JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Assessment of the Nutritional Values of Genetically Modified Wheat, Corn, and Tomato Crops

EUGENIA VENNERIA,* SIMONE FANASCA, GIOVANNI MONASTRA, ENRICO FINOTTI, ROBERTO AMBRA, ELENA AZZINI, ALESSANDRA DURAZZO, MARIA STELLA FODDAI, AND GIUSEPPE MAIANI

Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione, 00178 Roma, Italy

The genetic modification in fruit and vegetables could lead to changes in metabolic pathways and, therefore, to the variation of the molecular pattern, with particular attention to antioxidant compounds not well-described in the literature. The aim of the present study was to compare the quality composition of transgenic wheat (*Triticum durum* L.), corn (*Zea mays* L.), and tomato (*Lycopersicum esculentum* Mill.) to the nontransgenic control with a similar genetic background. In the first experiment, Ofanto wheat cultivar containing the tobacco *rab1* gene and nontransgenic Ofanto were used. The second experiment compared two transgenic lines of corn containing *Bacillus thuringiensis* "Cry toxin" gene (PR33P67 and Pegaso Bt) to their nontransgenic forms. The third experiment was conducted on transgenic tomato (*Lycopersicum esculentum* Mill.) containing the *Agrobacterium rhizogenes rolD* gene and its nontransgenic control (cv. Tondino). Conventional and genetically modified crops were compared in terms of fatty acids content, unsaponifiable fraction of antioxidants, total phenols, polyphenols, carotenoids, vitamin C, total antioxidant activity, and mineral composition. No significant differences were observed for qualitative traits analyzed in wheat and corn samples. In tomato samples, the total antioxidant activity (TAA), measured by FRAP assay, and the naringenin content showed a lower value in genetically modified organism (GMO) samples (0.35 mmol of Fe^{2+} 100 g^{-1} and 2.82 mg 100 g^{-1}, respectively), in comparison to its nontransgenic control (0.41 mmol of Fe^{2+} 100 g^{-1} and 4.17 mg 100 g^{-1}, respectively). On the basis of the principle of substantial equivalence, as articulated by the World Health Organization, 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.

KEYWORDS: GMO; nutritional values; antioxidants; total antioxidant activity; carotenoids; mineral composition

INTRODUCTION

The concept of risk assessment of genetically modified organisms (GMOs) was first discussed at the Asilomar Conference in 1975. This concept that allows for the comparison of a final product to one having an acceptable standard of safety is an important element of a GM food safety assessment. This principle was elaborated by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and Organization for Economic Cooperation and Development (OECD) in the early 1990s and referred to as "substantial equivalence". The principle suggests that GM foods can be considered as safe as conventional foods when key toxicological and nutritional components of the GM food are comparable, "substantially equivalent", to the conventional food (within naturally occurring variability) and when the genetic modification itself is considered safe (*1*). Quoting the OECD publication, the concept "embodies the idea that existing organisms used as food, or as a source of food, can be used as the basis for comparison when assessing the safety of human consumption of a food or food component that has been modified or is new" (*2*). Currently, the substantial equivalence in food is tested by chemical analysis of known macro/micronutrients (*3*).

Genetic engineering could result in potential toxicity, possible antibiotic resistance from GM crops, potential allergenicity and carcinogenicity from consuming GM foods, and alteration in nutritional quality of foods, in particular regarding the composition of critical macro-, micro-, and antinutrients. Foreign genes might alter the nutritional value of foods in unpredictable ways by decreasing levels of some nutrients while increasing levels of others. This will cause a difference between the traditional strain and the GM counterpart (*4*).

* To whom correspondence should be addressed. Fax: +390651494550. E-mail: venneria@inran.it.

10.1021/jf8010992 CCC: $40.75
Published on Web 09/10/2008

Table 1. Comparison of Fatty Acid Composition (Expressed as %) of Conventional and GM Ofanto Wheat Cultivars Containing the Tobacco *rab1* Gene[a]

experiment	treatment	palmitic (%)	stearic (%)	oleic (%)	linoleic (%)	sat/insat (%)
Ofanto	conventional	13.3 ± 2.8	2.2 ± 0.5	25.0 ± 11.8	59.5 ± 9.5	0.18
	GMO	12.0 ± 4.1	2.3 ± 0.1	23.8 ± 3.5	62.0 ± 1.4	0.17
significance		ns	ns	ns	ns	ns

[a] The values are expressed as mean ± standard deviation (SD) of nine replicates. ns = nonsignificant.

In addition, there is a lack of evidence regarding the substantial equivalence of antioxidant bioactive compounds, such as carotenoids, polyphenols, and total antioxidant activity.

In relation to the hypothesis that genetic modification in fruit and vegetables could lead to changes in metabolic pathways and, therefore, to the variation of molecular pattern, with particular attention to antioxidant compounds, we carried out a preliminary study to characterize nutritional parameters of further interest with respect to those commonly analyzed and not well-described in the literature. Those parameters have been used, subsequently, as markers to assess substantial equivalence between traditional products and the GM counterpart.

The overall purpose of this work is to define and quantify possible changes in nutritional values of GM products compared to their nontransgenic controls. We focused our attention on three different crops cultivated more in Italy and the Mediterranean area: wheat, corn, and tomato plants.

Conventional and genetically modified crops were analyzed for fatty acids content, total phenols, polyphenols, carotenoids, total antioxidant activity, and mineral composition.

MATERIALS AND METHODS

Plant Materials and Experimental Setup. In this study, the nutritional composition of transgenic plants were compared to a nontransgenic control, which were grown in similar growing conditions. Transgenic and nontransgenic plants were of the same genetic background. Three experiments were carried out during the 2005 growing season on three typical species of the Mediterranean area: wheat, corn, and tomato.

Experiment 1. Wheat (*Triticum durum* L.) samples were collected from field trials conducted at the Experimental Institute for Cereals, Foggia, Italy. The Ofanto cultivar containing the tobacco *rab1* gene that codifies for a GTP-binding protein (*5*) was compared to its isogenic line in a complete randomized block design with four replicates.

Experiment 2. Corn (*Zea mays* L.) samples were used. GM events PR33P67 and Pegaso Bt are derived from Monsanto's MON 810 corn. Both contain the *Bacillus thuringiensis* "Cry toxin" gene for insect resistance, especially lepidopteran. The experiment (PR33P67) was conducted in a field of 4 ha, situated at the INRAN experimental farm of Landriano, Italy. PR33P67 and PR33P66 were provided by Seeds Emporda, S.L. in Girona, Spain, and Pegaso BT samples were provided by Advanta Seeds Industry in Saragoza, Spain. Treatments were arranged in a randomized complete block design with four replicates. The experimental unit consisted of plots of 3600 m^2.

