Article

Tomato Landraces May Benefit from Protected Production—Evaluation on Phytochemicals

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Abstract: Plant genetic resources (PGRs) serving as a wide genetic pool of tomato germplasm can provide a solid base for recent breeding efforts to increase consumer acceptance towards the taste and the phytonutrient properties of novel tomato varieties. Old varieties and landraces were abandoned by producers due to unfavorable phenotypic characteristics; however, their high adaptability and nutritional properties are inevitably valuable. This study aims to investigate the impact of open-field vs. protected production on various bioactive compound parameters and on the antioxidant status of seven indeterminate-type tomato PGRs in an organic production system for two years (2015–2016). Genotype main effect plus genotype × environment interaction (GGE) biplots were created for visualizing the which-won-where concept of the PGRs investigated. The GGE analysis revealed that the phytonutrient content of certain PGRs is less dependent on location and more influenced by differences in microclimatic conditions. “Balatonboglár”, “Mátrafüred” and “Fadd” PGRs performed better in a polytunnel, while Tarnaméra provided better results in an open field. “Máriapócs” and “San Marzano” showed a relative independence from production location in terms of their measured phytonutrient values. These findings enrich the nutritional datasets of tomato landraces, which support the in situ conservation and utilization of PGRs in breeding programs.

Keywords: plant genetic resource (PGR); fruit quality; antioxidants; genotype × environment interaction; GGE biplot; phytonutrient

1. Introduction

Tomatoes (Solanum lycopersicum L.) are one of the most frequently consumed vegetables worldwide. More than 180 million tons of tomatoes were grown worldwide in 2019 [1]. This vegetable is an important ingredient in modern diet trends and is used globally for its color and taste. Tomato is a rich source of several health-improving compounds including vitamins, carotenoids, flavonoids and phenolic acids [2,3].

Increased interest in organic tomato production implies the evaluation of the nutritional quality of organic tomatoes. Organic farming is a certified agricultural method with the goal of producing food by decreasing environmental impact. It supports the sustainable use of natural resources like water, soil and energy and the maintenance of ecological balances and biodiversity [4].
To optimize productivity on organic farms, farmers need varieties bred to fit their environmental conditions, management and customers’ preferences [5]. In spite of having large variability of landraces and cultivars, world tomato production concentrates on a few modern genotypes with increased yield rates but less flavor and nutritional value [6–8]. In the USA, it is estimated that 95% of varieties grown on organic farms are not bred for organic environments [9].

Local landraces or traditional cultivars selected for specific regions may also be a very suitable genetic pool to improve tomato crop production [6,8]. Due to the high genetic diversity, traditional varieties and landraces can be used for obtaining new varieties. In addition, they may have an important role in long-term food security and could contribute to the development of local economies [10,11]. Other advantages of local varieties and landraces are the superior taste and their suitability for organic production [12].

Consumers expect fresh vegetable during the whole season. To extend the growing season and increase vegetable quality, organic growers produce tomato in polytunnels and in open fields. Organic growers can benefit from the lower disease pressure and higher marketable yields achieved in high tunnels [13,14]. Polytunnels protect crops from severe environmental stress, thereby supporting the production of tomato landraces, as these varieties often have softer fruit and are more susceptible to diseases; splitting and cracking occurs more frequently than for hybrid varieties [15,16].

The information on the cultivation practices is important for growers, because the cultivation technique could influence the nutritional value of tomatoes. Despite higher yields and higher aesthetic appeal in polytunnels, little is known about the nutritional quality of the produce [17].

Scientific interest in the phenolic compounds of food has increased nowadays, due to their possible beneficial effects on human health [3]. Their content depends on ripening, growing conditions, cultivars and other agricultural and pedoclimatic factors. For this reason, the same plant species could have different content in phenolic compounds, both quantitatively and qualitatively [3,18].

Zhao and colleagues [19] examined the accumulation of phenolic compounds in lettuce and found that it was suppressed in varieties grown in high tunnels. Similar results were observed in young green- and red-leaf lettuce varieties grown in high tunnels [20]. Romani et al. [21] showed that lettuce grown in a greenhouse which received approximately 27% less light intensity than those in an open field had a reduced concentration of many individual polyphenols and total phenolic compounds. High-tunnel cultivation of red raspberry resulted in reduced levels of carotenoids, including β-carotene, lutein and zeaxanthins, in the fruits, compared to open-field culture [22]. Asensio et al. [23] observed that the concentrations of chlorogenic acid, caffeic acid, ferulic acid, total phenolic content, lycopene, β-carotene, ascorbic acid and vitamin C were significantly higher in tomatoes grown in open fields rather than in greenhouses. Wooley et al. [17] showed that lettuce and tomato cultivars grown in high tunnels had a higher N (protein) level compared to those grown in the open field. Lettuce grown in a high tunnel also had higher concentrations of S and Zn. However, high-tunnel cultivation of lettuce suppressed the accumulation of many micronutrients such as Mg, Fe, Cu and Mn and many phenolic compounds including chlorogenic acid, chicoric acid and luteolin-7-glucoside. Quality parameters related to sensory properties were analyzed by Cebolla-Cornejo et al. [24] in a collection of four traditional varieties and two tomato hybrids grown in different environments, in a greenhouse and in an open field. Protected cultivation tended to show lower sugar concentration (fructose and glucose) but similar acid contents (citric, malic and glutamic acids). The decreased levels of sucrose equivalents indicated that protected cultivation reduces the sensory quality. The study of Healy et al. [9] compared 19 tomato varieties in organic greenhouse and field conditions. They found that tomatoes grown in a hoop house had significantly higher yield, lower disease severity and higher °Bx (total soluble solids) than those grown in an open field. It was determined that management (hoop house versus field) had significantly more influence over the examined traits than other variables (year, variety or market class). Lee
et al. [25] investigated the effect of high-tunnel cultivation in comparison to conventional open-field production on 41 volatiles from four tomato varieties. They found that levels of β-damascenone were higher in the high-tunnel tomatoes and geranylacetone was higher in open-field tomatoes.

The composition of bioactive compounds of vegetables is variable, both qualitatively and quantitatively. The content of these substances can also be affected by the environmental and nutritional conditions of crops (agronomic conditions), as well as treatments made during the handling of vegetables at the post-harvest stage and processing [9,23,24,26].

Higher-order organisms have many defense mechanisms against free radicals and reactive oxygen species. The first line of defense is the enzymatic defense system. The non-enzymatic system is activated when oxidative stress is exerted. A properly functioning redox homeostasis is a key factor in the adaptation to biotic and abiotic stress factors (e.g., thermal stress, stress caused by pH changes, UV irradiation, water and nutrient availability or pathogen pressure), since the generation of reactive oxygen species and the associated cell damage are not only related to external factors (e.g., pathogens, contaminants and environmental stresses) but also to the plant’s metabolism itself (e.g., photosynthesis) [27,28].

Important enzymes involved in maintaining redox homeostasis include ascorbate peroxidase (AsPOX), glutathione-S-transferase (GST) and peroxidase (POX), enzymes which have been identified in several plants. These enzymes use various substrates to neutralize H$_2$O$_2$ and catalyze the detoxification of several toxic organic compounds [29,30]. As certain types of peroxidases alter the composition of the cell wall, it is an important parameter which may be useful for monitoring plant development [31]. Because of this, changes of enzyme activity under different conditions are regularly monitored by researchers. In the study of Barka [32], UV-C irradiation, which can be used to extend the shelf life of certain fruits, increased the AsPOXs and reduced other enzymes’ (such as superoxide-dismutase (SOD) activity in treated tomatoes. The increased AsPOX activity is due to the ROS generated by photo-oxidation [32]. In an experiment on greenhouse-grown tomatoes, POX enzyme activity in water stress-treated plants increased [33]. Postharvest hot air treatments on tomato fruits led to increased enzyme activity of GST [34].

