

Determination of gene escape and fruit quality characteristics in transgenic melon (*Cucumis melo* L. var. *inodorus*)

Yeşim YALÇIN MENDİ^{1,*}, Nebahat SARI¹, Asiye AKYILDIZ², İlknur SOLMAZ¹, Ceren ÜNEK¹,
Okan ÖZKAYA¹, Sedat SERÇE³

¹Laboratory for Biotechnology, Department of Horticulture, Faculty of Agriculture, Çukurova University, 01330 Balcalı, Adana - TURKEY

²Department of Food Technology, Faculty of Agriculture, Çukurova University, 01330 Balcalı, Adana - TURKEY

³Department of Horticulture, Faculty of Agriculture, Mustafa Kemal University, Antakya, Hatay - TURKEY

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Abstract: Gene escape and fruit quality characteristics of transgenic melons (*Cucumis melo* L. var *inodorus* cv. 'Kırkağaç 637') resistant to zucchini yellow mosaic virus (ZYMV) and control plants were investigated under greenhouse conditions. No significant differences were observed between transgenic and transgenic × control genotypes, with regard to rind thickness, fruit cavity length, fruit cavity width, total soluble solids, pistil scar diameter, and peduncle length. Fruit characters, including fruit weight, fruit width, fruit length, fruit flesh thickness, and peduncle diameter were significantly different. These results indicate that transgenic × control genotypes had higher values than transgenic (T4 and T20) genotypes, regarding fruit weight, fruit width, fruit length, fruit flesh thickness, and peduncle diameter. Significant differences were not observed between transgenic (T4 and T20), control, and transgenic × control genotypes in terms of L-ascorbic acid, malic acid, citric acid, sucrose, glucose, or fructose, but differences were observed for fruit total acidity. Esters, lactones, and alcohols were aroma components, but none differed significantly between the transgenic and control genotypes. The results show that there was 100% gene escape in the control plants within 10 m of the transgenic plants, while there was 70% gene escape in plants 12.5, 15, and 17.5 m from the transgenic plants under greenhouse conditions.

Key words: Transgenic, GMO, melon, plant, aroma components

Transgenik kavunda gen kaçışı ve meyve kalite kriterlerinin belirlenmesi

Özet: Bu çalışmada, Kabak Sarı Mozaik Virüsüne (ZYMV) karşı dayanıklı transgenik 'Kırkağaç 637' kavun çeşidinin ve kontrol bitkilerinin tel sera koşulları içerisinde gen kaçışları ve meyve kalite özellikleri incelenmiştir. İstatistik analizler sonucunda, transgenik ve transgenik × kontrol melezlemesi sonucu elde edilen meyvelerdeki kabuk kalınlığı, çekirdek evi yüksekliği, çekirdek evi çapı, suda çözünebilir kuru madde, mühür çapı ve meyve sapının boyu bakımından fark bulunmadığı tespit edilirken; meyve ağırlığı, meyve çapı, meyve yüksekliği, meyve eti kalınlığı, meyve sapının çapı açısından ise fark bulunmuştur. Sonuç olarak, melez genotipe ait meyvelerin, transgenik genotiplerin meyvelerine (T4 ve T20) göre meyve ağırlığı, meyve çapı, meyve yüksekliği, meyve eti kalınlığı ve meyve sapı çapı bakımından daha yüksek değerler aldığı belirlenmiştir. Transgenik (T4 ve T20), kontrol ve melez (transgenik × kontrol) genotiplerde L-askorbik asit, malik asit,

* E-mail: yesimcan@cu.edu.tr;

sitrik asit, sakkaroz, glikoz ve früktoz açısından istatistiki olarak fark bulunmazken, toplam asitlik bakımından farklılıklar tespit edilmiştir. Denemede öngörülen aroma bileşikleri analizleri sonucunda esterler, alkoller ve laktonlar saptanmış olup, uygulamalar arasında fark bulunmamıştır. Araştırma sonucunda, tel sera koşullarında transgenik bitkilerden yaklaşık 10 m uzaklıkta bulunan kontrol bitkilerinde %100 gen kaçıışı görülürken; 12.5, 15 ve 17.5 m uzaklıktaki bitkilerde ise %70 oranında gen kaçıışı belirlenmiştir.

