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Changes in Carotenoid and Ascorbic Acid Contents in Fruits of Different Tomato Genotypes Related to the Depletion of UV-B Radiation

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The aim of the present study was to investigate if the depletion of UV-B radiation affected the most representative carotenoids as well as the ascorbic acid content in tomato fruits, harvested at both breaker and firm red stages. To do this, three tomato genotypes, DRW 5981, HP 1, and Esperanza, were grown inside a greenhouse either covered with polyethylene transparent to UV-B or depleted of UV-B by a special covering film. The antioxidant properties of the fruits were evaluated on the water-insoluble fractions according to the ABTS method. UV-B effect on antioxidant activity was negligible in DRW and HP 1 genotypes, whereas it was detrimental in Esperanza at both ripening stages. This genotype seems to have a negligible capability of accumulating carotenoids and a great susceptibility to detrimental effects of UV-B; conversely, the DRW genotype shows high carotenoid levels under sunlight conditions and a further promotion by UV-B. On the other hand, the HP 1 mutant displays an intermediate behavior and represents the only genotype favored by UV-B with respect to ascorbic acid accumulation.

KEYWORDS: ABTS; antioxidant properties; ascorbic acid; carotenoids; fruits; greenhouse; *Lycopersicon esculentum*; tomato; UV-B

INTRODUCTION

In recent years the content of healthy compounds has become, for consumers, a crucial parameter of the quality of fruits and vegetables.

Recent epidemiological studies pointed out a positive correlation between the intake of fruits and vegetables and prevention of degenerative diseases as well as aging (*1*). The increasing attention to the nutritional and healthy value of vegetal foods is related to the inability of animal organisms to protect them against the oxidative process by endogenous resources. The antioxidant compounds, being easily oxidable, are able to prevent the cell injury caused by the active oxygen species and radicals formed during aerobic reactions or due to exogenous stress (*2*, *3*).

The most representative antioxidant compounds in tomato fruits are β-carotene and lycopene, the contents of which are affected by ripening stage. Indeed, β-carotene represents the precursor of vitamin A, which is essential to the diet of animals as an antioxidant, whereas lycopene is not present in all vegetables. Tomato and its byproducts represent the most important source of this antioxidant compound in the human diet. Lycopene has recently emerged as an efficient radical quencher, consequently capable of fighting the reactive oxygen species and of avoiding cell injury. Singlet oxygen is quenched by lycopene at a rate of almost twice that of β-carotene (*4*). In addition to its antioxidant properties, lycopene has also been shown to induce cell to cell communication and modulate hormonal, immune system, and other metabolic pathways, which may also be responsible for the beneficial effects (*5*, *6*).

These properties, in addition to the consideration that lycopene is one of the most present carotenoids in the diet of European and North American people, emphasize the nutritional importance of this compound.

β-Carotene is present in all tomato byproducts and plays a similar protective role. β-Carotene has been shown to protect lipids from free radical autoxidation and to be an effective quencher of singlet oxygen. It is generally believed that the simultaneous presence and synergic action of lycopene, β-carotene, and other antioxidant compounds are required for the prevention of degenerative diseases.

The limited caloric supply of tomato fruits and their great content in minerals, vitamins, and antioxidant compounds such as ascorbic acid, carotenoids, and flavonoids make the tomato

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fruit an ideal food according to modern nutritional opinions. Therefore, tomato can be considered to be a "functional food" because it is capable of providing additional physiological benefits as well as meeting basic nutritional requirements. The ripening stage of the tomato affects its antioxidant properties in terms of antioxidant compound content. During ripening, tomato fruit undergoes a wide range of biosynthetic as well as degradative reactions that dramatically affect the final fruit composition. These changes are highly coordinated, taking place in every subcellular compartment, and are regulated by plant hormones and modified by genetic and environmental factors (*7*). At the breaker stage no more than 10% of surface color is pink or red; at the firm red stage >90% of surface color shows red (*4*). The production of the red color of the ripe fruit is due to the degradation of chlorophyll and the high accumulation of carotenoids as lycopene and β-carotene as the chloroplasts are trasformed to chromoplasts (*7*). In addition to the genetic background of the plants, even growing conditions represent a pivotal factor that affects the antioxidant content of the tomato fruit. Light plays a fundamental role in determining the final content of carotenoids.

In leaf tissue it has been reported that in addition to light intensity, even the quality of light radiation is believed to be of main importance in carotenogenesis, through the action of phytochrome and UV receptors (*8*). Less attention has been paid to the role played by light quality on carotenogenesis in fruits (*9*). The carotenoid contents of tomato fruits produced in an open field, in a glasshouse, or in a plastic tunnel were compared (*10*); unfortunately, the experimental design does not consent to discriminate between the effects of light quantity, light quality, and temperature.

In the present research, three commercial tomato plants, DRW 5981, Esperanza, and the photomorphogenic mutant HP 1, were grown until complete fruit ripening inside two greenhouses where two different light conditions were used: one represented by the whole sunlight spectrum and the other deprived of the UV-B region (280−320 nm). The fruits harvested at two different ripening stages were analyzed to evaluate the influence of the genotype and UV-B radiation on their antioxidant properties, in terms of the most representative carotenoids β-carotene and lycopene and of ascorbic acid, which represents, together with phenolic compounds, the main water-soluble antioxidant of tomatoes. To obtain more insights on the influence of UV-B radiation on the synthetic pathway of carotenoids, the precursors phytoene and phytofluene were quantified as well.

