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The Antioxidant Profile Evaluation of Some Tomato Landraces with Soil Salinity Tolerance Correlated with High Nutraceutical and Functional Value

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Received: 23 March 2020; Accepted: 31 March 2020; Published: 2 April 2020

Abstract: Romania has a wide variety of local landraces and heirloom genotypes. Our study aims to assess the performance of twenty halotolerant tomato landraces, collected from areas with medium and high levels of soil salinity, in terms of the accumulation of antioxidant compounds in fruits and to cluster them according to their nutraceutical components. The tomatoes used in the study were harvested once they had attained full ripeness and then analyzed for lycopene (Lyc), ascorbic acid content (AsA), total phenolic content (TPC), and total antioxidant capacity (TAC). The results revealed major differences between genotypes in terms of nutraceutical values. According to principal component analysis, the tomato landraces were grouped into five clusters, characterized by different proportions of compounds with antioxidant activity. The high/moderate nutritional values of Lyc, TAC, TPC, and AsA were obtained from varieties taken from local lands with high soil salinity, over 6.5 dS m⁻¹. These findings support the idea that metabolites and secondary antioxidants are involved in the process of stress adaptation, thereby increasing salinity tolerance in tomatoes. Our results show that there are tomato landraces with a tolerance of adaptation to conditions of high soil salinity and provide information on their ability to synthesize molecules with antioxidant functions that protect plants against oxidative damage.

Keywords: tomato cultivars; salinity tolerance; antioxidant activity; lycopene; ascorbic acid; total polyphenols content

1. Introduction

The commercial production of tomato (Solanum lycopersicum L.) in the developed regions of the world mostly concerns modern varieties of the fruit, and, more often than not, genetically uniform F1 hybrids that have a high yield, greater tolerance to diseases, and a long shelf life are chosen [1].
Landraces represent an important alternative, as they constitute a reservoir of genetic diversity, with their important abiotic stress tolerance and high fruit quality. Tomato landraces contain valuable alleles uncommon in highlight germplasms [2]; therefore, these local populations represent a valuable resource of genetic traits that can be used in breeding programs for the improvement of the crop [3].

Intensive tomato cultivation technologies require genotypes with good productivity, handling, transport, and storage properties, while the nutraceutical properties are passed on to the secondary level. Nevertheless, consumption of the traditional plant foods that have antioxidant content naturally occurring may be a better strategy to improve the human health status than the consumption of artificial antioxidant products [4]. From this perspective, an appropriate selection of tomato cultivar would help to achieve a higher antioxidant intake with the potential to produce significant health benefits. Considering the growing demand for tasty tomatoes and being rich in phytochemicals, a detailed characterization of the biologically active compounds completed with total antioxidant capacity evaluation should be performed. That is why the tomato landraces and their relatives are of great importance in breeding programs [5–7]. Besides, landraces have low requirements for inputs, which contribute to the development of environmentally-friendly technologies [8–10].

The genetic resources of cultivated plants that come from the soil salinity-affected areas have a major importance due to drought tolerance. The salinity is associated with the physiological drought that may induce the growth of the bioactive compound content with antioxidant properties [11,12]. The information about genetic variability specific to local populations is assumed to be limited [13–15] because traditional locally grown cultivars should not be considered rigorously homogenous.

The tomato taste is correlated with some bioactive components. Some authors consider that AsA (ascorbic acid), TAC (total antioxidant capacity), and TPC (total phenols content) have a direct impact on tomato taste [5], while others assign the taste to the ratio between sugar content and acidity [16–19]. The high content in valuable phytonutrients depends equally on technological, genetic, and storage factors [16,20–25]. Besides dietary fibers and carbohydrates, compounds such as lycopene, β-carotene, ascorbic acid, and polyphenols provide high levels of antioxidants. For this reason, the consumption of raw or processed tomatoes contributes to good activity into an organism by maintaining oxidative stress at a low level [26,27].

Lycopene is the most important and recognized phyto bioactive-compound of tomatoes. It is a carotenoid pigment that is less bio-available compared to β-carotene and lutein [28,29]. In addition to lycopene, the tomato fruits contain vitamins A and C, other carotenoids whose action interacted with those of polyphenols, resulting in an overall benefit on human health [30,31]. The ripening stage of tomato fruits represents a decisive factor regarding the establishment of nutritional values of thereof. From this point of view, the tomatoes harvested at technological ripeness revealed low quantities of lycopene while the content of ascorbic acid was variable, depending on genotype [32].

The aim of this paper is to characterize some tomato local landraces, originating from areas with saline soils located in western Romania (Banat region), concerning the total antioxidant capacity and the potential of biosynthesis and accumulation of some antioxidant biocomponents, such as phenolic compounds, lycopene and ascorbic acid.

In this work, we test the hypothesis that local tomato landraces originating from areas with soil salinity show a higher biosynthesis capacity of some compounds with antioxidant activity, and this property is maintained even under cultivation on non-saline soil conditions.