Experiment 3. Transgenic tomato (*Lycopersicum esculentum* Mill.) containing the *Agrobacterium rhizogenes rolD* gene for increasing the resistance to pathogens and yield (*6*) was derived from Tondino cultivar. The experiment was carried out at the experimental farm of Agrobios, Metaponto, Italy. A complete randomized block experiment with four replicates was used to compare the transgenic tomato to its control cv. Tondino. The crops management followed commercial practices used in South Italy.

Molecular Analysis of Samples. DNA was extracted from wheat and maize samples using a CTAB-based protocol. Wheat and corn seeds were powdered in a Waring blender, and 2 g were processed for DNA extraction. Potential cross-contamination between GMO and control flours was checked by real-time polymerase chain reaction (PCR) using as standards the genomic DNA extracted from single GM and control seeds (equivalent to 100 and 0% GMO, respectively). Primers, TaqMan probes, and PCR conditions were those reported by Kuribara et al. (*7*) for maize and Terzi et al. (*8*) for wheat. Cross-contamination was less than 1% in all wheat and maize samples.

RolD and control isogenic tomato samples were confirmed by means of PCR using as a template the genomic DNA extracted from leaves of singular fruit clusters before processing into pulp. In this case, DNA was extracted with the DNeasy Plant Mini Kit (Qiagen, Milan, Italy), and primers and PCR conditions were those reported by Mauro et al. (*9*).

Chemicals and Standards. The organic solvents used for the separation of carotenoids, α-tocopherol, ascorbic acid, and polyphenols were of high-performance liquid chromatography (HPLC) grade and purchased from Carlo Erba, Milan, Italy. Other organic solvents and chemicals used in the extraction procedures were of analytical grade (Sigma). For standard regression lines, pure standards purchased from Sigma were used.

Compositional Analyses. Compositional analyses were conducted to measure the fatty acid composition, unsaponifiable fraction of antioxidants, total phenols, polyphenols, vitamin C, carotenoids, antioxidant activity of the hydrophilic and lipophilic fractions, and finally, the mineral content.

Extraction and Quantification of Fatty Acids. The fatty acids composition was extracted and detected according to the EU regulations (*10*). The transmethylated samples were performed in duplicate, and 2 μL of each sample solute in *n*-hexane were injected into a capillary gas chromatograph Hewlett-Packard (Hewlett-Packard, Palo Alto, CA)

Table 2. Comparison of Carotenoids (Lutein and Zeaxantin, Expressed as μg 100 g^{-1}), Total Phenols (Expressed as mg 100 g^{-1}), Hydrophilic Antioxidant Activity (Measured by FRAP Assay and Expressed as mmol of Fe^{2+} 100 g^{-1}), Lypophilic Antioxidant Activity (Measured by the Crocin Bleaching Inhibition Method and Expressed as K_a/K_c) of Conventional and GM Ofanto Wheat Cultivars Containing the Tobacco *rab1* Gene[a]

experiment	treatment	lutein and zeaxanthin (μg 100 g^{-1})	total phenols (mg 100 g^{-1})	FRAP (mmol of Fe^{2+} 100 g^{-1})	K_a/K_c
Ofanto	conventional	77.4 ± 11.8	7.3 ± 1.0	0.52 ± 0.15	0.40 ± 0.19
	GMO	61.3 ± 12.8	5.9 ± 0.3	0.44 ± 0.12	0.36 ± 0.13
significance		ns	ns	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns = nonsignificant.

Table 3. Comparison of Unsaponifiable Fraction (Expressed as %) of Conventional and GM Ofanto Wheat Cultivars Containing the Tobacco *rab1* Gene[a]

experiment	treatment	squalene (%)	α-tocoferol (%)	campesterol (%)	β-sitosterol (%)
Ofanto	conventional	4.5 ± 1.0	9.9 ± 7.9	33.7 ± 1.8	51.9 ± 6.8
	GMO	4.2 ± 1.5	8.4 ± 3.3	34.3 ± 1.2	53.0 ± 3.1
significance		ns	ns	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns = nonsignificant.

Table 4. Comparison of Mineral Composition (P, K, Ca, and Mg, Expressed as mg 100 g^{-1} of DW) of Seeds from Conventional and GM Ofanto Wheat Cultivars Containing the Tobacco *rab1* Gene[a]

experiment	treatment	P (mg 100 g^{-1})	K (mg 100 g^{-1})	Ca (mg 100 g^{-1})	Mg (mg 100 g^{-1})
Ofanto	conventional	334.5 ± 7.9	492.6 ± 8.8	31.9 ± 6.8	161.9 ± 6.8
	GMO	333.9 ± 4.3	498.1 ± 9.2	33.0 ± 3.1	163.0 ± 3.1
significance		ns	ns	ns	ns

[a] The values are expressed as mean ± SD of four replicates. ns = nonsignificant.

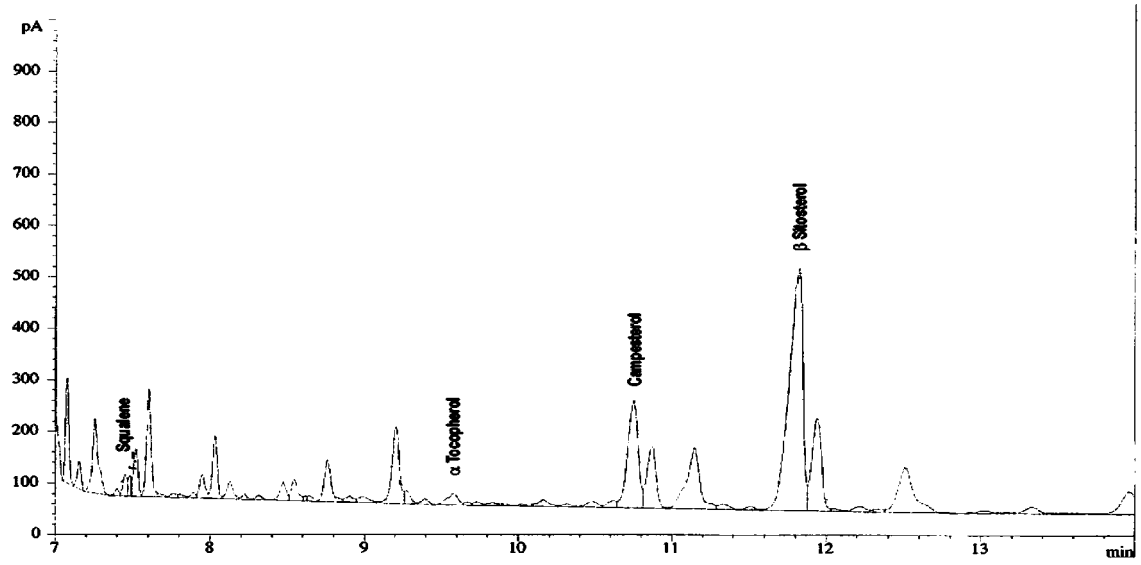

Figure 1. Representative chromatogram of the unsaponifiable fraction of conventional Ofanto wheat cultivar. The unnamed peaks have to be considered unknown.