According to these studies, various stress factors affect the activity of these antioxidant enzymes. As a preliminary conclusion, stress effects (e.g., heat, water deficit and photo-oxidation) activate the defense mechanisms of the plants, thus increasing the activity of enzymes responsible for the elimination of ROS. Based on this, we can deduce whether the greenhouse- or open field-grown plants were exposed to more stress.

Following the approach of Eberhart and Russel [35], the interaction of genotype and environment was recently analyzed by the additive main effects and multiplicative interaction (AMMI) model developed by Zobel et al. [36] and the genotype main effects and genotype × environment interaction effects (GGE) model published by Yan et al. [37]. According to the latter, genotype and genotype × environment interaction (GEI) are the most important factors which influence the variation in the evaluation criteria, such as yield or nutritional value [38]. In comparison with AMMI, GGE contains both the genotype effect and its interaction with the environment in a more accurate way; a valuable functionality of the GGE method is the which-won-where pattern [39]. GGE biplot analysis has proved to be a useful tool for the aggregative evaluation of breeding lines and PGRs [40,41] and for the selection of ideal environments [42–44] for different crops.

Regardless of the widely and generally mentioned higher nutritional value of PGRs, the availability of the data on bioactive compounds regarding Hungarian tomato landraces is limited [45,46], especially in relevance to different production systems. In this study, the phytonutrient profiles consisting of phenolics, antioxidants and enzyme activities of seven Hungarian tomato PGRs and a commercial variety grown in organically certified open-field and polytunnel conditions were investigated throughout two years. Our findings contribute to the determination of ideal environments for tomato PGR production in order to maximize the amount of useful bioactive compounds of tomato fruits.
2. Materials and Methods

The open-field experiment was carried out at Tahitótfalu (47°45′14.08″ N, 19°6′7.78″ E), while the polytunnel was set at Szignetmonostor (47°41′44.99″ N, 19°5′47.18″ E). Both locations are situated on Szentendre Island, 100 m above sea level, north of Budapest, Hungary, and are certified as organic for more than 10 years.

An Easyweather (iMetos) weather station was used in the open field for recording temperature, humidity and precipitation each hour. In the polytunnel, a Voltcraft DL-120TH datalogger was applied for measuring temperature and humidity each hour. Minimum, average and maximum temperature was calculated and visualized on the basis of 10-day periods (decades).

The experimental locations have alluvial soil. Soil analyses were done by the Soil Conservation Directorate of Velence, Hungary. Basic soil parameters are shown in Table 1.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>pH</th>
<th>SOM (%)</th>
<th>N (ppm)</th>
<th>P (ppm)</th>
<th>K (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>OF</td>
<td>7.27</td>
<td>2.58</td>
<td>18</td>
<td>146</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>7.47</td>
<td>2.31</td>
<td>20.6</td>
<td>250</td>
<td>372</td>
</tr>
<tr>
<td>2016</td>
<td>OF</td>
<td>7.32</td>
<td>2.8</td>
<td>24.9</td>
<td>120</td>
<td>439</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>7.42</td>
<td>2.53</td>
<td>176</td>
<td>643</td>
<td>562</td>
</tr>
</tbody>
</table>


The polytunnel hosting the experiment was 40 m long and 9 m wide. The plots were arranged in a randomized block design, in three replications. One plot contained 12 plants in a two-row alignment; one PGR/variety was represented by a total of 36 plants. For the open-field experiment, a randomized complete block design was set with four replications. One plot consisted of 10 plants in a two-row alignment [47].

2.1. Plant Materials

Based on previous studies, seven indeterminate-type Hungarian tomato PGRs were selected for the investigation of their phytonutrient value in the two different environments (open field and polytunnel) in two consecutive years (2015–2016). San Marzano, a commercial open-pollinated indeterminate-type variety, was used as control. The propagation material of the PGRs was provided by the National Centre for Biodiversity and Gene Conservation, Tápiószele, Hungary. The selected PGRs vary in fruit type, color and shape (Table 2).

<table>
<thead>
<tr>
<th>Code</th>
<th>Catalogue no.</th>
<th>Origin/ Variety Name</th>
<th>Type</th>
<th>Fruit Shape (^1), Size (^2)</th>
<th>Fruit Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>RCAT030566</td>
<td>Balatonboglár</td>
<td>fresh cons., processing</td>
<td>circular, M</td>
<td>red</td>
</tr>
<tr>
<td>C</td>
<td>RCAT030275</td>
<td>Cegléd</td>
<td>fresh cons.</td>
<td>circular, M</td>
<td>yellow</td>
</tr>
<tr>
<td>F</td>
<td>RCAT030373</td>
<td>Fadd</td>
<td>fresh cons.</td>
<td>rectangular, M</td>
<td>red</td>
</tr>
<tr>
<td>MR</td>
<td>RCAT030731</td>
<td>Máriapecs</td>
<td>fresh cons.</td>
<td>circular, S</td>
<td>red</td>
</tr>
<tr>
<td>MT</td>
<td>RCAT057656</td>
<td>Mátrafüred</td>
<td>processing</td>
<td>heart-shaped, L</td>
<td>light red</td>
</tr>
<tr>
<td>TA</td>
<td>RCAT030370</td>
<td>Tarnaméra</td>
<td>processing</td>
<td>cylindrical, M</td>
<td>red</td>
</tr>
<tr>
<td>TO</td>
<td>RCAT030184</td>
<td>Tolna County</td>
<td>processing</td>
<td>slightly flattened, L</td>
<td>red</td>
</tr>
<tr>
<td>SA</td>
<td>-</td>
<td>San Marzano (^3)</td>
<td>fresh cons., processing</td>
<td>cylindrical, M</td>
<td>red</td>
</tr>
</tbody>
</table>

\(^1\) According to UPOV TG 44/11 (International Union for the Protection of New Varieties of Plants 2001) [48].
\(^2\) S: small fruit size (10–80 g), M: medium fruit size (80–150 g), L: large fruit size (>150g), \(^3\) Maintained by SAIS Società agricola italiana sementi.

2.2. Instrumental Measurements

Approximately 1500 g of fruit samples were harvested from each PGR and variety in the period of peak harvest in August of both years. The fruits were collected in the stage of biological ripening (S6) [49] without any visible sign of infection or any other
disorder. Stems were removed, and after washing, fruits were homogenized by a laboratory homogenizer with no dilution. The homogenates were frozen in Falcon tubes until instrumental analyses.

The instrumental measurements were conducted in the laboratory of the Department of Dietetics and Nutrition Science, Faculty of Health Sciences, Semmelweis University, Hungary. Total soluble solids (TSS) were measured by a digital refractometer (Hanna Instruments HI96801) according to Codex Alimentarius 558/93 [50]; data are provided in °Bx. Titratable acid content (TAC) was determined by titration with 0.1 NaOH in the presence of phenolphthalein indicator, data are provided in citric acid equivalent percentage [51]. Sugar-acid ratio (SA) was calculated by dividing TSS by TAC values. SA values were transformed to SA_or, which defines the negative divergence from the ideal sugar-acid ratio.

For colorimetric measurements, a Konica Minolta CR-400 tristimulus colorimeter (Tokyo, Japan) was used, results were recorded in CIE LAB system using L*, a* and b* dimensions. Hue (h°) and chroma (C*) values were calculated according to McGuire [52]. Measurements were carried out on the homogenates in three replications.

