horticulturae

Article

Phytochemical Profile and Antioxidant Activity of Some Open-Field Ancient-Tomato (*Solanum lycopersicum* L.) Genotypes and Promising Breeding Lines

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Citation: Laayouni, Y.; Tlili, I.; Henane, I.; Ali, A.B.; Égei, M.; Takács, S.; Azam, M.; Siddiqui, M.W.; Daood, H.; Pék, Z.; et al. Phytochemical Profile and Antioxidant Activity of Some Open-Field Ancient-Tomato (*Solanum lycopersicum* L.) Genotypes and Promising Breeding Lines. *Horticulturae* **2023**, *9*, 1180. https://doi.org/10.3390/horticulturae9111180

Academic Editors: Maribel Ovando-Martínez, Ramón Francisco Dórame Miranda and Carmen Lizette Del Toro Sánchez

Received: 1 October 2023
Revised: 24 October 2023
Accepted: 25 October 2023
Published: 28 October 2023

Abstract: Tomato landraces have progressively faded into obscurity, making way for new hybrids and elite tomato cultivars. This study presents a comprehensive evaluation of the agronomic attributes, physicochemical properties, and functional traits across seven cultivars, comprising two high-pigment varieties, 'HLT−F81' and 'HLT−F82', as well as five underutilized ancient-tomato genotypes considered as landraces. Most of the studied genotypes exhibited satisfactory horticultural and processing traits. The average fruit weight ranged from 73.3 g in 'Rimone' to 91.83 g in 'HLT−F81', while the soluble solids content ranged from 4.66 °Brix in 'Justar' to 6.08 °Brix in 'HLT−F81'. The functional quality and the content of most antioxidants, as well as the antioxidant activity in both hydrophilic and lipophilic fractions, proved to be the most discriminating parameters among the tomato genotypes. The content of β−carotene and lycopene spanned from 2.94 mg kg^{-1} fw in 'Rio Grande' to 13.94 mg kg^{-1} fw in 'HLT−F82' and 227.8 mg kg^{-1} fw in 'HLT−F81', yielding large variations compared to 'Rio Grande'. The total phenolic content ranged from 139.83 mg GAE kg^{-1} fw in 'Rimone' to 352.41 mg GAE kg^{-1} fw in 'HLT-F81', while the flavonoid content varied from 136.16 mg RE kg^{-1} fw in 'Justar' to 311.23 mg RE kg^{-1} fw in 'HLT-F82'. The presence of tocopherol isomers was genotype-dependent, with a higher content in lines carrying the high-pigment mutations. Among the tested tomato genotypes, the high-pigment tomato line 'HLT−F81' achieved the highest hydrophilic and lipophilic antioxidant activity values. This study primarily focused on the recovery and valorization of tomato genetic resources and landraces. It also aimed to identify desirable horticultural (yield, low-input, and low-water demand), processing (°Brix and titratable acidity), and quality (rich antioxidant berries) traits for introgression into new tomato cultivars better suited to the evolving climate conditions of the near future.

Keywords: high-pigment tomato lines; ancient-tomato genotypes; agronomic characteristics; functional quality

1. Introduction

Tomato (*Solanum lycopersicon* L.) represents a significant agricultural crop on a global scale, with a total production of 189 M tons and coverage of approximately 5 M hectares in 2021 [1]. These berries provide a substantial amount of fiber, vitamins, and antioxidants, including lycopene [2]. The carotenoids synthesized and stored in tomato berries, especially lycopene, serve as free-radical scavengers protecting cells and tissues against various age-related diseases. Additionally, there is a growing body of evidence supporting lycopene's protective role in emerging health issues, such as the skeletal muscle metabolism [3], spleen-induced injuries [4], neuroprotection, and hypocholesterolemic activities [4,5]. In addition to lycopene, tomatoes also contain various phenolic compounds, flavonoids, and vitamins with high antioxidant activity [6]. These compounds play a crucial role in combating free radicals, which are responsible for various chronic diseases [7,8].

Recent years have witnessed a notable increase in the awareness and interest in tomato landraces and heirlooms cultivars. This is evident from the increasing number of publications and studies dedicated to this specific topic worldwide [9–17]. This trend is driven by both farmers, who appreciate the low-input and water requirements of local tomato cultivars, and consumers, who value the old-fashioned tomato flavor and associated health benefits [16,18].

The quality of tomato berries is generally evaluated on the basis of various physicochemical attributes, including color indexes, soluble solids, and pH [19], in addition to the content of several compounds, such as: (1) carotenoids, primarily lycopene, responsible for the red color of tomatoes [2,6,20]; (2) phenols and flavonoids [21]; (3) R,R,R−α−Tocopherol, the biologically active form of vitamin E [22–26]; (4) vitamin C [6,27,28]; (5) antioxidant activity, an essential indicator reflecting the strength of lipophilic and hydrophilic antioxidants present in a given sample, and their synergistic and/or antagonistic interactions [29].

Landraces represent a critical repository and safety valve of genetic diversity, owing to their distinctive traits, including tolerance/resistance to abiotic stress, and superior flavor and fruit quality compared to widely grown genotypes. Therefore, the local germplasm serves as a valuable resource of desirable traits that can be introgressed into new cultivars that are more suitable and resilient in the face of a constantly and rapidly changing climate [9].

Previously, significant variability has been observed among tomato landraces and heirlooms in terms of yields, physicochemical traits, disease resistance, and fruit functional quality. Additionally, some studies assessing old tomato genotypes and heirlooms have suggested that certain tomato landraces exhibit higher levels of soluble solids and better functional quality compared to commercial tomato hybrids, all while maintaining similar yields. This is particularly relevant in the context of today's changing climate [30–33]. Furthermore, tomato landraces have also proven to be well-suited for various emerging farming systems, including aridoculture [11], low-input practices [34], organic cultivation [35], and urban agriculture [36].

In Tunisia, small-scale farmers have historically cultivated ancient-tomato cultivars and old genetic varieties. However, these traditional seeds are gradually disappearing, increasingly replaced by highly productive hybrid-tomato cultivars suitable for both the fresh market and processing [6,31,32,37–41]. Additionally, the horticultural performance and fruit quality of most of these older genotypes have not been thoroughly evaluated within the framework of the conventional farming system. Therefore, this study aims to assess the agronomic traits and functional quality of various tomato landraces, which include two high-pigment tomato lines and five ancient-tomato genotypes grown under the conventional farming system over a two-year period, in 2021 and 2022.

2. Materials and Methods

Seven determinate open-pollinated cultivars were used in this experiment, comprising two high-pigment tomato lines (Figure 1): 'HLT−F81', homozygous, with the light-responsive high-pigment (hp) mutations hp-2^{dg}, and 'HLT−F82', carrying an UHLY mutation, both selected by the laboratory of Vegetable Crops of the National Agricultural Research Institute of Tunisia using conventional breeding techniques on the basis of their high-phytochemical profiles with respect to most ordinary tomato cultivars. Those open-pollinated lines were the output of a breeding program targeting the development of tomato genotypes with enhanced nutritional quality. Five local tomato cultivars, namely, 'BSP', 'Justar', 'Rimone', 'Salba', and 'Rio Grande', considered as local cultivars and genetic resources in Tunisia, were also used. The different ancient genotypes are progressively disappearing and cultivated only in small geographic areas. 'BSP' was widely grown in Tunisia. 'Justar' is tolerant or resistant to nematodes, and 'Rimone' to *Pseudomonas*. 'Salba' was selected by the National Agricultural Research Institute of Tunisia (INRAT) for firmness, and therefore its resistance to field over-maturity, and was kept at the laboratory of Horticulture. The traditional tomato variety 'Rio Grande' was one of the most cultivated cultivars in Tunisia for almost 3 decades.