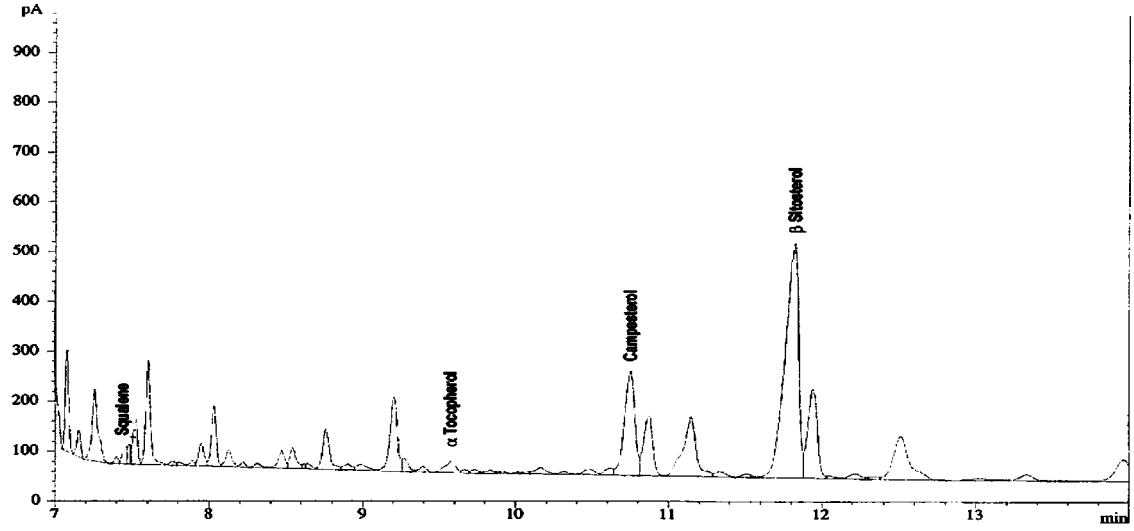

Figure 2. Representative chromatogram of the unsaponifiable fraction of GM Ofanto wheat cultivar containing the tobacco *rab1* gene. The unnamed peaks have to be considered unknown.

Table 5. Comparison of Fatty Acid Composition (Expressed as %) of Conventional and GM Corn Cultivars Containing the "Cry Toxin" Gene[a]

experiment	treatment	palmitic (%)	stearic (%)	oleic (%)	linoleic (%)	sat/insat (%)
Pegaso	conventional	11.21 ± 1.29	1.76 ± 0.21	29.07 ± 0.42	57.97 ± 1.75	0.15
	GMO	8.65 ± 1.42	1.53 ± 0.32	30.76 ± 1.19	59.06 ± 1.85	0.11
significance		*	ns	ns	ns	ns
PR33P66	conventional	8.09 ± 0.19	0.66 ± 0.02	26.06 ± 0.50	64.35 ± 0.05	0.09
PR33P67	GMO	7.88 ± 0.41	0.66 ± 0.01	24.72 ± 0.05	65.57 ± 0.46	0.09
significance		ns	ns	*	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns, nonsignificant; *, significant at $p \leq 0.01$.

equipped with a flame ionization detector (FID). A capillary column SP-2330 (Supelco), 30 m × 0.32 mm × 0.2 μm, was used. The column temperature was held at 50 °C for 0.5 min, then raised to 160 °C at 10 °C/min, held for 0.5 min, and then raised at 250 °C at 2 °C/min; the detector temperature was 250 °C; and the injector temperature was 220 °C. Helium was used as a carrier gas at a 2 mL/min flow rate. The results were elaborated by comparison to a fatty acids standard chromatogram.

Extraction and Quantification of Unsaponifiable Fraction. The unsaponifiable fraction of each oil was extracted and analyzed by capillary gas chromatography (GC) (*11*). The amount of squalen, α-tocopherol, β-sitosterol, and campesterol, for each sample, has been expressed as the percentage of the total area of the peak recorded and compared to a standard mixture.

Extraction and Quantification of Total Phenols. The extraction of total phenols in corn and wheat were performed according to the methods used by Halvorsen (*12*), with some modifications. Samples were extracted into 80% aqueous methanol (5 mL/g), mixed for 3 min, and centrifugated at 3600 rpm at 4 °C for 5 min. The extraction was repeated twice into 50% aqueous methanol, and the supernatants were combined and analyzed. The amount of total phenolics was determined with the Folin−Ciocalteu reagent, using a modified method of Coseteng

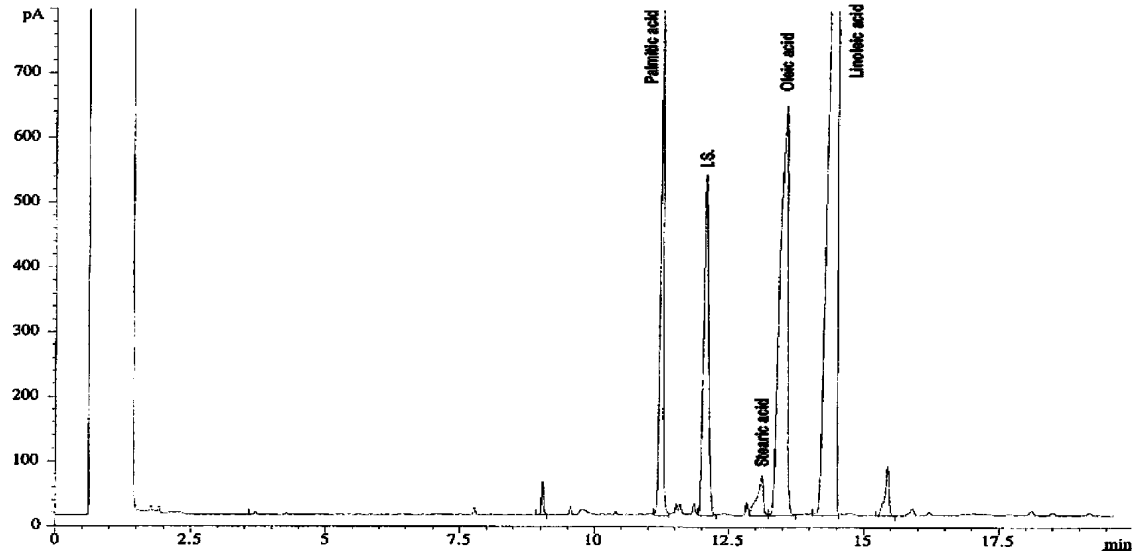

Figure 3. Representative chromatogram of the fatty acid of coventional Pegaso corn cultivar. IS = internal standard. The unnamed peaks have to be considered unknown.