Anahtar sözcükler: Transgenik, GDO, kavun, bitki, aroma maddeleri

Introduction

The melon *Cucumis melo* L. is one of the most economically important and widely cultivated crops in the world, and has been the subject of intense research for decades. Turkey is a secondary center of melon diversification and the second largest producer after China, producing 1.9 million t annually (FAOSTAT 2007). It is the most morphologically diverse species in the genus *Cucumis* (Kirkbride 1993) and differs widely in fruit size, morphology, and taste, as well as vegetative traits and climatic adaptation (Silberstein et al. 2003; Nuñez-Paleniús et al. 2008). As in other plant species, the most important limiting factors in the production of melon are diseases and pests. Diseases and pests decrease crop yield, and cause loss of product and crop quality in some plant varieties. Among the diseases and pests, viruses have an important place because they do not immediately struggle.

Along with the great number of viruses that affect melons, it has been shown that the most common viruses in Turkey are zucchini yellow mosaic virus (ZYMV) and cucumber mosaic virus CMV (Yılmaz and Davis 1984, Yılmaz et al. 1992). Studies conducted in Turkey on some commonly grown melon genotypes reported that all of the varieties used in the experiments were sensitive to ZYMV and that this sensitivity resulted in death, especially in the varieties 'Yuva' and 'Hasanbey' (Sari et al. 1994).

Although melon fruit and plant improvement via traditional hybridization has led to the generation of improved new varieties, this method of new plant development is relatively slow and limited to a restricted gene pool. For that reason biotechnological approaches have been successfully applied in melon (Nuñez-Paleniús et al. 2008). Regeneration studies on melons began in the 1980s. Two different melon lines of the Charentais type were used as a source of hypocotyl and flower stalk explants, and studies were performed on callus formation in different media, as well as shoot stimulation from callus (Abak and

Dumas de Vaulx 1980). Later, most melon research continued in the areas of regeneration (Lehsem 1989; Niedz et al. 1989; Chee 1991; Garcia-Sogo et al. 1991; Gaba et al. 1996, 1999) and transformation (Fang and Grumet 1990; Valles and Lasa 1994; Bordas et al. 1997; Fuchs et al. 1997; Hokanson et al. 1997; Guis et al. 2000; Pang et al. 2000; Curuk et al. 2003).

One of the most widely discussed issues involves the potential for engineered genes to move via pollen into populations of crop wild relatives; an additional concern expressed by the public is that transgenes could somehow behave differently than native genes. There have been several reports on gene escape in cucurbits and other species (Colwell et al. 1985; Ellstrand 1988; Ellstrand and Hoffman 1990; Dale 1992; Raybould and Gray 1994; Tabie et al. 1994; Rajapakse et al. 1995; Rissler and Mellon 1996; Hokanson et al. 1997; Venneria et al. 2008).

Yalcin-Mendi et al. (2004) optimized the conditions for plant regeneration and gene transfer in the melon variety 'Kirkagac 637', a melon of economic importance in Turkey. They obtained melon lines (T4 and T20) that are resistant to this virus, which causes great economic loss by transferring the genes of *Agrobacterium tumefaciens* that provide resistance to ZYMV. Yalcin-Mendi et al. (2007) examined plant height, main stem diameter, number of nodes, leaf width, leaf length, and petiole length in plants of transgenic and non-transgenic 'Kirkagac 637' melon seeds in the first part of the same experiment. Significant differences were observed between transgenic and non-transgenic plants, in terms of plant height, main stem diameter, number of nodes, and petiole length.

