Watermelon transformation with *Zucchini yellow mosaic virus* coat protein gene and comparison with parental cultivar

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Abstract – The objective of this work was to transfer *Zucchini yellow mosaic virus* coat protein (ZYMV-CP) and neomycin phosphotransferase II (NPT II) genes to the watermelon 'Crimson Sweet' (CS) genome, and to compare the transgenic progenies T1 and T2 with the nontransformed parental cultivar for morphological, pomological, growth and yield characteristics. The ZYMV-CP gene was transferred by *Agrobacterium tumefaciens*. The presence of the gene in transgenic T0, T1 and T2 plants was determined by polymerase chain reaction, and the results were confirmed by Southern blot. Two experiments were performed, one in the winter-spring and the other in the summer-autumn. In both experiments, the hypocotyl length of transgenic seedlings was significantly higher than that of nontransgenic parental ones. In the second experiment, the differences between transgenic and nontransgenic individuals were significant concerning fruit rind thickness, flesh firmness, fruit peduncle length, size of pistil scar, and a* values for fruit stripe or flesh color. Transferring ZYMV-CP gene to CS genome affected only a few characteristics from the 80 evaluated ones. The changes in rind thickness, flesh firmness and flesh color a* values are favorable, while the increase in the size of pistil scar is undesirable. The transgenic watermelon line having ZYMV-CP gene and the parental cultivar CS are very similar.

Index terms: *Citrullus lanatus*, comparative safety analysis, genetic modification, neomycin phosphotransferase II, nontransgenic watermelon, transgenic watermelon.

Transformação de melancia com o gene da capa proteica do *Zucchini yellow mosaic virus* e comparação com a cultivar parental

Resumo – O objetivo deste trabalho foi transferir o gene da capa proteica do *Zucchini yellow mosaic virus* (ZYMV-CP) e o gene neomycin phosphotransferase II (NPT II) para o genoma da melancia 'Crimson Sweet' (CS), e comparar as progênies T1 e T2 com a cultivar parental não transformada, quanto às características morfológicas, pomológicas, de crescimento e de produção. O gene ZYMV-CP foi transferido por meio da transformação com *Agrobacterium tumefaciens*. A presença do gene nas plantas transgênicas T0, T1 e T2 foi determinada pela reação de polimerase em cadeia, e os resultados foram confirmados pelo "Southern blot". Dois experimentos foram realizados, um no inverno-primavera e outro no verão-outono. Nos dois experimentos, o comprimento do hipocótilo das plântulas transgênicas foi significativamente maior do que nas plântulas não transgênicas. No segundo experimento, as diferenças entre as plantas transgênicas e não transgênicas foram significativas quanto à espessura da casca das frutas, firmeza da polpa, comprimento do pedúnculo, tamanho da cicatriz do pistilo, e valores a* da cor das listras ou da polpa. A transferência do gene da capa proteica ZYMV-CP ao genoma CS afetou apenas algumas características, das 80 avaliadas. As alterações na espessura da casca, firmeza e cor da polpa são favoráveis, enquanto o aumento no tamanho da cicatriz do pistilo é indesejável . A linhagem transgênica de melancia com o gene ZYMV-CP e a cultivar parental CS são muito similares.

Termos para indexação: *Citrullus lanatus*, análise de segurança comparativa, modificação genética, neomycin phosphotransferase II, melancia não transgênica, melancia transgênica.

Introduction

Transgenic crop production in the world is increasing. The leading countries in transgenic crop production are USA (66.8 million ha), Brazil (25.4 million ha), Argentina (22.9 million ha), India (9.4 million ha), Canada (8.8 million ha) and China (3.5 million ha) (International Service for the Acquisition of Agri-Biotech Applications, 2010). The concepts of substantial equivalence or comparative safety analysis are accepted for transgenic crop safety assessment (Organisation for Economic Co-Operation and Development, 1993; Food and Agriculture Organization, 1996; Barros et al., 2010). Since concerns over the transgenic crops safety raised, comparisons of transgenic crop compositions and performances with

those of conventionally bred crops, under field and protected cultivation, have been detailed (Shewry et al., 2007). In this sense, the genetically modified crop is compared to its conventional genotypes for agronomic and phenotypic variation, and by compositional analysis which includes analysis of macro- and micronutrients, as well as of toxins and antinutrients (Barros et al., 2010).