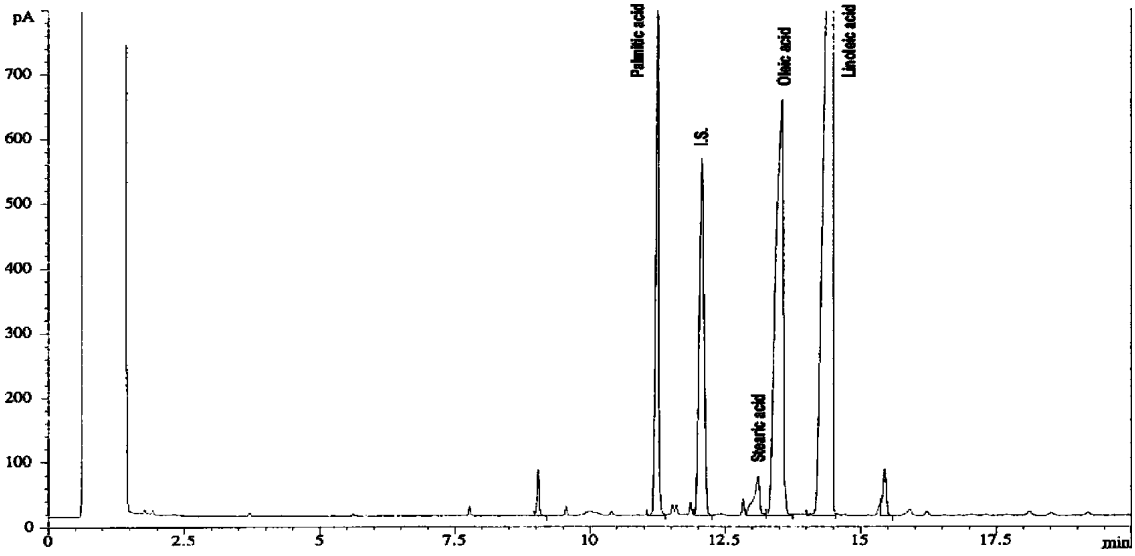

Figure 4. Representative chromatogram of the fatty acid of GM Pegaso corn cultivar containing the "Cry toxin" gene. IS = internal standard. The unnamed peaks have to be considered unknown.

Table 6. Comparison of Carotenoids (Lutein and Zeaxantin and β-Criptoxantina, Expressed as μg 100 g^{-1}), Total Phenols (Expressed as mg 100 g^{-1}), Hydrophilic Antioxidant Activity (Measured by FRAP Assay and Expressed as mmol of Fe^{2+} 100 g^{-1}), Lypophilic Antioxidant Activity (Measured by the Crocin Bleaching Inhibition Method and Expressed as K_a/K_c) of Conventional and GM Corn Cultivars Containing the "Cry Toxin" Gene[a]

experiment	treatment	lutein and zeaxantin (μg 100 g^{-1})	β-criptoxantina (μg 100 g^{-1})	total phenols (mg 100 g^{-1})	FRAP (mmol of Fe^{2+} 100 g^{-1})	K_a/K_c
Pegaso	conventional	1268 ± 427	535 ± 43	6.69 ± 0.45	0.89 ± 0.05	0.05 ± 0.02
	GMO	1331 ± 216	516 ± 38	6.03 ± 0.16	0.89 ± 0.13	0.05 ± 0.03
significance		ns	ns	ns	ns	ns
PR33P66	conventional	1131 ± 187	393 ± 18	5.78 ± 0.52	1.22 ± 0.12	0.25 ± 0.06
PR33P67	GMO	1222 ± 201	376 ± 53	5.74 ± 0.62	1.12 ± 0.03	0.16 ± 0.05
significance		ns	ns	ns	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns = nonsignificant.

et al. (*13*). To 100 μL of each sample, 500 μL of Folin−Ciocalteau's reagent and 2.65 mL of water were added and incubated for 60 min at dark. The absorbance of all samples was measured at 640 nm using a Beckman DU 7400 spectrophotometer. Results were expressed as milligrams of caffeic acid equivalent per gram of dry weight.

Extraction and Quantification of Polyphenols. Phenolics were hydrolyzed to obtain total free forms and extracted as described by Hertog et al. (*14*). Briefly, polyphenols were extracted from 1 g of homogenized tomatoes with ethyl acetate after acidic hydrolysis with HCL−methanol (1:1, v/v) at 90 °C for 2 h. Quantitative analysis was performed using an ESA series (MODEL 580) of HPLC solvent delivery module, an ESA 5600 eight-channel coulometric electrode array detector, and an ESA coularray operating software that control the equipment and perform data processing (ESA, Chemsford, MA). A Supelcosil LC-18 column (25 × 4.6 cm, 5 μm) with a Perisorb Supelguard LC-18 (Supelco, Milan, Italy) was used. The volume injected was 30 μL. Chromatography was performed at 30 °C and 1 mL/min flow rate using 0.02 M sodium phosphate adjusted with 85% ortophosphoric acid to pH 2.8 (solvent A) and methanol (solvent B). The eluent flow rate was maintained at 1 mL min^{-1}, and the setting

Table 7. Comparison of the Unsaponifiable Fraction (Expressed as %) of Conventional and GM Corn Cultivars Containing the "Cry Toxin" Gene[a]

experiment	treatment	squalene (%)	α-tocoferol (%)	campesterol (%)	β-sitosterol (%)
Pegaso	conventional	5.09 ± 0.82	5.14 ± 2.55	31.51 ± 0.58	58.26 ± 2.62
	GMO	5.60 ± 1.02	3.80 ± 1.43	31.17 ± 0.34	59.42 ± 2.07
significance		ns	ns	ns	ns
PR33P66	conventional	6.53 ± 0.89	4.70 ± 1.96	31.18 ± 0.69	57.58 ± 2.03
PR33P67	GMO	5.50 ± 0.75	3.68 ± 0.39	34.69 ± 0.48	56.14 ± 1.21
significance		ns	ns	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns = nonsignificant.