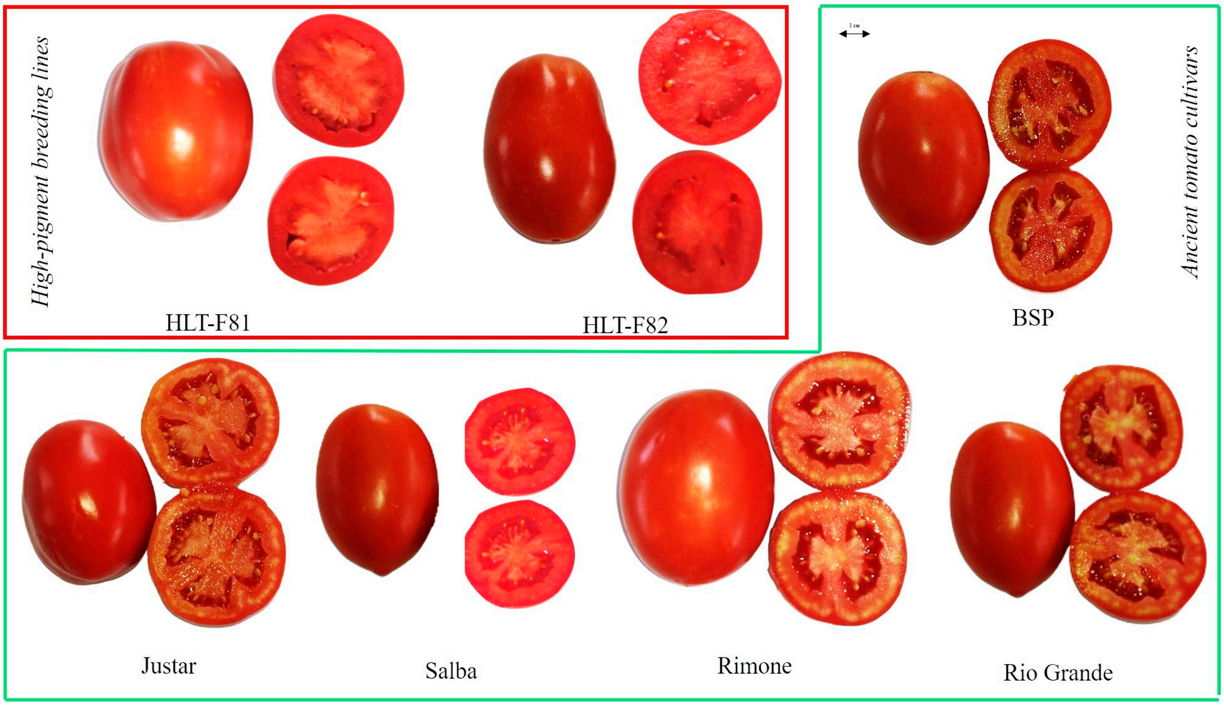

Figure 1. Whole fruits and transversal sections of red-ripe tomato berries of high-pigment tomato lines and ancient-tomato genotypes harvested from the open-field trials.

Tomato trials were performed under open-field conditions during the 2021 and 2022 growing seasons at the National Agricultural Research Institute of Tunisia in Ariana governorate, northern Tunisia (36°50′39″ N 10°11′30″ E), characterized by a typical Mediterranean climate. During the 2021 and 2022 growing seasons, the minimum temperature ranged between 10–25 °C and 11–27 °C, the relative humidity ranged between 55–89% and 51–87%, and the rainfall ranged between 1.2–56.8 mm and 0–33.8 mm, respectively.

Sowing was carried out in a clay–loamy substrate with a pH (7.72), EC (0.19 mS cm^{-1}), and mineral and organic composition appropriate for tomato cultivation. Transplanting was performed in double rows matching 3 plants m^{-2} using a spacing of 40 cm and 150 cm within and between rows, respectively. For each growing year, tomato genotypes were grown in three replicated plots.

Irrigation was applied using 4 L h^{-1} drippers positioned at 40 cm intervals lengthwise along the irrigation line. The agricultural practices consisted of synthetic fertilization via an irrigation line using 190 kg N ha^{-1}, 135 kg P_2O_5 ha^{-1}, 431 kg K_2O ha^{-1}, and 75 kg MgO ha^{-1}, as outlined in Ilahy et al. [28].

2.1. Fruit Sampling

Tomato fruits were collected from each plant at the commercial red-mature-ripening stage. A sample of 20–23 healthy fresh tomato fruits were picked from each block and delivered to the laboratory. Three samples were completed when the tomato berries reached the desirable red-ripe stage. The selected tomato fruits were washed with deionized water, cut into small pieces, and homogenized in a laboratory blender (Waring Laboratory Science, Torrington, CT, USA). The obtained homogenates were kept at −20 °C and used to assess the carotenoid, total phenol, flavonoid, vitamin C, and tocopherol contents, as well as the hydrophilic and lipophilic antioxidant activity, during the following days to avoid nutrient degradation/oxidation.

2.2. Evaluation of the Main Agronomic Characteristics

The marketable yield was evaluated considering the weight of the fruit free from physiological disorders, insect pests, and disease infestation, such as rotting, cracking, and sun scalding. Regarding the average fruit weight, a random sample of 1 kg of marketable fruits was used, and the average fruit weight was deduced by dividing the weight of the sample by the number of the fruit in the same sample. Fruit length, diameter, and pericarp thickness were measured using a Vernier Caliper and averaged over 6 fruits. Some droplets of filtered freshly made juice were put on the prism of an Atago PR-100 digital refractometer equipped with automatic temperature adjustment to determine the soluble solids concentration. Titratable acidity was measured as a percentage of citric acid after titrating the diluted juice with 0.1 M sodium hydroxide solution until a pH of 8.1 was reached. The redness (a^*) and yellowness (b^*) color indices were estimated using a Minolta CR-400 (Minolta Corp., Osaka, Japan), and the (a^*/b^*) ratio was derived as a result.

2.3. Evaluation of Carotenoid Content

Carotenoids were determined using the procedure outlined by Daood et al. [42]. In brief, 2 g of tomato homogenate was crushed using a mortar and pestle, mixed with 20 mL of methanol for 2 min, and the top layer was placed into a flask. In a graduated cylinder, 50 mL of dichloroethane was combined with 10 mL of methanol and softly stirred before and after by adding a few distilled-water drops. The mixture was filtered through a separating funnel and left to evaporate at 70 °C until completely evaporated. A volume of 5 mL of analytical-grade methanol was mixed with a volume of 5 mL of pigment eluents and poured into the same flask, then gently mixed, sonicated, filtered using a 0.22 m membrane syringe, and finally injected into the HPLC (Hitachi Chromaster, Tokyo, Japan) system comprised of a 5110 pump, a 5210 autosampler, and a 5430 diode. Carotenoids were separated using a Nucleodur C-18, 3 m, 240 4.6 mm column (Machery Nagel, Dürer, Germany) with a gradient elution of (A) water, (B) methanol, and (C) 10:55:35 methanol–isopropanol–acetonitrile. The elution began with 8% A in B, progressed to 100% B in 3 min, 100% C in 30 min, remained isocratic for 5 min, and then again to 8% A in B in 5 min. The flow rate was fixed at 0.6 mL min^{-1}, and the column oven temperature was 35 °C. The quantitative determination was carried out by integrating each peak area at the highest absorption wavelength produced by a diode array detector and comparing it to that of the internal standards of β−carotene and apocarotenal (Sigma-Aldrich, Budapest, Hungary). The retention times were 6.14, 21.56, 22.33, 25.81, and 30.27 min for lutein, lycoxanthin, β−carotene, γ−carotene, and lycopene, respectively (Figure 2).

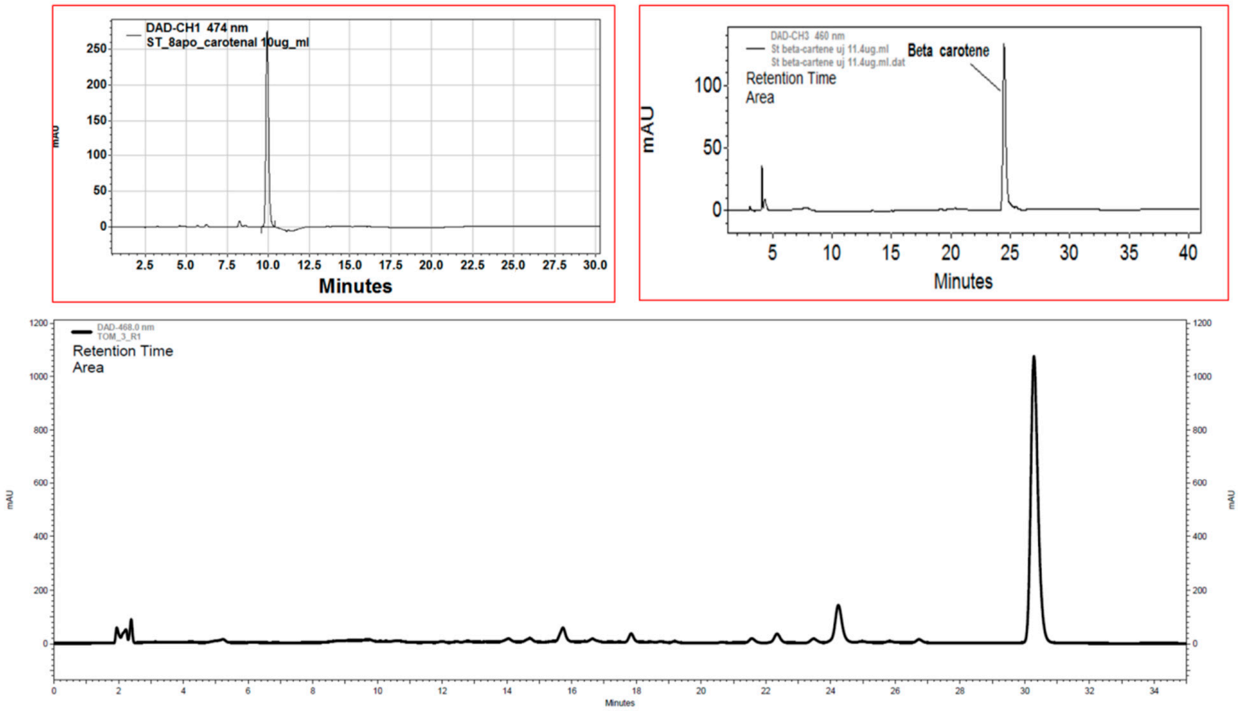

Figure 2. Carotenoid separation of 'HLT−F81' tomato extracts and chromatograms of standard substances (highlighted in red color).