There are several reports for detailed comparisons of genetically modified crops and those of conventional counterparts in cereals. According to Venneria et al. (2008), no significant differences were observed for qualitative traits analyzed in wheat and corn as fatty acids content, unsaponifiable fraction of antioxidants, total phenols, polyphenols, carotenoids, vitamin C, total antioxidant activity, and mineral composition. It was reported that the effect of transgenesis on transcriptome was lower than that of conventional breeding concerning the wheat grain (Baudo et al., 2006; Shewry et al., 2007; Ammann, 2011). Batista et al. (2008) have concluded that plant mutagenesis may induce more transcriptomic changes than transgene insertion in rice. Coll et al. (2009) showed that gene expression profiles of transgenic maize plants MON810 and its near-isogenic nontransgenic maize genotypes were more similar than those of conventional lines. According to Barros et al. (2010), the differences in transcript/protein/metabolite profiles between locations (environment) were higher than the difference between Bt-maize and nontransgenic cultivar, and growing seasons had a stronger overall effect in the transcriptome, proteome and metabolome of the maize genotypes than the genetic modification.

To our knowledge, unlike cereals, there are a few reports comparing transgenic and their nontransgenic originating conventional cultivars in fruit and vegetable crops. Catchpole et al. (2005) reported that except for targeted traits, genetically modified potatoes appear substantially equivalent to traditional cultivars. According to Jiao et al. (2010), the composition in transgenic papayas exhibited great similarity to the one in nontransgenic counterparts. Venneria et al. (2008) showed that genetically modified events are nutritionally similar to conventional tomato varieties. Yalçın-Mendi et al. (2010) have reported that there was no significant difference between transformed (with ZYMV-CP) melon line and nontransformed control cultivar, except for fruit total acidity. However, there are no reports on the detailed comparison of transgenic watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] line with its nontransgenic originating cultivar.

The objective of this study was to transfer *Zucchini yellow mosaic virus* coat protein (ZYMV-CP) and neomycin phosphotransferase II (NPT II) genes to watermelon cultivar Crimson Sweet (CS) genome, and to compare the transgenic T1 and T2 generations with nontransformed plants of the same cultivar (parental cultivar) for morphological, pomological, growth and yield characteristics.

Materials and Methods

Nontransgenic watermelon cultivar Crimson Sweet - CS (Bursa tohumculuk A.Ş., Turkey) was used. For genetic transformation, watermelon seed coats were removed, and the seeds were washed in 70% ethanol for 5 min, rinsed three times with sterile distilled water, and surface-disinfected in a solution of 1.2% sodium hypochlorite with two drops of Tween 20 per 100 mL, for 40 min. After rinsing, seeds were further washed for 15 min in 500 mg L^{-1} cefotaxime. MS medium (Murashige & Skoog, 1962) was used for seed germination in a growth room at 27±1°C, in the dark. Cotyledons were excised from four-day-old seedlings and cut across to make two explants per cotyledon, and then were trimmed along all edges. Diferent media were used for regeneration, shoot elongation and rooting, as follows: RM–MS salts, modified MS vitamins, 30 g L^{-1} sucrose, 2.25 mg L^{-1} BA, as in Compton & Gray (1993); SE–MS salts, modified MS vitamins, 20 g L^{-1} sucrose; and SR–MS salts, modified MS vitamins, 20 g L^{-1} sucrose, 0.186 mg L^{-1} NAA or 250 mg L^{-1} cefotaxime. Media were solidified with 8 g L^{-1} agar, and sterilized at 121°C for 15 min. In vitro cultures were incubated at 27±1°C, with a 16-hour photoperiod of 60–70 μmol m^{-2} s^{-1} photon flux during regeneration-selection, elongation-selection and rooting.

Agrobacterium tumefaciens strain EHA 105 having the plasmid pCIB10, which contained NPT II and ZYMV-CP genes (Fang & Grumet, 1993), was used for transformation. A colony of this bacterium was incubated for 18 hours in 2 mL liquid LB medium Merck, VM491085542 (Merck, Gibbstown, NJ, USA), with 50 mg L^{-1} kanamycin at 28±1°C, and shaken at 125–140 rpm. The generated culture was stored at

-80°C after the addition of 174 µL dimethyl sulfoxide. Two hundred microliters of this culture were added to 2 mL of liquid LB with 50 mg L^{-1} kanamycin, and shaken (as above) for 5 hours. Then, the culture was added to 40 mL liquid LB, without antibiotics, prior to inoculation. Approximately 10 mL of this diluted culture were used for inoculation of 100 cotyledon explants. The cotyledons of seedlings were transferred to the *Agrobacterium* culture in a sterile Petri dish. The explants were excised in the *Agrobacterium* culture (approximately 10 min), inoculated for 10 min with occasional agitation, and then distributed on RM medium without antibiotics, and co-cultivated for two days in the growth room in the dark. Then, they were washed three times with sterile distilled water, and placed in 90x15-mm Petri dishes (approximately 20 explants per dish) with the adaxial side onto RM medium containing 75 mg L^{-1} kanamycin and 400 mg L^{-1} timentin (Research Triangle Park, Durham, NC, USA), and cultured for three weeks. The explants were subcultured for three weeks in the RM medium with 100 mg L^{-1} kanamycin and the same concentration of timentin.