Table 8. Comparison of Mineral Composition (Expressed as mg Kg^{-1} DW) of Seeds from Conventional and GM Corn Cultivars Containing the "Cry Toxin" Gene[a]

experiment	treatment	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
Pegaso	conventional	0.31 ± 0.03	0.36 ± 0.04	0.0052 ± 0.001	0.12 ± 0.03
	GMO	0.29 ± 0.02	0.38 ± 0.03	0.0048 ± 0.001	0.13 ± 0.04
significance		ns	ns	ns	ns
PR33P66	conventional	0.30 ± 0.04	0.37 ± 0.02	0.0050 ± 0.001	0.12 ± 0.03
PR33P67	GMO	0.33 ± 0.03	0.36 ± 0.04	0.0053 ± 0.001	0.15 ± 0.05
significance		ns	ns	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns = nonsignificant.

Table 9. Comparison of Carotenoids (Lycopene, β-Carotene, and Lutein, Expressed as μg 100 g^{-1}), Total Antioxidant Activity (Measured by FRAP and TRAP Assays and Expressed as mmol of Fe^{2+} 100 g^{-1} and mmol of Trolox 100 g^{-1}, Respectively) of Conventional and GM Tomato Cultivars Containing the *Agrobacterium rhizogenes rolD* Gene[a]

treatment	lycopene (μg 100 g^{-1})	β-carotene (μg 100 g^{-1})	lutein (μg 100 g^{-1})	FRAP (mmol of Fe^{2+} 100 g^{-1})	TRAP (mmol of trolox 100 g^{-1})
conventional	1079 ± 352	1281 ± 135	182 ± 64	0.41 ± 0.01	0.36 ± 0.02
GMO	1014 ± 280	1614 ± 355	150 ± 11	0.35 ± 0.02	0.36 ± 0.02
significance	ns	ns	ns	*	ns

[a] The values are expressed as mean ± SD of nine replicates. ns, nonsignificant; *, significant at $p \leq 0.01$.

potentials were set at 60, 120, 200, 340, 480, 620, 760, and 900 mV. The linear gradient was started at 13% solvent B, then increased to 40% within 13.5 min, then to 90% within 25.5 min, reaching the final conditions of 100% within 3 min later, then returned to 13% solvent B within 3 min, and maintained at that condition for 4 min.

Extraction and Quantification of Carotenoids. Corn, wheat, and tomato carotenoids were determined as described by Sharpless et al. (*15*). This method is used by the National Institute of Standards and Technology (NIST). Briefly, approximately 1 g of sample was combined with 3 mL pf tetrahydrofuran (THF) and 2.7 mL of methanol. The mixture was saponified for 30 min in a 40 °C water bath after adding 0.3 mL of a 40% (w/v) methanolic KOH solution, and then, 0.15 g of ascorbic acid was added to neutralize the KOH. The analytes were extracted with three 15 mL portions of hexane−diethyl ether (50 + 50). The organic phase was combined and evaporated under a stream of nitrogen, and the residue was redissolved in 10 mL of ethanol. For the analysis, 0.1 mL of the sample was evaporated and reconstituted in 0.5 mL of mobile phase (50% methanol, 45% acetonitrile, and 5% tetrahydrofuran). A total of 50 μL of reconstituted extract was injected on a Waters Nova Pack C18 column (3.9 × 150 mm), 4 μm, at a flow rate of 1 mL min^{-1}. The extracts were analyzed by a Perkin-Elmer ISS 200 series HPLC system. The eluents were methanol/acetonitrile/tetrahydrofuran (50:45:5).The peaks were detected with a variable spectrophotometric detector (Perkin-Elmer LC-95, Norwalk, CO) connected to a personal computer Pe Nelson mod 1020 (Perkin-Elmer). The detection wavelengths were 450 and 292 nm.

Extraction and Quantification of Vitamin C. Total ascorbic acid (AA + DHAA) was extracted and quantified by the HPLC system according to the method of Margolis et al. (*16*). Briefly, 1 g of sample was suspended in 5 mL of water. To the sample was added 1 mL of 0.5 mol/L dibasic potassium phosphate containing 100 g/L of 1,4-dithiothreitol (DTT). The sample was vortex-mixed for 15 s and kept at room temperature for 30 min, and 1 mL of acqueous MPA (400 g/L metaphosphoric acid) was added. After the suspension was centrifuged (1000*g* for 30 min at 5 °C), the clear phase was transferred to 1.8 mL vials. Chromatographic separation was carried on a 250 × 4.6 mm Capcell Pak NH_2 column (Shiseido, Tokyo, Japan), using ESA series HPLC, equipped with a eight-channel coulometric electrode array detector and an ESA coularray operating software that control the equipment and perform data processing (ESA, Chemsford, MA). The setting potential was 0, 100, 200, 300, and 400 mV. The column was equilibrated at 40 °C at a flow rate of 0.8 mL/min, with a solvent composed of 0.680 g of monobasic potassium phosphate, 200 mL of water, 800 mL of acetonitrile, and 7.5 mL of concentrated phosphoric acid. The injection volume was 30 μL.

Antioxidant Activity of the Lypophilic Fraction. The antioxidant capacity of each unsaponifiable fraction was estimated by the crocin bleaching inhibition method (*17*). This method is based on the crocin bleaching as a result of its oxidation by a source of radicals, 2,2′-azobis(2,4-dimethylvalero nitrile) (AMVN). This reaction can be monitored by recording, for 10 min, the corresponding decrease of absorbance at 443 nm. The reaction with the crocin alone gives us the bleaching rate V_0, and when an antioxidant or pseudo-antioxidant compound is added, it reacts with the free radicals and, as a consequence, the crocin bleaching rate (V_a) is reduced, according to the competitive reaction equation

$$\frac{V_o}{V_a} = 1 + \frac{K_a}{K_c}\frac{[\text{pseudo-antioxidant}]}{[\text{crocin}]}$$

where K_c and K_a are the respective absolute second-order constants. The slope K_a/K_c has been calculated by means of the [pseudo-antioxidant]/[crocin] versus V_0/V_a linear regression plot. Its value indicates the relative capacity (antioxidant capacity) of different molecules to interact with the ROO• radicals. Reactions were carried out at 40 °C. After AMVN was added in the toluene reaction solution, the bleaching rate of crocin was recorded after 10 min. Blanks without crocin were run to rule out spectral interferences between compounds and crocin. In the toluene reaction solution, containing 40 mM AMVN and 0.24 mM crocin, each single unsaponifiable fraction was tested. Each kinetic analysis was compared to kinetic crocin bleach containing only AMVN (with a bleaching rate of V_0) and used for the calculations according to the competitive reaction equation.