2.4. Evaluation of Total Phenolic and Flavonoid Contents

The extraction and measurement of the total phenolic content was performed using the method described by Martínez -Valverde et al. [43]. An amount of 5 mL of 80% methanol and 50 µL of 37% HCl were combined with 0.3 g of tomato homogenate and extracted for 2 h at 4 °C and at 300 rpm before centrifuging for 20 min at 10,000× *g*. The Folin–Ciocalteu reagent was applied to a 50 µL supernatant sample, an absorbance at 750 nm was measured using a Cecil BioQuest CE 2501 spectrophotometer (Ceil Instruments Ltd., Cambridge, UK), and the total phenolic content was reported as mg of gallic acid equivalent (GAE) kg^{-1} fw. Furthermore, the Asami et al. [44] approach was employed to adjust the acquired results due to the sugar–phenolic compound interference.

Zhishen et al. [45] established a technique for determining the flavonoid concentration. As a result, a 0.3 g sample was extracted with methanol, and a volume of 50 µL was diluted with distilled water to obtain 0.5 mL; 30 µL of 5% $NaNO_2$ was poured, followed by 60 µL of $AlCl_3$ (10%) and 200 µL of NaOH (1 M) after 5 and 6 min, respectively. The samples' absorbances were measured at 510 nm using a Cecil BioQuest CE 2501 spectrophotometer (Ceil Instruments Ltd., Cambridge, UK), and the findings were reported as mg of rutin equivalent (RE) kg^{-1} fw.

2.5. Evaluation of Total Vitamin C Content

The total vitamin C (AsA + DHA) content was extracted and quantified on 0.2 g samples of homogeneous tomato juice according to Kampfenkel et al. [46]. The absorbance was measured using a spectrophotometer (Cecil BioQuest CE 2501) at 525 nm and expressed in mg kg^{-1} fw. The standard curve's linear measurement ranged from 0 to 700 mol AsA.

2.6. Evaluation of Tocopherol Content

The procedure of Abushita et al. [47] was adopted for the extraction of tocopherol using n−hexane. The analysis was conducted by the mean of an HPLC (Hitachi Chromaster, Tokyo, Japan). The system was composed of a model 5110 gradient pump, a model 5210 autosampler, and a 5440 fluorescence detector. The Nucleosil 5 mm (250 × 4.6 mm i.d.)

column was also used for separation with a mobile phase (99.5:0.5 n−hexane: ethanol). The excitation and emission wavelengths were detected at 295 nm and 320 nm, respectively, as outlined in Duah et al. [48]. The isomers of α−, β−, and γ−tocopherols were determined by means of external standards (Sigma-Aldrich, Budapest, Hungary) and cochromatographed with the samples. The retention times were 16.75, 14.41, and 13.34 min for α−, β−, and γ−tocopherols, respectively (Figure 3).

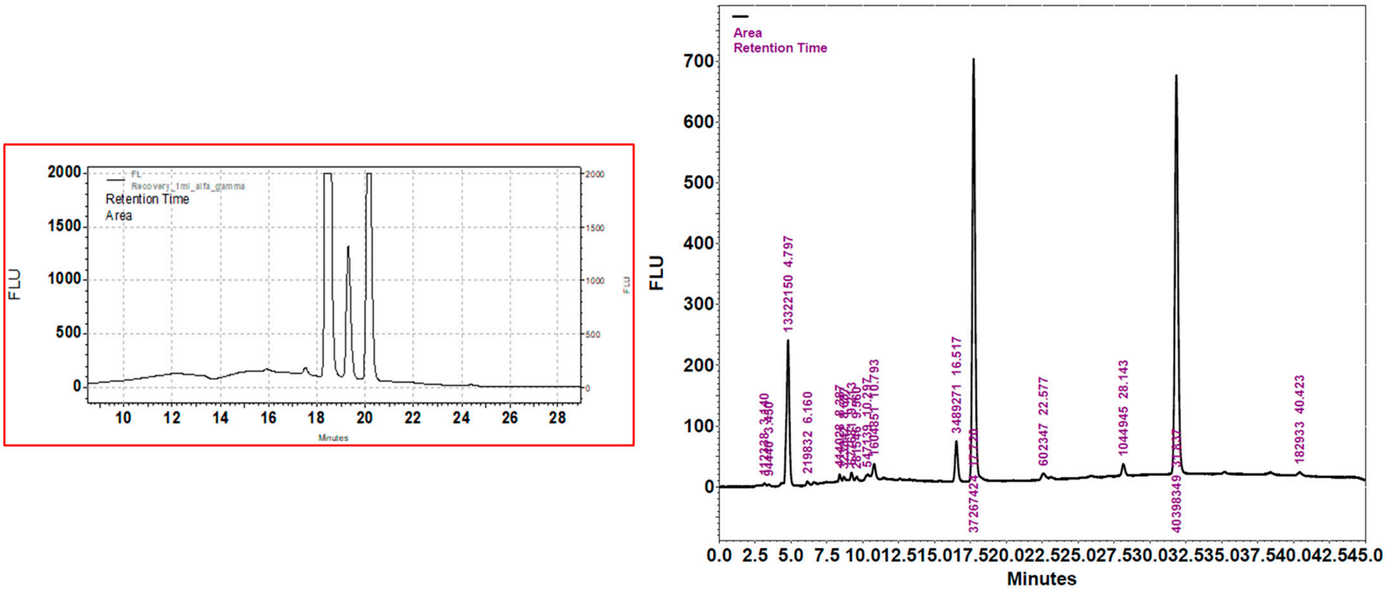

Figure 3. Tocopherol isomer separation of 'HLT−F82' tomato extracts and chromatograms of standard substances (highlighted in red color).

2.7. Evaluation of the Antioxidant Activity

The antioxidant activity of the hydrophilic and lipophilic fractions (HAA and LAA, respectively) was assessed utilizing the Trolox Equivalent Antioxidant Capacity (TEAC method), in agreement with Miller and Rice-Evans [49]. The choice of the methodology was due to its reproducibility and high fidelity when analyzing complex matrices. The extraction of hydrophilic and lipophilic antioxidants was performed on 0.3 g of the tomato sample extracted with methanol (50%) or acetone (50%), respectively, at 4 °C under continuous shaking (300 rpm) during a half-day. Tomato-homogenate samples were prone to centrifugation at 10,000× *g* for 7 min, and the collected supernatants were used for the determination of the antioxidant activity at 734 nm using a Cecil BioQuest CE 2501 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). The antioxidant activity was calculated and expressed as µM of Trolox 100 g^{-1} of fw.

2.8. Statistical Analysis

The variations affecting the agronomic, physicochemical, and functional quality of ancient-tomato genotypes and high-pigment breeding lines were assessed by variance (ANOVA) analysis. The means were compared using the Duncan test ($p < 0.05$) when a significant difference was noticed. The statistical evaluations were performed with the IBM SPSS Statistics software for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA). Correlations were generated by means of Pearson's correlation coefficient (r). Principal component analysis (PCA) biplot PC1 vs. PC2 of the main bioactive classes of molecules (total contents) identified in the different tomato lines under investigation was performed using the XLSTAT software (Addinsoft, Paris, France).

