

Article

Assessment of Tomato (*Solanum lycopersicum*) Landraces for Their Agronomic, Biochemical Characteristics and Resistance to *Phytophthora infestans*

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Abstract: Genetic diversity in crop plants is the *conditio sine qua non* for sustainable agriculture and long-term food security. Our research carried out the morphological, agronomic, and physico-chemical characterization and resistance to late blight of 35 tomato landraces from seven countries. These landraces have been approved and appear in the Official Catalog of Varieties. The International Plant Genetic Resources Institute (IPGRI) descriptors have been used to describe the tomato's morphological and agronomic characteristics. For the physico-chemical characteristics, the dry matter, the pH, and the carotenoid content (lycopene, lutein, and β -carotene) were analyzed. Carotenoids were monitored by high-performance liquid chromatography (HPLC). The results showed that the morphological diversity of landraces was very high. Three landraces of remarkable commercial value have shown increased resistance to late blight caused by *Phytophthora infestans*, one of the most damaging diseases of tomato. Also, six landraces had a lycopene content exceeding 100 $\mu\text{g/g}$ sample. The carotenoid content ranged between 0.769 (Marmande-FR 166) and 140.328 mg kg^{-1} FW (Răscruți). The landrace with the highest β carotene content was PT 308 with 65.499 mg kg^{-1} FW, while the lowest values were registered for Marmande-FR 166 with 0.105 mg kg^{-1} FW. The present study provides essential information on the morphological and agronomic qualities of these tomato landraces and their lycopene and other carotenoid content. The results are discussed in light of the importance of tomato landraces in meeting the preferences of different producers and consumers, the choice of the most suitable landraces for specific pedoclimatic conditions, and the supply of carotenoid pigment sources for the pharmaceutical industry. Our research responds to humanity's great global challenges: preserving agricultural biodiversity, protecting the environment by identifying pest-resistant varieties, and also protecting consumer health by finding important sources of antioxidants.

Keywords: carotenoids; landraces; late blight; lycopene; tomato

1. Introduction

Over time, humans have been creators of agronomic genetic diversity. However, genetic erosion has affected many cultivated species with the industrialization of agriculture. The disappearance of landraces in cultivated plants became more pronounced, especially in the second half of the 20th century [1]. The Convention on Biological Diversity, adopted at the 1992 Earth Summit in Rio de Janeiro, opened the possibility of more efficient conservation of plant genetic resources. This document was adopted in 2001 at the International Treaty on Plant Genetic Resources for Food and Agriculture and signed by members of the Food and Agriculture Organization (FAO), headquartered in Rome, Italy. The European Union (EU) was also a signatory to this treaty and has adopted several directives (for example: Directives 2008/62/EC, 20 June 2008; 2009/145/EC 26, November 2009; and 2010/60/EU, 30 August 2010) designed to stimulate the conservation of landraces [2]. According to art. 2 of the Directive 2008/62/EC, the “Landrace” is defined as a set of populations or clones of a plant species which are naturally adapted to the environmental conditions of their region. Landraces are also called with many terms like “conservation varieties”, “farmer varieties”, “local varieties”, “primitive varieties”, “local populations”, “peasant varieties” or “traditional varieties” [3]. They have a significant impact on human food security, on the adaptation of agriculture to global climate change, and on human and environmental health. They are also valuable genetic sources for creating new varieties. In addition, landraces have essential features such as high nutritional value, resistance to abiotic stress, and resistance to disease and pest attack. Also, they constitute the basis of many traditional foods, directly contributing to the nutritional and culinary diversity. At the European level, they can be certified as traditional products and, through agrotourism, they could be a source for local economic development initiatives. Landraces and traditional products provide cultural identity to the area from which they come [4–10].

Landraces are essential for organic farming because they are well adapted to local conditions, they are resistant to pest attack, and do not require high-energy inputs [11–14].

Genetic resistance to pathogens and pests is one of the fundamental qualities of a variety to be used in sustainable agriculture systems. High resistance implies using low amounts of pesticides associated with increased health for both the environment and the consumer. In Romania and other countries in Eastern Europe, the disease with the most significant economic importance in tomatoes is still the late blight caused by *Phytophthora infestans* (Mont.) de Bary. Without adequate phytosanitary treatments, the production can be compromised in years with favourable conditions for the evolution of tomato late blight.

Tomatoes are one of the most important vegetable crops in the world. This is due to the taste qualities, the various ways of capitalization, and the possibility of having fresh fruit all year round. The pharmaceutical industry also uses this species to extract lycopene—an anticancer substance [15,16]. As for the other cultivated species, genetic diversity in tomatoes has been affected by the industrialization of agriculture. There are no data on the number of tomato varieties worldwide or in Europe, but it is one of the most important horticultural crops worldwide (FAOSTAT, 2016)—<http://www.fao.org/faostat> (accessed on 27.07.2022). Bauchet and Causse (2012) [17] noted that tomatoes represent 14% of world vegetable production.

In Romania, the Suceava Bank for Plant Genetic Resources owns 218 landraces and varieties of tomatoes. The official catalogue of varieties in Romania [18] included, in 2020, 77 tomato varieties, of which 64 were suitable for fresh consumption and 13 for the processing industry. The germplasm collection of the University of Agricultural Sciences and

Veterinary Medicine Cluj-Napoca includes 87 landraces of tomatoes, mainly from Romania.

The main bioactive compounds in tomato fruit are carotenoids, predominantly lycopene, with well-documented and recognized health-promoting properties [19]. Carotenoids are a class of secondary metabolites with solid antioxidant properties in plants and certain microorganisms. They are important dietary sources of vitamin A [20]. The structure of carotenoids is typically made by long chains, which confer yellow, orange, and red colours to the organisms producing them, and mammals cannot biosynthesize them, and consequently not by the human organism. The primary source of carotenoid intake for humans is the consumption of fruits and vegetables. In this regard, tomatoes are recognized as a primary source of lycopene, containing 2.62–60.40 mg/100 g fresh weight [21]. The range of lycopene content can vary greatly, depending on numerous factors such as variety, ripening stage, cultivation technology, and geographic location.

The most abundant carotenoids in plasma include lycopene, β -carotene, and lutein [22,23]. Tomato fruits count for up to 85% intake of lycopene in humans. Lycopene can confer preventive effects related to cardiovascular diseases, diabetes, and cancer through its antioxidant, antimicrobial, and anti-inflammatory mechanisms. Along with lycopene, tomato fruits contain, even if in smaller amounts, β -carotene, and lutein. β -carotene is the main dietary precursor of vitamin A, and several studies have shown that β -carotene may increase immunological function and possess the antioxidant capacity.

Furthermore, human blood beta-carotene concentrations are inversely correlated with the risk of type 2 diabetes and obesity. Lutein plays a crucial role in the prevention of eyes diseases, as the yellow colour of the macula lutea of the primate retina contains lutein, zeaxanthin, and meso-zeaxanthin [24]. Along with its function as a blue light filter and as an antioxidant in the retina, lutein can also influence immunological and inflammatory responses elsewhere in the body.

The objective of this study was to assess 35 landraces of tomatoes from Romania, comparing them to those originating from other countries regarding their organoleptic characteristics, carotenoids content, resistance to late blight disease, and adapted to pedoclimatic conditions in Cluj-Napoca, Romania. The results of the present study contribute to information on the possibilities of using landraces according to the consumer's preferences, pedoclimatic conditions, and the pharmaceutical industry's interest in capitalizing on lycopene and other carotenoid pigments.