MATERIALS AND METHODS

Chemicals. L-Ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and butylated hydroxytoluene (BHT) were purchased from Aldrich. Celite Filter Cel was purchased from Fluka. The solvents (HPLC grade) were all from Mallinckrodt Baker. Trichloroacetic acid (TCA), dithiothreitol (DTT), sodium hydroxide (NaOH), *N*-ethylmaleimide (NEM), and metaphosphoric acid were all from Sigma. For standard reference regression lines pure β-carotene and pure lycopene purchased from Sigma were used.

Spectrophotometric measurements were recorded using a UV−vis Shimadzu 2100 instrument equipped with a Peltier electronic temperature control and magnetic stirring.

Plant Material. Three tomato (*Lycopersicon esculentum*) genotypes were used: the hybrid Esperanza F1 (previously named DRW 3226) characterized by low lycopene content; the hybrid DRW 5981 (characterized by high lycopene content), kindly provided by De Ruiter Sementi; and the high pigment 1 (HP 1), a monogenic recessive mutant in Ailsa-Graig background characterized by higher anthocyanin and carotenoid contents in all tissues (*11*). Although the nature of the *hp* mutation is still unclear, it has been demonstrated that the HP 1 mutant shows exaggerated phytochrome responses; therefore, it was proposed that the HP 1 mutation is associated with an amplification step in the phytochrome−transduction chain (*12*−*14*).

Growth Conditions. The experiment was carried out in the spring and summer of 2001. Seeds of the three tomato genotypes were sown, in plug trays with a peat/perlite (3:1 v/v) medium, four times (each separated by 1 week), and the fruits were harvested at the same time at two ripening stages. Plants were grown in a heated glasshouse under natural light conditions, were watered daily, and received 20N−20P−20K, 1 g L^{-1} soluble fertilizer once a week. About 45 days after sowing, when the seedlings had reached the stage of four true leaves, the tomato plants were transplanted into pots containing the same medium and fertilized weekly with 20N−8P−16K. The tomato plants were kept in the glasshouse (UV-B-free) until a week before the breaker stage of the first truss of fruits, and then the plants were transferred, in May, into two greenhouses characterized by different light conditions. In the first greenhouse, covered by polyethylene film, the plants were subjected to the whole sunlight spectrum conditions, whereas in the second greenhouse covered by polyethylene film stabilized with UV-B absorber, the plants were kept in the absence of UV-B. Tomato harvesting was performed at the breaker and firm red stages (*4*), and the ripeness stage was characterized in accordance to the procedure reported by Grierson and Kader (*7*). For each genotype and each light condition five samples were collected. Tomatoes from each maturity stage had about the same size and weight. Whole fruits for each sample were frozen by liquid nitrogen and stored at −80 °C until analysis. Fresh tomatoes were homogenized, and chemical and biochemical parameters were determined separately on three groups of fruits at each ripening stage, consisting of 20 fruits chosen at random from each sample.

Analytical and Productive Parameter Determination. The following measurements were made on each sample: number of fruits for each branch (g), weight of fruits (g), weight of branch (g), pH of fruits, and soluble solids by refractometer (Atago) results reported as Brix degrees at 20 °C.

Extraction and Quantification of Ascorbic Acid. For the determination of ascorbic (AA) and dehydroascorbic (DHA) acids, tomato fruits were homogenized with liquid nitrogen and quartz sand, resuspended in 5% metaphosphoric acid (1:2.5 w/v), and centrifuged at 23700*g* for 20 min. An aliquot of supernatant was added to 10% TCA (w/v) and, after the addition of 5 M NaOH, the mixture was centrifuged at 14300*g* for 2 min. The quantitative determination was carried out according to the method of Okamura (*15*). For the total amount of AA + DHA, samples were incubated with 10 mM DTT, and then 0.5% of NEM was added. Samples were incubated at 37 °C for 60 min, and the absorbance was read at 525 nm.

Extraction and Quantification of Carotenoids. Carotenoids were extracted according to the method of Tonucci (*16*) with some modifications (*17*). Tomatoes were cut into quarters and homogenized in an Ultra-Turrax homogenizer. The homogenized sample was extracted with tetrahydrofuran (THF) stabilized with 0.01% BHT (*18*); magnesium carbonate for buffering the acidic environment and Celite (Celite Filter Cel, Fluka) as a filter aid were added to the homogenized sample, each at 10% of the weight of the sample. The THF extracts were combined, and the volume was reduced by two-thirds under vacuum at 35 °C on a rotary evaporator. Components of the combined extracts were portioned into dichloromethane (25 mL) and NaCl-saturated water (15 mL) in a separating funnel. The water layer was washed with dichloromethane until carotenoids were completely removed. The organic layers were combined, and the volume was reduced to ∼2−4 mL under vacuum at 35 °C. The residue was then filtered through a 0.22 μm filter and injected into the column (Phenomenex Prodigy LC-18 ODS, 250 × 4.6 mm, 5 μm with guard column Phenomenex AJO-4287 C-18 ODS).