For antioxidant assays, the supernatant of homogenates was used after centrifugation at 2000 × g. The FRAP assay was done according to Benzie and Strain [53] and samples were measured spectrophotometrically on 593 nm. The results were provided in ascorbic acid (AA) equivalent using mg AAE L⁻¹ dimension [54]. The FRAP reagent and the calibration curve were prepared every two hours to minimize issues regarding AA instability. The DPPH method was conducted according to Molyneux [55]. An amount of 100 µL of supernatant was added to 3.9 mL of 6 × 10⁻⁵ M DPPH solution, kept in the dark for 20 min, and then the absorbance was recorded spectrophotometrically on 517 nm. Values were provided in inhibition percentage (I%) dimension. Total polyphenolic content (TPC) was measured according to Singleton and Rossi [56], using Folin–Ciocalteu’s reagent. A standard curve based on gallic acid (GA) concentrations was used; results were expressed in mg GAE L⁻¹ dimension. The ABTS assay was measured according to Salah et al. [57]. The reaction mixture contained 10 µL of sample; 20 µL of 3.50 mg/mL myoglobin in 50 mM, pH 7.4, 9% NaCl and 1% glucose containing potassium-phosphate buffer; 150 µL of 1 mg ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) and 25 µL 3% H₂O₂ in 0.1 M pH5 citrate buffer. After shaking for 5 min at 37 °C, alkaline stop solution was added and measured at λ = 405 nm against the trolox calibration curve. The CUPRAC assay was measured following the methodology of [58]. An amount of 1 mL 10⁻² M CuCl₂, 1 mL 7.5 × 10⁻³ M neocuproine solution, 1 mL 1 M pH 7.4 NH₄Ac buffer, 100 µL sample and 1 mL distilled water was mixed and incubated for 30 min in the dark at room temperature, and then the absorbance values were read at λ = 450 nm; values were calculated to trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalents. Antioxidant activity was measured in five technical repetitions in the case of all five assays.

Lycopene content was measured spectrophotometrically in five technical repetitions by the method of Fish et al. [59]; acetone (with 0.05% BHT), ethanol and hexane were mixed and used for extraction. Absorbance in the hexane layer was measured at λ = 503 nm against hexane as blank solution; the results were expressed in mg/100 g dimension.

Enzyme activities were measured spectrophotometrically, in the presence of the adequate chromogen, expressed per unit time, based on the work of Venisse et al. [60]. After the determination of protein content, enzyme activities were expressed in mg/protein.

First, tomato sample homogenates were suspended with the enzyme extraction mixture in extraction vials and centrifuged in a microcentrifuge. The supernatants were used to perform the measurements. The extraction mixture contained sodium phosphate buffer pH 7.5, polyethylene glycol as an emulsifier, phenylmethlysulfonyl fluoride (PMSF) as a serine protease inhibitor, polyvinylpiprolidone (PVPP) as a decolorizing agent and Triton X-100 as a permeabilizing agent. The measurements were performed within 2 h after the extraction, in three replicates, against reaction mixture blinds.
For ascorbate peroxidase (AsPOX) determination, the absorbance was measured at $\lambda = 290$ nm at timepoint 0 and 10 min. The decrease of the absorbance was proportional to the amount of oxidizing ascorbic acid. For glutathione-S-transferase (GST) determination, the absorbance was measured at $\lambda = 340$ nm at timepoints 0 and 10 min. The decrease of the absorbance was proportional to the amount of the conjugate of glutathione and 1 chloro 2,4-dinitrobenzene (CDNB). For peroxidase (POX) quantification, the absorbance was measured at $\lambda = 470$ nm at timepoints 0 and 10 min. The decrease of the absorbance was proportional to the amount of tetraguaiacol forming.

2.3. Statistical Analysis

Three-way multivariate analysis of variance (MANOVA) models were built to evaluate the effects of year (2015, 2016), location (open field, polytunnel) and plant genetic resource (PGR) (Balatonboglár (B), Cegléd (C), Fadd (F), Mátravölgy (MR), Mátrafüred (MT), Tarnaméra (TA), Tolna County (TO), San Marzano (SA)), together with factor interactions. The dependent variables were set to sensory parameters (total soluble solids (TSS), total acid content (TAC) and their ratio (SAR)); the antioxidant capacity variables (TPC, FRAP, DPPH, ABTS and CUPRAC); color parameters (hue ($h^\circ$) and chroma ($C^*$)) as well as enzyme activity parameters (GST, AsPOX and POX). Multivariate outliers were detected by Mahalanobis distances. In two cases (TAC and GST), two extreme outlier values were winsorized to decrease their biasing effects. The normality of the high sample-size model residuals was accepted in each case by their skewness and kurtosis, as their absolute values were below two and four, respectively. Having significant overall MANOVA test, follow-up univariate three-way ANOVA models were run for all dependent variables, separately, with Bonferroni’s correction in order to avoid familywise error inflation. The homogeneity of the variances was tested by Levene’s test and variance ration test, and we concluded that this assumption was slightly violated in some cases. Therefore, for significant factor effects, Games–Howell’s post hoc test was applied to separate homogeneous groups, since this post hoc test can successfully manage the problem of inhomogeneous variances.

Finally, a three-way ANOVA model was used to test the effect of the same three factors on the lycopene content (Lyc) of the samples. The assumptions were tested the same way as before, and again, Games–Howell’s post hoc test was applied thereafter.

Genotype plus genotype $\times$ environment biplot analysis was applied for the visualization of $G + (G \times E)$ effect on the investigated parameters of the PGRs. The biplot is capable of showcasing the mean performance and the stability of the PGRs and the interaction with the environmental factors defined by years and locations [37,39]. In addition, GGE biplots support plant breeding and PGR utilization efforts by providing well-interpreted charts for the selection of best phenotypes for a given environment.

GGE biplot analysis was run with the freely available GGEBiplotGUI [61] package of R-project (version R-3.3.3) [62]. The settings of the model were row-metric preserving (SVP = 2), no data transformation (‘Transform = 0’), scaling by standard deviation and tester-centered $G + GE$.

Only those parameters were involved into the GGE analysis, where there was a notable positive relation between the amount of the compound and the related dietary benefits. These traits were TSS, Lyc, TPC, FRAP, CUPRAC, ABTS, DPPH, AsPOX, POX and GST. Total acidity was excluded from the analysis, while sugar-acid ratio values were transformed into deviation from optimal sugar-acid ratio ($SAR_{or}$) according to the following formula:

$$SAR_{or_n} = -|SAR_n - SAR_i|$$

where $SAR_{or_n}$ is the calculated relative sugar-acid ratio, $SAR_n$ is the sugar-acid ratio of the sample (TSS TAC$^{-1}$) and $SAR_i$ is the ideal sugar-acid ratio, defined as 8.5 according to Helyes et al. [63]. In this way, $SAR_{or}$ indicates the negative divergence from the ideal value and, therefore, is suitable for the GGE analysis together with the other selected parameters. The traits analyzed with GGE have different characteristics; TSS and $SAR_{or}$ contain information about the taste of PGRs, while the other parameters are related to
the non-enzymatic (TPC, FRAP, CUPRAC, ABTS, DPPH) and enzymatic (AsPOX, POX, GST) antioxidant status of the tomato samples. In order to analyze the bioactive traits of PGRs in an aggregated way, a previously applied methodology of Csambalik et al. [46] was employed.

3. Results

3.1. Weather Conditions

The summer of 2015 was warm and dry, while the vegetative period of 2016 was rather humid, with moderate average temperature (Figure 1).

![Temperature and Humidity Charts](image)

**Figure 1.** (a) Polytunnel and (b) open-field temperature (mean ± sd) (°C) and relative humidity (%) dataset of tomato PGR experiment conducted in 2015 and in 2016, presented from the date of the planting time to the end of the season, grouped in decades. Precipitation was measured only in the open fields.
3.2. Fruit Quality Parameters

The MANOVA overall test revealed that the variation in the studied parameters significantly differs between the levels of all the three factors—year, location and PGR (Wilk’s $\lambda = 0.048$, Wilk’s $\lambda = 0.036$ and Wilk’s $\lambda < 0.001$, respectively)—and for all two-way and three-way interactions (Wilk’s $\lambda < 0.200$), all with $p < 0.001$. The follow-up univariate ANOVA tests were significant for all variables—TSS, TAC and SAR—considering the effect of year ($F(1;64) = 32.00$, $F(1;64) = 1157.40$ and $F(1;64) = 518.51$, respectively, with $p < 0.001$). The location effect was significant for TSS ($F(1;64) = 1705.28$, $p < 0.001$) and SAR ($F(1;64) = 265.84$, $p < 0.001$) but not for TAC ($F(1;64) = 5.14$, $p = 0.08$). The PGR effect was significant again for all variables ($F(7;64) = 774.11$, $F(7;64) = 804.45$ and $F(7;64) = 244.66$ for TSS, TAC and SAR, respectively, with $p < 0.001$). Meanwhile, all two-way and three-way interactions were significant, for each of TSS, TAC and SAR ($p < 0.001$). The detailed post hoc test comparisons are presented in Table 3.