2. Results

2.1. Morphological Traits

The data reported in Table S1 show that only two landraces (originating from Austria and Romania) out of 35 investigated (5.8% of the entire collection) exhibited a determinate growth pattern. The remaining 33 landraces showed an indeterminate growth. The predominant colour of the fruit at maturity was red, that has been observed in 25 out of 35 landraces belonging to our collection (71.4%). The red combinations were: red-orange, red-green, and red with green streaks. The fruit colour was orange in four landraces (11.5%). The other colours present on the outer surface of the ripe fruits were: orange-yellow (8.6%), pink (5.7%), and yellow (2.9%). An excellent fruit shape variability was also observed. In 18 of the 35 landraces (51.4%), the shape of the fruit was round, mostly slightly flattened. The other fruit shapes, ordered according to their frequency, were oval (17.1%), cordiform (11.4%), and pear-shaped (5.7%). Five landraces had specific fruit shapes: ellipsoidal (FR 141), cylindrical-conical (FR 163), Kapia pepper shape (San Marzano—IT 173), donut shape (KP 103), and flattened shape (Purple Calabash—FR 132). Ribbed fruits were found in 8 of the 35 landraces studied (22.9%).

It is well known that the weight of the fruit depends on the type of fruit. For example, fruits of cherry varieties (*Solanum lycopersicum* subsp. *cerasiforme*—Dunal) are tiny and weigh between 2 and 23 g per fruit. The weight of the fruit of normal size varies very much, comprising between 33 and 550 g. In the present study, the following landraces were noted for their high fruit weights: Danamari (450 g), Cassiana (376 g), and Aussi (235 g).

Fruit production/plant had extensive intervals ranging from 1.3 kg/plant in the CJ 360 (cherry variety) case to 4.8 kg/plant, which was obtained for the Danamari variety (AB 343). The yield of landraces in the production of vegetables keeps the same order mentioned in production kg/plant. Furthermore, 54.3% of the investigated landraces had yields below 2 kg/plant and 40% between 2 and 4 kg/plant. Higher productions (4 kg/plant) were obtained only for the Danamari and Cassiana varieties which originated from two landraces (SJ 373 and AB 343). The days from emergence to fruit ripening ranged from 47 days (KP 103) to 70 days (KP 111). According to the IPGRI-developed descriptors for tomatoes, reaching maturity is defined as when 50% of the plants have at least one ripe fruit. Most landraces studied needed 50–60 days germination to adulthood. The earliest landraces were Gregori Altai (GA 157), SJ 371-R, BZ 315, KP 103, and Marmande (FR 166).

In our experiment, 15 of the 35 landraces investigated showed a medium resistance to late blight (42.9%). In contrast, mostly cherry-type, 34.3% of landraces showed a high or very high resistance to the same disease. Only three landraces (SJ 457165, BZ 315, and PT 308) with regular fruits showed increased resistance to late blight disease. Furthermore, SJ 457165 has become the Chandona-approved variety.

2.2. Biochemical Parameters

The results for lycopene, β -carotene, lutein, and total carotenoid content (expressed as mg kg⁻¹ of fresh weight—FW) of the analyzed local tomato varieties are presented in Table S1.

The total carotenoid values ranged between 0.769 and 140.328 mg kg⁻¹ FW. The landraces with the highest carotenoid concentration (above 100 mg kg⁻¹ FW) were: Răscruți (140.33 mg kg⁻¹ FW), FR 309 (140.06 mg kg⁻¹ FW), Aussi—AUS 135 (135.45 mg kg⁻¹ FW), ChRm (124.93 mg kg⁻¹ FW), Gregori Altai—RUS 157 (118.21 mg kg⁻¹ FW) and KP 162 (112.78 mg kg⁻¹ FW). The landraces with the lowest carotenoid concentration were Marmande (FR 166), FR 163, and KP 111, with a total carotenoid content of 0.769, 0.849, and 1.040 mg kg⁻¹ FW for the tomato sample, respectively.

The values for the lycopene content ranged from 0.664 to 129.29 mg kg⁻¹ FW. The highest amounts (more than 100 mg kg⁻¹ FW) were observed in FR 309 (129.297 mg kg⁻¹ fw), followed by Răscruți (120.158 mg kg⁻¹ FW) and ChRm (100.722 mg kg⁻¹ FW). The lowest observed values were as follows: 0.664 mg kg⁻¹ FW (Marmande), 0.706 mg kg⁻¹ FW (FR 163), 0.916 mg kg⁻¹ FW (KP 103) and 0.933 mg kg⁻¹ fw (KP 111). The β -carotene content of the studied landraces showed a wide range from 0.105 mg kg⁻¹ FW (Marmande—FR 166) to 65.499 mg kg⁻¹ FW (PT 308). Furthermore, the PT 308 landrace stood out from all the other varieties investigated, considering that the next value, in descending order, was 20.85 mg kg⁻¹ FW (CJ 360).

The β -carotene/lycopene ratio showed a different trend among the landraces tested, with higher levels found in PT 308, ChG, and KP 103 (7.98, 3.58, and 1.04, respectively), compared to the lowest level recorded in FR 141 (0.02) landrace.

The lutein content ranged from 0.769 to 140.328 mg kg⁻¹ FW. The highest amount of lutein was determined in Black Sea Man—RUS 128 (8.708 mg kg⁻¹ FW) landrace, followed by ChG (6.967 mg kg⁻¹ FW), Răscruți (6.123 mg kg⁻¹ FW), Chandona—SJ 457165 (6.028 mg kg⁻¹ FW) and ChRm (6.003 mg kg⁻¹ FW) landraces.

2.3. The Influence of Origin on Tomato Fruit Characteristics

Data analysis performed by one-way ANOVA revealed differences depending on the origin of tomato varieties for each studied character. The Austrian tomato variety had the highest lycopene content, and there were significant differences comparing all the groups of tomato varieties studied (Table 1). This parameter showed values over 70 for the TH and RU tomato varieties but decreased below 25 for the Italian tomato variety and even below 10 for Greece. In contrast, lutein content was significantly higher in the Greece tomato variety and the RO, RU, and TH ones. All other tomato varieties showed a lutein content ranging from 2.02 to 2.68, significantly lower than the others. The β -Carotene and total carotene content presented a different trend; for β -Carotene, significantly higher values were recorded in the PT308 tomato variety originating from Greece, while for the entire carotene range, the Austrian tomato variety, which the only one which showed values greater than 135 mg kg⁻¹ FW. The RU and TH tomato varieties had under 100 units of total carotene but no significant differences compared to the Austrian one. The only tomato variety with a significantly lower pH value was the KP311 from TH, while for the other tomato varieties, this parameter was over 4.07.