The objective of the present study was to investigate the effects of this foreign gene (ZYMV) on fruit quality characters by comparing transgenic plants with control plants, and monitoring gene escape from transgenic to control plants under greenhouse conditions.

Materials and methods

We used 2 melon lines (T4 and T20) derived from 'Kirkagac 637' that have the ZYMV resistance gene (Yalcin-Mendi et al. 2004) and control 'Kirkagac 637' plants obtained from the Aegean Agricultural Research Institute. In all, 18 transgenic and 157 control seeds were germinated in a mixture of peat and perlite (2:1 v:v), according to a sowing plan created for use in the experiment. Seedlings belonging to the transgenic lines were identified by PCR to confirm the presence of the ZYMV coat protein. For DNA isolation from the leaf samples belonging to the plant material, 2-3 true leaves that had recently emerged from the germinated melon seeds were used. Genomic DNA was isolated according to Fulton et al. (1995). PCR was performed in 25- μ L reaction volumes containing 50 ng of DNA, 1 \times PCR reaction buffer, 2.5 mM of MgCl₂, 0.02 mM dNTP mixture, 2.5 μ mol of each primer, 1 unit of Taq DNA polymerase, and distilled water, overlaid by a drop of mineral oil. The primers for the PCR reaction used to detect the ZYMV coat protein gene were 5' AGATC TAAAT AACA AATCTC AACAC 3' and 5' AGATCTCTGC AGCCC TTTT TTTT 3', as per Fang and Grumet (1990). The mixtures were assembled at 0 °C and transferred to a thermal cycler, and then were pre-cooled to 4 °C. Amplification was carried out in a Perkin Elmer Cetus 480 DNA thermocycler, with an initial denaturation step of 2 min at 94 °C, 40 s at 95 °C, 40 s at 55 °C, and 5 min at 72 °C. The amplification products (approximately 1.1 kb) were separated by electrophoresis in 2% agarose gel in 1 \times TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) for 3 h at 70 V. The gel was stained for 20 min with ethidium bromide and the fragment patterns were photographed (Polaroid 667 film) under UV.

Seedlings of the transgenic 'Kirkagac 637' lines (T4 and T20) and control 'Kirkagac 637' were transferred to a greenhouse. While the plants were growing in the greenhouse, 1-m row spacing and 0.5-m plant spacing within the rows were used. In the experiment 6 of the T4 transgenic plants, 12 of the T20 transgenic plants, and 157 of the non-transgenic (control) plants were used. Beehives were used in the greenhouse to facilitate pollination.

Fruit weight (g), fruit width (cm), fruit length (cm), fruit flesh thickness (cm), rind thickness (mm), fruit

cavity length (cm), fruit cavity width (cm), total soluble solids (%), pistil scar diameter (mm), peduncle length (cm), and peduncle diameter (mm) were measured. Length measurements were made with a ruler and digital compass (Mitutoyo CD-15D), and total soluble solids were analyzed by a hand-held refractometer (Atago). The fruit types tested included self-pollinated transgenic plants and fruits formed by controlled pollination of transgenic plants with control plants at defined distances. Three fruits were used in each replication and the experiment had 3 replications. Quality characters, such as glucose, fructose, sucrose, L-ascorbic acid, malic acid, citric acid, and total acidic ratios, were determined in the fruits formed by self-pollination in transgenic plants, in fruits formed by controlled pollination with control plants at defined distances, and the fruits of control plants. For the determination of aromatic compounds, 3 plants each were randomly chosen from the self-pollinated T20 and T4 lines, and from the random crossings and controlled crossings, and then their fruits were analyzed. For determining the aromatic compounds, the liquid-liquid extraction method commonly used for plant products was used and the solvent dichloromethane (CH₂Cl₂) was used for extraction (Priser et al. 1997). Gas chromatography-mass spectrometry (GC/MS) analysis was carried out using an Agilent 6890 N-5973 N GC/MS. The column used was a J & W fused silica DB-wax capillary column (60 m, 0.25 ID, 0.25 film). For identification, the library flavors NIST and Wiley were used.