The analytical separation of carotenoid extracts was achieved by HPLC CLASS M10 (Shimadzu) connected to a UV-DAD (SPD-M10A) (*19*). The mobile phases used for the determination were (A) constituted by acetonitrile, hexane, methanol, and dichloromethane (4:2:2:2) and (B) constituted by acetonitrile. The flow was 0.8 mL/min, and the gradient was linear.

Table 1. General Characteristics of the Fruits of the Three Hybrid Tomato Lines DRW 5981, HP 1, and Esperanza at Different Ripening Stages[a]

	control tunnel		no UV-B tunnel	
	breaker	well ripe	breaker	well ripe
	Line DRW 5981			
no. of fruits	3.6 ± 0.54 a	2.4 ± 0.54 b	4.2 ± 0.83 a	2.6 ± 1.34 b
wt of fruits, g	66.15 ± 31 a	61 ± 17.3 a	76.5 ± 21.1 a	71.3 ± 27.7 a
wt of branch, g	226.8 ± 74 a	151.2 ± 69 b	330.6 ± 143 a	175.9 ± 77 b
pH	4.07 ± 0.05 b	4.28 ± 0.04 a	4.02 ± 0.04 b	4.32 ± 0.06 a
refractive grade	5.4 ± 0.34 a	5.26 ± 0.46 a	5.66 ± 0.11 a	5.39 ± 0.92 a
	Line HP 1			
no. of fruits	6.6 ± 1.67 a	3 ± 0.70 b	7.4 ± 0.89 a	4.2 ± 1.64 b
wt of fruits, g	41.66 ± 7.2 a	49.12 ± 15.4 a	44.3 ± 6.7 a	36.1 ± 8.3 a
wt of branch, g	284.2 ± 105 a	150 ± 61.49 b	323 ± 27.6 a	103 ± 29.6 b
pH	4.04 ± 0.02 b	4.24 ± 0.01 a	4.12 ± 0.03 b	4.26 ± 0.08 a
refractive grade	4.93 ± 1 a	3.93 ± 0.11 a	5.4 ± 0.69 a	4.4 ± 1.4 a
	Line Esperanza			
no. of fruits	4.8 ± 1.3 ab	3.4 ± 0.89 c	5.6 ± 1.34 a	3.8 ± 0.44 bc
wt of fruits, g	90.1 ± 17.7 a	96.36 ± 31.7 a	76.5 ± 9.84 a	96.6 ± 4.85 a
wt of branch, g	433.9 ± 138 a	322.7 ± 107b	420 ± 65 ab	367 ± 47 ab
pH	4.013 ± 0.06 bc	4.13 ± 0.03a	3.99± 0.02 c	4.12 ± 0.04 a
refractive grade	4.86 ± 0.11 a	4.13 ± 0.11 b	4.46 ± 0.3 ab	4.4 ± 0.4 a

[a] Values shown are mean ± SD of five determinations. For each parameter and each line, different letters indicate significantly different values at $P = 0.05$, following two-way ANOVA.

Identification of the peaks in the HPLC chromatogram of the carotenoid extract from tomatoes was carried out by comparison of UV−vis spectra and retention times of eluted compounds with pure standard for lycopene and β-carotene at 450 nm, for phytofluene at 350 nm, and for phytoene at 290 nm (*20*). Furthermore, to quantify phytofluene and phytoene, their respective peak areas were compared with the ones of standard lycopene at known concentrations, established by the molar extinction coefficient in acetone reported in the literature and corrected by the molar extinction coefficient relative at each compound (*21*).

Antioxidant Activity. The ABTS method (*22*, *23*) was employed to assess the antioxidant activity of the water-insoluble fraction, with some modifications. The pulp resulting from centrifugation of tomato homogenate was extracted with 10 mL of CH_2Cl_2, centrifuged at 1500 rpm for 5 min (4 °C), and filtered, and the supernatant was recovered; this extraction step was repeated three times, and the supernatant fractions were pooled. The extract (water-insoluble fraction) was used for the test. The results are expressed as equivalent millimolar Trolox per 100 g of fresh tissue.

Statistics. Values shown in the figures are means of three replications, and the data were analyzed by two-way ANOVA to test the significance of the observed differences following the LSD test. The significance of the *F* ratio following the ANOVA analysis is reported in each figure: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns = not significant.

RESULTS

Productive and Chemical Parameters. The number, weight, pH, and soluble solids of the tomato fruits of each genotype determined at breaker and firm red stages did not differ with the light conditions (**Table 1**). On the other hand, the stage of ripening appeared as the only factor influencing the number of fruits and the weight of branch, which increased with the ripening of the fruits, and the pH, which, conversely, showed a trend to decrease, ranging from 3.99 to 4.32 °Brix.