Table 1. Difference between tomatoes parameters classified by origin

Tomato Parameters					
Class *	Lycopene (mg kg ⁻¹ FW **)	Lutein (mg kg ⁻¹ FW **)	β -Carotene (mg kg ⁻¹ FW **)	Total carotene (mg kg ⁻¹ FW **)	pH
AU	126.40 ± 3.78 a	2.02 ± 0.25 b	7.03 ± 0.25 de	135.45 ± 3.26 a	4.29 ± 0.02 a
FR	41.21 ± 6.83 cd	2.20 ± 0.26 b	5.49 ± 0.81 e	48.90 ± 7.38 c	4.07 ± 0.04 ab
GR	8.21 ± 5.81 d	3.76 ± 0.27 ab	65.50 ± 0.71 a	77.46 ± 5.22 bc	4.11 ± 0.03 ab
IT	22.48 ± 4.13 cd	2.68 ± 0.23 ab	6.76 ± 0.60 de	31.93 ± 6.07 c	4.28 ± 0.02 a
RO	59.94 ± 4.26 bc	3.94 ± 0.27 a	12.25 ± 0.65 bc	76.13 ± 4.50 bc	4.16 ± 0.02 a
RU	82.33 ± 6.21 b	4.80 ± 1.00 a	9.70 ± 0.22 cd	96.83 ± 6.10 ab	4.23 ± 0.02 a
TH	70.06 ± 5.26 bc	3.65 ± 0.29 ab	15.65 ± 0.24 b	89.36 ± 4.90 abc	3.92 ± 0.03 b
<i>F test</i>	6.18	4.83	92.85	5.79	2.51
<i>p.val</i>	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.03

* Country of origin: France (FR), Greece (GR), Italy (IT), Romania (RO), Russia (RU), Thailand (TH).

** FW—fresh weight. Within each column, different letters refer to differences among countries of origin (LSD tests, $\alpha = 0.05$).

2.4. The Influence of Lycopene on Tomato Fruit Characteristics

The use of Lycopene classes as a group method provides a deeper analysis of the characteristics of fruits. Fruit weight did not show significant differences related to the Lycopene content, meaning that varieties with different yield potential had the same Lycopene content. These results could benefit farmers who want to combine yield and a specific Lycopene content. Lutein and β -Carotene also showed significant differences, mostly due to their very low content in class 1 and very high content in class 2 compared with the other Lycopene classes (Table 2). Total carotene was significantly affected by the Lycopene classes, with a minimum range in class 1 and a maximum achieved in class 5. The pH values do not vary considerably between classes, the only significantly higher value being associated again with class 5.

Table 2. Differences in tomato parameters for varieties classified by lycopene content

Tomato Parameters					
Class*	FFW ** (g)	Lutein (mg kg ⁻¹ FW **)	β -Carotene (mg kg ⁻¹ FW **)	Total Carotene (mg kg ⁻¹ FW **)	pH
1	110.75 ± 4.27 a	0.35 ± 0.18 b	0.33 ± 0.11 c	1.48 ± 0.31 e	4.12 ± 0.06 ab

Tomato Parameters					
Class*	FFW ** (g)	Lutein (mg kg ⁻¹ FW **)	β-Carotene (mg kg ⁻¹ FW **)	Total Carotene (mg kg ⁻¹ FW **)	pH
2	68.00 ± 12.01 a	4.03 ± 0.81 a	25.22 ± 1.07 a	34.00 ± 10.89 d	4.07 ± 0.02 b
3	131.00 ± 31.71 a	2.88 ± 0.29 a	11.64 ± 0.99 b	47.23 ± 2.56 c	4.16 ± 0.04 ab
4	91.33 ± 18.28 a***	3.93 ± 0.25 a	10.07 ± 0.64 b	83.85 ± 1.99 b	4.10 ± 0.03 b
5	113.60 ± 19.69 a	3.75 ± 0.52 a	11.59 ± 1.08 b	131.79 ± 2.35 a	4.24 ± 0.04 a
<i>F test</i>	0.01	24.99	0.72	571.65	1.30
<i>p.val</i>	0.94	<i>p</i> < 0.001	13.83	<i>p</i> < 0.001	3.62

* Class based on lycopene content of fruit (mg kg⁻¹ fresh weight): 1=<1; 2=1.1–10; 3=10.1–50; 4=50.1–100; 5=>100.

** FFW—Fresh fruit weight. Within each column, different letters refer to differences among lycopene content (LSD tests, α = 0.05).

2.5. The Influence of Tomato Fruit Characteristics on Late Blight Disease

Assessment of local tomato varieties to late blight resistance

Isolations performed from symptomatic tissues in vitro allowed us to isolate colonies morphologically resembling *P. infestans*. Microscopical observations, based on morphological features, confirmed that the obtained colonies belonged to *P. infestans*. The sporangia and the sporangiophores were characteristic of *P. infestans* [25,26].

Resistance to late blight disease was a factor for grouping and analyzing the fruit characteristics of the tested varieties. High resistance values to late blight disease were registered for the types with significantly lower fruit weights. An interesting phenomenon of a maximum weight was reported for the medium-resistant varieties. Low disease resistance was observed in high fruit weights varieties, with 20–25 g lower fruit weights than the maximum potential. Another interesting result was that the Lycopene content was similar among resistance classes; the class represented the only exception with a very low resistance, where the lycopene content was only 23.50. A higher lutein content was associated with the high and very high resistance classes. The β-carotene presented a maximum value in the high resistant class while the total carotene had maximum values in the low resistant class, with less significant differences. In addition, the fruit pH could be associated with disease resistance; lower values are recorded in higher resistant varieties and the maximum in lower ones. Interestingly, the resistance to late blight disease and the extremes of pH variation was registered in high and low classes, which sustains the need for future research for both very high and very low resistant tomato varieties (Table 3).

Table 3. Difference between tomato parameters classified by resistance to *P. infestans*.

Tomato Parameters						
Class *	Fresh Fruitweight ** (g)	Lycopene (mg kg ⁻¹ FW **)	Lutein (mg kg ⁻¹ FW **)	β-Carotene (mg kg ⁻¹ FW **)	Total carotene (mg kg ⁻¹ FW **)	pH
very high	14.88 ± 1.27 c	57.56 ± 6.20 a	4.17 ± 0.36 a	12.57 ± 0.87 b	74.29 ± 6.67 a	4.12 ± 0.03 bc
high	69.25 ± 11.72 bc	41.43 ± 6.33 ab	4.63 ± 0.38 a	26.18 ± 7.05 a	72.25 ± 1.14 a	4.04 ± 0.03 c
medium	147.63 ± 20.48 a	58.13 ± 6.01 a	2.71 ± 0.22 b	7.26 ± 0.65 c	68.11 ± 0.65 a	4.09 ± 0.03 bc
low	127.00 ± 20.88 ab	70.56 ± 13.53 a	3.34 ± 0.98 ab	7.55 ± 1.45 bc	81.45 ± 14.87 a	4.34 ± 0.02 a
very low	122.33 ± 16.99 ab	23.50 ± 5.49 b	1.87 ± 0.25 b	9.16 ± 1.98 bc	34.53 ± 6.23 b	4.22 ± 0.06 ab
<i>F test</i>	7.33	2.73	5.60	10.79	2.71	5.90
<i>p.val</i>	<i>p</i> < 0.001	0.03	<i>p</i> < 0.001	<i>p</i> < 0.001	0.08	<i>p</i> < 0.001

* Class of "resistance to *P. infestans*": not infected: (0%); very low (1–20% infected tissue); low (21–40% infected tissue); medium 41–60% infected tissue), high 61–80% infected tissue) and very high

(>81% infected tissue). The ranges from the resistance classes, expressed in percentages, mean the values of the degree of attack (calculated according to established formulas in the field of plant protection). ** FFW—fresh fruit weight. Within each column, different letters refer to differences among tomato parameters classified by resistance to *P. infestans* (LSD tests, $\alpha = 0.05$).