2. Materials and Methods

2.1. Plant and Soil Analyses

Twenty halotolerant tomato landraces, collected from local farmers of the country-side situated in areas with soils affected by different levels of sodicity from western Romania (Banat region) were analyzed. Saline-sodic soils are high in exchangeable sodium and low in total soluble salts. The level of ESP (exchangeable sodium percentage) in these soils is 15 or more, which tends to destroy their
structure by dispersing the particle, and electrical conductivity (EC) is over 4 dS m$^{-1}$. Most saline soils in the collecting area have a clayed loamy texture, medium glomerular structure and moderately to high salinization level determined by using an EC-meter (model consort C933, producer De Bruine Instruments bvba, Belgium) determination using the EC 1:2w/w method [33] (Table 1).

Seeds and soil samples were collected in the period 2012–2015, and the specimen is available at Plant Physiology Department, Faculty of Horticulture and Forestry, Timisoara. Previously, each local landraces was characterized in morpho-physiologically and genetically manner [34], for each of them was drawn up an identification sheet in which source, specific cultivation technology, productivity, shape, and color of tomato fruits were noticed. Based on productivity traits, high tolerance to salinity and minimum growth requirements proven in the summer of 2015, twenty tomato landraces were selected for assessment of nutraceutical traits (Table 1).

The genotypes were open field cultivated in a plain site located on the northern side of Timisoara (45°78′N; 21°21′E), on cambichernozem soil [35]. The soil had the following physico-chemical characteristics: clay 402 g kg$^{-1}$; sand 330 g kg$^{-1}$; loam 268 g kg$^{-1}$; organic matter 26.8 g kg$^{-1}$; pH 6.26; total N 2 g kg$^{-1}$; available P$_2$O$_5$ 20.52 mg kg$^{-1}$; exchangeable K$_2$O 117 mg kg$^{-1}$; sulfates (mobile in water) 105.6 mg kg$^{-1}$; sodium (mobile in water) 366.7 mg kg$^{-1}$; calcium (water-soluble) 270.5 mg kg$^{-1}$; magnesium (soluble in water) 60.8 mg kg$^{-1}$.

A randomized complete block experimental design with three replicates was used in the field during the spring–summer season. Plants were transplanted at the four-leaf stage on 18 April 2017 in plots of 24 m$^2$ (6 x 4 m) at 1.66 plants m$^{-2}$. The average monthly temperatures ranged between 11.6 °C in April to 27.4 °C in July. Plants were spaced 1.2 m between the rows and 0.5 m within the row and watered with a drip irrigation system. Plants were trained with canes and cultivated using a traditional horticultural practice in the area for local tomato varieties. All genotypes have an indeterminate plant growth type.

| Genotype Code | Site          | GPS Coordinates (lat/long) | Soil EC(dSm$^{-1}$) | Fruit Shape 1 | Tomatoes Weight Average (g) 2 | Full Ripeness Color 3 |
|---------------|---------------|---------------------------|---------------------|---------------|-------------------------------|----------------------|
| CN26          | Crai Nou      | 45°29′17″N/21°0′1″E       | 6.86                | flattened     | 352.47                        | light red            |
| PN            | Peciu Nou     | 45°36′54″N/21°0′1″E       | 5.63                | flattened     | 184.66                        | light red            |
| Gi            | Giera         | 45°25′21″N/20°57′25″E     | 5.25                | circular      | 124.18                        | red                  |
| L-189a        | Lovrin        | 45°57′03″N/20°46′32″E     | 5.02                | obovate       | 73.75                         | light red            |
| C-102         | Cruceni       | 45°28′23″N/20°52′44″E     | 7.04                | circular      | 133.55                        | light red            |
| Pe            | Periam        | 46°01′41″N/20°53′35″E     | 6.86                | circular      | 295.76                        | red                  |
| Gr            | Gradinari     | 45°06′16″N/21°34′59″E     | 4.38                | circular      | 273.00                        | light red            |
| DV            | Dudesiti Vechi| 46°04′55″N/20°26′55″E     | 5.80                | obovate       | 164.92                        | red                  |
| Ch            | Cheglevici    | 46°6′40″N/20°26′56″E      | 6.04                | flattened     | 264.54                        | red                  |
| C-60pr12      | Cherestur     | 46°7′60″N/20°22′60″E      | 5.65                | ovate         | 150.64                        | light red            |
| Ch-165        | Cheglevici    | 46°6′40″N/20°26′56″E      | 6.29                | circular      | 105.44                        | red                  |
| Li            | Livezile      | 45°23′09″N/20°1′02″E      | 6.44                | cilindric     | 133.62                        | yellow               |
| L-189b        | Lovrin        | 21°0′2′3″E/20°46′32″E     | 6.58                | flattened     | 136.11                        | light red            |
| Ru            | Rudna         | 45°29′54″N/21°0′31″E      | 4.50                | obovate       | 81.73                         | red                  |
| SS180         | Sannartini Sarbesc | 45°36′23″N/20°57′38″E | 4.47                | cordate       | 303.09                        | red                  |
| T673          | Tarnova       | 45°20′06″N/22′00′08″E     | 4.11                | flattened     | 309.57                        | red                  |
| T370          | Tarnova       | 45°20′06″N/22′00′08″E     | 4.23                | flattened     | 348.42                        | red                  |
| IM/pusta      | Jeece Mare/Pusta | 45°50′51″N/20°54′08″E | 4.18                | flattened     | 392.60                        | red                  |
| SS            | Sannartini Sarbesc | 45°36′23″N/20°57′38″E | 4.30                | flattened     | 185.59                        | light red            |
| CN-254        | Crai Nou      | 45°29′17″N/21°0′1″E       | 7.21                | obovate       | 150.33                        | red                  |

1 According to UPOV (International Union for the Protection of New Varieties of Plants) classification in tomatoes fruit shape [36]; 2 data were collected from 15 fruits; 3 USDA (United States Department of Agriculture) tomato ripeness color chart.
2.2. Tomatoes Samples Preparation

From each genotype, samplings were taken at different harvesting times only when tomatoes were at a fully physiological ripening stage. Fifteen fruits were randomly taken from each replication in order to compose the average tomato sample. Evaluations of shape, weight, and colors, as well as chemical analysis, were done. The fruits were stored in polyethylene bags and kept in freezing conditions at −18 °C until performing the chemical analysis. All analyses were carried out in triplicate.