Regenerating parts of the explants were excised and transferred to SE medium with 125 mg L^{-1} kanamycin and 400 mg L^{-1} timentin, and cultured for six weeks, at three-week intervals. To stimulate root formation, shoots growing on kanamycin were first transferred to SR medium in test tubes (25x150 mm) and incubated for two weeks. Then, the shoots were rooted on SR medium without NAA, but supplemented with 250 mg L^{-1} cefotaxime, which stimulates root formation (Çürük et al., 2005). Test tubes which had the putative transformants were gradually opened in the growth room, and the plants were transplanted to the plastic pot containing 0.2 L substrate, composed of 66% peat Potgrond H (Klasmann-Deilmann, GmbH, Geeste, Germany) and 34% perlite.

For ZYMV-CP gene detection, polymerase chain reaction (PCR) was performed. One hundred miligrams of plant tissue were grinned in liquid nitrogen, and DNA was isolated (Gusmini & Wehner, 2010). For each sample, 4 µL suspended DNA (approximately 100 ng) were used for 21 µL reaction mixture. The reaction mixture was composed of 2.5 µL PCR buffer (x10), 1.5 µL of 25 mmol L^{-1} $MgCl_2$, 0.5 µL of 10 mmol L^{-1} dNTPs, 14.3 µL double distilled water, 1 µL each of primer 1 (5 µmol L^{-1}, 5'-AAGTCTAGAAAATAACAAATCTCAACA-3') and primer 2 (5 µmol L^{-1}, 5'-AATGAGCTCTTTTTTTTAGGCTTG-3'), and 0.2 µL Taq DNA polymerase (Fermentas, Vilnius, Lithuania). In the PCR reaction, final concentrations of PCR buffer, $MgCl_2$, dNTPs, primers, and Taq DNA polymerase were x1, 1.5 mmol L^{-1}, 200 µmol L^{-1} (each dNTP), 0.2 µmol L^{-1} (each primer), and one unit, respectively. Each PCR reaction consisted of an initial denaturation at 95°C for 2 min (1 cycle), and denaturation at 95°C for 45 s, annealing at 50°C for 1 min, extention at 72°C for 90 s (30 cycles), and a final extention at 72°C for 5 min (1 cycle).

The presence of ZYMV-CP gene in PCR–positive plants, and the absence of the gene in nontransgenic parental plants were detected by Southern blot. For Southern blot analysis, a nonradioactive probe of ZYMV-CP gene was prepared. The pCIB10 plasmid was isolated from 5 mL *Agrobacterium* culture that was grown for 20 hours under the mentioned conditions, combining the protocol by Helms (1990) and Spin Columns of GeneJET Plasmid Miniprep Kit (Fermentas, Vilnius, Lithuania). The ZYMV-CP gene (approximately 1.2 kbp) was amplified by PCR with 1 µL of suspended plasmid DNA (30 ng). The PCR product was run on 0.8% agarose gel, and the ZYMV-CP gene was gel-extracted following the manufacturer's instructions Silica Bead DNA Gel Extraction Kit #K0513 (Fermentas, Vilnius, Lithuania). The gel-extracted DNA was labeled using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's recommendations.

For Southern blot analysis, DNA was prepared from watermelon leaves (Levi & Thomas, 1999). Approximately 10 µg of genomic DNA was restriction-digested with *Xba*I, following the manufacturer's instructions, (Fermentas, Vilnius, Lithuania), run on 1% agarose gel at 30 V for 8 hours, and blotted onto a charged nylon membrane NP0HY00010 (GE Infrastructure Water & Process Technologies, Vista, CA, USA), using a vacuum blotter 785 (BIO-RAD, Hercules, CA, USA). DIG–labeled ZYMV-CP probe and blots of restriction-digested watermelon DNA onto charged nylon membrane were hybridized, washed and detected according to the manufacturer's manual Roche, 11585614910 (Roche, Maslak, Istanbul, Turkey). The membrane was

prehybridized in DIG Easy Hyb for 30 min at 44°C, with gentle agitation at 14 rpm in a hybridization oven HB-1000 Hybridizer, UVP (Laboratory Products, Cambrige, UK). The DIG–labeled ZYMV-CP probe which had been stored at -20° was denaturated by incubation in boiling water for 5 min and, then, by rapidly cooling in ice-water for 2 min. After that, the membrane was hybridized with this probe for four hours or overnight at 44°C and 14 rpm. After hybridization, the wash procedure of the nylon membrane was carried out in two steps. In the first step, the membrane was washed twice with a 2xSSC/0.1% SDS solution, for 5 min at 15–25°C, under constant agitation at 20–30 rpm. In the second step, it was washed twice with a 0.5xSSC/0.1% SDS solution for 15 min at 67°C. All the incubations made for detection were performed at 30ºC with agitation at 40–50 rpm. The membrane was agitated in washing buffer (0.1 mol L^{-1} maleic acid, 0.15 mol L^{-1} NaCl (pH:7.5), 0.3% Tween 20), and incubated for 40 min in a blocking solution and 30 min in an antibody solution; then, it was washed again twice with the washing buffer for 20 min. After that, the membrane was equilibrated for 5 min in detection buffer (0.1 mol L^{-1} Tris-HCl, 0.1 mol L^{-1} NaCl, pH:9.5). Then, 1 mL of CSPD ready-to-use was applied on the membrane. Hybridization signals were detected by exposing the membrane to an X-ray film at 15–25°C for 60 min.