2.6. The Influence of Fruit Weight on Tomato Fruit Characteristics

Using fruit weight classes as a grouping factor makes a deeper analysis of differences between varieties possible. It was observed that there were varieties with an average weight higher than 500 g, followed by a significantly lower value of 235 in the 4th class. The differences were maintained significantly between the rest of the types. However, for Lycopene content, the use of this as a grouping factor showed similar values (ranging from 52.67 to 57.61) in the case of classes 1, 2 and 5, and over 126 units in the 4th class, which was the only class with significant values. Lutein content was associated with a lower weight of fruits, and this result was identical to what was obtained for β -Carotene. The total carotene followed the Lycopene trend, with a maximum of 135.45 in the 4th class and significant differences from the other classes. Weight as a grouping factor normalized the pH values; all varieties presented similar values without significant differences between classes (Table 4).

Table 4. Difference between tomatoes parameters classified by fresh fruit weight

Class *	Tomato Parameters				pH
	Lycopene (mg kg ⁻¹ FW **)	Lutein (mg kg ⁻¹ FW **)	β -Carotene (mg kg ⁻¹ FW **)	Total Carotene (mg kg ⁻¹ FW **)	
1	55.63 ± 5.60 b	4.34 ± 0.33 a	13.49 ± 0.93 a	73.45 ± 5.93 b	4.10 ± 0.03 a
2	57.61 ± 5.51 b	3.37 ± 0.32 b	11.64 ± 2.26 ab	72.61 ± 5.72 b	4.13 ± 0.03 a
3	39.06 ± 9.00 b	2.25 ± 0.31 c	6.91 ± 1.20 b	48.23 ± 9.79 c	4.13 ± 0.03 a
4	126.40 ± 4.97 a	2.02 ± 0.31 c	7.03 ± 0.91 ab	135.45 ± 3.98 a	4.29 ± 0.03 a
5	52.67 ± 3.27 b	2.36 ± 0.04 bc	10.42 ± 0.95 ab	65.45 ± 2.73 bc	4.20 ± 0.03 a
<i>F test</i>	4.26	15.26	2.97	4.35	1.82
<i>p.val</i>	0.990.003	$p < 0.001$	0.09	0.002	0.18

* Class "average weight (g)": W1: 2–23, W2: 50–95, W3: 100–190, W4: 201–450, W5: 507–519. ** FW—fresh weight. Within each column, different letters refer to differences among tomato parameters classified by resistance to *P. infestans* (LSD tests, $\alpha = 0.05$).

2.7. PCA Ordination of Tomato Fruit Characteristics as Influenced by Experimental Factors

All data were projected on bifactorial PCA ordinations to evaluate the differences in variety pools and extract the most suitable variety groups based on two characters. The PCA grouped by the intersection of origin x *P. infestans* resistance shows a reasonable projection of data around the center of ordination (Figure 1). In the case of the origin, RO varieties are located in the center of the ordination, with an equal position compared to RU, TH, FR, and GR varieties. For this factor, AU and IT varieties are located opposite places on PA, sustaining the unique combination of fruit characteristics. In the case of *P. infestans* resistance, the graph shows the central position of medium resistant varieties, the correlation of wide and very high varieties with Axis 1, and the correlation with Axis 2 in the case of low and very low resistant varieties, respectively. The RO varieties are located in all of the quadrats of PCA, which sustain both their variable resistance to disease and the specificity of fruit characteristics of each variety. The best fit of *P. infestans* on the PCA graph supports the further use of this parameter for the deeper exploration of resistance in combination with fruit weight and lycopene.

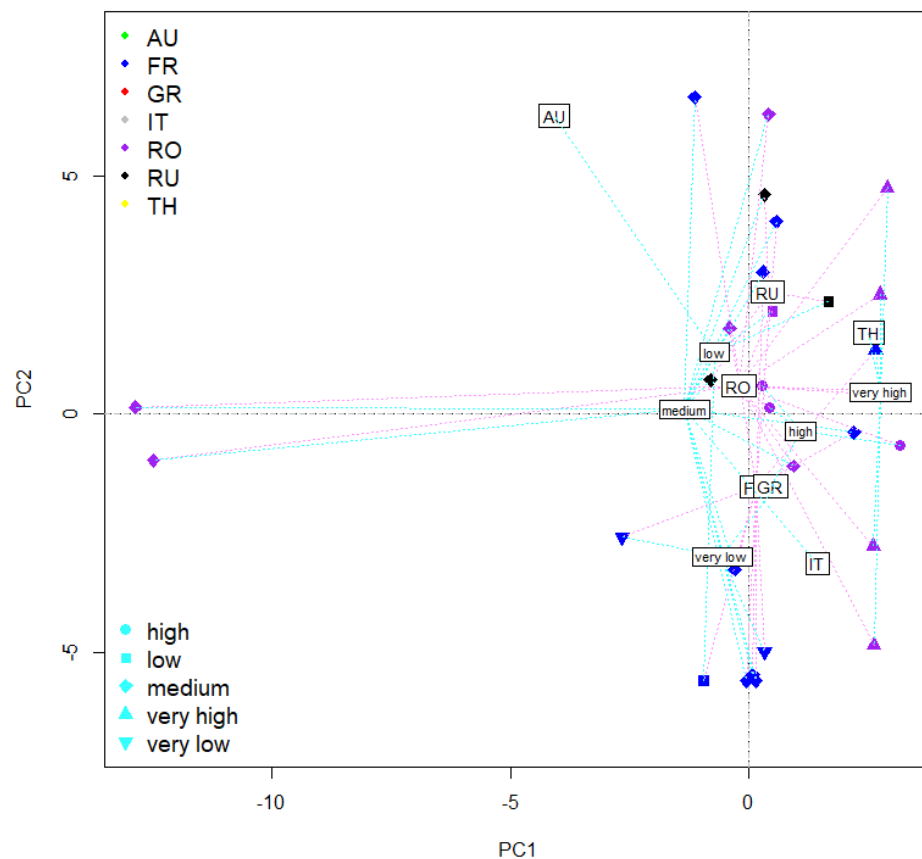


Figure 1. PCA of origin \times resistance to *P. infestans*. (Variance explained by Axis: Axis 1 = 80.00%; Axis 2 = 18.47%, Total = 98.88%).

The letters show the acronym of the name for each country: AU–Austria; FR–France; GR–Greece IT–Italy; RO–Romania; RU–Russia; TH–Thailand. The geometrical figures represent the intensity of the late blight disease attack, which was assessed on the following scale: not infected: 0%; very low (1–20% infected tissue); low (21–40% infected tissue); medium (41–60% infected tissue), high (61–80% infected tissue); very high (>81% infected tissue).

The use of *P. infestans* resistance as a parameter on fruit weight makes visible the differences of various groups, which is essential for selecting the best-suited and adapted varieties for a farmer or industry's needs (Figure 2). The middle of the Origin \times Weight PCA is occupied by three fruit weight classes, 2–23, 50–95, and 507–519. This group comprises the highest and the lowest values registered for fruit weight. Most of the very high resistant varieties are located above the middle of the graph, with one exception, which shows a unique feature within the fruit weight class 201–450. Besides, most of the fruits with weights between 50–95 g showed to be medium resistant to *P. infestans*, while varieties with the weight of fruits ranging between 201–450 g demonstrated to be high and very high resistance to the disease. Two varieties within 100–190 g showed a very high resistance to late blight. However, two varieties within the same weight class showed low resistance. All the varieties included in the class weight of 2–23 showed very high resistance to *P. infestans*.