2.3. Chemical Analysis

The chemical analysis consisted of assessing of TAC, TPC, AsA, and Lyc content from each tomatoes landrace. Prior analysis, the frozen samples were kept in refrigeration condition (4–6 °C) for 6 h and then homogenized in a Bosch Blender (MMB42G0B, 700 W, Germany) for 1 min. Three replicates were prepared from each average sample.

2.3.1. Extract Preparation

Briefly, 10 g of blended tomato sample was mixed with 20 mL ethyl alcohol 70% (v/v) for 2 h at 25 °C, then, the mixture was filtered and the clear extract was used for the analysis of TAC and TPC.

2.3.2. Reagents and Equipment

All chemicals and reagents were analytical grade or purest quality purchased from Merck and Fluka. Deionized water was used.

TAC Evaluation

TAC of tomatoes was evaluated by ferric reducing antioxidant power (FRAP) assay, according to the method described by Benzie and Strain [37]. This method supposed the reduction of Fe$^{3+}$-TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) complex to ferrous form at low pH. The ferrous tripyridyltriazine complex has an intense blue color monitored at a wavelength of 593 nm. An aqueous solution of Fe$^{2+}$ with a concentration in the range of 0.1 to 0.8 mM/L was used for the calibration curve preparation. The absorption was measured at $\lambda = 593$ nm after 15 min of incubation at 25 °C using the UV–vis spectrophotometer SPECORD 205 (Analytic Jena, Germany). TAC was expressed as μM Fe$^{2+}$ equivalents·100 g$^{-1}$ FW (fresh weight).

TPC Determination

TPC in tomato samples was evaluated following the Folin–Ciocalteu colorimetric method described by Singleton and Rossi [38]. For analysis, it was used the tomato ethanol extracts diluted 1/10 with bidistilled water. For calibration curve preparation, 0.5 mL aliquot of aqueous gallic acid solution with a concentration in the range 0.2–1.2 μM/mL were mixed with 2.5 mL Folin–Ciocalteu reagent (diluted ten-fold with bidistilled water) and 2.0 mL sodium carbonate (7.5%). The absorption was read at $\lambda = 750$ nm after 2 h of incubation at 20 °C. TPC in mg gallic acid equivalents (GAE)-100 g$^{-1}$ FW was calculated.

AsA Content

The AsA content of tomato samples was measured on the base of the AOAC method [39] by titration with 2,6-dichlorophenolindophenol sodium salt. For this purpose, 10 g of blended tomato sample was mixed with 10 mL bidistilled water for 2 h at 25 °C, then, the mixture was filtered, and the clear extract was used for analysis. Further, 5 mL of extract was diluted with 10 mL bidistilled water, then 1 mL HCl 1N was added and the mixture was titrated with 2,6-dichlorophenolindophenol sodium solution 1 mM in an acid medium (pH = 4). The results are expressed as mg ascorbic acid·100 g$^{-1}$ FW.
Determination of Lyc Content

Lyc was extracted from tomato samples with a hexane–ethanol–acetone (2:1:1) mixture in agreement with the method described by Sharma and Le Maguer [40]. Briefly, 1 g of tomato blended sample was mixed with 25 mL of the previously mentioned mixture and then placed on a rotary mixer for 30 min. Further, 10 mL of bidistilled water was added, and the mixture was stirred for another 2 min. The obtained mixture solution was separated into two distinct polar and non-polar layers. The absorbance was measured at 502 nm, using hexane as a blank. The Lyc content of tomato samples was calculated on the base of its specific extinction coefficient (E 1%, 1 cm) of 3150 at 502 nm [41]. The Lyc concentration was expressed as mg 100 g$^{-1}$ FW.

2.4. Statistical Analysis

The experimental data were statistically processed using ANOVA, and the means were compared using the multiple range test [42]. The significance of differences was expressed based on letters, being considered as significant the differences between genotypes marked with different letters.

The clustering of genotypes was carried out using the UPGMA (unweighted pair group method with arithmetic mean), with the NEIGHBOR program of PHYLIP package, version 3.5c. [43]. Average intra and inter-cluster distances (D2) were estimated [44], and the percent contribution of each character to the total divergence was calculated by ranking each character on the basis of transformed uncorrelated values. To display the performance of each landrace for each of four nutritional traits in a single graph, the basic principle of the biplot technique was used [45,46].

3. Results

3.1. Fruit Morphological Traits

All the measured fruit morphological traits (shape, weight, and color), showed a large range of phenotypic variation among the 20 tomato landraces.

