectively

Table 4. Means for transgenic 'Galia' (TGH-AS-1 and TGH-AS-2), wild-type 'Galia' and 'Gal-52' melons, fall 2004.

Treatment	Fruit no./ plant	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Flesh thickness (mm)	SSC (°Brix)	Flesh firmness (N)	Days to harvest
TGH-AS-1	1.0b ^z	1312	153	134	30	7.4	23.2	53a
TGH-AS-2	1.1b	1535	163	140	32	8.9	23.4	51ab
Galia	1.1b	1513	159	140	33	8.1	24.3	47bc
Gal-52	1.3a	1515	153	141	34	7.3	30.2	44c
Significance	*	NS	NS	NS	NS	NS	NS	*

^z Means separated within each column by Duncan's multiple range test (P=0.05)

* and ^{NS} Significant and non significant

Figures

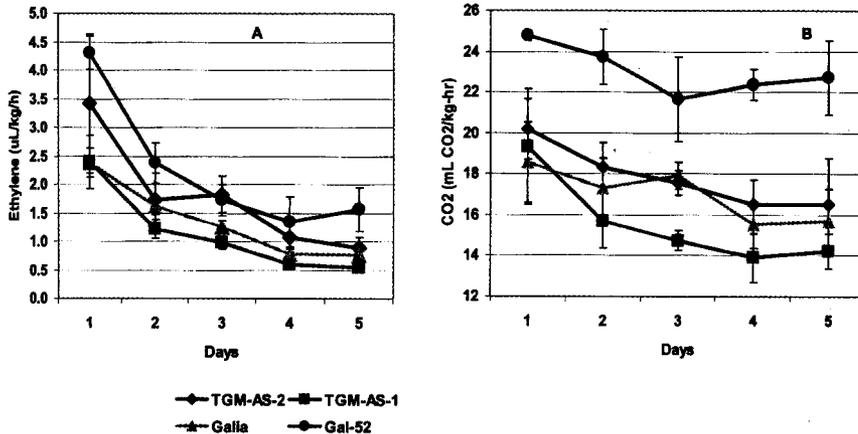


Fig. 1. Ethylene evolution (A) and respiration (B) for transgenic 'Galia' (TGH-AS-1 and TGH-AS-2), wild-type 'Galia' and 'Gal-52' melons stored five days at 20°C, spring 2004. Each point represents a mean of three replications \pm SE.