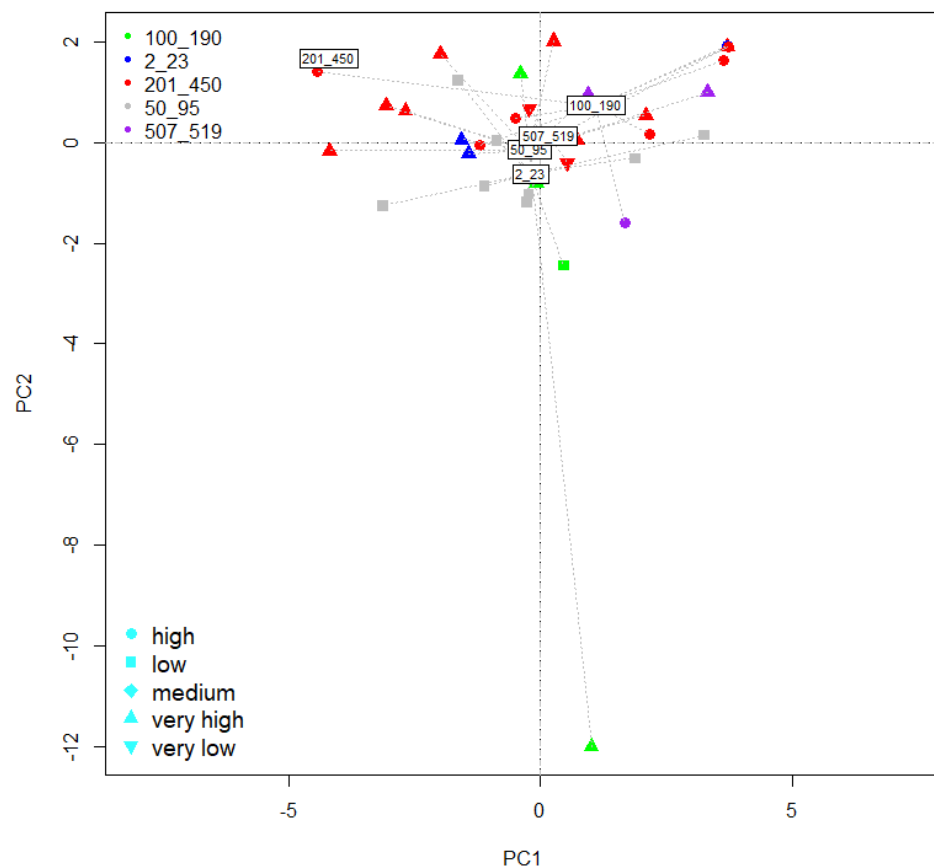


Figure 2. PCA of origin \times fruits weight interaction. (Variance explained by Axis: Axis 1 = 94.24%; Axis 2 = 5.63%, Total = 99.87%). The numbers represent the intervals of weight: 2–23 g; 50–95 g; 100–290 g; 201–190 g; 201–450 g; 507–519 g. The geometrical figures represent the intensity of the late blight disease attack, which was assessed on the following scale: not infected:0%; very low (1–20% infected tissue); low (21–40% infected tissue); medium 41–60% infected tissue), high 61–80% infected tissue); very high (>81% infected tissue).

The best PCA projection was the combination of *P. infestans* resistance \times Lycopene content as parameters (Figure 3), where all lycopene classes were correlated with Axis 2, starting with a condensed group of varieties with a content of less than 1 unit of lycopene and in a more close area the group with 1.1–10 units of lycopene. The varieties in this area showed low and very high resistance to *P. infestans*. All varieties with a medium content of lycopene (10.1–50) exhibited a medium to very high resistance to the disease, and their position was located below the middle of the ordination. Varieties with 50.1–100 units of lycopene had all types of resistance, which supported the idea of different susceptibility for the medium classified varieties. The highest lycopene content was associated with medium resistance, but two varieties with low and very high resistance were placed opposite the other. For the 10.1–50 and 50.1–100 classes, there were two varieties with medium resistance but with unique features (T32 and T33).

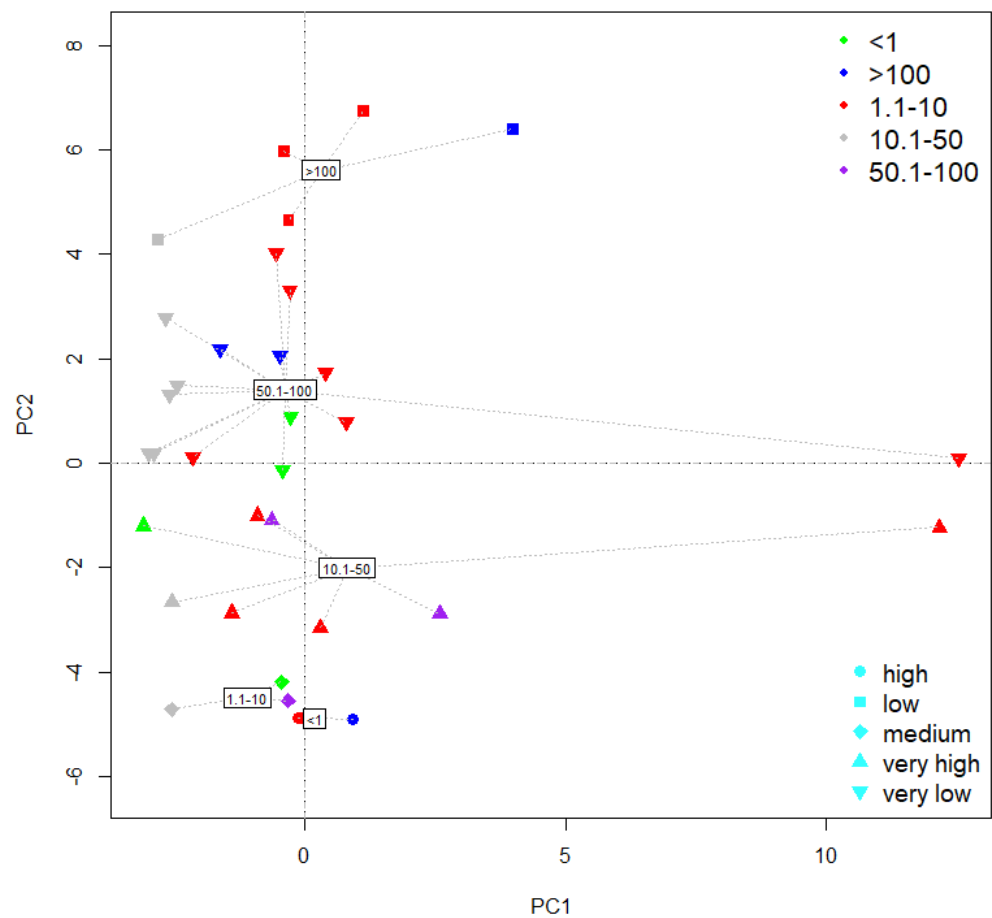


Figure 3. PCA of *P. infestans* \times lycopene interaction. (Variance explained by Axis: Axis 1= 89.00%; Axis 2= 9.86, Total= 99.18%). The numbers represent the intervals of lycopene quantities/sample: <1 mg/kg; between 1.1–10 mg/kg; between 10.1–50 mg/kg; 50.1–100 mg/kg; >100 mg/kg. The geometrical figures represent the intensity of the late blight disease attack, which was assessed on the following scale: not infected: 0%; very low (1–20% infected tissue); low (21–40% infected tissue); medium 41–60% infected tissue), high 61–80% infected tissue) and very high (>81% infected tissue).