3. Results

3.1. Agronomic Characteristics

The main agronomic attributes (Table 1) and physicochemical traits (Table 2) of different high-pigment breeding lines and ancient-tomato genotypes, including fruit shape, average fruit weight, pericarp thickness, fruit length, fruit diameter, soluble solids content, pH, titratable acidity, color indexes a^* and b^*, as well as the ratio (a^*/b^*), exhibited significant variations between the tested ancient-tomato genotypes and high-pigment breeding lines ($p < 0.05$). In open-field conditions, the tomato genotypes displayed a determinate growth habit, appearing vigorous with excellent foliage coverage. Notably, the high-pigment breeding lines exhibited darker foliage and darker green immature fruit compared to the ancient-tomato genotypes (Figure 4). All genotypes yielded satisfactorily, ranging from 88 to 91 t ha^{-1} in 'Salba' and 'Justar', respectively. 'HLT−F82', 'BSP', and 'Rio Grande' produced oval-shaped tomato berries, while 'HLT−F81', 'Justar', 'Rimone', and 'Salba' produced similar squared tomato berries. All of these tomato fruit shapes are suitable for both fresh consumption and processing.

Table 1. Agronomic attributes of high-pigment tomato breeding lines and ancient-tomato genotypes grown under open-field conditions during two growing seasons. Data are expressed as mean ± S.E. with six replicates.

Cultivar	Fruit Shape	Marketable Yield (t ha^{-1})	Average Fruit Weight	Pericarp Thickness (mm)	Fruit Length (mm)	Fruit Diameter (mm)
HLT−F81	Squared	80.83 ± 2.08 a	91.83 ± 5.50 a	0.796 ± 0.06 c	6.45 ± 0.21 c	5.53 ± 0.32 bc
HLT−F82	Oval	78.26 ± 3.40 a	82.50 ± 4.20 ab	1.16 ± 0.055 ab	7.14 ± 0.31 bc	7.16 ± 0.55 a
BSP	Oval	89.00 ± 5.69 a	78.50 ± 4.84 ab	1.21 ± 0.04 ab	7.24 ± 0.28 bc	5.11 ± 0.14 c
Justar	Squared	82.88 ± 2.22 a	76.81 ± 4.15 ab	1.31 ± 0.10 a	6.60 ± 0.30 c	6.36 ± 0.22 ab
Rimone	Squared	79.33 ± 3.73 a	73.33 ± 3.95	1.04 ± 0.05 b	7.60 ± 0.20 ab	5.61 ± 0.17 bc
Rio Grande	Oval	88.10 ± 5.09 a	81.53 ± 4.90 ab	0.78 ± 0.02 c	7.57 ± 0.14 ab	5.30 ± 0.23 c
Salba	Squared	77.00 ± 4.64 a	81.3 ± 6.74 ab	1.22 ± 0.10 ab	8.12 ± 0.33 a	5.43 ± 0.19 c

Lower case letters indicate mean separation within genotypes by LSD test, $p < 0.05$.

Table 2. Physicochemical properties of high-pigment tomato breeding lines and ancient-tomato genotypes grown under open-field conditions during two growing seasons. Data are expressed as mean ± S.E. with six replicates.

Cultivar	Soluble Solids (°Brix)	pH	Titratable Acidity (%)	a^*	b^*	a^*/b^*
HLT−F81	6.08 ± 0.30 a	4.44 ± 0.05 a	0.477 ± 0.042 a	29.83 ± 1.35 a	19 ± 0.57 b	1.58 ± 0.11 a
HLT−F82	5.76 ± 0.27 a	4.29 ± 0.07 ab	0.445 ± 0.018 ab	26.17 ± 1.51 b	24.66 ± 1.05 a	1.06 ± 0.06 b
BSP	5.68 ± 0.22 a	4.24 ± 0.08 b	0.438 ± 0.025 ab	24.50 ± 0.957 bc	24.03 ± 0.6 a	1.02 ± 0.05 b
Justar	4.66 ± 0.16 b	4.35 ± 0.06 ab	0.390 ± 0.022 b	24.4 ± 0.428 bc	23.0 ± 1.15 a	1.08 ± 0.07 b
Rimone	4.78 ± 0.18 b	4.22 ± 0.03 b	0.381 ± 0.023 b	24.33 ± 0.760 bc	24.36 ± 1.11 a	1.01 ± 0.05 b
Rio Grande	5.43 ± 0.16 a	4.30 ± 0.05 ab	0.416 ± 0.01 ab	22.67 ± 0.558 c	24.0 ± 1.12 a	0.95 ± 0.055 b
Salba	5.62 ± 0.23 a	4.20 ± 0.03 b	0.385 ± 0.022 b	23.0 ± 1.15 bc	23.0 ± 0.68 a	1.00 ± 0.068 b

Lower case letters indicate mean separation within genotypes by LSD test, $p < 0.05$.

The average fruit weight varied from 73.3 g in 'Rimone' to 91.83 g in 'HLT−F81'. Pericarp thickness ranged from 0.796 mm in 'HLT−F81' to 1.31 mm in 'Justar'. The high-pigment tomato genotype 'HLT−F82' and both ancient-tomato cultivars 'BSP' and 'Salba' exhibited pericarp thicknesses statistically similar to that of 'Justar'. Fruit length spanned from 6.45 cm in 'HLT−F81' to 8.12 cm in 'Salba', while fruit diameter ranged from 5.11 cm in 'BSP' to 7.16 cm in 'HLT−F82'.

Figure 4. Immature berries of 'HLT−F81' (dark green color; (**left**)), 'HLT−F82' (**middle**), and 'Rio Grande' (light green color; (**right**)) showing differences in pigmentation at early stages of development.

The soluble solids content (Table 2) exhibited a range from 4.66 °Brix in 'Justar' to 6.08 °Brix in 'HLT−F81'. The tested tomato genotypes were sorted in two groups based on their soluble solids content, with 'HLT−F81', 'HLT−F82', 'BSP', 'Rio Grande', and 'Salba' forming the group with the highest soluble solids content, while 'Justar' and 'Rimone' formed the group with the lowest soluble solids content. The values of the pH ranged from 4.20 in 'Salba' to 4.44 in 'HLT−F81', with 'BSP' and 'Rimone' showing statistically similar pH values to 'Salba'.

Titratable acidity varied significantly among the tested tomato genotypes, ranging from 0.385% in 'Salba' to 0.477% in 'HLT−F81'. 'Justar' and 'Rimone' showed titratable acidity levels similar to that of 'Salba'. The color reading a^*, which indicates the intensity of the red color, spanned from 22.67 in 'Rio Grande' to 29.83 in 'HLT−F81', with the highest values observed in both high-pigment tomato lines compared to the ancient-ordinary-tomato genotypes. The yellowness of the tomato berries, expressed by the color reading b^*, ranged from 19 in 'HLT−F81' to 24.66 in the high-pigment tomato line 'HLT−F82'. As a result, the (a^*/b^*) ratio, useful for characterizing the quality and maturity of tomato berries, varied from 0.95 in 'Rio Grande' to 1.58 in 'HLT−F81'.

3.2. Carotenoid Content

In this study, lutein, lycoxanthin, and ϒ− and β−carotene (minor carotenoids), as well as lycopene (major carotenoid) and total carotenoid contents, varied significantly between tomato genotypes ($p < 0.05$) (Figure 5a,b). Lutein content was the lowest among all the detected carotenoids and ranged from 0.28 mg kg^{-1} fw in 'Rimone' to 1.64 mg kg^{-1} fw in 'HLT−F81'. Variations ranging from 67% to 480% were noticed in different tomato genotypes with respect to 'Rimone'. The ϒ−carotene content was higher in all genotypes compared to lutein and ranged from 0.47 mg kg^{-1} fw in 'Rimone' to 1.93 mg kg^{-1} fw in 'HLT−F82', with variations ranging from 57 to 304% higher compared to 'Rimone'. Lycoxanthin content ranged from 0.99 mg kg^{-1} fw in 'Rio Grande' to 4.80 mg kg^{-1} fw in 'Justar', with registered variations ranging from 58% to 382% higher compared to 'Rio Grande'. The content of β−carotene and lycopene ranged from 2.94 to 76 mg kg^{-1} fw in 'Rio Grande' to 13.94 mg kg^{-1} fw in 'HLT−F82' and 227.8 mg kg^{-1} fw in 'HLT−F81', respectively. Variations of 4–373% and 15–199% were noticed for β−carotene and lycopene compared to 'Rio Grande', respectively. Total carotenoids ranged from 81.9 mg kg^{-1} fw in 'Rio Grande' to 243.32 mg kg^{-1} fw, detected in the high-pigment tomato line 'HLT−F81'. The ancient-tomato genotype 'Justar' showed similar total carotenoid content to the high-pigment line 'HLT−F82', carrying an unidentified high-pigment mutation. Regarding total carotenoids, variation ranging from 15% to 197% higher compared to 'Rio Grande' was noticed.