In 2015, TSS values ranged between 3.63 and 6.03 in an open field (OF) and between 4.6 and 5.93 in a polytunnel. San Marzano did not deviate from PGRs in any location. With the exception of MR, polytunnel samples had significantly higher TSS values in comparison with those from the open field. MR showed the highest values in both locations. In the second year, the values were between 3.73 and 5.6 in an open field, while the TSS was higher in the polytunnel, ranging between 6.73 and 4.1. San Marzano had moderate values in this year, which were significantly lower than MR in the open field, as well as compared to C, F, MR, MT and TO in the polytunnel. The effect of year was significant in the majority of cases and both locations. However, this influence was not clear; the first year showed higher values in an open field, while 2016 seemed to enhance the TSS of the polytunnel samples.

With regards to total acid content (TAC), significant differences were measured between locations in 2015 in the case of C, F, MT and SA. With the exception of the latter, the open field was more favorable for this trait. MR had the significantly highest TAC mean value in this year on open field, followed by SA and TO, which overlapped with B and MT. F significantly deviated from all other plant materials. In the polytunnel, MR again had the significantly highest mean TAC values, while SA and F, had the lowest TAC content. In 2016, except F, all the PGRs showed significant differences between locations in terms of TAC. In the case of B, C, MT, SA and TA, in the open field, TAC was elevated, while for MR and TO, the polytunnel was more favorable. In most cases, the second year revealed significantly higher mean TAC values. Similar to the outcome of 2015, the acid content of MR in 2016 was also significantly higher than that of SA and the other PGRs in an open field. SA also significantly deviated from all the PGRs, except TO. Samples from the polytunnel showed approximately the same trend, with SA being ranked lower.

Consequently, sugar-acid ratios (SAR) were generally and, in most cases, significantly higher in 2015. In this year, the polytunnel results were significantly lower for C and F, while this location was more favorable for B, SA, TA and TO. The commercial variety did not deviate from most of the PGRs in an open field, while in the polytunnel, it overlapped with every variety except TA. In 2016, locations did not deviate significantly in the case of F and TO. For the other PGRs, with the exception of MR, the polytunnel seemed to contribute significantly to higher SAR mean values. SA, together with MR and TO showed significantly lower SAR values in both locations.

For hue (ho) and chroma (C*), the three-way overall MANOVA model revealed significant differences for year, location and variety (Wilk’s $\lambda = 0.001$, Wilk’s $\lambda = 0.002$ and Wilk’s $\lambda < 0.001$, respectively) and for all two-way and three-way interactions (Wilk’s $\lambda < 0.009$), all with $p < 0.001$. Similarly, the follow-up univariate tests were all significant (year: $F(1;64) = 10.177.11$, $p < 0.001$; $F(1;64) = 956.19$, $p < 0.001$; location: $F(1;64) = 1215.80$, $p < 0.001$; $F(1;64) = 8084.30$, $p < 0.001$) with all two-way and three-way interactions for both variables C* and ho ($p < 0.001$). We provide the post hoc test comparisons in Table 1.
Table 3. Fruit quality parameters (mean ± sd) of the seven Hungarian tomato PGRs investigated and the control variety, in an open field and in a polytunnel, in 2015 and in 2016.

<table>
<thead>
<tr>
<th>PGR Code</th>
<th>Location (° Bx)</th>
<th>TSS (g/L)</th>
<th>TAC</th>
<th>SAR</th>
<th>C*</th>
<th>h*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>OF</td>
<td>3.63 ± 0.06</td>
<td>3.67 ± 0.06</td>
<td>0.29 ± 0.01</td>
<td>0.38 ± 0.01</td>
<td>12.64 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>4.67 ± 0.12</td>
<td>4.23 ± 0.06</td>
<td>0.30 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>15.34 ± 0.37</td>
</tr>
<tr>
<td>C</td>
<td>OF</td>
<td>4.50 ± 0.10</td>
<td>4.63 ± 0.06</td>
<td>0.25 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>17.95 ± 0.87</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>4.70 ± 0.00</td>
<td>5.67 ± 0.06</td>
<td>0.45 ± 0.20</td>
<td>0.32 ± 0.00</td>
<td>13.01 ± 1.27</td>
</tr>
<tr>
<td>F</td>
<td>OF</td>
<td>4.63 ± 0.06</td>
<td>3.73 ± 0.06</td>
<td>0.19 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>24.52 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>4.73 ± 0.06</td>
<td>5.20 ± 0.17</td>
<td>0.21 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>22.14 ± 0.46</td>
</tr>
<tr>
<td>MR</td>
<td>OF</td>
<td>6.03 ± 0.15</td>
<td>5.60 ± 0.10</td>
<td>0.42 ± 0.01</td>
<td>0.49 ± 0.01</td>
<td>14.53 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>5.93 ± 0.06</td>
<td>6.43 ± 0.12</td>
<td>0.41 ± 0.01</td>
<td>0.59 ± 0.00</td>
<td>14.44 ± 0.45</td>
</tr>
<tr>
<td>MT</td>
<td>OF</td>
<td>4.53 ± 0.06</td>
<td>4.40 ± 0.00</td>
<td>0.27 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>16.96 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>5.90 ± 0.00</td>
<td>5.50 ± 0.00</td>
<td>0.37 ± 0.01</td>
<td>0.21 ± 0.03</td>
<td>16.12 ± 0.32</td>
</tr>
<tr>
<td>SA</td>
<td>OF</td>
<td>4.63 ± 0.06</td>
<td>4.43 ± 0.06</td>
<td>0.29 ± 0.01</td>
<td>0.44 ± 0.01</td>
<td>16.10 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>4.90 ± 0.10</td>
<td>4.60 ± 0.00</td>
<td>0.25 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>19.53 ± 0.29</td>
</tr>
<tr>
<td>TA</td>
<td>OF</td>
<td>4.60 ± 0.10</td>
<td>4.30 ± 0.00</td>
<td>0.24 ± 0.01</td>
<td>0.32 ± 0.00</td>
<td>19.30 ± 0.95</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>4.60 ± 0.00</td>
<td>4.10 ± 0.00</td>
<td>0.23 ± 0.01</td>
<td>0.26 ± 0.01</td>
<td>19.66 ± 1.04</td>
</tr>
<tr>
<td>TO</td>
<td>OF</td>
<td>4.40 ± 0.00</td>
<td>3.90 ± 0.00</td>
<td>0.33 ± 0.01</td>
<td>0.40 ± 0.02</td>
<td>13.39 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>4.77 ± 0.06</td>
<td>5.43 ± 0.00</td>
<td>0.33 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>14.32 ± 0.57</td>
</tr>
</tbody>
</table>

Legend: TSS: total soluble solids, TAC: titratable acid content, SAR: sugar-acid ratio, C*: chroma value, h*: hue value, OF: open field, PT: polytunnel, B: “Balatonboglár”, C: “Cegléd”, F: “Fadd”, MR: “Máriapócsa”, MT: “Matrafűred”, SA: “San Marzano”, TA: “Tamaméra”, TO: “Tolna”. Data highlighted with grey are significantly higher in the comparison of locations within a year; data in bold are significantly higher in the comparison of years within a location. Different lower- and upper-case superscript letters within columns are for significant differences among PGRs in an open field and in a polytunnel, respectively (Games–Howell, \( p < 0.05 \)).
The impact of year and the genetic background on the chroma values is obvious; in the second year, C* values were significantly higher in the case of all PGR × location combinations, with the exception of TA samples in the polytunnel. In 2015, open-field conditions were significantly more favorable for MT, TA and TO, while for B and MR grown in polytunnels, C* values of the samples were significantly elevated. This pattern was consistent in the next year for B, MR and TA. Note that in 2016, all samples deviated significantly both in terms of C* and h°.