Antioxidant Activity of the Hydrophilic Fraction. The antioxidant activity was measured by total radical-trapping antioxidant parameters (TRAP) assays for tomatoes and by ferric reducing-antioxidant power (FRAP) for corn, wheat, and tomatoes. The extraction method followed the procedure reported by Pellegrini et al. (*18*) for tomatoes. Corn and wheat extracts for FRAP analyses were obtained according to the method of Halvorsen et al. (*12*), previously described.

To obtain tomato extracts, the edible portion of tomato was homogenized in a blender. A total of 1 g of the homogenized sample was extracted with 4 mL of water under agitation for 15 min at room temperature and centrifugated at 1000*g* for 10 min and the supernatant (water-soluble fraction) was collected. The extraction was repeated with 2 mL of water. The two supernatants were combined and used directly for the TRAP and FRAP assays. The residue was extracted using 4 mL of acetone under agitation for 15 min at room temperature and centrifugated at 1000*g* for 10 min, and the supernatant (liposoluble fraction) was collected. The extraction was repeated with 2 mL of acetone. The two supernatants were combined and used directly for the TRAP and FRAP assays. TRAP and FRAP values of tomatoes were obtained summing the values of the water- and liposoluble fractions. The TRAP was determined according to the method of Ghiselli et al. (*19*) that was based on the protection provided by antioxidants on the fluorescence decay of *R*-phycoerythrin (lag phase) during a controlled peroxidation reaction. Briefly, 120 μL of diluted tomato extract sample

Table 10. Comparison of Vitamin C and Phenolic Compounds (Caffeic Acid, Naringenin, Quercetin, Chlorogenic Acid, and Rutin) Contents of Conventional and GM Tomato Cultivars Containing the *Agrobacterium rhizogenes rolD* Gene[a]

treatment	vitamin C (mg 100 g^{-1})	caffeic (mg 100 g^{-1})	naringenin (mg 100 g^{-1})	quercetin (mg 100 g^{-1})	chlorogenic (mg 100 g^{-1})	rutin (mg 100 g^{-1})
conventional	37.29 ± 2.6	0.09 ± 0. 03	4.17 ± 0.86	0.23 ± 0.02	1.68 ± 0.17	1.68 ± 0.57
GMO	40.45 ± 1.3	0.10 ± 0.05	2.82 ± 0.49	0.25 ± 0.01	1.98 ± 0.57	1.18 ± 0.11
significance	ns	ns	*	ns	ns	ns

[a] The values are expressed as mean ± SD of nine replicates. ns, nonsignificant; *, significant at $p \leq 0.01$.

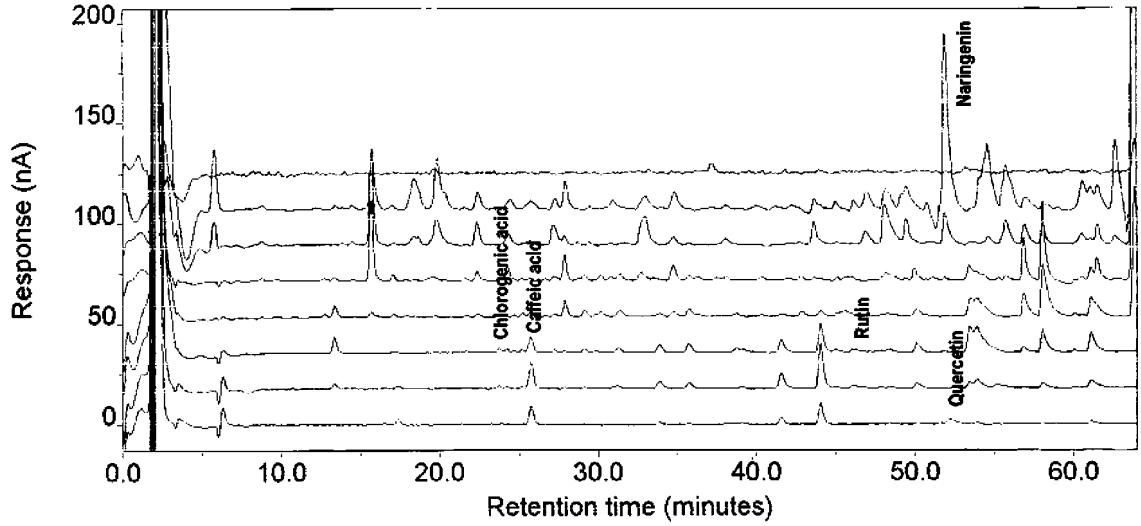

Figure 5. Representative chromatogram of phenolic compounds obtained after hydrolysis of the extract of coventional tomato cultivar. The unnamed peaks have to be considered unkown.

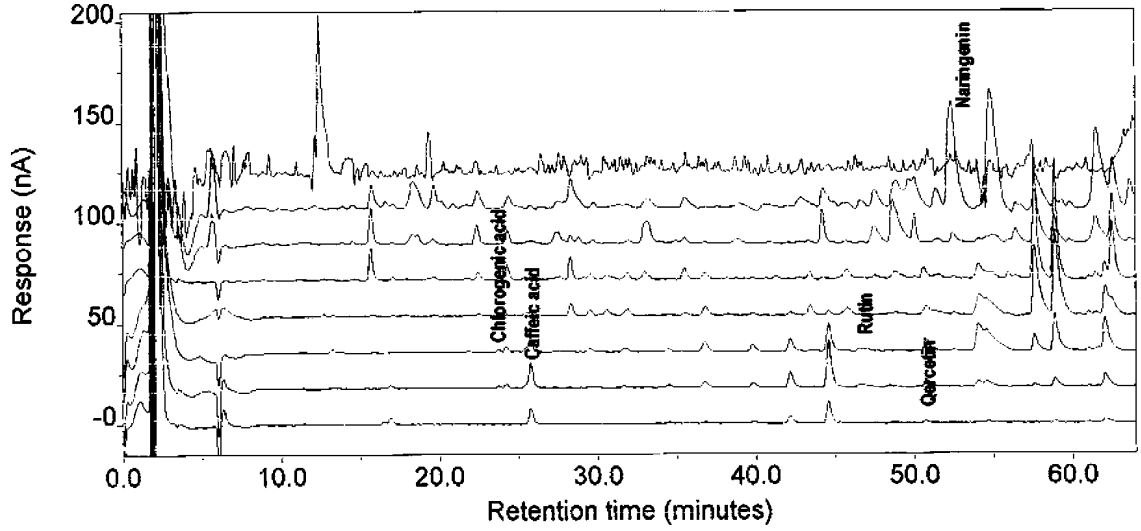

Figure 6. Representative chromatogram of phenolic compounds obtained after hydrolysis of the extract of GM tomato cultivar. The unnamed peaks have to be considered unkown.