To determine the control of gene escape the seeds obtained from fruits randomly pollinated by bees were taken out and dried. The plantlets needed for gene isolation were provided by germinating these seeds under in vitro conditions. To calculate gene escape we measured the distance between the plants ascertained by tests to be transgenic and the genotypes randomly pollinated by bees that contained the 1000-bp band coding the ZYMV coat protein gene (Fang and Grumet 1993; Yalcin-Mendi et al. 2004) according to PCR.

Statistical analysis was performed using the SAS program. While averages and most of the other defining statistics were calculated using the TABULATE procedure, for variance analysis tables the GLM procedure was used. In the variance analysis tables, for units that had a difference at the 5% significance level average comparisons were again made with Duncan's test at the 5% significance level.

Results

Fruit characteristics

Significant differences were not observed between transgenic and transgenic × control genotypes, in terms of rind thickness, fruit cavity length, fruit cavity width, total soluble solids, pistil scar diameter, or peduncle length (Figures 1 and 2). However, the fruit characters, including fruit weight, fruit width, fruit length, fruit flesh thickness, and peduncle diameter were significantly different (Table 1). The results of fruit quality characters show that fruit weight was 2706 g for the transgenic × control genotype, 1816 g for T20, and 1956 g for T4. Fruit width ratios were 17.6 cm, 15.5 cm, and 16.8 cm for the transgenic × control genotype, T20, and T4 lines, respectively. Fruit length was 18.9 cm for the transgenic × control, 16.0 cm for T20, and 17.0 cm for T4. Fruit flesh thickness was 3.3 cm, 2.6 cm, and 2.7 cm for the transgenic × control genotype, T20, and T4, respectively. Rind thickness was 10.2 mm for the crossing of transgenic × control, 8.4 mm for T20, and 10.3 mm for T4. Fruit cavity length was 11.2 cm, 10.4 cm, and 10.4 cm, for the crossing of transgenic × control, T20, and T4, respectively. Fruit cavity width was 8.7 cm, 9.0 cm, and 8.9 cm for the crossing of transgenic × control, T20, and T4, respectively. Total soluble solids were 7.0% for the transgenic × control genotype, 7.8% for T20, and 7.8% for T4. Pistil scar diameter was 17.5 mm, 22.3 mm, and 22.6 mm for the transgenic × control genotype, T20, and T4, respectively. Fruit peduncle length was 2.6 cm for the transgenic × control genotype, 4.1 cm for T20, and 2.2 cm for T4. Fruit peduncle diameter was 7.4 mm, 9.2 mm, and 7.6

mm for the transgenic × control genotype, T20, and T4, respectively (Figures 1 and 2).

Fruit quality

Significant differences were not observed between the fruit of transgenic, control, and transgenic × control genotypes, in terms of L-ascorbic acid, malic acid, citric acid, sucrose, glucose, and fructose (Table 2). However, the amount of total acidity varied between genotypes (T20, T4, and transgenic × control.). In the fruits of the transgenic T20 genotype L-ascorbic acid (4.0 mg 100 g⁻¹), malic acid (18.3 mg 100 g⁻¹), citric acid (124.1 mg 100 g⁻¹), sucrose (31.10 mg g⁻¹), glucose (11.76 mg g⁻¹), and fructose (12.02 mg g⁻¹) values were determined; total acidity was 1.20 g kg⁻¹.

In the fruits of transgenic T4 plants, L-ascorbic acid (4.0 mg 100 g⁻¹), malic acid (24.3 mg 100 g⁻¹), citric acid (121.8 mg 100 g⁻¹), sucrose (26.69 mg g⁻¹), glucose (12.8 mg g⁻¹), fructose (12.7 mg g⁻¹) values were determined (Table 2); total acid was 1.10 g kg⁻¹. The sucrose (19.69 mg g⁻¹), glucose (11.25 mg g⁻¹), and fructose (16.69 mg g⁻¹) contents in cross-bred fruits were within wider limits relative to transgenic T20 and T4. L-ascorbic acid content in fruits was 2.7 mg 100 g⁻¹. While the malic acid content was 24.6 mg 100 g⁻¹, the citric acid content was 121.6 mg 100 g⁻¹; total acid was 0.89 g kg⁻¹.