According to the growth of the putative transgenic shoots on selection medium supplemented with kanamycin, and to PCR analysis, four transformation events were obtained from transformation experiments. In vitro regenerated plants from one of these events, which were morphologically the most similar to their parental cultivar, were selected as primary transgenic (T0). Then, the seeds of T1 generation were obtained by self-pollination of T0 plants. Transgenic T1 plants were selected by PCR for transgene ZYMV-CP. The seeds of T2 generation were similarly produced by self-pollination of the PCR-positive plants of T1 generation. The transfer of the genes was confirmed by Southern blot detection in T1 and T2 generations. The growth and self-pollination of the plants were carried out in the glasshouse, and described as follows.

To compare transgenic and nontransgenic watermelon genotypes, two experiments were carried out in the winter-spring (first experiment) and summer-autumn (second experiment) growing seasons in 2009, at the Agricultural Research Station of Mustafa Kemal University, Antakya, in the Southeastern Mediterranean Region of Turkey. Plants were grown in a glasshouse with steel structure, with 10 m span width, 4 m gutter height, 5.5 m ridge height, continuous roof ventilation windows (2x10 m) and two exhaust fans. The ventilation windows of the glasshouse were closed with a 50-mesh net. The exhaust fans were on, when the inside temperature was higher than 28°C. The glasshouse was heated with a radiator using the central heating system, when the inside temperature was lower than 17°C. When air temperature in the glasshouse was higher than 35°C, an inside screen XLS 15 Firebreak (AB Ludving Svenson, Sweden) was used to reduce the temperature. Nontransformed CS and T1 (transgenic) seeds for the first experiment, and nontransformed CS and T2 (transgenic) seeds for the second experiment were sown in the aforementioned substrate, in January 10 and July 18 of 2009, respectively. The PCR-negative plants of nontransformed CS, and the positive ones of transgenic generations were transplanted to plastic pots containing 16 L of the mentioned substrate, and grown in the glasshouse at a density of 1.33 plants m^{-2} (1.5x0.5 m).

The experiment was performed in a completely randomized design, with three replicates per treatment and 10 plants per replicate. Unless otherwise noted, measurements were made on eight plants per replicate. The plants were irrigated by hand as needed. The total amount of applied N-P_2O_5-K_2O was 200-120-300 kg ha^{-1}. Since insect activity was very low due to the net use (with 50 meshes) on the ventilation windows, the flowers were self-pollinated to obtain fruit. During the first experiment, average minimum and maximum air temperatures inside the glasshouse were approximately 15.7 and 30.6ºC in January, 18.2 and 30.3ºC in February, 19.5 and 32.1ºC in March, 21.3 and 37.3ºC in April. In the second experiment, the average minimum and maximum temperatures were approximately 28.1 and 41.1ºC in July, 26.8 and 38.5ºC in August, 22.6 and 35.4ºC in September, 20.0 and 35.2ºC in October, 20.0 and 27.2ºC in November. The photoperiod during both experiments was the natural daylength.

The measurements of hypocotyl and cotyledon length (cm) and width (cm), and the observation (absent or spotted) of cotyledon spotty status were made in January 26 and July 26 of 2009, in the first

and second experiments, respectively. Stem thickness (mm), main stem length (cm), and number of branches and nodes in the main stem were determined three weeks before harvest. Measures were performed on two fully developed leaves of each plant for petiole length (cm), and for blade width (cm) and length (cm), and observations were done for blade blistering or spotting (absent or present), being the leaf blade secondary lobing classified as weak (3), intermediate (5) or strong (7), according to the International Union for the Protection of New Varieties of Plants (2004)

From each replicate, two plants at flowering were used for the measurements of fresh and dry weigh (g), and leaf area (cm^2 per plant), using a leaf area meter Li-cor 3100 (Li-cor, Lincoln, NE, USA). The leaves, roots and stems-branches of the plants were weighted separately, before and after drying at 65°C for two days. Node number of the first male and female flower, and time (day) of male and female flowering were recorded. Leaf and flower measurements or observations were made on two leaves or flowers per plant from each replicate. For pomological analysis, seven or eight fruit were used in each replicate. Petal width (mm) of male, and petal height (mm), and ovary length (mm) and width (mm) of female flowers were measured. Petal apex shape of female flower (acute, 3; rounded, 5; obtuse, 7) according to TC Resmi Gazete (1998), and pubescence of the ovary (weak, 3; intermediate, 5; strong, 7) as per the International Union for the Protection of New Varieties of Plants (2004) were determined. Pollen viability (%) was determined using 1% triphenyl tetrazolium chloride. Pollens were germinated in a medium containing 20% sucrose, 100 mg L^{-1} H_3BO_3, 300 mg L^{-1} $Ca(NO_3)_2.4H_2O$, 200 mg L^{-1} $MgSO_4.7H_2O$, 100 mg L^{-1} KNO_3 and 0.2% agar (Şensoy et al., 2003).