Fruit shape is one of the most important qualities which can be determined with the naked eye, being used to identify local tomato populations. Globally, there is a huge variety of fruit shapes on tomatoes, specific to different landraces, a fact reported by many studies conducted in different geographic areas and time periods [2,15,36,47–52].

As for the traits related to color and flesh color of fruits, ten genotypes had red fruit, nine genotypes had a light red color, and one had yellow fruits.

Regarding the tomato fruit weight, a number of eight genotypes had large fruits ($\geq$200 g), of which five were very large ($\geq$300 g), ten landraces had average fruits weighing between 100 and 200 g, and only two formed small fruits (below 100 g). The comparative analysis of the form of fruit and their weight shows that six of the local populations with high weight fruit have a flattened shape.

These results are also in line with other studies [53] confirming that, in the process of improving tomatoes, people prefer to increase the size and mass of the fruit, causing and modifying the round shape (wild species) with a flattened one in the most forms cultivated for fresh consumption or elongated for industrialization. On the other hand, some studies [15] found that, in the case of some local Italian and South American tomatoes populations, the flattened form of the fruit has been associated with a small and average weight (50–150 g). Obviously, the fruit mass is a genetically controlled process, but it depends to a large extent on the specific pedo-climatic conditions and applied technology.

Therefore, the study of genetic variability in local tomato landraces will be able to provide additional information on the genetic, physiological, and biochemical mechanisms that are based on the correlation of the shape, size, and weight of the fruit, thus contributing to the identification of the alleles and new ecotypes with superior properties in terms of productivity, adaptation, quality, and nutritional value.
3.2. Antioxidants and Nutraceutical Component Analysis

Antioxidants are redox buffers that interact with ROS (reactive oxidative species) and can manifest as a metabolic interface that regulates adaptation responses or programmed cell death [54]. The low values of \( F \): 120 and 126, for significant differences at \( p < 0.01 \), show that there is a lower variability between landraces for TPC and AsA.

3.2.1. Assessment of TAC

Statistical data analysis concerning the performance of TAC of tomato fruit samples reveals significant differences between landraces (Table 2). The quantity and proportion between bioactive compounds with antioxidant capacity depend on the plant’s genotype and post-harvest storage conditions [55,56]. The reducing ability recorded for the 20 landraces varies from 561.61 to 240.75 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW with an amplitude of variation of 320.86 between landraces (Table 2).

Table 2. Total antioxidant capacity (TAC) of tomato fruit samples.

| Genotype Code | FRAP \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW |
|---------------|------------------------------------------|
| CN-26         | 413.27 ± 3.63 d                          |
| PN            | 305.44 ± 2.99 g                          |
| Gi            | 349.03 ± 3.76 f                          |
| L-189a        | 386.49 ± 3.91 e                          |
| C-102         | 420.97 ± 2.13 d                          |
| Pe            | 451.76 ± 1.36 c                          |
| Gr            | 384.03 ± 3.19 e                          |
| DV            | 411.36 ± 2.02                            |
| Ch            | 274.00 ± 1.53 i                          |
| C-60pr12      | 240.75 ± 1.51 j                          |
| Ch-165        | 304.67 ± 1.65 g                          |
| Li            | 415.14 ± 1.99 d                          |
| L-189b        | 506.51 ± 2.65 b                          |
| Ru            | 300.05 ± 2.42 gh                         |
| SS-180        | 289.21 ± 1.58 h                          |
| T-673         | 287.27 ± 1.60 hi                         |
| T-370         | 407.56 ± 2.31 d                          |
| IM/pusta      | 307.69 ± 1.62 g                          |
| SS            | 387.05 ± 1.92 e                          |
| CN-254        | 561.61 ± 4.37 a                          |
| Mean          | 370.19 ± 10.48                           |
| Cochran’s C Test | 0.145; \( p = 1.00 \)                     |
| Bartlett’s Test  | 1.300; \( p = 0.974 \)                   |
| LSD5%         | 13.78                                   |

Data are mean ± SE, \( n = 3 \). Values within columns with different letters are significantly different (\( p = 0.05 \)).

The high value of TAC was determined for CN-254, 561.61 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW, followed by L-189b with 506.51 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW. Both landraces have recorded statistically higher differences related to mean. In the second category fall the local populations Pe (451.76 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW), C-102 (420.97 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW), and CN-26 (413.27 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW). The lowest value of FRAP (240.75 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW) was determined for genotype C-60pr12. It should be noted that all five cultivars of the first two categories were collected from the areas with high concentrations of soil salinity between 7.21 dS m\(^{-1} \) for CN-254 and 6.58 dS m\(^{-1} \) at L-189b.

The recorded TAC values are in agreement with those reported by other authors who have used the same method. Thus, mean values of TAC of 506 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW [57] were reported, while other authors reported limits between 387 and 493 \( \mu M \) Fe\(^{2+} \) 100 g\(^{-1} \) FW [31] for tomatoes fruits.
Phenolic compounds play an important role in the antioxidant capacity of tomato fruit. In addition, other bioactive compounds such as ascorbic acid and lycopene, the main tomato carotenoid, play a major role in the antioxidant capacity of tomato fruit samples. The ascorbic acid is considered the most important water-soluble antioxidant, with a significant contribution to the antioxidant cellular defense against oxidative stress. FRAP assay also measures the antioxidant capacity of ascorbic acid besides that of phenolic compounds. The phenolic compounds act synergistically with ascorbic acid in order to preserve and regenerate the antioxidant species. Lycopene is one of the most powerful antioxidants among the dietary carotenoids. The total antioxidant capacity of tomato fruit is the result of a combination of different compounds having synergistic and antagonistic effects.