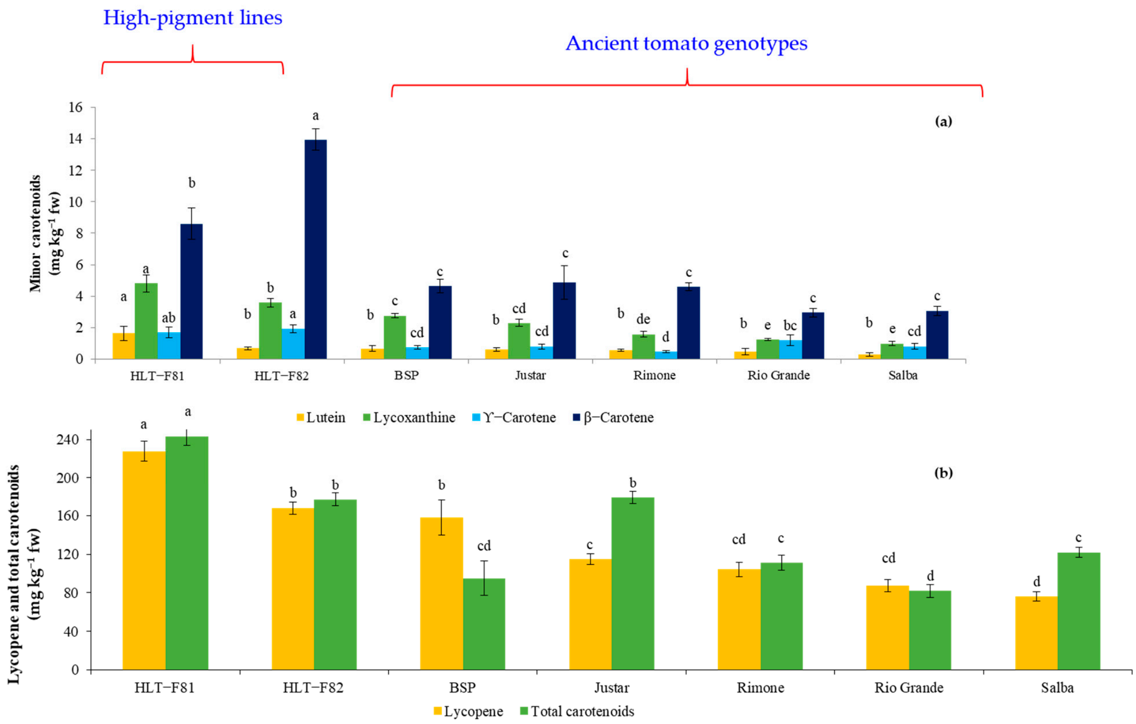

Figure 5. (**a**) Lutein, lycoxanthin, and ϒ− and β−carotene (minor carotenoids), as well as (**b**) lycopene and total carotenoids (mg kg^{-1} fw) in high-pigment tomato breeding lines and the ancient genotypes of tomato. Values represent mean ± standard error of six replicates (2021 and 2022 sampling data). Histograms marked with the same letters are not significantly different (LSD test, $p < 0.05$).

3.3. Total Phenolic and Flavonoid Contents

The content of total phenolics and flavonoids varied significantly between tomato genotypes ($p < 0.05$) (Figure 6). Total phenolic content ranged from 139.83 mg GAE kg^{-1} fw in "Rimone" to 352.41 mg GAE kg^{-1} fw in 'HLT−F81'. Flavonoid content ranged from 136.16 mg RE kg^{-1} fw in 'Justar' to 311.23 mg RE kg^{-1} fw in 'HLT−F82'. The high-pigment tomato lines showed significantly higher contents of total phenolics and flavonoids with respect to the ancient-tomato genotypes, which exhibited an almost-similar content of both compounds. Regarding total phenolics and flavonoids, variations ranging from 1% to 152% and from 2% to 112%, respectively, higher compared to 'Justar' were detected in different tomato genotypes.

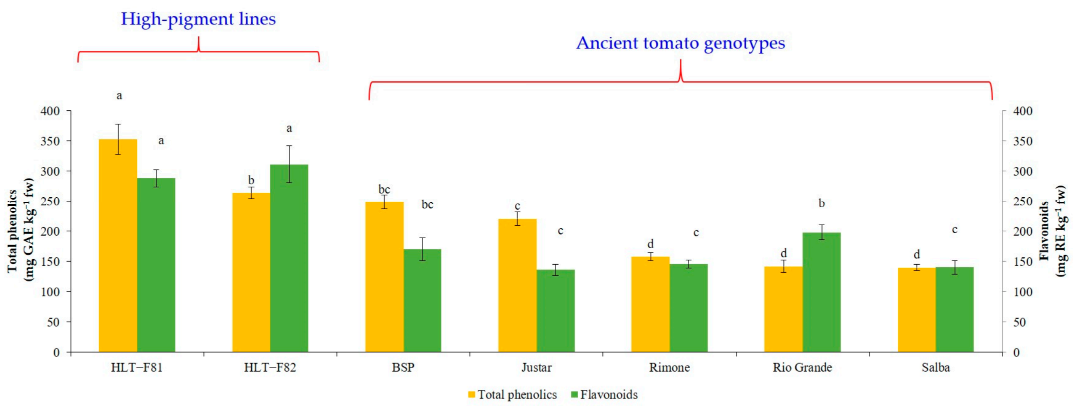

Figure 6. Total phenolics (mg GAE kg^{-1} fw) and flavonoids (mg RE kg^{-1} fw) in high-pigment tomato breeding lines and the ancient genotypes of tomato. Values represent mean ± standard error of six replicates (2021 and 2022 sampling data). Histograms marked with the same letters are not significantly different (LSD test, $p < 0.05$).

3.4. Tocopherol Content

In this study, the content of all tocopherol isomers and their total varied significantly among tomato genotypes ($p < 0.05$) (Figure 7). All tocopherol isomers were only detected in the high-pigment tomato line 'HLT−F81' and the ancient-tomato genotype 'BSP'. It is worthy to underline that the total content of tocopherol was strongly correlated to the α−Tocopherol content rather than the other forms. The α−Tocopherol content ranged from 5.45 mg kg^{-1} fw in 'Salba' to 15.26 mg kg^{-1} fw in 'HLT−F81' and was the highest (14.7–15.26 mg kg^{-1} fw) in both high-pigment tomato breeding lines with respect to the ancient-ordinary-tomato genotypes (5.45–8.55 mg kg^{-1} fw). The content of β−tocopherol exhibited lower variability and ranged from 0 mg kg^{-1} fw to 0.57 mg kg^{-1} fw in high-pigment tomato lines 'HLT−F82' and 'HLT−F81', respectively. The ϒ−tocopherol isomer was only detected in 'HLT−F81' and 'BSP', with values ranging from 0.63 to 0.26 mg kg^{-1} fw, respectively. The total tocopherol content was highest is the high-pigment tomato lines, ranging from 14.7 to 16.2 mg kg^{-1} fw, while in the ancient-tomato genotypes, it ranged from 5.5 to 6.02 mg kg^{-1} fw.