Antioxidant Activity. The antioxidant activity of the water-insoluble fraction evaluated according to the ABTS assay changed depending on the genotype considered (**Figure 1**). The HP 1 mutant showed the greater antioxidant capability followed, in descending order, by the DRW 5981 and Esperanza lines. In these two latter genotypes, the ripening stage of the fruits

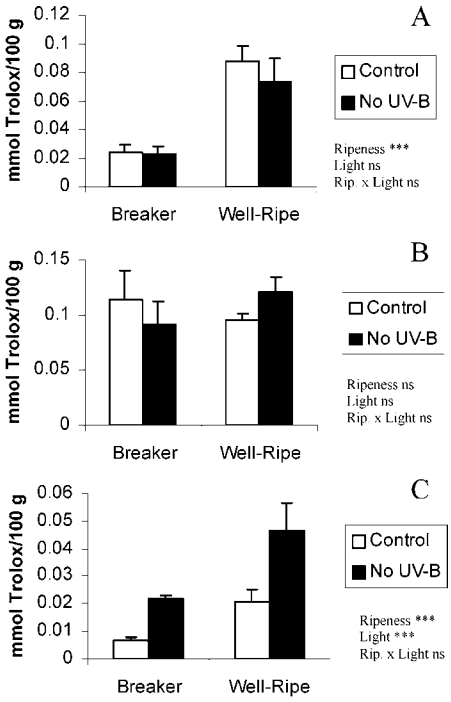

Figure 1. Antioxidant activity, expressed as millimoles of antioxidant reference Trolox/100 g of fresh weight, of the fruit of the three different tomato lines DRW 5981 (**A**), HP 1 (**B**), and Esperanza (**C**) at different ripening stages (breaker and well ripe) and different light conditions (with or without UV-B rays). For each tomato hybrid line the figure graphically presents the two-way ANOVA results according to Fischer's LSD test regarding the interaction between two factors of variation: ripening stage and light conditions. The SE is reported for each treatment. Significant differences between treatments are marked by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant.

dramatically affected the antioxidant capacity of the fruits. In fact, at the firm red stage their antioxidant activity was 3−4-fold greater than that at breaker stage. On the contrary, the ripening stage did not influence the antioxidant ability of HP 1 mutant, which showed already very high values at the breaker stage. The light conditions induced significant changes in the antioxidant capacity only in the Esperanza genotype, showing higher ABTS values in fruits ripened in a UV-B-free environment at both ripening stages (**Table 2**).

Ascorbic Acid. Ascorbic acid represents one of the main water-soluble antioxidants of tomatoes. In the fruits of each tomato genotype tested the reduced form of ascorbic acid (ASA) accounted for 77−99% of the total ascorbic acid content (AA) (**Figure 2**). The ANOVA analysis shows a significant influence of both light conditions and ripening stage on both ASA and AA content in all of the tomato lines. The interaction of the two factors was not significant only in the HP 1. Concerning the influence of the ripening stage on the accumulation of ascorbic acid, the breaker stage represents the growing phase at which the antioxidant compound showed higher values in comparison with the well-ripening one. In fact, the decrease of both the reduced ascorbic acid and total ascorbate was evident in all lines, DRW 5981 showing the highest reduction ranging from 61 to 66.9%. The depletion of UV-B radiation in the light growth conditions induced a drastic increase in the two forms of ascorbic acid in the DRW 5981 and Esperanza genotypes, whereas the HP 1 line showed an opposite behavior.

Quantification of Carotenoids in Tomato Fruits. The total carotenoid content of DRW 5981 and Esperanza fruits was significantly influenced both by the ripening stage and by the

Table 2. Antioxidant Activity and Carotenoid Components of Fruits of the Three Hybrid Tomato Lines DRW 5981, HP 1, and Esperanza at Different Ripening Stages[a]

	DRW 5981				HP 1				Esperanza			
	breaker		well ripe		breaker		well ripe		breaker		well ripe	
	control	no UV-B	control	no UV-B	control	no UV-B	control	no UV-B	control	no UV-B	control	no UV-B
antioxidant activity	0.02 ± 0.005	0.023± 0.005	0.088 ± 0.01	0.07 ± 0.016	0.114 ± 0.026	0.091 ± 0.0211	0.096 ± 0.005	0.12 ± 0.014	0.007 ± 0.001	0.022 ± 0.001	0.021 ± 0.0044	0.0467 ± 0.01
lycopene content	0.82 ± 0.165	1.16 ± 0.182	9.06 ± 1.77	4.81 ± 0.41	1.55 ± 0.05	1.02± 0.028	9.5 ± 0.09	11.7 ± 2.09	0.05 ± 0.02	0.553 ± 0.03	0.16 ± 0.02	1.94 ± 0.015
β-carotene content	0.36 ± 0.045	0.44 ± 0.015	0.97 ± 0.12	1.23 ± 0.54	1.33 ± 0.058	0.75 ± 0.05	2.10 ± 0.005	2.22 ± 0.445	0.04 ± 0.02	0.27 ± 0.02	0.13 ± 0.02	0.5 ± 0.03
phytoene content	0.33 ± 0.049	0.43 ± 0.051	1.22 ± 0.07	0.44 ± 0.025	0.72 ± 0.02	0.11 ± 0.02	0.68 ± 0.04	0.68 ± 0.19	0.46 ± 0.03	0.17 ± 0.02	0.28 ± 0.076	0.77 ± 0.026
phytofluene content	0.08 ± 0.025	0.27 ± 0.035	0.75 ± 0.09	0.23 ± 0.015	0.11 ± 0.02	0.07 ± 0.02	0.31 ± 0.005	0.41 ± 0.075	0.02 ± 0.015	0.06 ± 0.02	0.09 ± 0.01	0.25 ± 0.04
total carotenoids	1.6 ± 0.1	2.52 ± 0.19	12 ± 2.05	6.31 ± 0.56	3.66 ± 0.25	1.96 ± 0.025	12.6 ± 0.055	15 ± 2.8	0.59 ± 0.02	1.07 ± 0.04	0.67 ± 0.02	3.46 ± 0.06