The LSD Post-hoc test showed a relationship between the colour of the fruits in tomatoes and their lycopene quantity (Figure 4). The varieties grouped in the colour P-RG intervals showed significant differences compared to the O-Y interval.

Our results showed that the intensity of fruit colour in tomatoes was directly related to the lycopene content (Figure 4). In particular, the highest amounts of lycopene had been observed in fruits of red (R), pink (P), or reddish-orange (RG) compared to those of lighter colors like orange (O), yellow-orange (YO), and yellow (Y).

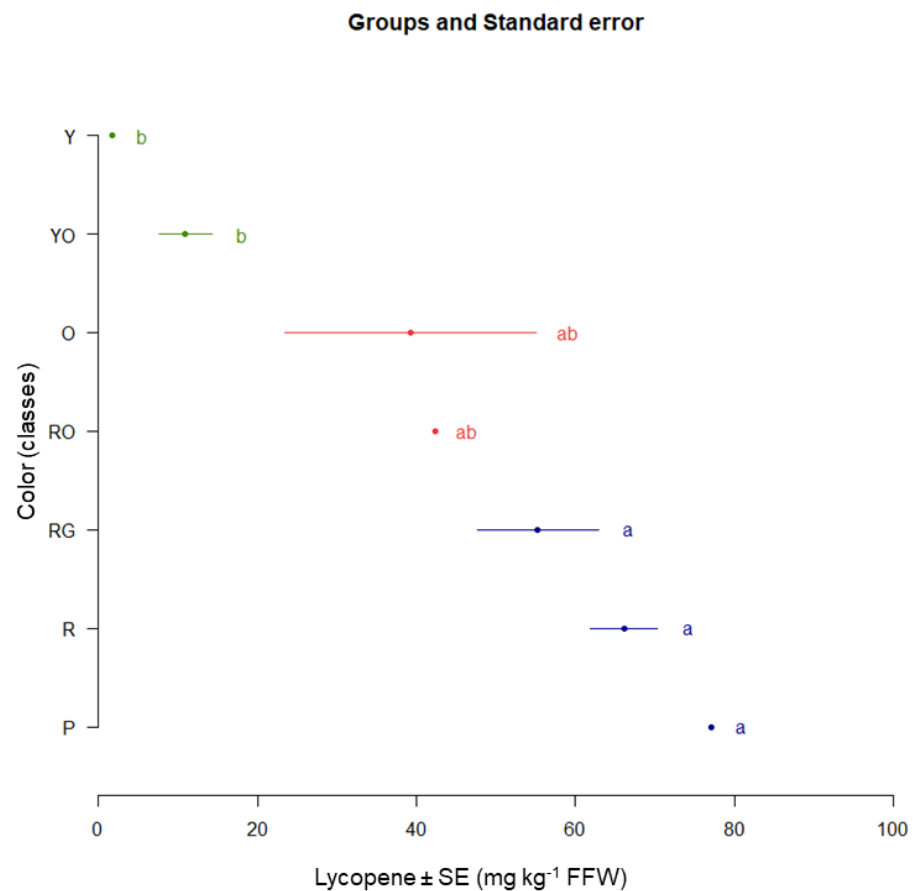


Figure 4. Differences between colour and lycopene content. Different letters show significant differences between varieties according to the LSD Post-hoc test, $p < 0.05$. Colour of the fruits: P = pink; R = red; RG = reddish-green; RO = redish-orange; O = orange; YO = yellow-orange; Y = yellow.

3. Discussion

3.1. Characteristics of Tomato Landraces

Tomato fruit quality and susceptibility to various diseases are influenced by a number of factors. This species will produce high quality fruit under conditions of abundant irradiation and mild temperature ranges. Low temperature and low light cause swelling and spot ripening [27,28]. K and Ca deficiencies produce blotchy ripening and blossom-end rot. The accessibility of the two elements in the plant depends on water absorption which in turn is influenced by light intensity, temperature, humidity and salinity [29,30]. Temperatures below 12°C and above 32°C inhibit lycopene synthesis [31].

In sensitive varieties and under high humidity conditions, tomato late blight attack can compromise the entire tomato crop. Therefore, finding new tomato varieties with resistance to *P. infestans* remains essential to reduce damage and reduce the amount of fungicides [32,33].

The collection and morphological, agronomic, and biochemical characterization of the germplasm is very important for agriculture. This way, we can choose the most suitable varieties for various growing areas. It also helps breeders to find the most suitable parents for new varieties. On-farm and ex-situ conservation of plant varieties can reduce genetic erosion and enhance sustainable agricultural systems' practice [3].

Numerous studies have shown that the most common tomato genotypes are those with indeterminate growth [34,35]. Sacco et al. 2015 [36] observed indeterminate growth in 77.2% of the genotypes considered. In our collection, however, only two landraces

(5.8%) with determinate growth patterns were found. Both landraces are of the cherry type.

Of the 35 landraces studied, the predominant colour of tomato fruits was red (71.4%). Similarly to our results, Rocha et al. (2010) [37] observed the red colour of the fruit in 25 of the 40 varieties of cherry tomatoes (62.5%) grown organically. Other studies showed the same predominance of red [38–40]. In addition to the various shades of red of the fruit, the above authors identified other colours, but in smaller proportions: pink, yellow, orange, and green, as well as all of their different combinations. Instead, Terzopoulos and Bebeli (2010) [41] mentioned that in the 34 landraces studied; the most common color was orange (34.7%).

Many studies on the shape of the fruit in tomatoes showed a very high genetic diversity [42,43]. Sacco et al. (2015) [36] studied 123 tomato genotypes from various geographical areas of the world and concluded that about 45% of the morphological variation was attributed to the shape of the fruit.

The main groups for classifying the shape of the fruit in tomatoes differed according to the author: round, slightly flattened, cylindrical, and round elongated [40]; globular, cordiform, cylindrical, pear-shaped, and slightly flattened [39]; slightly flattened, flattened and round [38]. However, in all the studies mentioned above, round and round-flattened shapes were predominant. In our experience, these forms were present in 51.4% of landraces. In smaller proportions, there were also landraces with oval, cordiform, pear-shaped, ellipsoidal, cylindrical-conical, Kapia pepper shapes, donut shapes, and flattened shapes.

In our study, tomato production ranged from 1.3 to 4.8 kg/plant. Our results are similar to those obtained by Scarano et al. (2020) [44], where, out of the ten landraces studied, five of them had an average production (2–4 kg/plant), four landraces had a high production (more than 4 kg/plant), and two had a low production (less than 2 kg/plant). Following the comparative study of 68 African tomato landraces, Tembe et al. (2018) [45] reported fruit weights between 0.565 and 2759 kg/plant, while Chávez-Servia et al. (2018) reported values between 1.28–4.15 kg/plant [46].