Sucrose, glucose, and fructose contents in the control plants were 24.86, 15.44, and 15.63 mg g⁻¹, respectively (Table 2). L-ascorbic acid content in the fruits was 5.7 mg 100 g⁻¹ and the malic acid content was 16.7 mg 100 g⁻¹; mean total acid was 0.91 g kg⁻¹.

Table 1. The comparisons of several pomological characteristics among fruits of 2 transgenic melon lines and transgenic × control cross.

	Fruit weight (g)	Fruit width (cm)	Fruit length (cm)	Fruit flesh thickness (cm)	Rind thickness (mm)	Length of fruit cavity (cm)	Width of fruit cavity (cm)	Total soluble solids (%)	Diameter of pistil scar (mm)	Length of peduncle (cm)	Diameter of peduncle (mm)
Transgenic × control	2706 a	17.6 a	18.9 a	3.3 a	10.2	11.2	8.7	7.0	17.5	2.6	7.4 b
T4	1956 b	16.8 ab	17.0 b	2.7 b	10.3	10.4	8.9	7.8	22.6	2.2	7.6 b
T20	1816 b	15.5 b	16.0 b	2.6 b	8.4	10.4	9.0	7.8	22.3	4.1	9.2 a

Mean separation was conducted using Duncan's test at 5%.

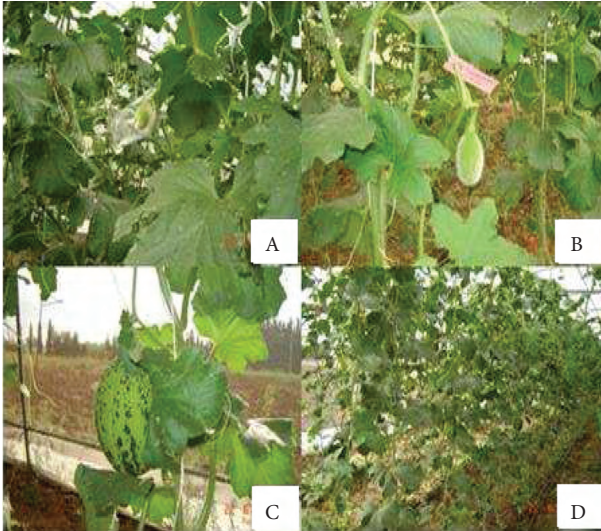


Figure 1. The melon fruits grown in screenhouse (A: self-pollinated flower; B: Young fruit, C: Fruit before maturation; D: General view from screenhouse) on the control plants pollinated by pollen from transgenic plants.