3.2.2. Assessment of TPC, Lyc, and AsA Content

The quantity and quality of phenolic compounds determined in tomato fruit varies in relation to the genotype but also depends on environmental and technology factors [58]. Data from Table 3 reveal that the TPC values range from 51.49 to 123.32 mg GAE·100 g−1 FW. The highest value was registered for C-102 (123.32 30 mg GAE·100 g−1 FW), followed by L-189b with 117.77 mg GAE·100 g−1 FW and CN-254 (114.89 mg GAE·100 g−1 FW), but the differences between them have no statistical significance.

The TPC values for the local tomato populations collected from the saline areas are higher compared to other studies [31,56,59,60]. TPC values recorded for industrial processing cultivars (green Ronaldo and red cherry Pera) were between 18.69 to 55.86 mg GAE·100 g−1 FW [56] and in the range 19.7–21.1 mg GAE·100 g−1 FW [61], while for fresh tomato consumption ranging from 50.86 to 53.88 mg GAE·100 g−1 FW were reported [31]. Also, Martinez-Valverde reported TPC values between 25.9 and 49.8 mg GAE·100 g−1 FW for determinations of some commercial varieties of tomatoes from supermarkets [59]. The lowest values of TPC in our cultivars were determined for IM/Pusta with 51.49 mg GAE·100 g−1 FW, followed by genotype Ch with 53.06 mg GAE·100 g−1 FW.

The value of TPC for our landraces (especially C-102, L-189b, and CN-254) are higher than those obtained by other researchers for different hybrids. The highest value of TPC for CN-254 justifies the large TAC value determined for this local population, poly-phenols being well known for their antioxidant activity [30,62]. By correlating the results to the fact that these local populations have adapted over time to high levels of soil salinity, we can assume that they have developed genetic, biochemical, and physiological mechanisms to synthesize some of the antioxidant-related co-agents, to allow survival under specific stress conditions. It seems that the high pressure of the soil salinity influenced the plants which were obliged to adapt by increasing the synthesis capacities of polyphenols and indirectly intensifying of antioxidant processes. These traits appear to be manifested in the conditions of their cultivation outside specific saline areas. Indeed in a recent work, it has been shown that the traditional variety of tomato improved cations homeostasis and increased sucrose content in the fruits as a part of the salt stress tolerance mechanism [63].

Raw tomatoes contain usually Lyc between 3 to 10 mg 100 g−1, but field-grown tomatoes appear to contain higher levels of this compound, ranging from 5.2 to 23.6 mg 100 g−1 FW than greenhouse-grown tomatoes (0.1 and 10.8 mg 100 g−1 FW) [31].

In our research, the Lyc content recorded a high variability of 13.13 mg 100 g−1 FW amplitude of variation (Table 3). The highest value of Lyc was determined for SS-180 (18.43 mg 100 g−1 FW) followed by Gr (17.37 mg 100 g−1 FW) and T-673 (16.61 mg 100 g−1 FW). At the same time, significantly lower mean values of the Lyc content were noticed for landraces Li (5.30 mg 100 g−1 FW), Ch-165 (6.79 mg 100 g−1 FW), and C-60pr12 (6.81 mg 100 g−1 FW) that have recorded statistically assured differences related to mean of experience.

Comparing our results concerning the Lyc content of tomato fruit with other studies [64–67], we found that the local populations collected by the salt-crops cultivated under non-saline conditions recorded higher values. Most previous studies reported values of Lyc content in fresh tomato fruits ranging from 3 to 10 mg 100 g−1 FW. Higher amounts of Lyc (around 25 mg 100 g−1 FW) were reported in the cherry tomatoes IIHR-249-1 line [68].
Although most research confirms the growth of Lyc content in salinity conditions [69–71], there are a few studies that infirm this hypothesis [23,72]. Therefore, the genetic requirements and the specific conditions in which the genotype was cultivated are decisive factors on the capacities of biosynthesis of Lyc, alongside other evident factors such as ripening stage and cultivation technologies (e.g., field/greenhouse, organic/non-organic) [32].

The results obtained through this research do not show a direct connection between the soil salinity level in the harvesting area and the amount of Lyc of the various local populations. Cultivars with high levels of Lyc come from areas with moderate levels of soil salinity (E_C ≤ 4.5 dS m⁻¹).

The introduction of Lyc as a phyto-compound in the human diet, due to multiple antioxidant properties, led to obtaining products enriched in this biomolecule [73]. From a nutritional point of view and based on recommendations on the consumption of 35 mg Lyc/day [74], this can be achieved by eating 200 g of tomatoes from SS 180 or GR local population for the daily requirement of an adult.

AsA is an antioxidant with an electron donor role in many important reactions [75,76]. It plays a key role in photosynthesis protection during salinity stress. It has been shown that on salinity stress conditions, mutations with deficiency of AsA synthesis accumulate very large amounts of H₂O₂, which coincides with an important decrease of the reduced AsA. The accumulation of H₂O₂ in the foliar apparatus of tomato plants determines the inhibition of photosynthesis by reducing the amount of chlorophyll, CO₂ assimilation, and decrease in PS II activities [54].