[a] Values shown are mean ± SD of five determinations.

light conditions. Even the interaction between the two factors represented a significant source of variation (**Figure 3−5**). In both genotypes, similar to the pattern found for antioxidant activity, the content of total carotenoids at the breaker stage was similar in fruits ripened in the presence or without UV-B energy. In the DRW 5981 hybrid the maximal content of carotenoids (12 and 6.31 mg/100 g of fresh weight in control and no UV-B tunnel, respectively) was observed at the firm red stage irrespective of light quality conditions. Similarly, the Esperanza hybrid showed enhanced levels of carotenoids at the firm ripe stage, reaching the higher amount (3.46 mg/100 g of fresh weight) if the fruits ripened in the absence of UV-B. When fruits of the DRW 5981 genotype were ripened under full sunlight, the contents of lycopene, β-carotene, phytoene, and phytofluene at firm red stage were markedly greater than at the breaker stage. The firm red fruits of the same genotype, ripened in the UV-B-free greenhouse, showed increased levels of only lycopene (1112%) and β-carotene (284%). Furthermore, at the firm red stage the levels of lycopene, phytoene, and phytofluene of DRW 5981 fruits ripened in the absence of UV-B dropped by 47, 63, and 68%, respectively, in comparison to fruits ripened in the control greenhouse. It is noteworthy that only the content of β-carotene was not changed as a consequence of the different light conditions.

The Esperanza hybrid showed a lower constitutive content of carotenoids with respect to the other two tomato genotypes examined (−94%). In more detail, the lowered carotenoid amount was due mainly to the low levels of lycopene (0.16 mg/100 g of fresh weight) and β-carotene (0.13 mg/100 g of fresh weight). During ripening a marked increase of the total carotenoid content in the UV-B-free conditions was exhibited by the Esperanza tomato hibryd, whereas, unlike the other two tomato lines, Esperanza fruits did not show an increase of carotenoids during ripening if plants were kept under full sunlight.

The HP 1 mutant showed the highest constitutive carotenoid content in comparison with the other two genotypes. Under both light conditions the fruits harvested at the firm ripe stage accumulated high levels of carotenoids, resulting in an increase of 4−5-fold in comparison with the breaker phase. The amount of total carotenoid of HP 1 tomato fruits was influenced mainly by ripening stage and to a lesser extent by the interaction between the ripening phase and light conditions. The light conditions alone did not influence carotenoid accumulation. The ripening stage affected the level of all the carotenoids, whereas light conditions influenced only the phytoene level (−84%) at breaker stage. Lycopene was affected only by ripening stage. At the breaker stage the presence of UV-B had a detrimental effect on the level of the precursor phytoene of 84% (**Table 2**).

DISCUSSION

Nowadays there is great interest in the improvement of the nutritional and antioxidant values of food crops. The protective actions of food crops against vascular diseases and certain kinds of cancer are ascribed to the contemporary presence of carotenoids, flavonoids, and vitamins.The antioxidant properties of tomato fruits are mainly related to carotenoid content, in particular lycopene and β-carotene, the accumulation of which generally increases with the ripening of fruits. From immature green to orange color stage of tomato fruits, PSY (phytoene synthase) mRNA is induced >25-fold, whereas the enzyme phytoene desaturase (PDS), which catalyzes the conversion from the precursor phytoene to ζ-carotene, increases <3-fold (*24*).