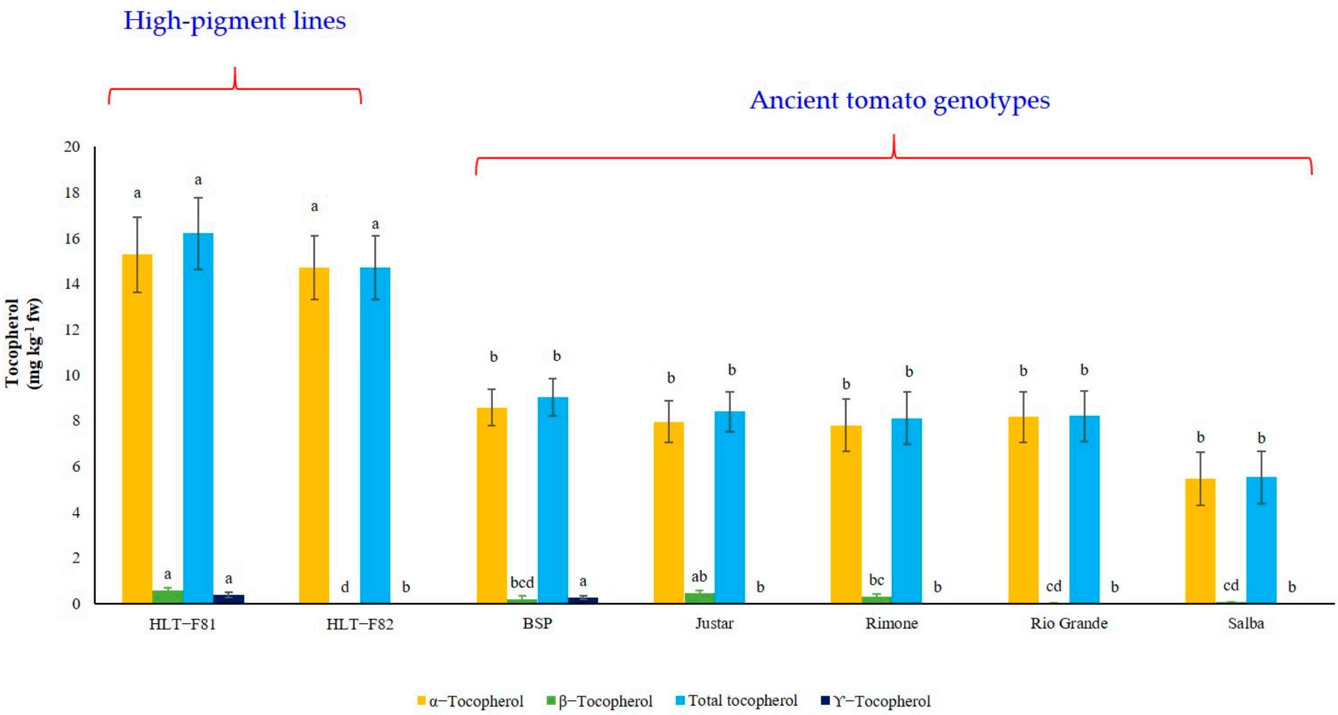

Figure 7. Tocopherol isomers their total content (mg kg^{-1} fw) in high-pigment tomato breeding lines and the ancient genotypes of tomato. Values represent mean ± standard error of six replicates (2021 and 2022 sampling data). Histograms marked with the same letters are not significantly different (LSD test, $p < 0.05$).

3.5. Total Vitamin C Content

In this study, the content of total vitamin C ranged from 155.85 mg kg^{-1} fw in 'BSP' to 256.43 mg kg^{-1} fw in the high-pigment tomato line 'HLT−F82' (Figure 8). The ordinary ancient genotypes 'Justar' and 'Salba' showed statistically similar content of total vitamin C to 'Rio Grande'. Compared to 'BSP', variations ranging from 9–37% to 29–64% were recorded in ancient-tomato genotypes and high-pigment tomato breeding lines, respectively.

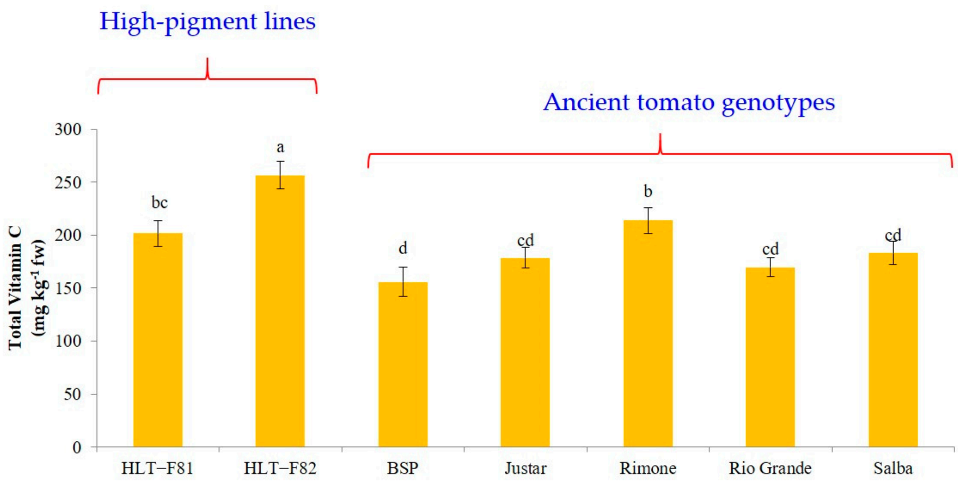

Figure 8. Total vitamin C content (mg kg^{-1} fw) in high-pigment tomato breeding lines and the ancient genotypes of tomato. Values represent mean ± standard error of six replicates (2021 and 2022 sampling data). Histograms marked with the same letters are not significantly different (LSD test, $p < 0.05$).

3.6. *The Antioxidant Activity (the Trolox Equivalent Antioxidant Capacity, TEAC Assay)*

The values of hydrophilic and lipophilic antioxidant activity varied significantly between the studied tomato genotypes ($p < 0.05$) (Figure 9). HAA and LAA values ranged from 111.56 µM Trolox 100 g^{-1} fw in 'Rio Grande' to 196.86 µM Trolox 100 g^{-1} fw in ''HLT−F81', and from 138.6 µM Trolox 100 g^{-1} fw in 'Rimone' to 265.0 µM Trolox 100 g^{-1} fw in 'HLT−F81'. Consequently, the high-pigment tomato line 'HLT−F81' obtained the highest HAA and LAA values among the tested tomato genotypes. Regarding HAA, compared to 'Rio Grande', variations ranging from 10 to 54% and from 61 to 77% were recorded among ancient-tomato genotypes and high-pigment breeding lines, respectively. Similarly, concerning LAA, compared to 'Rimone', variations ranging from 4 to 26% and from 33 to 91% were recorded among ancient-tomato genotypes and high-pigment breeding lines, respectively.

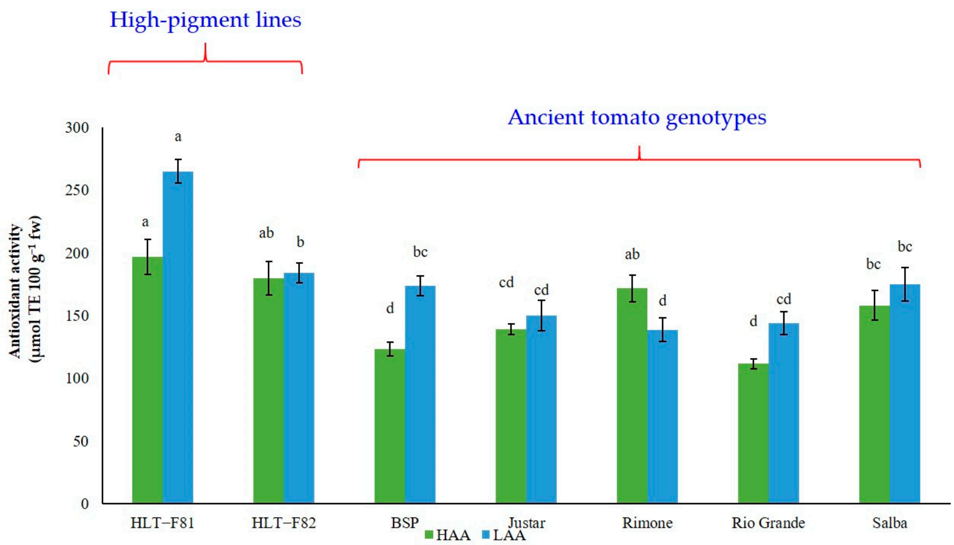

Figure 9. HAA and LAA (µmol TE 100 g^{-1} fw) in high-pigment tomato breeding lines and the ancient genotypes of tomato. Values represents mean ± standard error of six replicates (2021 and 2022 sampling data). Histograms marked with the same letters are not significantly different (LSD test, $p < 0.05$).

3.7. Correlation Analysis

Pearson correlation analysis was used to explore the interrelationships among the studied variables, with a significance level of $p < 0.01$ (Figure 10). Positive correlations were observed between the hydrophilic antioxidant activity and various hydrophilic antioxidants, including total phenols, total flavonoids, and vitamin C content. Similarly, lipophilic antioxidant activity displayed positive and significant correlations with most of lipophilic antioxidants, such as lycopene, β−carotene, γ−carotene, lutein, lycoxanthin, total carotenoids, α−tocopherol, β−tocopherol, γ−tocopherol, and total tocopherol content. Furthermore, color indexes a^* and the ratio (a^*/b^*) exhibited significant correlations with various quality traits of tomato berries, such as total phenolic, total flavonoid, lycopene, β−carotene, lutein, lycoxanthin, and total carotenoid content, as well as the antioxidant activity in both hydrophilic and lipophilic fractions. This suggests the potentiality of these color indexes in predicting the concentration of most of the mentioned traits.