When the hue values of the two years were assessed, PGR level differences were visible and none of the years could be highlighted as generally more favorable. Regarding location, B, MT and SA deviated significantly. In all three cases, values were higher in the case of open-field conditions.

### 3.3. Antioxidant Traits

Although comparison of antioxidant capacity measured in different years is generally irrelevant due to the high influence of variable weather parameters, it is visible here that the first year of the experiment was more favorable for the synthesis of antioxidant compounds in both locations. The advantage of executing several parallel antioxidant assays is that the selectivity of assays to certain compounds can be overcome.

The three-way MANOVA model resulted in significant overall differences in the variations of the investigated parameters for all the three factors (Wilk’s $\lambda = 0.013$, Wilk’s $\lambda = 0.410$ and Wilk’s $\lambda = 0.001$, for year, location and PGR, respectively) and for all two-way and three-way interactions (Wilk’s $\lambda < 0.600$), all with $p < 0.001$. According to the follow-up univariate ANOVA, the year effect was again significant for all dependent variables (TPC, FRAP, DPPH, ABTS and CUPRAC: $F(1,127) > 32.83$, $p < 0.001$). The location effect, however, was not significant for TPC and FRAP ($F(1,127) = 5.04$, $p = 0.13$; $F(1,127) = 0.10$, $p = 0.76$, respectively) while it was significant for all the other variables ($F_{\text{DPPH}}(1,127) = 82.73$, $p < 0.001$; $F_{\text{ABTS}}(1,127) = 87.71$, $p < 0.001$ and $F_{\text{CUPRAC}}(1,127) = 9.90$, $p < 0.05$).

As for the PGR effect, we again found significant differences for all variables—TPC, FRAP, DPPH, ABTS and CUPRAC ($F(7,127) > 23.52$, $p < 0.001$). Moreover, the two-way and three-way interactions were also significant for all variables (TPC, FRAP, DPPH, ABTS and CUPRAC, $p < 0.001$), except the two-way and three-way interactions with location for TPC and year × location interaction for DPPH and ABTS ($p > 0.10$). The detailed post hoc test comparisons are shown in Table 4.

In both years and locations, the highest TPC values were measured in the case of MR; however, it deviated significantly from SA only in 2016 in both production systems. In 2015, PGRs did not deviate from SA; B showed significantly lower levels of TPC than F and TA in an open field, while in the polytunnel, B, C and TA significantly underperformed the results of F. In 2016, B and F gave significantly lower TPC than SA in the open field, while in the polytunnel, SA had one of the lowest results among samples. No consequent significant differences were found when locations were compared within PGRs or the variety for both years. Single-year TPC data was significantly higher in the polytunnel in the cases of B, C, F, MT and TO, while in the cases of SA and TA, open-field conditions resulted in significantly higher TPC values in one of the years.

Regarding FRAP results, MR significantly exceeded SA in 2015 in both locations, while in 2016, it did so only under polytunnels. In 2015, all PGRs showed significantly lower FRAP results than the control variety in the open field, with the exception of MR. The same is true for the polytunnel, although the values of F were higher than those of SA and were grouped together with MR in this environment. In 2016, MR and SA fell into the same group in an open field, significantly deviating from the other PGRs. The FRAP results of the polytunnel samples were significantly lower from that of MR. In both years, the effect of location was significant in most cases; however, the effect was consistent only in the case of SA and TA, in favor of open-field conditions.
Table 4. Antioxidant capacity (mean ± sd) of the seven Hungarian tomato PGRs investigated and the control variety, measured by five different assays, in the open field and in the polytunnel, in 2015 and 2016.

<table>
<thead>
<tr>
<th>PGR Code</th>
<th>Location</th>
<th>TPC (mg GAE/L)</th>
<th>FRAP (mg AAE/L)</th>
<th>DPPH (%)</th>
<th>ABTS</th>
<th>CUPRAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>OF</td>
<td>35.40 ± 4.88 a</td>
<td>11.09 ± 1.56 ab</td>
<td>15.22 ± 2.41 ±</td>
<td>6.65 ± 0.34 ab</td>
<td>34.87 ± 1.79 b</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>41.31 ± 2.90 A</td>
<td>14.37 ± 3.59 AB</td>
<td>20.47 ± 1.32 A</td>
<td>5.25 ± 0.18 A</td>
<td>39.64 ± 0.34 BC</td>
</tr>
<tr>
<td>C</td>
<td>OF</td>
<td>44.92 ± 8.69 ab</td>
<td>12.79 ± 1.16 acd</td>
<td>16.96 ± 1.18 A</td>
<td>6.36 ± 0.20 ab</td>
<td>27.88 ± 0.45 a</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>48.33 ± 9.43 A</td>
<td>17.59 ± 1.97 Bc</td>
<td>20.27 ± 1.19 A</td>
<td>6.22 ± 0.17 A</td>
<td>41.83 ± 0.84 A</td>
</tr>
<tr>
<td>F</td>
<td>OF</td>
<td>64.72 ± 8.22 A</td>
<td>13.22 ± 1.00 ade</td>
<td>31.76 ± 2.53 h</td>
<td>6.56 ± 0.18 ab</td>
<td>60.63 ± 4.19 de</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>77.79 ± 10.91 A</td>
<td>18.21 ± 1.21 c</td>
<td>43.90 ± 2.4 c</td>
<td>5.19 ± 0.07 A</td>
<td>80.43 ± 2.32 f</td>
</tr>
<tr>
<td>MR</td>
<td>OF</td>
<td>91.25 ± 24.92 a b</td>
<td>19.70 ± 1.00 f</td>
<td>46.67 ± 2.79 d</td>
<td>12.22 ± 0.40 c</td>
<td>89.64 ± 1.53 f</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>91.24 ± 21.76 A</td>
<td>19.31 ± 0.78 C</td>
<td>43.70 ± 3.65 C</td>
<td>13.94 ± 1.33 B</td>
<td>80.01 ± 1.18 f</td>
</tr>
<tr>
<td>MT</td>
<td>OF</td>
<td>37.78 ± 16.40 A</td>
<td>11.24 ± 0.34 b</td>
<td>16.76 ± 4.24 a</td>
<td>6.78 ± 0.38 b</td>
<td>39.28 ± 0.30 c</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>56.09 ± 10.10 AB</td>
<td>13.89 ± 1.07 B</td>
<td>21.19 ± 3.17 A</td>
<td>5.19 ± 0.43 A</td>
<td>39.28 ± 1.12 B</td>
</tr>
<tr>
<td>SA</td>
<td>OF</td>
<td>56.39 ± 8.54 A</td>
<td>15.20 ± 0.92 a</td>
<td>38.45 ± 1.43 a</td>
<td>11.81 ± 0.23 c</td>
<td>68.03 ± 1.88 B</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>58.19 ± 13.37 AB</td>
<td>10.53 ± 1.25 A</td>
<td>32.78 ± 2.45 A</td>
<td>5.20 ± 0.25 A</td>
<td>68.30 ± 0.90 B</td>
</tr>
<tr>
<td>TA</td>
<td>OF</td>
<td>63.02 ± 9.06 A b</td>
<td>14.03 ± 0.46 bde</td>
<td>29.55 ± 2.29 b</td>
<td>6.53 ± 0.50 a</td>
<td>56.05 ± 1.12 d</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>51.94 ± 9.4 A</td>
<td>9.82 ± 0.54 A</td>
<td>21.21 ± 4.92 A</td>
<td>4.62 ± 0.43 A</td>
<td>44.63 ± 0.61 D</td>
</tr>
<tr>
<td>TO</td>
<td>OF</td>
<td>45.79 ± 5.33 ab</td>
<td>14.93 ± 1.65 bde</td>
<td>15.60 ± 0.53 a</td>
<td>8.00 ± 0.86 b</td>
<td>30.91 ± 1.03 b</td>
</tr>
<tr>
<td></td>
<td>PT</td>
<td>58.62 ± 7.08 AB</td>
<td>13.55 ± 2.26 AB</td>
<td>16.51 ± 2.13 A</td>
<td>7.79 ± 3.26 AB</td>
<td>34.22 ± 0.48 A</td>
</tr>
</tbody>
</table>