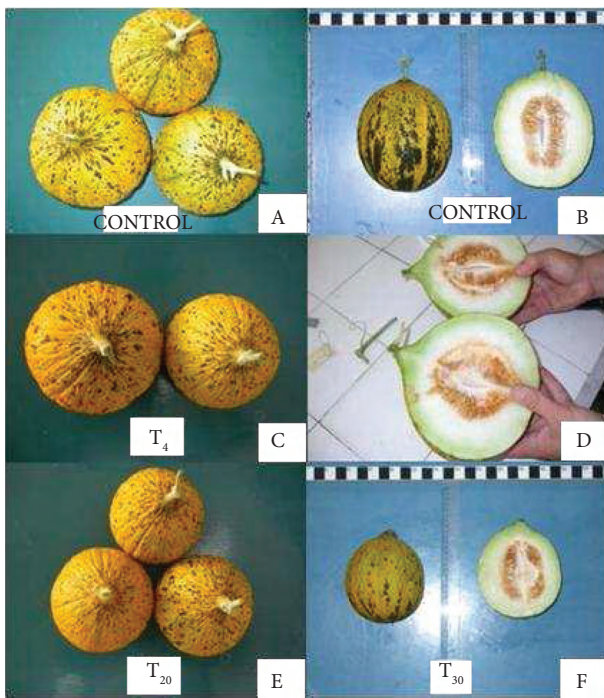


Figure 2. The melon fruits grown in screenhouse (A-B = non-transgenic fruits; C-D = the fruits of T4; E-F = the fruits of T20).

Aroma compound

Extraction results show that in the melons given the code numbers T20(5), T20(6), T20(10), T4(6), T4(10), T4(12), TM5, TM25, TM41, KT4(1), KT4(2), KT4(6), KT20(3), KT20(6) and KT20(10) no differences were observed in the aroma compounds, as ascertained by aroma analysis. The general aroma compounds were screened and when the compounds observed in the extraction results were examined, it was determined that the following esters, alcohols, and lactones were in the melons: benzyl acetate, ethyl butyrate, and isoamyl acetate, benzyl alcohol and 2-octanol alcohol, and γ -undecalactone, γ -hexalactone, and γ -dodecalactone.

Gene Escape

To determine the control of gene escape the seeds of fruits (approximately 20) obtained from flowers randomly pollinated by bees were extracted and dried. The plantlets needed for gene isolation were obtained by germinating these seeds under in vitro conditions. PCR reaction results show that while 16 plant genomes contained a 1000-bp gene sequence, 4 plants did not have 1000-base pair bands. When plants within rows were examined it was observed that even though 4 plants that were in rows 20 (10 m distance), 25 (12.5 m distance), 30 (15 m distance), and 35 (17.5 m distance) were not pollinated by pollen that came from transgenic plants, in all the other plants in these rows the 1000-base double band was observed, which was also observed in the transgenic plants.

In the control plants 100% gene escape was observed within 10 m of transgenic plants, while 70% gene escape was observed in control plants at distances of 12.5, 15, and 17.5 m from transgenic plants under screenhouse conditions. The gene escape results were proven by the existence of the 1000-base double band in the transgenic plants, while non-transgenic plants did not have this fragment.

Discussion

The concept of risk assessment that facilitates comparison of a final product to one with an acceptable standard of safety is an important element of genetically modified organisms (GMO) food safety assessment. This principle was elaborated by the Food and Agriculture Organization (FAO), World Health

Table 2. Comparisons of the L-ascorbic acid, malic acid, citric acid, sucrose, glucose, fructose, and total acidity amounts among fruits of 2 transgenic melon lines and transgenic × control cross.

Source	L-ascorbic acid (mg 100 g ⁻¹)	Malic acid (mg 100 g ⁻¹)	Citric acid (mg 100 g ⁻¹)	Sucrose (mg g ⁻¹)	Glucose (mg g ⁻¹)	Fructose (mg g ⁻¹)	Total acidity (g kg ⁻¹)
Control	5.7	16.7	120.9	24.86	15.44	15.63	0.91 b
Transgenic × control	2.7	24.6	121.6	19.69	11.25	16.69	0.89 b
T4	4.0	24.3	121.8	26.69	12.80	12.77	1.10 ab
T20	4.0	18.3	124.1	31.10	11.76	12.02	1.20 a

Mean separation was conducted using Duncan's test at 5%.

Organization (WHO), and Organization for Economic Cooperation and Development (OECD) in the early 1990s, and is referred to as “substantial equivalence”. The principle suggests that GMO foods can be considered as safe as conventional foods when key toxicological and nutritional components of a GMO food are comparable—substantially equivalent—to the conventional food (within naturally occurring variability) and when the genetic modification itself is considered safe (Venneria et al. 2008).