Fruit weight (kg), length (cm), and width (cm) were measured at maturity. Fruit shape (flattened, 1; round, 2; broad elliptical, 3; elliptical, 4; pyriform, 5; oblong, 6) was classified in conformity with the European Cooperative Programme for Plant Genetic Resources (2008). Shape of apical and basal parts of the fruit (flat, 1; flat to rounded, 2; rounded, 3; rounded to conical, 4; conical, 5) were recorded according to TC Resmi Gazete (1998) and the International Union for the Protection of New Varieties of Plants (2004). Grooves and marbling on fruit were observed as absent or present. Flesh firmness was measured in newtons (N), using a penetrometer (Nippon Optical Works Ca, LTD, Tokyo, Japan) fitted with a 5 mm diameter probe, and fruit rind thickness (mm) were determined. Fruit peduncle length (cm) and diameter (mm), and insertion size of peduncle (mm) were measured just before harvest. Number of seeds per fruit, and size of pistil scar (mm) were recorded. The watermelon fruit quality properties, namely malic acid (%) (Sadler, 1994), pH and total soluble solid (°Brix) (Çürük et al., 2009), were measured. Ground and stripe colors of fruit skin, and colors of fruit flesh and leaf blade were determined according to Cielab (L*a*b*) colour space, using a Chroma Meter Minolta CR-300 (Minolta, Osaka, Japan), and L*, a*, b*, C*, h^o values were recorded (McGuire, 1992).

The ploidy levels of nontransgenic and transgenic T0 plants of CS were analyzed by flow cytometry, using leaf samples (Arumuganathan & Earle, 1991). The ploidy level (diploid, triploid or tetraploid) of transgenic T1 or T2 plants were determined observing flower, leaf, pollen and seed characteristics. Reproductive system (monoecious or andromonoecious) and plant growth habit (bushy or runner) were recorded. Seed characteristics, namely mean weight (mg per seed), testa ground color, and patches at hilum, were determined. Total yield (kg m^{-2}) was taken from each plot and weighted on a weekly basis once fruit began to ripen.

Data analyses were performed by using SAS (SAS Institute, 1999). Data were subjected to a general linear model analysis (GLM) ANOVA, and means were compared by the least significant difference (LSD) test.

Results and Discussion

The flow cytometry analysis showed that the ploidy levels of nontransformed and transgenic T0 plants were diploid.The PCR detection showed that T0, T1 and T2 plants had the ZYMV-CP gene (Figure 1 A), and none of the nontransformed control plants contained the gene. In T1 and T2 populations, 71% (57/80) and 83% (50/60) of the plants (respectively) contained the ZYMV-CP gene according to PCR amplification.

Since *Xba*I only cuts on one side of the ZYMV-CP gene, the other *Xba*I side should come from the plant genome. Southern blot analysis showed that nontransformed control plants did not possess the ZYMV-CP gene, and plants that were selected by PCR

contained the ZYMV-CP gene as multiple copies of T-DNA integrated in the plant genome (Figure 1 B).

In both experiments, spots on cotyledons, and blisters or spots on leaf blade were absent in nontransgenic control and transgenic T1 or T2 plants. Parental control, and transgenic T1 and T2 plants were diploid, their reproductive systems were monoecious, and the growth habits were runner. Regarding these traits, differences between genotypes were not significant.

In the first experiment, there was no significant difference between transgenic T1 and control plants concerning stem, branch, leaf, and flower fresh and dry weight parameters (Table 1). Seedling hypocotyl length and leaf blade width of transgenic T1 generation were respectively 22.04 and 7.77% higher than those of nontransgenic control (Table 2). However, seedling cotyledon width and length of transgenic T1 were 16.95 and 16.48% lower than those of parental control (Table 2).