Legend: TPC: total polyphenol content, FRAP: ferric reducing antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazil, ABTS: 2,2'-azino-bis (3-ethylbenothiazoline-6-sulfonic acid), CUPRAC: cupric ion reducing antioxidant capacity. OF: open field, PT: polytunnel, B: "Balatonboglár", C: "Cegléd", F: "Fadd", MR: "Máriapócs", MT: "Mátrafüred", SA: "San Marzano", TA: "Tarnaméra", TO: "Tolna". Data highlighted with grey are significantly higher in the comparison of locations within a year; data in bold are significantly higher in the comparison of years within a location. Different lower- and upper-case superscript letters within columns are for significant differences among PGRs in the open field and in the polytunnel, respectively (Games–Howell, p < 0.05).
DPPH results showed a somewhat similar pattern, with the significantly higher results given by MR. This difference is significant from the control variety in 2015 in both locations, while in 2016, MR was grouped together with SA in the polytunnel. Meanwhile, the open-field value showed a radical drop. In 2015, together with MR, the DPPH value of F significantly exceeded that of SA in the polytunnel. Location-based comparisons of DPPH results showed inconsistencies again; however, in the case of TA, open field was more favorable, while the DPPH values were higher in polytunnels in the case of C in both years.

The ABTS values of MR were the highest among samples; however, this difference was significant only in a few cases. In 2015, results of MR and TA exceeded that of B, MT and TO significantly in the open field, while in the polytunnel, MR and F deviated from SA and the other PGRs. In 2016, MR deviated from SA and TA in the open field; B, C and F showed significantly lower ABTS results here. Regarding polytunnel results, the best-performing MR did not deviate from SA, while the other PGRs together formed a different group. Differences between locations were insignificant in most cases within samples. However, the results of MT and TA were consistent throughout years: open-field results were significantly higher.

The CUPRAC results of MR were significantly higher in both environments, deviating from the control variety. In both locations, significantly lower values were shown by B, C and TO in 2015. In the second year, the same three PGRs showed the lowest values, significantly deviating from SA in both locations. When locations were compared, inconsistent results were found. Several PGRs show significant differences among locations; however, instead of location, the interaction of location × year seemed to be the determinant factor here as well. In the case of B, C and F, polytunnels resulted in higher CUPRAC values in 2015, while in 2016, open field values were significantly higher than those of the polytunnel. MR showed significant differences in all four environments; in 2015, the open field was more favorable, while in 2016, the polytunnel was more favorable for CUPRAC values.

Based on the three-way ANOVA model, considering the lycopene content, the year effect was not significant (F(1;64) = 0.02, p = 0.88) while the location and PGR effects were significant (F(1;64) = 18.89, p < 0.001; F(1;64) = 136.22, p < 0.001, respectively). Meanwhile, all two-way and three-way interactions were significant (p < 0.001), except that of year × location (p = 0.69).

The enzyme activity values of individual tomato PGRs showed high variability, including their heterogeneities, depending on locations and years (Table 3). The three-way overall MANOVA model resulted in significant factor effects on the studied parameters (Wilk’s λ = 0.047, Wilk’s λ = 0.090 and Wilk’s λ = 0.014, for year, location and PGR, respectively) and interaction effect (Wilk’s λ < 0.012 for all two-way and three-way interactions, all with p < 0.001). The follow-up univariate ANOVA revealed significant effects for all variables (AsPOX, GST and POX) in cases of all the three factors—year, location and PGR (year: F_{AsPOX}(1;64) = 715.61; F_{GST}(1;64) = 371.41; F_{POX}(1;64) = 548.09, all with p < 0.001; location: F_{AsPOX}(1;64) = 55.27, p < 0.001; F_{GST}(1;64) = 7.98, p < 0.05; F_{POX}(1;64) = 449.30, p < 0.001; PGR: F_{AsPOX}(1;64) = 74.15; F_{GST}(1;64) = 7.57; F(1;64) = 75.21, all with p < 0.001)—together with all two-way and three-way interactions for all variables (AsPOX, GST and POX, p < 0.001). The post hoc test comparisons are shown in Table 5.

The AsPOX enzyme activity showed significantly higher values in 2016 in the case of almost all PGRs. In 2015, the highest values were observed for F, grown in the open field, while in 2016, the highest values were observed for MR, also grown in the open field. In 2015, there were significant differences among the AsPOX activity values of two PGRs’ open field-grown and one polytunnel-grown samples, and in 2016, there were significant differences in the case of almost all PGRs’ polytunnel-grown samples.
Table 5. Lycopene content and enzyme activity (mean ± sd) of the seven Hungarian tomato PGRs investigated and the control variety, measured by five different assays, in the open field and in the polytunnel, in 2015 and 2016.

<table>
<thead>
<tr>
<th>PGR Code</th>
<th>Location</th>
<th>Lycopene (mg/100 g)</th>
<th>AsPOX (µg)</th>
<th>GST (µg)</th>
<th>POX (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: AsPOX: ascorbate peroxidase, GST: glutathione-S-transferase, POX: peroxidase, OF: open field, PT: polytunnel, B: “Balatonboglár”, C: “Cegled”, F: “Fadd”, MR: “Marispočo”, MT: “Mátraházas”, SA: “San Marzano”, TA: “Tarnaméra”, TO: “Tolna”. Data highlighted with grey are significantly higher in the comparison of locations within a year, data in bold are significantly higher in the comparison of years within a location. Lower- and upper-case superscript letters within columns denote homogenous subgroups of PGRs in the open field and in the polytunnel, respectively (Games–Howell, p < 0.05).
For the GST enzyme, the differences were not as large; however, the enzyme activity values were also higher in 2016. The highest activity mean value was measured in 2015 for C grown in an open field, while in 2016, it was measured in MT, also grown in an open field. In 2015, there were significant differences in terms of location in the case of C, where the open-field sample showed higher enzyme activity, while in the case of B and F, the polytunnel-grown samples showed higher values. In 2016, half of the open field-grown samples showed significant differences in their GST enzyme activity values.

In general, the activity of POX enzymes was also higher in the 2016 samples, but in this year, there were larger differences between the samples grown in the open field and in the polytunnel. In 2015, the highest mean activity value was observed in the open field-grown MR, while in 2016 it was observed in the polytunnel-grown F sample. There were significant differences among the POX activity values of every polytunnel-grown sample in 2016; however, in 2015, only the polytunnel-grown sample of B, and the open field-grown sample of TO, showed significant difference compared to the sample from the other location.

3.4. GGE Analysis of the Tomato PGR Phytonutrient Dataset

In this study, four environments were identified by the interaction of two years and two locations. This acts as a valuable foundation for the which-won-where assessment of the investigated PGRs from the point of view of their bioactive compounds. The application of the GGE model enables the evaluation of all measured parameters in an aggregated manner, and allows the formulation of recommendations regarding the optimal production environment of the tomato PGRs investigated.

The analysis of variance revealed significant effects of the environment, genotype and genotype × environment on the investigated parameters, which justifies the existence of genotype × environment interaction (GEI).

A GGE biplot was created based on the phytonutrient dataset of the investigated PGRs in different years and locations, i.e., environments (Figure 2). The first two principal components explain 60.72% of the total variance of the GGE model (PC1 = 40.23% and PC2 = 20.49%). The mean performance of the PGRs is demonstrated along PC1; the GEI is defined by PC2, which accounts for the variability in the different measured components of a given sample. Lower variability is referred to as stability. The datasets of the two years deviate with minimal overlapping, which might be explained by the differences in weather conditions. As the investigated phytonutrient traits—especially those with antioxidant properties—are highly influenced by weather conditions, the comparison between years is rather theoretical; the analysis of the datasets by year can provide more realistic information about the ideal production environments of the investigated PGRs. The cluster of the second year shows higher stability, i.e., lower distance from the average environment coordination (AEC). When location is investigated within year, no sub-clusters can be highlighted in the first year. Rather, the data points representing locations of the same PGRs are relatively close to each other, with the exception of B. In contrast, the dataset of the second year mostly deviates by the average axis along the AEC; except for B, C, F and MR, PGRs produced under the polytunnel showed higher values in all the parameters measured.