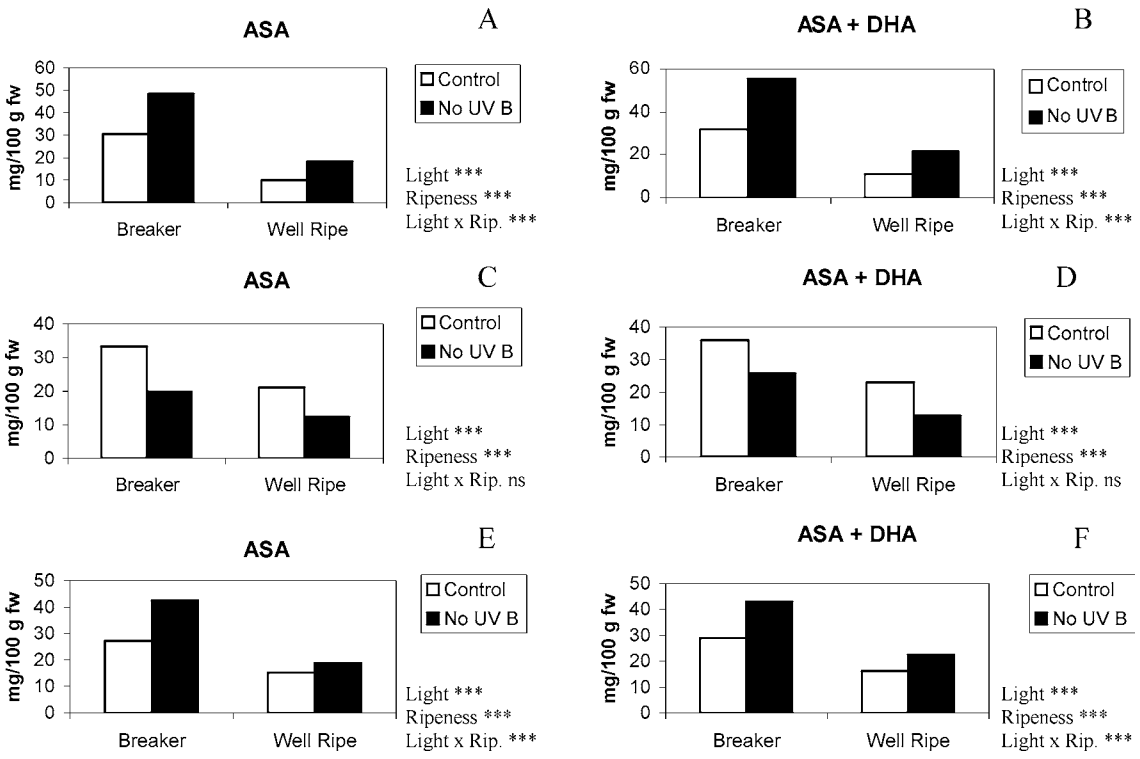

Figure 2. Changes in the content of ascorbic acid (ASA) and total ascorbate (ASA + dehydroascorbic acid), expressed as milligrams of ASA and ASA + DHA/100 g of fresh weight, of the fruit of the three different tomato lines DRW 5981 (**A**, **B**), HP 1 (**C**, **D**), and Esperanza (**E**, **F**) at different ripening stages (breaker and well ripe) and different light conditions (with or without UV-B rays). For each tomato hybrid line the figure graphically presents the two-way ANOVA results according to Fischer's LSD test regarding the interaction between two factors of variation: ripening stage and light conditions. The SE is reported for each treatment. Significant differences between treatments are marked by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant.

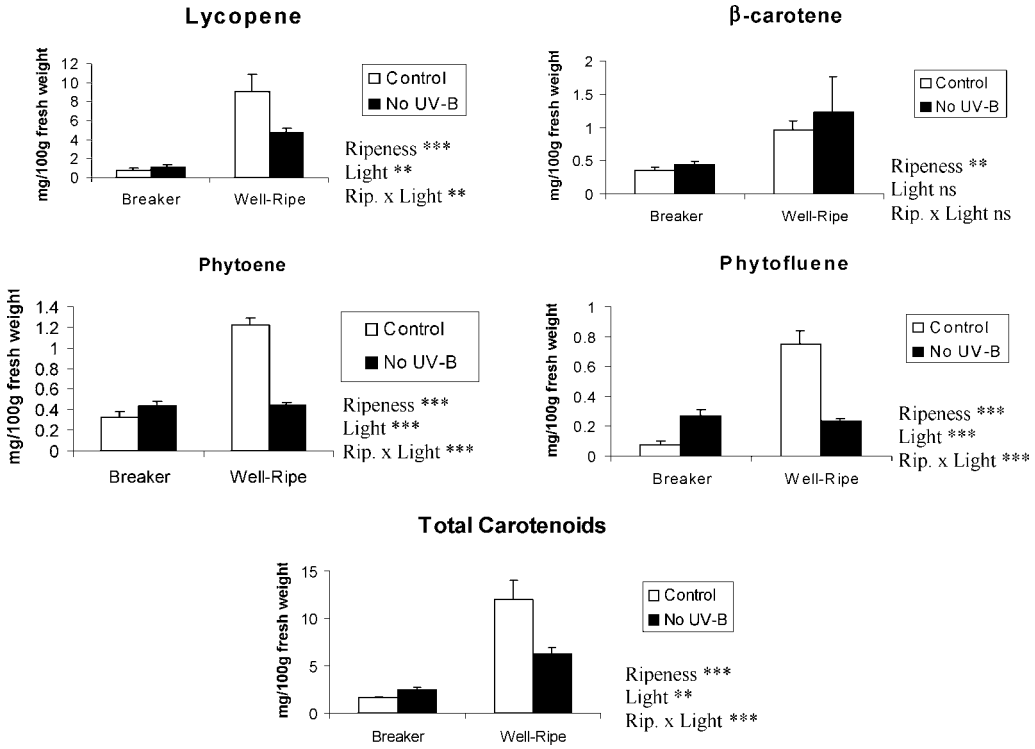

Figure 3. Changes in the content, expressed as milligrams of carotenoid/100 g of fresh weight, of principal carotenoids investigated in tomato fruits of DRW 5981 hybrid genotype at different ripening stages and as result of different light conditions. For each carotenoid white bars represent control values and black bars UV-B-depleted samples, respectively. The figure graphically presents the two-way ANOVA results according to Fischer's LSD test regarding the interaction between two factors of variation: ripening stage and light conditions. The SE is reported for each treatment. Significant differences between treatments are marked by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant.