In the second experiment, fruit grooving or marbling, patches at hilum were absent, and testa ground color was brown in nontransgenic control or transgenic T2 seeds. The differences between transgenic T2 and nontransgenic control concerning stem, branch, leaf, flower, and fruit parameters (Table 3 and 4), and seed, fresh and dry weight, total yield, and cotyledon parameters (Table 5) were not significant. However, hypocotyl length of transgenic T2 seedling was 8.30% higher than that of nontransgenic control. Moreover, flesh firmness, size of pistil scar, and flesh color a* value of genetically modified T2 fruit were respectively 7.19, 11.69 and 8.16% greater than those of control fruit. Fruit rind thickness and peduncle length of

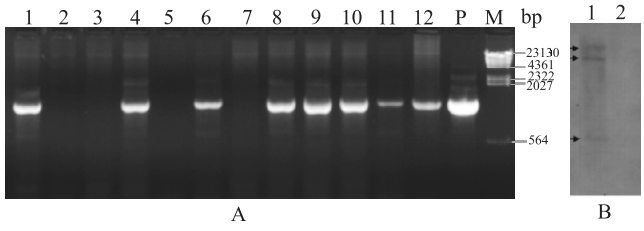

Figure 1. Molecular analysis: A, ethidium bromide stained agarose gel showing the amplification by PCR of the ZYMV-CP gene present in transgenic plants. Samples from transgenic plants (lanes 1, 4, 6 and 8–11), escapee plants (lanes 2, 3, 5 and 7), positive control (lane 12, transgenic watermelon), positive control of pCIB10 plasmid (lane P), and molecular size markers (lane M) are indicated. Molecular weight markers (bp) are indicated on the side of the gel. B, Southern blot analysis of T2 progeny. The PCR fragment of ZYMV-CP gene, amplified from the pCIB10, was used as a probe. Total genomic DNA (10 µg), from a transformed plant (lane 1) and a nontransformed control plant (lane 2), was digested with *Xba*I.

Table 1. Values of stem, branch, leaf, flower, and fresh and dry weight parameters of nontransgenic 'Crimson Sweet' (CS) watermelon and T1 progeny with transgene ZYMV-CP (T1)[1].

Parameter	Genotype		CV
	CS	T1	(%)
Stem thickness (mm)	10.71a	11.22a	7.78
Main stem length (cm)	420.79a	413.54a	6.81
Node number in the main stem	31.27a	29.79a	4.45
Branch number	6.36a	6.88a	12.12
Degree of secondary lobing in leaf blade[2]	5.42a	4.42a	16.34
Leaf blade length (cm)	12.41a	12.99a	4.92
Leaf area (cm^2 per plant)	4,762.70a	2,999.00a	24.24
Petiole length (cm)	8.88a	8.67a	1.16
Node number of the first male flower	12.00a	11.33a	6.99
Node number of the first female flower	15.67a	15.00a	8.41
Petal height of female flower (mm)	10.80a	11.18a	2.86
Petal apex shape of female flower[3]	5.83a	5.29a	12.37
Petal width of male (mm)	24.83a	25.24a	7.36
Pollen viability (%)	93.37a	95.30a	1.14
Pollen germination (%)	75.05a	76.42a	3.93
Ovary length (mm)	3.25a	3.44a	3.27
Ovary width (mm)	3.50a	3.35a	5.47
Pubescence of ovary [2]	4.63a	5.13a	6.53
Root fresh weight (g per plant)	32.61a	22.45a	45.38
Leaf fresh weight (g per plant)	154.04a	92.07a	22.55
Shoot fresh weight except leaves (g per plant)	107.33a	64.92a	30.96
Root dry weight (g per plant)	3.16a	1.08a	72.72
Leaf dry weight (g per plant)	13.83a	9.02a	26.72
Shoot dry weight except leaves (g per plant)	8.33a	5.16a	39.92
Total fresh weight (g per plant)	293.98a	179.44a	27.65
Total dry weight (g per plant)	25.31a	15.26a	34.47

[1]Means of each parameter followed by equal letters, in the rows, do not differ by LSD test, at 5% probability. [2]According to the International Union for the Protection of New Varieties of Plants (2004): 3, weak; 5, intermediate; and 7, strong. [3]According to TC Resmi Gazete (1998): 3, acute; 5, rounded; and 7, obtuse.

Table 2. Values of seedling and blade parameters of nontransgenic 'Crimson Sweet' (CS) watermelon and T1 progeny with transgene ZYMV-CP (T1)[1].

Parameter (cm)	Genotype		CV
	CS	T1	(%)
Hypocotyl length	5.40b	6.59a	5.21
Cotyledon width	1.77a	1.47b	3.82
Cotyledon length	2.67a	2.23b	6.02
Leaf blade width	13.52b	14.57a	2.62

[1]Means of each parameter, followed by equal letters in the rows, do not differ by LSD test, at 5% probability.

transgenic T2 individuals were 7.96 and 10.88% lower than those of control individuals. Stripe color a* value of the nontransgenic control fruit was higher than that of transgenic T2.