In the first year, the most determinant factors were TPC, CUPRAC, ABTS and FRAP, which might refer to the relatively higher heat stress of that year. The low proximity of antioxidant assays to each other also refers to their interactions, which supports the validity of the outcomes. In the second year, enzyme activity assays and sugar-acid ratio seem to influence the position of PGRs in the GGE biplot. Due to the distant position of TSS, acid content might be more determinant here. It is also worthwhile to mention that lycopene is closer to the data cluster of the second year, mainly characterized by lower average temperature. Within this cluster, lycopene shows more relevance towards those data points, which represent the antioxidant capacity of the PGRs produced in the polytunnel.
The phytonutrient dataset of the investigated PGRs in 2015 is visible in Figure 3. The total variance of the GGE model is explained by the first two PCs in 48.3 and 17.43%, respectively (total of 65.76%). Here, the arrowed line is referred to as average tester coordinate (ATC), which crosses the origin of the biplot [38].

The phytonutrient value of PGRs is evaluated using this line; proximity to the arrow sign indicates higher results. Intersection with the other axis is referred to as the average value of the whole dataset. Distance from the ATC represents the stability of a given PGR in terms of measured parameters; longer vectors characterize PGRs with lower stability. The position of the arrow on the ATC defines the parameters of an “ideal sample”, concentric rings demonstrate the relation of PGRs towards this point.

When PGRs are evaluated in different environments (year × location) along the AEC, most of their data points are positioned consequently, i.e., under or over average in both years. F and MT are exceptions: their open-field data point in the second year is far below average. TO is a counterexample for this: here, all data points are under average, except the polytunnel point. In the case of TA, the data pairs are above average in 2015 and slightly under average in 2016.

The phytonutrient dataset of the investigated PGRs in 2015 is visible in Figure 3. The total variance of the GGE model is explained by the first two PCs in 48.3 and 17.43%, respectively (total of 65.76%). Here, the arrowed line is referred to as average tester coordinate (ATC), which crosses the origin of the biplot [38].

The phytonutrient value of PGRs is evaluated using this line; proximity to the arrow sign indicates higher results. Intersection with the other axis is referred to as the average value of the whole dataset. Distance from the ATC represents the stability of a given PGR in terms of measured parameters; longer vectors characterize PGRs with lower stability. The position of the arrow on the ATC defines the parameters of an “ideal sample”, concentric rings demonstrate the relation of PGRs towards this point.


When PGRs are evaluated in different environments (year × location) along the AEC, most of their data points are positioned consequently, i.e., under or over average in both years. F and MT are exceptions: their open-field data point in the second year is far below average. TO is a counterexample for this: here, all data points are under average, except the polytunnel point. In the case of TA, the data pairs are above average in 2015 and slightly under average in 2016.

The phytonutrient dataset of the investigated PGRs in 2015 is visible in Figure 3. The total variance of the GGE model is explained by the first two PCs in 48.3 and 17.43%, respectively (total of 65.76%). Here, the arrowed line is referred to as average tester coordinate (ATC), which crosses the origin of the biplot [38].

The phytonutrient value of PGRs is evaluated using this line; proximity to the arrow sign indicates higher results. Intersection with the other axis is referred to as the average value of the whole dataset. Distance from the ATC represents the stability of a given PGR in terms of measured parameters; longer vectors characterize PGRs with lower stability. The position of the arrow on the ATC defines the parameters of an “ideal sample”, concentric rings demonstrate the relation of PGRs towards this point.
The ATC divides the dataset: open-field data are over, while the polytunnel data are under it. The first two PCs explain 57.68% of the total variance of the GGE model (PC1 is responsible for 35.05%, while PC2 for 22.63%). Similar to the first year, both location data are relatively farther from the ideal sample, i.e., are in the same concentric ring, but they are relatively far from each other. The proximity of the datasets relevant to the locations, although not without overlapping each other (Figure 4); the ATC divides the dataset: open-field data point of TA, SA, as well as both data points of F. The worst performing PGRs are TA and MT in polytunnel in this year. The second year shows some divergence of the phytonutrient dataset of the investigated tomato PGRs in 2015. Red oval circles divide datapoints of locations. Legend: green data points, first character: 1: 2015, 2: 2016; second character: 1: open field, 2: polytunnel, B: “Balatonboglár”, C: “Cegléd”, F: “Fadd”, MR: “Máriapócs”, MT: “Mátrafüred”, SA: “San Marzano”, TA: “Tarnaméra”, TO: “Tolna”, TSS: total soluble solids, TAC: titratable acid content, SA_or ratio: relative sugar-acid ratio, C*: chroma value, h*: hue value, TPC: total polyphenolic content, FRAP: ferric reducing antioxidant power, DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC: cupric ion reducing antioxidant capacity, AsPOX: ascorbate peroxidase, GST: glutathione-S-transferase, POX: peroxidase.

When the dataset is split by year and presented along the ATC, it is visible that sub-clusters of locations cannot be defined without overlapping. It seems that location has less impact on the bioactive compounds and other measured parameters of PGRs than the genotype itself. Data points of the same PGR from different locations are more or less close to each other; this proximity might refer to a relative independence from environment. The shortest distance is shown in the case of TO and MR, while the highest is in the case of C, TA, B and MT, which means that their data points from different locations show one ring distance.

When the absolute distance from the ideal sample is evaluated, MR performs the best among the investigated PGRs. Its data points for both environments are within the first concentric circle. MR is followed by TA in the open field in the third ring. The fourth ring contains the open-field data point of TA, SA, as well as both data points of F. The worst performing PGRs were B and C grown in the open field in this year.

In comparison with the first year’s GGE, the second year shows some divergence of the datasets relevant to the locations, although not without overlapping each other (Figure 4); the ATC divides the dataset: open-field data are over, while the polytunnel data are under it. The first two PCs explain 57.68% of the total variance of the GGE model (PC1 is responsible for 35.05%, while PC2 for 22.63%). Similar to the first year, both location data points of the individual PGRs are mostly in the same distance from the ideal sample, i.e., are in the same concentric ring, but they are relatively farther from each other. The proximate are the data points of MR, followed by C and TO. The highest distances are...
visible in the case of F and MT data points. The two data points of SA show a three-ring distance, while in the case of others it does not exceed one ring.


In this year, PGR data points are relatively farther from the ideal sample; however, MR again scored as best in both locations, followed by the open-field data point of SA in the third concentric ring. Most of the PGR × location data points are in the fifth and sixth circle, beyond the average axis on the ATC. The worst performing PGRs are TA and MT in polytunnels and B in both locations.

4. Discussion

The climatic conditions of the two experimental years showed differences both in the open field and in the polytunnel. The phytonutrient dataset of the investigated PGRs, visualized in a GGE biplot, shows minimal overlapping in terms of years, which predicts that traits with high environmental influence cannot be compared in terms of years. The summer of 2015 was warm and dry, while in 2016 there was a humid, temperate vegetative period. The temperature in the polytunnel was significantly higher, which impacted the investigated fruit quality and antioxidant parameters.

4.1. Assessment of PGR Phytonutrient Status

The TSS of investigated PGRs and the variety were higher in polytunnel production in both years. These results agree with Healy et al. [9], who found that tomatoes grown in a hoop house had higher total soluble sugars than those grown in an adjacent field.
Total acid content results were generally higher in open-field production, which is in agreement with the results of Asensio et al. [23]. This was more pronounced in 2016, and for some PGRs, seasonal variations were observed, which supports the results of Fibiani et al. [64], which showed that acid content remarkably depends on years.