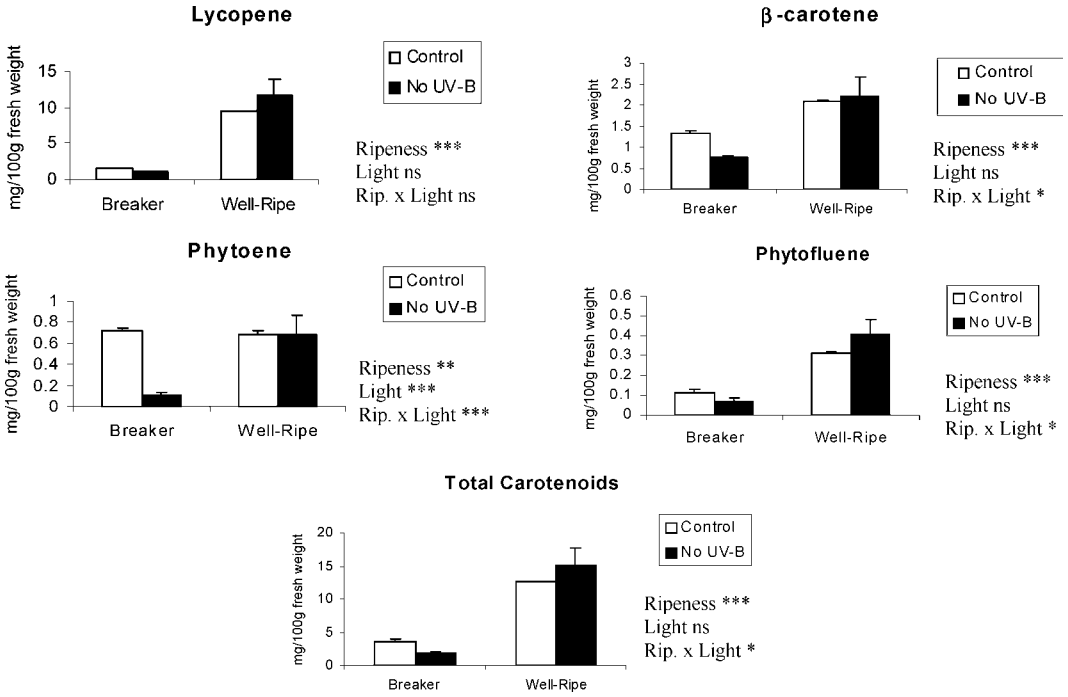

Figure 4. Changes in the content, expressed as milligrams of carotenoid/100 g of fresh weight, of principal carotenoids investigated in tomato fruits of HP 1 hybrid genotype at different ripening stages and as result of different light conditions. For each carotenoid white bars represent control values and black bars UV-B-depleted samples, respectively. The figure graphically presents the two-way ANOVA results according to Fischer's LSD test regarding the interaction between two factors of variation: ripening stage and light conditions. The SE is reported for each treatment. Significant differences between treatments are marked by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant.