The major environmental risks associated with the release of GMOs to the environment include changes in genome organization and gene flow to other crops or wild relatives (Rissler and Mellon 1996). The risk of gene flow from GMOs to relatives was significant and relevant in centers of origin of wild and domesticated plants, which are usually centers of high genetic diversity. Gene flow between crops and their wild relatives is widely documented in the literature (Ellstrand and Hoffman 1990) and is of special concern, particularly when a new combination of genes is involved (Arriaga et al. 2006). One of the most widely discussed issues involves the potential for engineered genes to move via pollen into populations of crop wild relatives; an additional concern expressed by the public is that transgenes could somehow behave differently than native genes (Colwell et al. 1985; Ellstrand 1988; Ellstrand and Hoffman 1990; Raybould and Gray 1994, Hokanson et al. 1997).

Among the *Cucurbitaceae*, *C. melo* is one of the most important cultivated cucurbits. It is grown primarily for its fruit, which generally has a sweet aromatic flavor, with great diversity in size (50 g-15

kg), flesh color (orange, green, white, and pink), rind color (green, yellow, white, orange, red, and gray), shape (round, flat, and elongated), and dimension (4 cm-200 cm) (Nuñez-Palenius et al. 2008).

In the present study gene escape and fruit quality characteristics—fruit weight, fruit length, fruit width, fruit flesh thickness, rind thickness, fruit cavity length, fruit cavity width, total soluble solids, pistil scar diameter, peduncle length, peduncle diameter, glucose, fructose, sucrose, L-ascorbic acid, malic acid, citric acid, and total acidic ratios—were examined. Additionally, esters, lactones, and alcohols in the transgenic ‘Kirkagac 637’ melon variety resistant to ZYMV and control plants under screenhouse conditions were investigated. Significant differences were not observed between transgenic and control plants, in terms of rind thickness, fruit cavity length, fruit cavity width, total soluble solids, pistil scar diameter, or peduncle length. Fruit characters, including fruit weight, fruit width, fruit length, fruit flesh thickness, and peduncle diameter were significantly different. Fruit quality characters observed in the present study show that fruit weight ranged between 1816 g (T20) and 2706 g (transgenic × control), fruit width ranged between 15.5 cm (T20) and 17.6 cm (transgenic × control), fruit length ranged between 16.0 cm (T20) and 18.9 cm (transgenic × control), and fruit flesh thickness varied between 2.6 cm (T20) and 3.3 cm (transgenic × control). These results show that transgenic × control genotypes had higher values than transgenic T4 and T20 genotypes, regarding fruit weight, fruit width, fruit length, fruit flesh thickness, and peduncle diameter. Significant differences were not observed

between the transgenic and control genotypes, in terms of L-ascorbic acid, malic acid, citric acid, sucrose, glucose, or fructose, but total acid of fruit differed. Total acid content in transgenic genotypes (T4 and T20) was higher than in the control and transgenic \times control genotypes.

Yalcin-Mendi et al. (2007) examined plant morphological characters in transgenic and non-transgenic 'Kirkagac 637' melon seeds in the first stage of the same experiment, and statistical differences were observed between transgenic and non-transgenic plants, in terms of plant height, main stem diameter, number of nodes, and petiole length.

In the present study, esters, lactones, and alcohols were determined to be aroma components, but there were no differences between the transgenic and non-transgenic melons. It is known that these 3 compounds can be stored in many locations in fruit and vegetable tissues, including fatty acids, sugars, amino acids, carotenoids, and their related structures (Buttery 1981). Studies have shown that these substances are common to all applications and that no other compounds were observed. When esters, alcohols, and lactones in the application results were examined, they were in accord with other compounds produced and observed in melon, and there were no differences between applications (Aubert et al. 2005; Aubert and Pitrat 2006).