Regarding differences between transgenic watermelon line with nontransgenic parental cultivar CS, only four of the 36 characteristics investigated in the first experiment were significant. In the second experiment, only seven out of 80 were significant. The differences between transformed and nontransformed genotypes, with regard to cotyledon width and length or leaf blade width, were not obvious, since the differences were significant in the first experiment (Table 2), but not in the second (Table 3 and 5). This may be attributed to the difference in environmental conditions between the growing seasons (winter-spring and summer-autumn), when the experiments were carried out. It has been reported that three profiling technologies were used to compare two transgenic maize lines with the respective control line, and the effect of growing conditions as an additional environmental effect was also evaluated by comparing the Bt-maize line with the control line, in three different locations in one growing season (Barros et al., 2010). The authors have concluded that environmental factors caused more variation in different transcript/protein/metabolite profiles than the genotypes.

In both experiments, in the present work, hypocotyl length of transgenic watermelon seedlings was significantly higher than that of the nontransgenic parental ones (Table 2 and 5). In the second experiment, the differences between transgenic and nontransgenic

Table 3. Values of stem, branch, leaf, and flower parameters of nontransgenic 'Crimson Sweet' (CS) watermelon and T2 progeny with transgene ZYMV-CP (T2)[1].

Parameter	Genotype		CV
	CS	T2	(%)
Stem thickness (mm)	8.49a	8.75a	5.05
Main stem length (cm)	218.17a	219.92a	2.49
Node numbers in the main stem	30.50a	30.46a	5.66
Branch number	11.38a	10.92a	3.99
Degree of secondary lobing in leaf blade[2]	5.79a	5.96a	3.88
Leaf blade length (cm)	11.18a	11.18a	2.89
Leaf blade width (cm)	10.85a	10.92a	4.10
Leaf blade color L* value	42.42a	42.16a	1.45
Leaf blade color a* value	-16.94a	-17.18a	-15.35
Leaf blade color b* value	19.18a	19.41a	21.99
Leaf blade color C* value	25.62a	25.94a	19.05
Leaf blade h° value	131.88a	132.04a	1.52
Leaf area (cm^2 per plant)	7,500.00a	4,516.00a	22.69
Petiole length (cm)	6.96a	7.04a	6.55
Time of male flowering (day)	32.25a	32.29a	1.32
Node number of the first male flower	10.33a	11.00a	10.12
Time of female flowering (day)	32.96a	33.21a	1.12
Node number of the first female flower	14.67a	15.00a	5.50
Petal height of female flower (mm)	10.70a	10.90a	3.92
Petal apex shape of female flower[3]	5.71a	5.42a	3.04
Petal width of male (mm)	19.60a	19.04a	4.05
Pollen viability (%)	92.60a	93.53a	1.27
Pollen germination (%)	68.12a	78.57a	7.50
Ovary length (mm)	2.95a	2.97a	3.76
Ovary width (mm)	3.40a	3.43a	4.44
Pubescence of ovary[2]	5.17a	5.25a	7.06

[1]Means followed by equal letters, in the rows, do not differ by LSD test, at 5% probability. [2]According to the International Union for the Protection of New Varieties of Plants (2004): 3, weak; 5, intermediate; and 7, strong. [3]According to TC Resmi Gazete (1998): 3, acute; 5, rounded; and 7, obtuse.

Table 4. Values of fruit parameters of nontransgenic 'Crimson Sweet' (CS) watermelon and T2 progeny with transgene ZYMV-CP (T2)[1].

Fruit parameter	Genotype		CV
	CS	T2	(%)
Shape[2]	2.52a	2.65a	6.09
Width (cm)	13.24a	13.24a	3.81
Length (cm)	14.52a	13.32a	4.68
Weight (kg)	1.42a	1.24a	9.86
Shape of apical part[3]	3.67a	3.77a	6.84
Shape of basal part[3]	3.54a	3.69a	6.30
Peduncle diameter (mm)	7.44a	7.65a	1.97
Size of insertion of peduncle (mm)	15.82a	16.07a	0.92
Ground color L* value	66.04a	64.87a	2.52
Ground color a* value	-16.27a	-18.07a	-5.36
Ground color b* value	26.06a	28.27a	3.61
Ground color C* value	30.78a	33.23a	4.35
Ground color h° value	122.00a	122.62a	0.66
Stripe color L* value	41.65a	41.23a	2.91
Stripe color b* value	20.90a	23.25a	5.39
Stripe color C* value	26.58a	29.30a	4.57
Stripe color h° value	128.49a	127.67a	0.73
Flesh color L* value	42.70a	42.46a	4.33
Flesh color b* value	14.94a	16.00a	6.38
Flesh color C* value	23.45a	25.24a	3.45
Flesh color h° value	39.44a	39.21a	3.54
Malic acid (%)	0.1477a	0.1483a	1.32
Total soluble solid (°Brix)	7.17a	7.62a	7.41
pH	5.14a	5.16a	0.52