Table 11. Comparison of Mineral Composition (Expressed as mg 100 g^{-1} DW) of Fruits from Conventional and GM Tomato Cultivars Tondino Containing the *Agrobacterium rhizogenes rolD* Gene[a]

treatment	N (mg 100 g^{-1})	P (mg 100 g^{-1})	K (mg 100 g^{-1})	Ca (mg 100 g^{-1})	Mg (mg 100 g^{-1})
conventional	2.20 ± 0.06	0.45 ± 0.06	3.41 ± 0.06	0.22 ± 0.02	0.41 ± 0.02
GMO	2.23 ± 0.03	0.50 ± 0.05	3.40 ± 0.03	0.20 ± 0.03	0.40 ± 0.03
significance	ns	ns	ns	ns	ns

[a] The values are expressed as mean ± SD of four replicates. ns = nonsignificant.

was added to 2.4 mL of phosphate buffer (pH 7.4) with 375 μL of double-distilled water and 75 μL of 2,2-azobis(2-amidinopropane)dihydrochloride (ABAP), and then the reaction kinetic was studied at 37 °C for 45 min by a Perkin-Elmer LS-50 B luminescence spectrometer. Antioxidant activity was expressed as equivalent millimolar 6-hydroxy-2,5,7,8-tetramethylchlorman-2-carboxylic acid (Trolox) per 100 g of fresh weight (FW).

The FRAP method followed that of Benzie and Strain (*20*) through the use of a Beckman DU 7400 spectrophotometer equipped with a thermostatically controlled cell-holder. The method is based on the reduction of the Fe^{3+}−2,4,6-tripyridyl-*s*-triazine (TPTZ) complex to ferrous at low pH. Briefly, 900 μL of working FRAP reagent prepared daily was mixed well with 30 μL of diluted sample, and the absorbance was recorded at 595 nm after 30 min of incubation at 37 °C. Antioxidant activity was expressed as equivalent millimolar Fe^{2+} per 100 g of FW.

Mineral Analysis. To obtain fruit dry matter (DM), the samples were dried in a thermo-ventilated oven at 80 °C for 72 h. Dry samples

were ground in a Wiley mill to pass through a 20-mesh screen and analyzed for N, P, K, Ca, and Mg. The nitrogen concentration was determined after mineralization of vegetative material with sulfuric acid by the Regular Bremmer method (*21*); P, K, Ca, and Mg were determined by the dry-ashing method at 400 °C for 24 h. Subsequently, ash was dissolved in HCl (1:25, v/v), and the supernatant was assayed using an inductively coupled plasma (ICP, Iris, Milano, Italy) emission spectrophotometer (*22*).

Statistical Analyses. Data were statistically analyzed by analysis of variation (ANOVA) using the SPSS software package (SPSS 12.0).

RESULTS AND DISCUSSION

The safety assessment of genetically modified crops has relied on a comparative approach that focuses on similarities and differences between the food and the feed derived from a genetically enhanced crop and its conventional counterpart.

Experiment 1: Nutritional Composition of Wheat. In this experiment, the nutritional composition of transgenic wheat Ofanto holding a mutant form of the tobacco *rab1* gene, was evaluated. Genetic manipulation of durum wheat by tobacco *rab-1* genes that codifies for a GTP-binding protein influences the trafficking of gluten proteins through the secretor system by up- or downregulating the transport step from the endoplasmic reticulum (ER) to the Golgi apparatus. This manipulation modifies functional properties of gluten protein (*5*). For some authors, the availability of transgenic wheat varieties, with high-quality low-content gluten, may be a useful alternative to the addition of wet gluten to improve end-use quality performance for certain food products. This may help curb the frequent resort to such a practice, which is known to underline the greater incidence of gluten intolerance or celiac disease recorded in the western world (*23*). The plant material used in this experiment lead to a high uniformly of plants for both transgenic samples and isogenic control within the experimental unit.

The levels of fatty acids in the wheat of transgenic Ofanto were similar to those observed in the grain of the nontransgenic control (**Table 1**). The fatty acids content was in the range reported in the literature, and it confirms the high content in wheat of linoleic acid (C18:2), 59.5% for conventional and 62.0% for GM, oleic acid (C18:1), 25 and 24% for conventional and GM event, respectively, and palmitic acid (C16:0), 13% for conventional and 12% for GM. A low level of stearic acid (C18:0) was observed (2.2 and 2.3% for conventional and GM, respectively), which is in accordance with published information (*24*).

The antioxidant activity is an important parameter in assessing the quality of products, and it is considered a new parameter to assess substantial equivalence. According to numerous studies, such antioxidant activity, as a measure of a global antioxidant system, appears to be closely related with the prevention of degenerative illness, such as different types of cancer, cardiovascular and neurological disease, and oxidative stress dysfunctions (*19, 25*). Besides vitamins A, C, and E, the most important natural occurring plant substances showing antioxidant activity are carotenoids and phenolic compounds, which, in different proportion and quantities, are to be found in cereals, fruit, and vegetables. Of these compounds, some are of a hydrophilic nature (e.g., ascorbic acid and phenols) and others are clearly lypophilic (e.g., carotenoids and α-tocopherol), and it is interesting to evaluate hydrophilic and lypophilic antioxidant activity.

The hydrophilic antioxidant activity of Ofanto transgenic wheat was similar to the nontransgenic control (0.44 versus 0.52 mmol of Fe^{2+} 100 g^{-1}), and the same trend was observed for lypophilic antioxidant activity (0.36 versus 0.4 K_a/K_c for GM and control, respectively), measured by the crocin bleaching inhibition method (**Table 2**).