Venneria et al. (2008) compared conventional and genetically modified wheat, corn, and tomato, in terms of fatty acids content, non-saponifiable fraction of antioxidants, total phenols, polyphenols, carotenoids, vitamin C, total antioxidant activity, and mineral composition. No significant differences were observed between the qualitative traits analyzed in the wheat and corn samples. On the basis of the principle of substantial equivalence, as articulated by the WHO, the Organization for Economic Cooperation and Development, and the United Nations Food and Agriculture Organization, these data support the conclusion that GM events are nutritionally similar to conventional varieties of wheat, corn, and tomato on the market today. These data also support our fruit quality character comparison results between transgenic and non-transgenic 'Kirkagac 637' melon.

Koga-Ban et al. (2004) compared genetically modified cucumber and conventional cucumber, in terms of flowers, stems, leaves, fruit, and plant type under greenhouse and field conditions, and no significant differences were observed between the transgenic and non-transgenic plants.

Among the raspberries, raspberry bushy dwarf virus (RBDV)-resistant transgenic and wild type 'Meeker' plants were grown in Oregon and Washington, and the fruits were harvested (Sarah et al. 2008). Year-to-year and site-to-site variations were observed in °Bx and titratable acidity, with Oregon raspberries having slightly higher °Bx and lower titratable acidity than Washington raspberries. Twenty-nine volatile compounds were quantified using stir bar sorptive extraction (SBSE) paired with gas GC-MS. There were very few differences in volatile concentrations between the transgenic varieties and the wild type 'Meeker'. The flavor compounds tested in that study did not differ between the transgenic lines and the wild type 'Meeker' raspberry; these findings also support the present results.

Pineapple plants transformed with the bar gene for bialaphos resistance were evaluated for transgene stability, gene expression, and tolerance to glufosinate ammonium, the active ingredient of the herbicide Basta (R) X, under field conditions. Results show that fruit characteristics and yield were not affected by transgene introduction and expression (Sripaoraya et al. 2006).

Environmental risk evaluation of transgenic melon plants introduced with the coat protein gene of cucumber mosaic virus was carried out in a closed and semi-closed greenhouse by Tabei et al. (1994). Morphological characteristics were compared in a closed greenhouse. Morphological characteristics of transgenic melon plants did not differ from those of non-transgenic melon plants. Wind pollination of transgenic and non-transgenic melon plants was investigated under artificial wind generated by an electric fan in a closed greenhouse. Pollen from transgenic or non-transgenic melon plants was not detected on germination medium at all distances from the plants. It was concluded that the pollen of melon, an entomophilous plant, did not disperse via wind and was generally dispersed only by insects.

Rajapakse et al. (1995) reported that a cultivated strawberry (*Fragaria × ananassa*) and its weedy relative (*F. virginiana*) represented a particularly suitable system for investigating transgene escape and its potential ecological consequences. In a test of potential gene flow, potted plants of *F. virginiana* were introduced into a strawberry farm. Several progeny of these wild plants were observed to have RAPD markers specific to the cultivar, suggesting that pollen from the cultivar fertilized ovules of the wild species. Hokanson et al. (1997) compared the pollen-mediated gene movement of native and engineered marker genes using melon plants that express dominant morphological and transgenic traits. Of the nearly 4600 seedlings screened for both morphological (presence of green vs. virescent cotyledons) and transgene movement (presence of the NPT II protein by ELISA), in no case was the NPT II gene observed in the absence of green cotyledons; however, 39 seedlings were green, but did not express NPT II as measured by ELISA.

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Similar to the studies given above, we observed gene escape by our ZYMV-resistant transgenic plants system. Our screening procedure was limited to a distance of 17.5 m; however, the results obtained and the literature suggest that escape is limited by the distance of bee migration. In the present study we did not observe significant differences between the transgenic and control plants; however, this should be investigated further in much greater detail.

In conclusion, government agencies must promote the establishment of biosafety guidelines, and the development of specific studies related to reproductive constraints and the effects of introgression in wild populations of plants in Turkey. As such, GMO studies can be conducted not only under greenhouse conditions, but also in restricted field areas governed by biosafety rules.

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