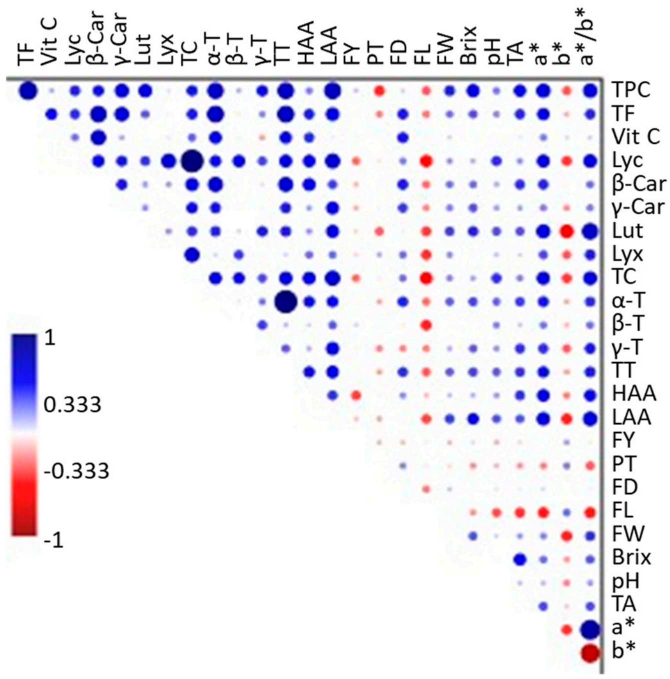

Figure 10. Pearson correlation matrix between agronomic, biochemical, and antioxidant attributes of the tomato lines under investigation. Blueish tones indicate higher positive correlations, whereas reddish tones point towards negative correlations. Larger circle diameters denote higher modules of the correlation coefficient (r). Nonsignificant correlations are shown as white intersections (significance was set at $p \leq 0.05$). TF: total flavonoids; Lyc: lycopene; β−Car: β−carotene; γ−Car: γ−carotene; Vit C: vitamin C; Lut: lutein; Lyx: lycoxanthin; TC: total carotenoids; α−T: α−tocopherol; β−T: β−tocopherol; γ−T: γ−tocopherol; TT: total tocopherols; HAA: hydrophilic antioxidant activity; LAA: lipophilic antioxidant activity; FY: fruit yield; PT: pericarp thickness; FD: fruit diameter; FL: fruit length; FW: fruit fresh weight; TA: titratable acidity; a^*: redness; b^*: yellowness.

3.8. Principal Component Analysis (PCA)

To gain a deeper understanding of the relationships between the high-pigment tomato breeding lines and the ancient genotypes, we utilized the biochemical data as input variables for the PCA analysis. The resulting biplot (PC1 vs. PC2) is shown in Figure 11. The first two principal components accounted for 78.852% of the observed variation, with the first component contributing 65.226% and the second 13.626%. PC1 exhibited positive correlations with total phenols, total flavonoids, vitamin C, total carotenoids, total tocopherols, as well as both hydrophilic and lipophilic antioxidant activities. Conversely, PC2 displayed positive correlations with vitamin C, total carotenoids, and both antioxidant activities, but it showed negative correlations with total phenols and total flavonoids.

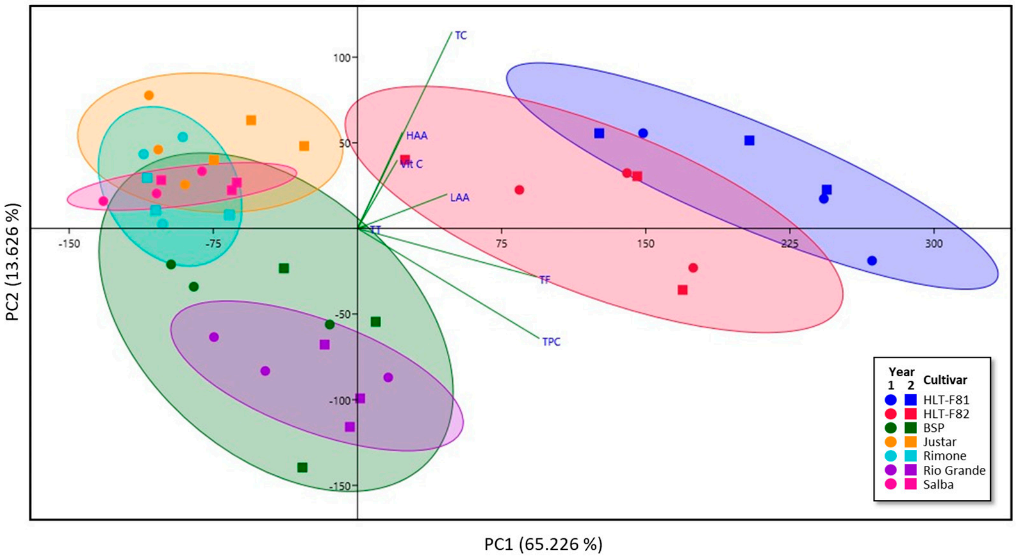

Figure 11. Principal component analysis (PCA) biplot PC1 vs. PC2 of the main bioactive classes of molecules (total contents) identified in the different tomato lines under investigation. The variance (%) explained by each PCA axis is given in brackets. The length of the vectors is correlated to their significance within each population. Between vectors, and between a vector and an axis, there is a positive correlation if the angle is <90°, whereas the correlation is negative if the angle reaches 180°. There is no linear dependence if the angle is 90°. Ellipses enclose the 75% confidence interval.

Regardless of the year of cultivation, both high-pigment breeding lines clearly clustered separately from the ancient-tomato genotypes, showing a positive score along PC1. Instead, the ancient genotypes fall within the negative sector of PC1. 'Justar', 'Rimone', and 'Salba' largely overlap with each other, occupying the upper-left quadrant of the graph. In contrast, 'BSP' and 'Rio Grande' are positioned in the lower-left quadrant, separate from the aforementioned varieties.

4. Discussion

The ancient-tomato genotypes demonstrated yields almost similar to the high-pigment tomato lines, suggesting their adaptation to the local climatic condition. This also highlights the choice of the varieties made in the past, mainly driven by the selection of cultivars suitable for both fresh markets and processing. The data further emphasize the importance of these genotypes thriving in the face of ongoing climatic changes, suggesting their potential utility not only in Tunisia but also in most Mediterranean countries. An intriguing point is that 'Salba', a cultivar selected for its higher firmness and resistance to field overripeness [50], exhibited a similar pericarp thickness to the high-pigment line 'HLT−F82' and the ancient genotypes 'Justar' and 'BSP'. This suggests their possible application for the same purpose, potentially resulting in a longer shelf-life and delayed fruit deterioration compared to currently commercialized tomato cultivars. These traits have been attributed to the higher wax content of the fruit cuticle, as discussed by Caiazzo et al. [51].

The yields of the high-pigment tomato lines and the ancient-tomato genotypes were comparable to those of other processing tomato cultivars commonly grown in Tunisia [6,28,52]. The yield, fruit shape, and average fruit weight of the tested tomato genotypes were in line with findings reported by several authors. For instance, Ronga et al. [53] recently evaluated the horticultural performance of modern and heirloom tomato genotypes cultivated in Italy. Their study revealed that the total yield was similar in heirloom and modern cultivars across different growing systems, thus emphasizing the consistent horticultural performances of heirlooms and ancient-tomato genotypes under the prevailing changes in climatic conditions.

Tripodi et al. [54] assessed twenty-one accessions from the two landraces 'San Marzano' and 'Re Fiascone'. Concerning morphological traits, they unveiled a broad range of variations. For instance, the fresh weight varied from 27.8 to 66.8 g in 'San Marzano' accessions and from 40.17 to 82.44 g in 'Re Fiascone' accessions. Similarly, fruit length ranged from 51.61 to 76.55 mm in 'San Marzano' accessions and from 47.83 to 67.0 mm in 'Re Fiascone' accessions. There were also differences in fruit width, with variations ranging from 28.94 to 40.07 mm in 'San Marzano' accessions and from 34.15 to 52.87 mm in 'Re Fiascone' accessions. Pericarp thickness exhibited variations from 4.17 to 8.47 mm among 'San Marzano' accessions and from 4 to 8.47 among 'Re Fiascone accessions. Furthermore, the data suggest that the variability among accessions of the same genotypes is mostly driven, not only by the genotype X environment interaction, but also by breeder selection and consumer preferences in different localities.

Regarding chemical traits, the findings highlight that most of the tested genotypes are well-suited for both the fresh market and processing. Despite slight variability in fruit shape, these genotypes showed almost identical chemical traits. The obtained values align with those reported by various authors. Tripodi et al. [54], for example, observed variation in soluble solids ranging from 3.47 to 5.97 °Brix in 'San Marzano' accessions and from 4.13 to 5.30 g in 'Re Fiascone' accessions. Additionally, pH values ranged from 3.22 to 4.81 in 'San Marzano' accessions and from 4.21 to 4.61 in 'Re Fiascone' accessions, while total acidity exhibited variations from 0.34 to 0.62 mEq% fw in 'San Marzano' accessions and from 0.32 to 0.49 mEq% fw in 'Re Fiascone'. In a similar vein, Athinodorou et al. [55] revealed substantial variations affecting several traits, such as pH values ranging from 4.55 to 4.67, total acidity ranging from 2.85 to 3.90 g L^{-1}, and total soluble solids ranging from 3.20 to 5.07 °Brix in different Cypriot tomato landraces. A critical factor in consumer demand for tomato fruits is the total soluble solids content and acidity characteristics.