In our research, the fruit mass of the 35 landraces varied between 33 and 450 g / fruit in regular tomatoes and between 2 and 23 g in cherry varieties. Ortiz and Izquierdo (1994) [47] reported that for the 20 tomato genotypes collected from different areas of Brazil and the Caribbean fruit weights were between 53 and 159 g/ fruit (tomatoes with average fruit).

The resistance of tomato varieties to diseases is an important concern of breeders and vegetable growers, especially regarding organic farming. It is well accepted that in organic farms, the restrictions relating to the use of pesticides are very high and therefore, the genetic resistance of the varieties used remains fundamental [48–50]. Boziné-Pullai et al. (2021) [51] reported a comparative study between the ten landraces of tomatoes and two commercial varieties, focusing on their different agronomic characteristics, including late blight resistance. The investigation concluded that landraces could successfully replace commercial varieties under organic farming conditions, even if grown outside the origin. Majid et al. (2008) [52] identified the genetic resources of blight resistance in *Solanum pimpinellifolium* and *S. habrochaites*. Our research confirms the increased resistance of cherry varieties to late blight disease. Therefore, eight out of the nine varieties of cherry tomatoes showed a “very high” resistance to late blight disease (89%), and one variety exhibited a “high” disease resistance. Instead, only three landraces with normal fruit were identified with “high” blight disease resistance (11.5%).

Differences in the β -carotene/lycopene ratio can be attributed to a possible different genetic background of the landraces tested, which might affect either the β -carotene or lycopene biosynthesis. In addition, variations in the β -carotene/lycopene ratio may be attributed to the different genetic background of the tested landraces, which could affect the β -carotene or lycopene biosynthesis.

3.2. Biochemical Characteristics of Tomato Landraces

Lycopene is the most important carotenoid present in tomatoes. The results of the lycopene content showed a wide variation between the examined local tomato varieties, ranging from 0.664 mg kg⁻¹ FW to 129.29 mg kg⁻¹ FW. These results were close to the values reported for traditional Italian landraces (43 to 120 mg kg⁻¹ FW, Lenucci et al., 2009 [53]; 78.6 mg kg⁻¹ FW, Fattore et al., 2016 [54]; 96.9 mg kg⁻¹ FW, Ilahy, 2011 [55]) and for some farmer' varieties in Portugal (94.9 mg kg⁻¹, Pinela et al., 2012 [56]). However, the lycopene values determined in the local studied varieties were higher than the values of commercial varieties of tomato from Spain (18.60–64.98 mg kg⁻¹ FW, Martinez-Valverde, et al., 2002 [57]) and Taiwan (20–30 mg kg⁻¹ FW, Chang et al., 2006 [58]). Furthermore, Fratianni et al. (2020) [59] analyzed lycopene using a spectrophotometry approach. They reported a high lycopene content (up to 218 mg kg⁻¹ of fresh product) in five traditional landraces of the tomato "Piennolo" of the Campania region (Southern, Italy).

The colour was generally an accurate indicator of lycopene content, with the yellow cultivar containing less lycopene than the red ones and two of the three red cultivars containing more than an orange cultivar [60]. In 92.3% of the local studied varieties with the intense red predominant colour of tomato fruits, the lycopene content ranged between 19.93 and 126.4 mg kg⁻¹ FW. Published values of ordinary red tomato cultivars ranged from 10 to 150 mg kg⁻¹ FW [61]. Hart and Scot (1995) [62] determined that the intense red tomato varieties were characterized by a lycopene content of 50 mg kg⁻¹ FW and the yellow tomato varieties of 10 times lower. In the present study, we observed that 70.1% of the intense red variety of tomatoes had a lycopene content higher than 50 mg kg⁻¹ FW.

A large variation in β -carotene content was observed in the studied landraces, ranging from 0.105 mg kg⁻¹ FW to 65.499 mg kg⁻¹ FW. Other studies reported higher and lower values for the β -carotene content of tomatoes. For example, Martí et al. (2016) [16] reported values ranging from 1 to 12 mg kg⁻¹ FW, while [63] obtained values of 2.3–28.3 mg kg⁻¹ FW using a method based on IR spectroscopy.

The values obtained for lutein ranged from 0 to 8.708 mg kg⁻¹ FW. Studies to quantify the content of carotenoids in tomatoes and their by-products showed that along with lycopene, the main lipophilic antioxidant in tomatoes, lutein was also present in appreciable amounts. Calvo (2005) [64] reported the data on lutein concentrations published by different authors (1.3 mg kg⁻¹ FW, Tee and Lim, 1991 [65]; 0.4–0.7 mg kg⁻¹ FW, Granade et al., 1990 [66]; 0.9 mg kg⁻¹ FW, Müller, 1997 [67]; 0.28–3.38 mg kg⁻¹ FW, Abushita et al., 2000) [68]). Aruna et al. 2009 [69] study reported 2.89 mg kg⁻¹ of edible portion. In addition, Montesano et al. (2012) [70] analyzed lutein from tomato by-products by HPLC–DAD and reported that lutein levels ranged from 9.9 to 10.5 mg kg⁻¹ dry weight (DW).

In conclusion, our research makes a valuable contribution to the conservation of the tomato (*Solanum lycopersicum*) genetic heritage, in the context of the increasingly accentuated genetic erosion caused by the industrialization of agriculture. Highlighting landraces with resistance to *Phytophthora infestans* creates the conditions to protect the environment, by reducing the amount of pesticides. Also, the identification of landraces with high content of lycopene, lutein and β -carotene supports human health, given the alarming increase in the incidence of degenerative diseases in the population of countries with developed economies.

4. Materials and Methods

4.1. Biologic Material and Experimental Conditions

Thirty-five local tomato varieties were collected from different sources: small farmers; small seed growers; various international events where seeds were exchanged between participants. Of the 35 local populations, 15 came from Romania (Alba, Cluj, Sălaj, and Buzău counties), 13 were from France (e.g., Kokopelli Peasant Confederation), three from Russia, and one from Greece, Italy, Austria, and Thailand, respectively.

Each local variety received a code and was tested, during 2019–2020, in the experimental fields at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, located in Cluj County, Romania. Tomatoes were grown in an organic cultivation system with minimal energy inputs.

Cluj-Napoca is located in the north western part of Romania, in the Transylvanian Hilly Depression. Cluj's climate is temperate continental. The average annual temperature is 8.2 °C, and the amount of annual precipitation is 557 mm. The experimental field was located on a preluvosol land with a high clay content.

The experimental tomato crops were organized in the open field, between May and September. In the experimental years 2019 and 2020, the temperatures during the vegetation period exceeded the multiannual average, and the precipitation recorded lower values compared to the multiannual values. However, massive late blight infections were achieved in both years due to the rainy periods in July–August, which lasted between 4 and 6 days.

4.2. Morphological, Agronomic Characterization and Assessment of Resistance to *P. infestans* of the Tomato Landraces

Passport and characterization descriptors were used to characterize the landraces, as recommended by the International Plant Genetic Resources Institute (IPGRI) [71]. In particular, the characterization descriptors included 91 characteristics: 66 morphological, 20 agronomic, and five biochemical.

The morphological description included 66 characteristics related to the vegetative organs, inflorescences, and fruits. The agronomic description was made through 20 features of the landraces. In addition, five Physico-chemical characteristics were used: dry matter, pH, lycopene, β -carotene, and lutein content.