The analysis of the results confirms that the above-presented genotypes with high synthesis capacity of the AsA have manifested very good qualities of TAC and TPC (Table 2). The results prove

Table 3. Lycopene (Lyc), ascorbic acid (AsA), and total phenolic content (TPC) of tomato fruit samples.

| Genotype Code | Lycopene mg 100 g⁻¹ FW | Ascorbic Acid mg 100 g⁻¹ FW | Total Phenols mg GAE 100 g⁻¹ FW |
|---------------|------------------------|-----------------------------|---------------------------------|
| CN26          | 10.72 ± 0.19 hi        | 16.93 ± 0.20 de             | 69.15 ± 1.28 f                  |
| PN            | 7.93 ± 0.12 k          | 15.21 ± 0.14 ij             | 92.88 ± 1.83 c                  |
| Gi            | 11.43 ± 0.17 g         | 15.94 ± 0.17 h              | 93.22 ± 1.71 c                  |
| L-189a        | 11.20 ± 0.13 gb        | 16.44 ± 0.19 fg             | 93.68 ± 2.18 c                  |
| C-102         | 12.57 ± 0.12 f         | 17.37 ± 0.16 bc             | 123.32 ± 2.10 a                 |
| Pe            | 12.51 ± 0.10 f         | 17.70 ± 0.18 b              | 91.76 ± 2.19 cd                 |
| Gr            | 17.37 ± 0.14 b         | 16.56 ± 0.14 ef             | 86.58 ± 1.67 cd                 |
| DV            | 12.29 ± 0.11 f         | 16.99 ± 0.16 cde            | 91.37 ± 1.70 cd                 |
| Ch            | 9.30 ± 0.08 j          | 13.98 ± 0.16 l              | 53.06 ± 0.73 g                  |
| C-60pr12      | 6.81 ± 0.06 l          | 13.17 ± 0.12 m              | 112.96 ± 1.74 b                 |
| Ch-165        | 6.79 ± 0.07 l          | 15.07 ± 0.13 ij             | 75.19 ± 1.53 ef                 |
| Li            | 5.30 ± 0.07 m          | 17.06 ± 0.11 cd             | 88.26 ± 2.12 cd                 |
| L-189b        | 10.73 ± 0.12 hi        | 19.23 ± 0.16 a              | 117.77 ± 2.53 ab                |
| Ru            | 12.87 ± 0.17 ef        | 14.81 ± 0.13 jk             | 89.29 ± 2.09 cd                 |
| SS-180        | 18.43 ± 0.15 a         | 14.54 ± 0.11 kl             | 73.19 ± 1.17 f                  |
| T-673         | 16.61 ± 0.13c          | 14.31 ± 0.13 l              | 71.79 ± 1.01 f                  |
| T-370         | 12.83 ± 0.11 ef        | 16.10 ± 0.18 gh             | 82.69 ± 1.83 de                 |
| IM/pusta      | 13.23 ± 0.10 e         | 15.30 ± 0.17 i              | 51.49 ± 1.19 g                  |
| SS            | 14.00 ± 0.13 d         | 16.72 ± 0.15 def            | 93.48 ± 1.46 c                  |
| CN-254        | 10.18 ± 0.08 i         | 20.15 ± 0.17 a              | 114.89 ± 2.16 ab                |
| means         | 11.65 ± 0.44           | 16.18 ± 0.22                | 88.30 ± 2.49                    |
| Cochran’s C Test | 0.128; p = 1.00    | 0.086; p = 1.00              | 0.102; p = 1.00                  |
| Bartlett’s Test | 1.191; p = 0.998     | 1.057; p = 1.00              | 1.162; p = 0.999                 |
| LSD5%         | 0.65                   | 1.56                        | 9.49                            |

Data are mean ±SE, n = 3. Values within columns with different letters are significantly different (p = 0.05).

Consequently, our results show that AsA content of tomato fruit has not a large amplitude, the determined quantities ranging from 13.17 to 20.15 mg 100 g⁻¹ FW. Particularly notable are CN-254 and L-189b landraces, having the highest values of AsA content of 20.15 and 19.23 mg 100 g⁻¹ FW, respectively, followed by Pe (17.70 mg 100 g⁻¹ FW) and C-102 (17.37 100 g⁻¹ FW).

The analysis of the results confirms that the above-presented genotypes with high synthesis capacity of the AsA have manifested very good qualities of TAC and TPC (Table 2). The results prove
that the AsA content is conditioned by genotype to a minimal extent. High AsA content was reported in F1 tomato hybrid for which heterosis effect was manifested, the maximum content determined of 36.3 mg 100 g\(^{-1}\) FW being over mid parental values [20,77].

It was demonstrated that AsA increase is linked to the adopted cultivation system. Studies on tomato growing technologies have highlighted the importance of nutrient availability for tomato plant requirements for AsA accumulation [78]. Therefore, there are numerous reports of increasing the content of AsA in tomato fruit subject to various types of stress [79–84], as well as some results reported in which stress resulted in the reduction of AsA [85,86]. The contradictory results can be attributed to the genetic differences regarding the sensitivity of the different genotypes to the oxidative stress manifested by salinity.