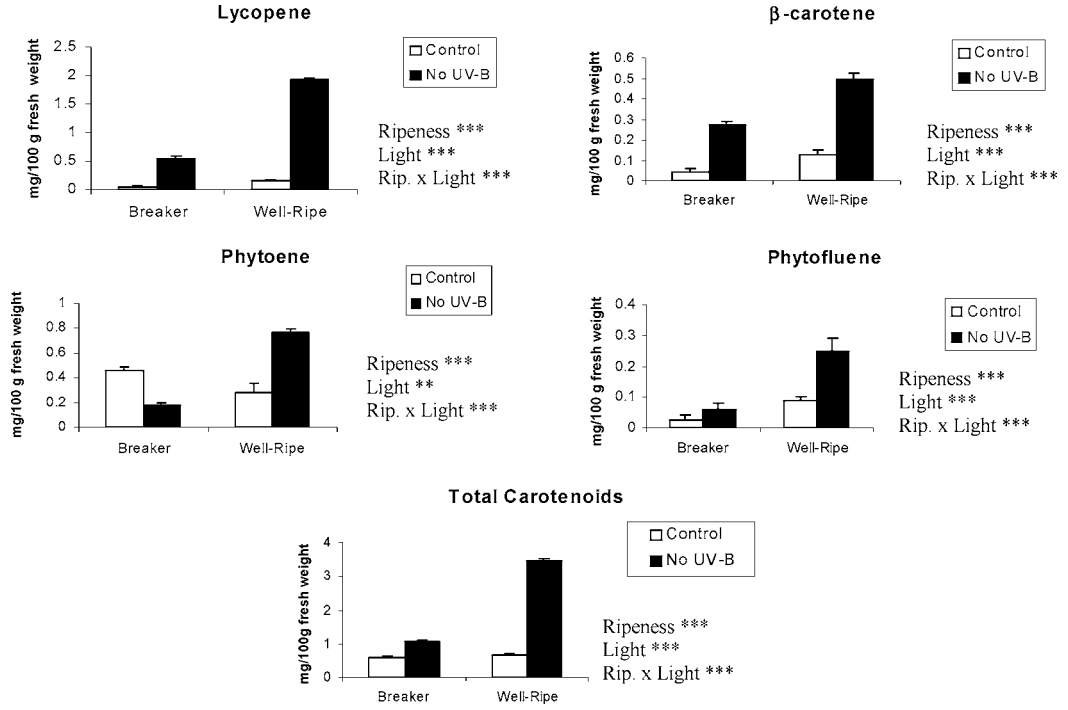

Figure 5. Changes in the content, expressed as milligrams of carotenoid/100 g of fresh weight, of principal carotenoids investigated in tomato fruits of Esperanza hybrid genotype at different ripening stages and as result of different light conditions. For each carotenoid white bars represent control values and black bars UV-B-depleted samples, respectively. The figure graphically presents the two-way ANOVA results according to Fischer's LSD test regarding the interaction between two factors of variation: ripening stage and light conditions. The SE is reported for each treatment. Significant differences between treatments are marked by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ns, not significant.

On the other hand, it is well established that light plays a pivotal role in the control of carotenoid biosynthesis in the leaves through the regulation of the first enzyme of the carotenoid biosynthetic pathway, that is, phytoene synthase (*8*, *25*, *26*). In leaf blades, besides light quantity also the quality of light radiation is believed to be of main importance in the control of carotenogenesis through the action of phytochrome and/or UV-B receptors (*25*, *26*).

Little attention has been paid to the influence of light signaling in fruit ripening, probably because the fruit of the model plant

Arabidopsis, a silique, is not suitable for ripening studies and does not have any commercial value. On the contrary, a large number of studies have been devoted to the ripening of tomato fruits; indeed, fresh tomato fruits are an important constituent of the human diet either directly or as tomato-based food products. Thus, the influence of light quality on carotenogenesis in fruits has been mainly studied in tomato, where a control by multiple phytochromes has been hypothesized (*9*, *24*) and differences in carotenoid content attributable to the presence of UV-B radiation have been detected previously. The results of our investigation provide insight into the possible role of the UV-B radiation present in the solar spectrum in the regulation of carotenoid biosynthesis in fruits of tomato plants under standard growing conditions. The three tomato genotypes Esperanza F1 (low lycopene), DRW 5981 (high lycopene), and HP 1 (high content of all carotenoids and flavonoids) showed different responses to the presence of UV-B radiation during the ripening phase.

With regard to the antioxidant activity, measured through the ABTS assay, the two hybrids DRW 5981 and Esperanza exhibited lower values in the fruits harvested at breaker stage in comparison to the well-ripe phase, whereas no change in this parameter was shown by HP 1 at the two ripening stages. The effect of UV-B radiation on antioxidant activity was negligible in DRW 5981 and HP 1 genotypes, whereas it was detrimental in the Esperanza genotype at both ripening stages. The fruits of the Esperanza genotype showed the lowest antioxidant activity and the lowest carotenoid content in all of the experimental conditions. Furthermore, Esperanza was the genotype showing the strongest detrimental effect of UV-B on carotenoid content and antioxidant activity. Therefore, it appears that the Esperanza genotype has a scarce light capability of accumulating carotenoids and a great susceptibility to detrimental effects of the UV-B waveband. Conversely, the DRW genotype shows high levels of carotenoids under visible light and a further promotion of their level by the UV-B waveband. On the other hand, the HP 1 mutant displays an intermediate behavior: high levels of carotenoids under visible light and a detrimental effect of UV-B. Because the HP 1 mutant, which is characterized by high levels of UV-B shielding pigments and is considered to be a mutant with exaggerated responsiveness to phytochrome, shows a detrimental effect of UV-B radiation, it seems that the negative effect of UV-B is not due to a damaging effect but rather to interference of UV-B signaling with phytochrome signal transduction pathways. The genetic backgrounds that determine low or high levels of carotenoids in Esperanza and DRW hybrids, respectively, are not available; therefore, it is not possible to draw reliable conclusions about the mechanisms that influence UV-B effects on the levels of carotenoids in tomato fruits of these two genotypes. In the case of Esperanza tomato, which has the lowest level of both antioxidant activity and carotenoids, a damaging effect mediated by UV-B cannot be ruled out.

High levels of carotenoids and high antioxidant capacity, with the related health benefits, are considered an adjunctive quality parameter of tomato. Therefore, to produce tomato fruits of high quality, it is necessary to take into account that a large part of tomatoes consumed are produced in greenhouses (with or without UV-B energy) and that the results of our research clearly indicate that UV-B exerts very different effects on carotenoid content in fruits of different genotypes of tomato. Consequently, the appropriate choice of tomato genotype as a function of the optical properties of the covering materials of the greenhouses can markedly influence the tomato's beneficial effects for human health and the market value. Accordingly, with reference to the tomato genotypes used in the present research it is possible to conclude that in order to maximize the parameters mentioned above, it is convenient to choose DRW 5981 for open air or UV-B-enriched growth environment, whereas HP 1 and Esperanza produce the maximal levels of carotenoids and antioxidant activity in UV-B-free greenhouses.

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