Three of the studied local varieties were patented in Romania by the State Institute for Testing and Homologation of Varieties in Bucharest. Two patented varieties came from Salaj County—Cassiana (SJ 373) and Chandona (SJ 457165)—and the third patented local variety originated from Alba County—Danamari (AB 343). These varieties met the Distinction, Uniformity, Stability (DUS) criteria and the requirements for variety patenting. As these varieties are present in the Official Catalogue of Varieties in Romania, they can be grown by any farmer.

Among all morphological and agronomic characteristics, we selected some of them for this paper, such as: the type of growth, colour, shape, and weight of the fruit, the number of days from emergence to fruit maturity, and the resistance to late blight disease caused by *P. infestans*.

The percentage of late blight disease attack was assessed by field observations. For this purpose, three hundred leaves and fruits from untreated tomato plants were randomly observed. To assess the percentage of late blight attack the following scale was used: not infected (0%); very low (1–20% infected tissue); low (21–40% infected tissue); medium (41–60% infected tissue), high (61–80% infected tissue) and very high (>81% infected tissue).

In addition, to identify the possible pathogen causal agent responsible for the observed symptoms on tomato plants, twenty symptomatic leaves and fruits were randomly collected from each variety and utilized for possible *P. infestans* isolation. For this purpose, symptomatic tissues were cut under laminar flow sterile conditions into small parts, surface-sterilized by soaking in a 70% ethanol solution for 1 min, in a 1% NaOCl solution for 1 min, in 70% ethanol solution for another 30 sec and finally rinsed in sterile water for 2 min. After sterilization, they were dried on sterile paper, cut into smaller parts and placed on Petri plates containing selective media [72]. Petri plates were then incubated at 20°C in the dark until growth could be detected. Subsequently, pure fungal cultures (PFCs) were made for all obtained isolates. For morphological identification, the PFCs were observed under a light microscope (Axioscope, Zeiss, Germany).

4.3. Determination of the Dry Matter Content and pH of Tomato Landraces

The moisture content of the samples was determined according to method No. 943.06 (section 31.1.10B) of the AOAC. Dry matter was determined for each of the 35 tomato cultivars. Thus, 1 g of fresh pulp was taken, after which the empty container was weighed, and the second weighing was carried out after drying the sample (container with dry sample). The procedure was repeated three times after which the average weight of each variety was taken. Briefly, weight loss of the pre-weighed samples, after oven drying for \approx 24 h at 105°C, was calculated. Subsequently, 5 g of sample were dissolved in 45 mL of distilled water, homogenized continuously with a magnetic stirrer, and the pH of each variety was measured using a digital pH Meter (InoLab 7110, Germany) at 22°C temperature.

4.4. Ultrasound-Assisted Extraction (UAE) of Carotenoids

Carotenoids were extracted from tomato fruits assisted by ultrasound, as described by Szabo et al., 2019 [73]. The Ultrasound Assisted Ultrasound-Assisted Extraction (UAE) method presents the advantages of reduced processing time and the possibility of using low processing temperatures to recover heat-sensitive compounds such as carotenoids. Briefly, a mixture of methanol/ethyl acetate/petroleum ether (1:1:1, v/v/v) was used to extract total carotenoids from each sample (1 g). Falcon tubes containing the sample and 10 mL solvent were placed in an ultrasonic unit (Elma Schmidbauer GmbH, Singen, Germany) for 10 min, centrifuged at 11,000 RPM at room temperature, and filtrated. The remaining residue was re-extracted four times more by applying the same protocol until the samples remained colorless. The extracts were collected in a separation funnel and were successively washed with sodium chloride solution (15%) and diethyl ether. The organic phase (upper layer), enclosing the targeted carotenoids, was dried over anhydrous sodium sulfate, and the solvent was removed by a rotary evaporator (Rotavapor R-124, Buchi, Flawil, Switzerland) at 35 °C and further analyzed by HPLC with a diode-array detector (DAD). The presented results are an average of three series of analyses.

4.5. Quantitative and Qualitative Analysis of Carotenoids by HPLC/DAD

The extracts were dissolved in 1 mL of ethyl acetate, passed through an MF-Millipore® Membrane Filter, 0.45 μ m pore size (Merck, Darmstadt, Germany), and injected into the HPLC/DAD system. Individual carotenoids, particularly lycopene, β -carotene, and lutein, were determined using an Agilent 1200 HPLC system coupled to a diode array detector (Agilent Technologies, Santa Clara, CA, USA), using a reversed-phase EC 250/4.6 Nucleodur 300–5 C18 ec. Column 5 m (Macherey-Nagel, place, Germany) as described in Katalin Szabo et al., 2021 [74]. The mobile phases consisted of mixtures of acetonitrile: water (9:1, v/v) with 0.25% triethylamine (A) and ethyl acetate with 0.25% triethylamine (B). The gradient started with 90% A at 0 min, decreasing to 50% A at 10 min; the A percentage decreased from 50% at 10 min to 10% A at 20 min. The flow rate was 1 mL/min, the chromatograms were registered at 450 nm, and the HPLC peaks were identified using carotenoid standards. Individual carotenoids were quantified using the calibration curve of the β -carotene standard (Sigma-Aldrich, Steinheim, Germany).

4.6. Statistical Analysis

Data analysis was performed with the R Studio software version.4.1106 (RStudio Team, 2019), sustained by the R platform (RCoreTeam, 2021). Due to the large database obtained by analyzing the 35 tomato varieties, an organizing procedure was performed before exploring the differences between our biological materials. Thus, we first grouped our varieties based on their origin, which consisted of 7 centers of Origin; the second type of grouping was the resistance to *P. infestans*, followed by the fruit weight and lycopene content. This approach permitted us to explore the potential of each group of varieties better and more profoundly in a specific context [75]. The final step was represented by

the projection of all results in a Principal Component Analysis (PCA) graph, based on each combination of grouping factors, with the PCA option from the “vegan” package [76–78].

Means and standard errors were extracted with the package “psych,” followed by ANOVA and LSD analysis with formulas included in the package “agricolae,” the three tests allowing complete analysis of the most important differences [79,80].

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy13010021/s1>, Table S1: Morphological and agronomic characteristics of tomato (*Solanum lycopersicum*) landraces were investigated during 2019–2020 at the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (Romania).

Author Contributions: Conceptualization, A.M. and V.C.A.; methodology, D.C.V., A.M., V.C.A., T.M., S.M.M., I.C., V.T., M.G.B., M.S., F.R. and O.B.; software, V.T. and V.C.A.; validation, A.M. and S.M.M.; formal analysis, T.M., A.M., S.M.M., M.G.B. and L.M.; investigation, V.C.A. and A.M.; resources, V.C.A. and A.M.; data curation, V.C.A.; writing—original draft preparation, A.M., V.C.A. and S.M.M.; writing—A.M., V.C.A., T.M. and S.M.M.; visualization A.M., S.M.M., V.C.A., V.T., I.C. and L.M.; supervision, A.M., S.M.M., I.C., M.S. and L.M.; project administration, A.M. and V.C.A.; funding acquisition, A.M. and V.C.A. First author (A.M.) and the corresponding author (V.C.A.) have contributed equally to this paper, both being considered as first authors. All authors have read and agreed to the published version of the manuscript.

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