It is known that there are universal mechanisms as responses to the action of stress factors, but their relative impacts can vary from one species to another and within the same species from one genotype to another, depending, actually, on specific metabolic background.

3.3. Comparison of Tomato Landraces for Nutraceutical Traits

Using the UPGMA method for 20 variables of tomato landraces, a dendrogram was designed that has identified five groups (clusters) on the basis of coefficients similarity for bioactive compounds (Figure 1). The first cluster brings together nine landraces: CN26, Gi, L-189a, DV, SS, T370, Pe, Gr, and C-102. These are characterized by higher TAC and AsA values, while TPC and Lyc have recorded values above landrace average. Three landraces are grouped in the second cluster, namely: PN, Ch-165, and Li; these recorded low values of Lyc but medium values of TAC, TPC, and AsA content.

In Cluster III, there are grouped five landraces characterized by a high Lyc, while TAC and AsA have lower values than average, and TPC has the lowest values. In Cluster IV, one single landrace is noticed; C-60pr12 has a high value of TPC but a low one of Lyc, while TAC and AsA contents are lower than mean values of landraces.

![Figure 1. Dendrogram of tomato landraces for quality traits.](image)

The landraces L-189b and CN-254 are grouped in Cluster V, which has recorded higher TAC, TPC, and AsA values, but medium Lyc content. Regarding the contribution of different traits to diversity between clusters, it was found that TPC is the most important by 45%, contributing to divergence; meanwhile, AsA has the lowest contribution, respectively, 13.5% (Table 4). Therefore, studied tomato landraces can be distinguished between each other to a much lesser extent in terms of AsA content.
Table 4. Cluster’s mean for five traits of tomato landraces and the contribution of each trait to the total divergence.

| Traits | Clusters | Times Ranked First | Contribution to Divergence (%) |
|--------|----------|--------------------|--------------------------------|
|        | I        | II                 | III                            | IV          | V          |
| TAC    | 401.28   | 341.75             | 291.77                         | 240.75      | 534.06     | 35        | 17.5      |
| TPC    | 91.79    | 85.44              | 67.76                          | 112.96      | 116.33     | 90        | 45.0      |
| Lyc    | 12.77    | 6.67               | 14.09                          | 6.81        | 10.46      | 48        | 24.0      |
| AsA    | 16.75    | 15.78              | 14.59                          | 13.17       | 19.69      | 27        | 13.5      |

It seems that this bioactive compound might not be important in landraces recognition and might oscillate depending on the tomato growing technology. Our observation is also supported by other researches regarding the accumulation of AsA in tomatoes; major differences have been found among the individual samples but not between tomato varieties. It seems that light exposure of tomato fruits directly affects the accumulation of phyto-compound [87]. Analyzing the contribution of different traits to inside cluster diversity, it was found that the highest diversity exists between the landraces Ch, IM/pusta, Ru, SS180, and T-673 grouped in Cluster III ($D^2 = 3.62$), while between landraces L-189b and CN 254 from Cluster V, a high similarity ($D^2 = 0.78$) was recorded to all nutritional components (Table 5).

According to the inter-cluster distances, it was observed that the landraces L-189b and CN254 from Cluster V differ significantly to the landraces from other clusters except for the nine landraces of Cluster I. Also, the landrace Ch-60pr12, characterized by low values of these quality traits, differs significantly to the landraces from Clusters I and III.

Table 5. Average intra- (bold diagonal) and inter-cluster (off diagonal) $D^2$ values.

| Cluster | Landraces | I     | II    | III   | IV     | V     |
|---------|-----------|-------|-------|-------|--------|-------|
| I       | CN-26; Gi; L-189a; DV; SS; T-370; Pe; Gr; C102 | 2.30  | 5.94  | 7.42  | 13.22 * | 8.71 |
| II      | PN; Ch-165; Li | 2.76  | 8.63  | 6.70  | 15.42 **|       |
| III     | Ch; IM/pusta; Ru; SS180; T-673 | 3.62  | 12.31 * | 26.36 ***|       |
| IV      | Ch-60pr12 | 0.00  | 28.31 ***|       |       |
| V       | L-189b; CN254 |       |       |       | 0.78   |

$\chi^2 = 9.49 \ (p = 0.05); \chi^2 = 13.28 \ (p = 0.01); \chi^2 = 18.47 \ (p = 0.001).$ The data show correlation index values; *$p < 0.05,$ **$p < 0.01,$ ***$p < 0.001.$

The four principal components account for the whole variability among the studied tomato landraces for the analyzed quality traits (Table 6). The first principal component (PC1) has a major contribution of 59.84% to the total variation. Only Lyc (0.144) contributed positively to PC1, while the other traits contributed negatively to this principal component.