These data showed that no correlation was observed between genetic manipulation and the antioxidant activity. Phenolic compounds represent the main water-soluble antioxidants in wheat and corn. The antioxidant activity is highly correlated to the total phenolic content measured by the Folin−Ciocalteu method. The effectiveness of carotenoids as antioxidants is also dependent upon their interaction with other coantioxidants, especially α-tocopherol and vitamin C (*26*).

The carotenoids content observed in wheat samples expressed as the sum of lutein and zeaxantin was not significantly different between the GM event and its control (77.4 ± 11.8 and 61.3 ± 12.8 μg 100 g^{-1} in conventional and GM events, respectively) and was in the range reported by Adom (*27*) that ranged from 26.41 to 170.5 μg 100 g^{-1}. The total phenols content in transgenic Ofanto wheat were comparable to those observed in the grain of the nontransgenic control (5.9 ± 0.3 and 7.3 ± 1.0 μg 100 g^{-1} in the GM event and its control, respectively) (**Table 2**).

α-Tocopherol is the most potent lipid-soluble antioxidant (*28*). The primary role of α-tocopherol in preventing free-radical-initiated peroxidative tissue damages is accepted by several investigators. Squalene is an intermediate of cholesterol metabolism and is secreted in human sebum, where it may protect the skin from ultraviolet radiation. Storm and colleagues (*29*) demonstrated the protective activity of squalene against radiation-induced injury in a mouse model.

The phytosterols play an important role in plant, especially in the membrane biology. Recent researches confirmed the biological functions of antioxidant activities of campesterol, stigmasterol, and sitosterol. Moreover, Finotti et al. (*17*) demonstrated that β-sitosterol has pro-oxidant activity, and it is important to study also these compounds to understand the balance between antioxidants and prooxidants compounds and, at list, their contribution to the total antioxidant activity.

These results demonstrate that the levels of unsaponifiable fraction (**Table 3**) and mineral content (**Table 4**) in the grains of GM Ofanto cultivar containing the tobacco *rab1* gene were comparable to those in the grains of the nontransgenic control. All of the results were in agreement with the literature data (*30, 31*). Representative chromatograms in **Figures 1** and **2** show the levels of the unsaponifiable fraction in conventional and GM Ofanto wheat cultivars.

Experiment 2: Nutritional Composition of Maize. Transgenic maize, *Zea mays* (L.), containing the endotoxin of *Bacillus thuringiensis* (Bt) subsp. *kurstaki*, was developed to control European corn borer (ECB), pests of maize (*32*). Infection may be associated with yield reduction (7−15% in Italy, 30% in Spain, 5−7% in Europe, and 9% in the rest of the world) (*33*), but could also lead to infections from *Fusarium*. Several *Fusarium* species are also able to produce mycotoxins, such as fumonisins, zearalenone, and trichothecenes, which may be dangerous for both human and animal health. The use of BT maize could carry the lessening of pesticides in Europe equal to 5.2 tons (*34*). In the current experiment, fusarium and others pathogens caused serious damage in conventional samples and the BT maize showed an higher yield, with an increasing of 13% compared to the isogenic control.

No significant differences for fatty acids content were observed between the GM event and its control (**Table 5**), and all of the values were in line with literature data (*35*).

Representative chromatograms in **Figures 3** and **4** show the levels of fatty acids in conventional and GM Pegaso corn cultivars.

The lutein and zeaxantin, β-criptoxantina, total phenols contents, and hydrophilic (measured by FRAP assay) and lypophilic antioxidant activity (measured by the crocin bleaching inhibition method) were not affected by the insertion of the "Cry toxin" gene in both cultivars (**Table 6**). The carotenoids contents measured in the current experiment were similar to those reported by Kurilich et al. (*36*). The insertion of the "Cry toxin" gene in both cultivars did not affect the squalene, α-tocoferol, campesterol, and β-sitosterol concentrations (**Table 7**).

No significant differences were observed for mineral concentrations (P, K, Ca, and Mg) between GM events (PR33P67 and Pegaso Bt) and their nontransgenic controls (**Table 8**). Similar trend was observed by Ridley and George in comparison studies between a glyphosate-tolerant and conventional corn and between rootworm-protected and conventional corn (*35*).

Experiment 3: Nutritional Composition of Tomato. Previous works have shown the importance of insertion of the *Agrobacterium rhizogenes rolD* gene in transgenic tomato (*Lycopersicum esculentum* Mill.) Tondino cultivar to increase the resistance to pathogens and yield (*6*). The *rolD* gene, coding for an ornithine cyclodeaminase, is involved in the biosynthesis of proline from ornithine. In accordance with Bettini et al. (*6*), the transgenic plants were found to flower earlier and showed an increased number of inflorescences and higher fruit yield. Moreover, the transformation of tomato plants with *rolD* could lead to an increased competence for defense response, as shown by toxin tolerance and increased expression of the systemic acquired resistance marker gene PR-1. The lycopene, β-carotene, lutein content, and total antioxidant activity (TAA) measured by the TRAP assay was not affected by the insertion of the *Agrobacterium rhizogenes rolD* gene (**Table 9**). The FRAP value was significantly higher in conventional tomato, and this could be attributed to a significant increment of naringenin content (**Table 10**).

Representative chromatograms in **Figures 5** and **6** show the levels of phenolic compounds obtained after hydrolysis of extracts of the conventional and GM tomato cultivars.

The carotenoids content measured in the current experiment were similar to those reported by several authors (*37*). The above-mentioned authors showed a lycopene content that ranged from 43 to 181 mg kg^{-1} and a β-carotene content in a range of 2.4−2.7 mg kg^{-1}. Abushita et al. (*37*) reported a lutein content that ranged from 77 to 338 μg 100 g^{-1} FW. No significant differences were observed for vitamin C, caffeic acid, quercetin, chlorogenic acid, and rutin content between the GM event and its control, while the naringenin content was significantly higher in the conventional sample (**Table 10**).

No significant differences were observed for mineral concentrations (N, P, K, Ca, and Mg) between the GM events and its nontransgenic control (**Table 11**), which is interesting from a nutritional point of view because fruits and vegetables usually contribute to 35, 24, and 11%, respectively, of the total K, Mg, and P of the dietary intake of humans (*38*).

Finally, in all experiments, it has been shown by targeted nutritional analysis that the genetic enhancement of conventional cultivar to produce GM events did not produce significant changes in any of nutritional and antioxidant components analyzed. On the basis of the principle of substantial equivalence as articulated by the World Health Organization, 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.

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Received for review April 7, 2008. Revised manuscript received June 11, 2008. Accepted July 22, 2008.

JF8010992