Regarding color indexes, the high-pigment line 'HLT−F82' exhibited the highest a^*/b^* ratio, a result consistent with recent findings by Ilahy et al. [6]. They observed that the a^*/b^* ratios of tomatoes grown in Tunisia increased by 33% in the high-lycopene lines compared to the conventional cultivar Rio Grande. The result is also in agreement with those of Hanson et al. [56] who noticed an increase of the a^*/b^* index in different double mutant high-pigment tomatoes when compared to conventional genotypes.

In this study, the high-pigment breeding line 'HLT−F81' showed higher lycopene and total carotenoid contents compared to the other tomato genotypes. The values obtained are in line with those previously reported for open-field-grown tomato berries, ranging from 52 to 236 mg kg^{-1} fw [31]. These results are also consistent with the data from Levin et al. [21] and Ilahy et al. [6], who reported values attaining 440 mg kg^{-1} fw in different tomato genotypes carrying high-pigment mutations. Similarly, Sumalan et al. [57], in their assessment of 20 halotolerant tomato landraces from local farmers in Romania, reported lycopene-content values ranging from 53 to 184.3 mg g^{-1} fw. Tripodi et al. [54] assessed twenty-one accessions of the two landraces 'San Marzano' and 'Re Fiascone' and revealed lycopene-content variation of 37.75–112.82 μg g^{-1} fw in 'San Marzano' accessions and of 27.28–91.02 μg g^{-1} fw in 'Re Fiascone' accessions. The authors also noted variations in the β−carotene content, ranging from 4.46 to 8.56 μg g^{-1} fw in 'San Marzano' accessions and of 3.55–16.29 μg g^{-1} fw in 'Re Fiascone' accessions.

Genetic tomato resources also exhibited a longer shelf-life and better storability at room temperature compared to currently grown commercial cultivars. Parisi et al. [58] highlighted that renowned Italian small-tomato landraces like "Vesuviano" or "Piennolo" can be stored at room temperature for 120 days without compromising quality. They noticed stable lycopene cis−isomer levels and a stable β−carotene/total lycopene ratio, indicating relatively stable isomerization activity and, consequently, an optimal ripening pattern throughout the storage period.

Athinodorou et al. [55] conducted a thorough phytochemical assessment of the Cypriot tomato germplasm, revealing large variations in both the lycopene and β−carotene contents, ranging from 14.2 to 58.5 mg kg^{-1} fw and from 4.9 to 9.2 mg kg^{-1} fw, respectively.

These findings were attributed to the high nutritional value resulting from the thermodrought adaptation and tolerance of these genotypes. The high-pigment tomato breeding line 'HLT−F81' appears well-suited for human nutrition and the development of nutritious processed-tomato products, since the consumption of 100 g of those berries provide 91% of the recommended daily intake (25 mg per capita) compared to 30% provided by 'Rio Grande' berries.

Concerning the total phenol and flavonoid contents, Tripodi et al. [54] assessed various tomato accessions from the landraces 'San Marzano' and 'Re Fiascone'. They revealed a wide variation in the total flavonoid content, with values ranging from 5.81 to 16.99 mg of quercetin 3−glucoside equivalents 100 g^{-1} fw in 'San Marzano' accessions and from 2.20 to 23.84 mg of quercetin 3−glucoside equivalents 100 g^{-1} fw in 'Re Fiascone' accessions. The variation was less pronounced for the total quercetin, total kaempferol, total rutin, and total naringenin contents. Similarly, Sumalan et al. [57] noticed large variability in tomato landraces from local farmers in western Romania, reporting a total phenolic content ranging from 51.49 to 123.32 mg GAE.100 g^{-1} fw. Athinodorou et al. [55] found large variations affecting the total phenolic content, which ranged from 45 to 88.7 mg GAE kg^{-1} fw in different Cypriot tomato landraces.

The higher production of tocopherols in high-pigment tomato breeding lines compared to the ancient-tomato genotypes is likely associated with the hp genes carried by such lines, leading to increased plastid biogenesis [59], and consequently the overproduction of other metabolites generally accumulating in plastids, such as tocochromanols [60–62]. This may be valuable in breeding new tomato genotypes with improved stress tolerance, as tocopherols have been correlated with stress tolerance in tomato cultivars, a desirable trait under current climatic conditions.

Regarding the vitamin C content, Tripodi et al. [54] revealed a large variation in the ascorbic acid content, which ranged from 10.17 to 21.91 mg 100 g^{-1} fw in 'San Marzano' accessions and from 11.44 to 22.48 mg 100 g^{-1} fw in 'Re Fiascone' accessions. Sumalan et al. [57], assessing various halotolerant tomato landraces from local farmers in Romania, revealed a lower ascorbic acid content, ranging from 13.17 to 20.15 mg 100 g^{-1} fw. Athinodorou et al. [55] revealed large variations affecting the total vitamin C content, ranging from 243.7 to 480.2 mg kg^{-1} fw, in different Cypriot tomato landraces. This discrepancy in content suggests further evidence that hp mutations dramatically increase many important antioxidants, including vitamin C, as previously reported by Mochizuki and Kamimura [63] and Mustilli et al. [64]. The high-pigment 'HLT−F82' might be useful in developing new cultivars with both high lycopene and vitamin C content.

The obtained data for antioxidant activity generally fall within the range typically reported for field-grown tomatoes, ranging from 129 to 489 μM TE 100 g^{-1} fw for HAA and from 50 to 228 μM TE 100 g^{-1} fw for LAA [6,31,32]. Recently, Sumalan et al. [57] noted variations from 240 to 561.61 μM Fe^{2+} 100 g^{-1} fw in different halotolerant tomato landraces harvested in western Romania. Additionally, the data for high-pigment tomato lines align with that of other genotypes carrying mutations that enhance functional quality, ranging from 219 to 572 μM TE 100 g^{-1} fw for HAA and from 134 to 540 μM TE 100 g^{-1} fw for LAA [6,31,32]. Siddiqui et al. [65] evaluated the antioxidant activity in different tomato genotypes carrying the hp-2^{dg}/+ or og^{c}/+ alleles using two different analytical methods. The results revealed a consistent trend using both methods. Tomato genotypes carrying the hp-2^{dg}/+ exhibited greater antioxidant activity compared to the ordinary-tomato genotypes and those carrying the og^{c}/+ mutation. This suggests variability depending on the type of mutation, even within high-pigment tomato lines. Generally, the higher range of hydrophilic and lipophilic antioxidant activity has been observed in berries of high-pigment tomato lines.

5. Conclusions

Despite the limited number of landraces included in this study due to their dwindling presence, the evaluation of both ancient-tomato genotypes and high-pigment lines in terms of horticultural performance and fruit quality underscores the inexhaustible resource of variability, with proven nutraceutical properties, of the traditional ordinary genotypes. The study also highlights the potential significance of most of these genotypes in conventional farming due to their low-input requirements and their adaptability to the prevailing climatic conditions in North Africa, characterized by rising temperatures and frequent heatwaves during the hot season.

Beyond horticultural performance, the study provides valuable insights into the functional quality of high-pigment and ancient-tomato genotypes that have adapted to high-temperature conditions during their growth cycle. These genotypes accumulate several desirable traits, as is evident when the data are correlated with the ongoing climatic conditions. However, further functional research is still necessary to establish the link between the tocopherol content and stress tolerance, particularly in high-pigment tomato lines.

Author Contributions: Conceptualization, Y.L., I.T., I.H., R.I. and T.R.; investigation, Y.L., I.T., I.H., M.É., S.T., A.B.A., M.A. and M.W.S.; data curation, M.S.L., H.D. and Z.P; formal analysis and validation, Y.L., I.T., I.H., R.I., L.H., T.R., S.T., H.D., Z.P. and M.S.L.; writing—original draft preparation, Y.L., I.H., I.T., M.É., S.T., M.A., M.W.S., H.D., Z.P., L.H., T.R., M.S.L. and R.I.; writing—review and editing, Y.L., I.H., I.T., M.É., S.T., M.A., M.W.S., H.D., Z.P., L.H., T.R., M.S.L. and R.I. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Saloua Taoueb Msellmi and Aziza Zoghlami from the National Agricultural Research Institute of Tunisia.

Conflicts of Interest: The authors declare no conflict of interest.

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