Table 6. Eigen vectors and eigen values of the first four principal components for quality traits of tomato landraces.

| Traits | PC1     | PC2     | PC3     | PC4     |
|--------|---------|---------|---------|---------|
| TAC    | -0.956  | 0.171   | 0.225   | -0.081  |
| TPC    | -0.737  | -0.253  | -0.627  | 0.000   |
| Lyc    | 0.144   | 0.959   | -0.244  | 0.000   |
| AsA    | -0.957  | 0.169   | 0.221   | 0.082   |

Eigen value | 2.394 | 1.041 | 0.552 | 0.013
Cumulative eigen value | 2.394 | 3.435 | 3.987 | 4.000
Proportion variance | 59.84 | 26.02 | 13.80 | 0.33
Cumulative variance | 59.84 | 85.86 | 99.67 | 100.00
The second principal component (PC2) accounted for 26.02% of the total variation, with positive support of Lyc (0.959), TAC (0.171), and AsA (0.169), while TPC (−0.253) has a negative involvement. The third principal component (PC3) showed 13.80% of the overall variation and was positively associated with TAC (0.225), and AsA, as well as TPC (0.627) and Lyc (−0.244), were negatively associated with PC3. The fourth principal component (PC4) depicted a low proportion of the whole variability (0.33%), indicating the strongest discriminatory power of these two principal components (Figure 2). The biplot reveals a broad dispersion of the landraces and explained 85.86% of the variability. Negative values at PC1 indicate landraces with high TAC, ascorbic acid, and TPC. In this regard were highlighted the landraces from Cluster V, L-189a, C-102, and CN-245 have the highest values for TAC, AsA, and TPC, but a medium Lyc content. Positive values for PC2 belong to landraces having a high Lyc amount, SS-180, T-673, IM/pusta, and Ru, from Cluster III. The negative values of PC2 associated with positive values of PC1 are characteristic of the landraces with low levels of these traits like PN, Ch-165, and Ch-60pr12, grouped in Clusters II and IV, respectively. The landrace Li shows a higher TAC compared to PN and Ch-165 from Cluster II, thus being the main contributor to intra-cluster diversity. According to the dendrogram (Figure 1), it was noticed that there are different landraces groups inside Cluster I. Thus, landrace Gr with a high Lyc content exceeds the average values for TAC and ascorbic acid content, while SS-180 and T-673 with a high content of Lyc are associated with low values for the other qualitative traits.

The nutritional value of tomatoes proven by the content in bioactive compounds with high value is influenced by several factors. Many studies on tomato cultivars highlighted that variation of both abiotic [23,29,60] and technological [10,25,78,88] factors have a decisive effect on increasing the nutritional value of tomatoes.

![Biplot of the first two principal components for 20 tomato landraces and four quality traits.](image)

**Figure 2.** Biplot of the first two principal components for 20 tomato landraces and four quality traits.

### 4. Conclusions

The results reveal that the 20 tomato landraces with tolerance to salinity have high potential in phyto-compound accumulation with high antioxidant levels. The ratio between these is different. Even if it is widely accepted nowadays that the idea of phyto-chemicals with high nutraceutical value depend on plants’ genetic information, environmental factors may alter the expression of these genes.
The research confirms the hypothesis that tomato landraces with tolerance to soil salinity have a higher ability to accumulate in ripe fruits large amounts of antioxidants such as phenolic compounds and carotenoids. The largest amounts of antioxidants were recorded in that local populations originating from the areas with a high level of soil salinity, whose electrical conductance was over 6.5 dS m\(^{-1}\).

Correlating the results to the fact that these tomato landraces have adapted over time to high levels of soil salinity, we can assume that they have developed genetic, biochemical, and physiological mechanisms to synthesize some of the antioxidant-related co-agents, to allow survival under specific stress conditions. Under the higher soil-salinity conditions, the plants were more obliged to adapt by increasing the synthesis capacities of polyphenols and indirectly intensifying of antioxidant processes. These traits seem to be manifested in the conditions of their cultivation outside specific saline areas. The compositional evaluation highlighted that tomato halo-tolerant landraces are an inexhaustible resource of variability with nutraceutical properties that have been proven. These resources can be exploited in breeding programs or could be cultivated in traditional farms that adopt ecological technology.

The results of the TAC, TPC, Lyc, and AsA determinations can be considered the cumulative response of the genetic fund interaction with all the interactive effects occurring during the maturation phases of the fruit. This approach can provide meaningful information on the modeling of the nutritional quality of tomato fruit and also provides interesting insights into the metabolic capacities of the old local populations that have adapted to the conditions of high soil salinity. However, additional functional research is still needed to link the direct determinations with genetic and metabolic analyses.

**Author Contributions:** Conceptualization, R.M.S. and R.L.S.; methodology, M.A.P., R.L.S., R.M.S., and L.C.; software, S.I.C.; validation, M.E.C., L.C., and I.R.; formal analysis, L.C.; investigation, R.L.S., S.I.C., R.M.S., D.M., and M.N.; resources, R.L.S.; data curation, D.M., M.N., and S.I.C.; writing—original draft preparation, R.M.S. and R.L.S.; writing—review and editing, M.E.C. and L.C.; project administration, R.L.S. and I.R.; funding acquisition, I.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by Romanian National Authority for Scientific Research, CNDI-UEFISCDI project number PN-II-PT-PCCA-2011-3.1-0965.

**Acknowledgments:** The authors thanks for the financial support for publishing through the project “Ensuring excellence in RDI activities within USAMVBT”, code 35FFE/2018 financed by the Ministry of Research and Innovation (MCI) through Program 1–Development of the national research and development system, Subprogram 1.2–Institutional performance, Institutional development projects–Projects to fund excellence in RDI; Special thanks to all persons from the villages who were very kind to let us visit their gardens and supplied us with the necessary plant material.

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

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