[1]Means of each parameter, followed by equal letters in the rows, do not differ by LSD test, at 5% probability. [2]According to the European Cooperative Programme for Plant Genetic Resources (2008): 1, flattened; 2, round; 3, broad elliptical; 4, elliptical; 5, pyriform; and 6, oblong. [3]According to TC Resmi Gazete (1998) and the International Union for the Protection of New Varieties of Plants (2004): 1, flat; 2, flat to rounded; 3, rounded; 4, rounded to conical; and 5, conical.

individuals were significant, concerning fruit rind thickness, flesh firmness, fruit peduncle length, size of pistil scar, and fruit flesh or stripe color a* values (Table 5). The variation in size of pistil scar in the transgenic line was undesirable. However, variations in some characteristics of the transgenic genotype were favorable, since they were related to fruit with less rind thickness, more flesh firmness and higher flesh color a* value. The differences between the transgenic watermelon line and its nontransformed originating cultivar may be a result of pleiotropic effect of transgenes, as reported by Montero et al. (2011).

Our results are consistent with previous publications available in the literature indicating high similarity of the transgenic genotypes to their nontransgenic counterparts. Jiao et al. (2010) have revealed that the transgenic papaya composition showed great similarity to the nontransgenic cultivar. Venneria et al. (2008) showed that nutritional values of transgenic genotypes were similar to nontransgenic control cultivars of wheat, corn, and tomato. No significant differences were reported between transgenic (with ZYMV-CP) and control melon genotypes, with regard to L-ascorbic acid, malic acid, citric acid, sucrose, glucose, fructose (Yalçin-Mendi et al., 2010). However, these authors have shown that fruit total acidity was significantly different in transgenic and control melon genotypes. According to Baudo et al. (2006) and Shewry et al. (2007), the genetic modification has a lower effect on the transcriptome of the wheat grain compared to traditional breeding. Coll et al. (2009) showed that the gene expression profiles of maize (MON810) and comparable non-genetically modified maize varieties cultured in the field are more similar than those of conventional lines.

Some reports in the literature indicate that there are great similarities between transgenic genotypes transformed with one or two intended transgenes, and nontransgenic parental lines (Baudo et al., 2006; Shewry et al., 2007; Venneria et al., 2008; Coll et al., 2009; Barros et al., 2010). However, when more than two intended genes or transcriptional factor genes are transferred to a plant genome, the similarity of transformed and nontransformed counterpart may not be so great, what demands a careful study, as reported by Batista et al. (2008). Based on the results reported here, it is not possible to conclude that all transgenic and nontransgenic crops are very similar; however, it is possible to produce transgenic genotypes which have a great similarity with their originating conventional cultivars.

Table 5. Values for parameters of seed, fresh and dry weight, total yield, seedling and fruit of nontransgenic 'Crimson Sweet' (CS) watermelon and T2 progeny with transgene ZYMV-CP (T2)[(1)].

Parameter	Genotype		CV
	CS	T2	(%)
Number of seeds per fruit	66.86a	46.02a	38.40
Seed mean weight (mg per seed)	29.57a	28.32a	6.23
Root fresh weight (g per plant)	29.70a	33.45a	31.12
Leaf fresh weight (g per plant)	157.36a	135.55a	18.47
Shoot fresh weight except leaves (g per plant)	225.95a	219.32a	16.60
Root dry weight (g per plant)	1.60a	1.37a	28.99
Leaf dry weight (g per plant)	13.17a	12.64a	24.63
Shoot dry weight except leaves (g per plant)	13.47a	12.20a	27.04
Total fresh weight (g per plant)	413.01a	388.31a	18.32
Total dry weight (g per plant)	28.24a	26.22a	24.36
Total yield (kg m^{-2})	1.90a	1.68a	10.53
Cotyledon width (cm)	1.71a	1.77a	3.44
Cotyledon length (cm)	2.14a	2.38a	4.81
Hypocotyl length (cm)	4.94b	5.35a	2.64
Fruit rind thickness (mm)	7.79a	7.17b	3.08
Fruit flesh firmness (Newton)	7.23b	7.75a	2.13
Fruit peduncle length (cm)	2.39a	2.13b	3.57
Size of pistil scar (mm)	6.50b	7.26a	4.64
Fruit flesh color a* value	18.02b	19.49a	1.58
Fruit stripe color a* value	-16.39a	-17.82b	-3.57

[(1)]Means of each parameter, followed by equal letters in the rows, do not differ by LSD test, at 5% probability.

Conclusions

1. Transferring *Zucchini yellow mosaic virus* coat protein (ZYMV-CP) gene to watermelon cultivar Crimson Sweet (CS) via *Agrobacterium tumefaciens* affects only a few traits from the 80 investigated ones.

2. Changes in rind thickness, flesh firmness, and flesh color a* value are favorable, while size of pistil scar is undesirable.

3. The transgenic watermelon line containing ZYMV-CP gene and its parental cultivar CS are very similar.

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