Color is the most important fruit quality factor for consumers. For this trait, no significant tendencies were observed between open-field and plastic-house production. With the exception of polytunnel data of TA, the C* values were significantly higher in 2016 for all PGRs and locations. As the color of a tomato is mainly determined by lycopene content [65], the synthesis of which is temperature-dependent [66], it seems that the lower average temperature of the second year was more favorable for this carotenoid.

The hue angle values provide information about the fruit color of the PGRs; with the exception of C, hue values were over 60°, which can be interpreted as red color [6]. Comparing with the results of Rodriguez-Burruezo et al. [6], all investigated PGRs had a high chroma value connected to hue angle value, indicating highly saturated fruit color.

It is widely known that the composition of bioactive compounds in vegetables is varied, and the content can be affected by the environmental and nutritional conditions of crops [9,23,24,26]. Even genotypically identical plant species can differ in phenolic content in terms of both qualitative and quantitative traits [18]. This is further supported by the outcomes of the present study: in the examination of the same variety of PGR for total polyphenolic content and for other parameters, values differed among locations and years.

Asensio et al. [23] showed that the concentrations of total phenolic content, lycopene, β-carotene, ascorbic acid and vitamin C were higher in tomatoes grown in an open field versus a polytunnel. Stewart et al. [67] found that polytunnel tomato samples accumulated less polyphenols. This is only partly in agreement with the results of the present study, although results are not totally consistent in some cases. In the hot summer of the first year, the total phenolic content and FRAP values were higher in polytunnel production, while in the next humid, temperate year, the open-field values were higher. Unfavorable abiotic environmental conditions supplemented with pathogen stress enhance the synthesis of phenolic compounds [68–71]; such harsh environments are likely to occur in the open field. Cano et al. [72] found that growing conditions have the highest influence on TPC, but the genotypic characteristics also count, which might explain the conflicting results of this study. Additionally, plant nutrition management also has an impact on polyphenol levels [73–75], which might be different in the open field and in protected cultivation due to water management characteristics.

Although it is irrelevant to compare the antioxidant status of the samples between years, due to the high influence of the environment, it is visible that, with the exception of DPPH, all assays showed more favorable results in 2015 for all plant materials and both locations; results deviated significantly in all cases by year. Different antioxidant assays have selective measuring preferences [76], therefore assessing by a single assay can be misleading; instead, parallel application of multiple antioxidant assays is suggested for the sufficient characterization of antioxidant status [77]. The aggregative assessment is well supported by the application of the GGE model; TPC, FRAP, ABTS and CUPRAC data points are all very close to each other, characterizing the dataset of the first year, while DPPH is located outside the datasets of both years, which might be explained by its different measuring mechanism, using an artificial radical.

In the case of lycopene content, the results were inconsistent between the two years. The accumulation of carotenoids, like that of lycopene, is reported to be influenced by abiotic stress, such as high or low temperature. It is widely known that lycopene synthesis is inhibited over 32 °C [66,78]; therefore, in the present experimental design, the polytunnel samples of 2015 were expected to produce the lowest values. However, this was justified only by two PGRs. Similar results were found also by Scarano et al. [79], with the conclusion that different genetic backgrounds of the tested landraces might affect the biosynthesis of carotenoids.
In terms of the measured AsPOX, GST and POX enzymes, almost all PGRs had higher activities in 2016, which may be due to the stress caused by the generally lower average temperature and higher humidity. All PGRs tended to have lower enzyme activity values in the crops grown in the open field; however, in the case of San Marzano, the enzyme activity values were lower in the case of the polytunnel-grown crops. The enzyme activities of MR were not affected by cultivation locations. However, it is important to note that the AsPOX and POX enzyme activity values were significantly higher in the case of almost all polytunnel-grown PGRs harvested in 2016, suggesting that the aforementioned environmental factors caused more stress to those crops, or the two enzymes are more sensitive to these in the examined cultivars. The latter can be supported by a study published in 2021, in which the POX activity of different varieties grown in a net house and in the open field was tested. For some varieties, the open-field crops had higher enzyme activity values, while for other varieties, the cultivation method had no effect on this parameter [3].

4.2. Identifying Ideal Environments for PGRs

Genotype plus genotype x environment analysis was successfully applied for determining ideal environments for selecting the best tomato varieties, from the perspective of their bioactive compounds [44]. Zhang et al. [42] applied a GGE biplot to assess the stability of proso millet yield and to evaluate the representativeness of domestic locations. Koundinya et al. [43] analyzed 40 eggplant genotypes in two environments, based on yield and phytochemical traits. The antioxidant status of 49 underutilized tomato PGRs was investigated using a GGE biplot; Adalid et al. [40] identified several PGRs with outstanding nutritional properties. These findings are in agreement with the results of the present study and demonstrate the applicability of the GGE biplot model for PGR screening.

In general, the year with lower precipitation can be characterized by higher antioxidant capacity, while the warmer and more humid year resulted in higher acid and lower total soluble solids content. However, the existence of different environments enables identifying specialists with outstanding phytonutrient properties.

The dataset reported also provides detailed information about the phytonutrient values of the investigated PGRs, which can be utilized for recommending ideal production technology for these Hungarian tomato landraces. Although the results for all investigated traits shows some inconsistencies among PGRs and locations over the years, general conclusions can be drawn based on the dataset. The TSS, TPC and lycopene values of Balatonboglár were higher under protected cultivation in both years. Additionally, all antioxidant assays showed significantly higher results for the sample from covered cultivation in the first year; the results for the second year were rather diverse. This is supported by the GGE results as well, which showed that the best year × location environment was the polytunnel in 2015, in terms of phytonutrient value. The data point representing this G × E is the closest to the “ideal variety” among the environments for Balatonboglár PGR.

Mátrafüred showed exactly the same pattern of B in terms of the measured parameters: TSS was significantly higher, while TPC and lycopene were insignificantly higher both years in the polytunnel. The majority of the antioxidant assays employed show higher results in covered cultivation. This is supported by the GGE biplot, where the data points representing the polytunnel results of the years is ranked before the open-field points in the AEC, suggesting, that in case of this PGR, location has higher impact on the investigated parameters than the season itself. As both polytunnel data points are in the fifth circle, the distance from the “ideal variety” is very similar.

Somewhat similar results were observed for Fadd, where TSS and TPC in both years, TAC and all antioxidant assays provided higher results under polytunnel cultivation in 2015; FRAP, DPPH and ABTS values deviated significantly between locations. Lycopene values were inconsistent: the first year was more favorable in the open field, while the second year significantly elevated the lycopene content in the polytunnel. The GGE biplot revealed that the best environment was the protected cultivation of the first year for the
phytonutrient values of Fadd, closely followed by the open field cultivation of the same year. However, due to its higher stability, the data point representing the first year and open-field cultivation is the closest to the “ideal variety”. In summary, the location of the first year had little impact on the investigated parameters of Fadd, while it was determinative in the second year.

Tolna generally performs better in polytunnel; in both years, the TSS, and in the second year, the TAC, of this PGR was significantly higher in this location. Regarding antioxidant assays, TPC and DPPH values were significantly higher in the case of polytunnel samples in the first year, while those of the FRAP and CUPRAC were insignificantly higher. The results of the second year were rather inconsistent. The GGE identified the polytunnel location of the first year as the most favorable environment for this PGR. This might be due to the fact that, with the exception of CUPRAC, all antioxidant assays measured higher results in the case of open-field samples in the second year, but the differences were insignificant in every case. The stability of the first-year, open-field and polytunnel data was indeed higher, which resulted a higher proximity to the “ideal variety”. It is also visible that the effect of the location was not drastic in the first year, but it was more pronounced in the second year. Based on TSS or lycopene, Tarnaméra performed equally on both locations; however, the results of assays show that the antioxidant capacity and activity of this PGR is higher in the open field, with relative independence from seasonal variations. The GGE analysis indicated the open-field cultivation in the first year as the best environment. Location seems to have intermediate impact on the investigated parameters, as the proximity of data points is one and two concentric circles in the first and in the second year, respectively.

Regarding Máriapócs PGR and San Marzano varieties, values are varying in open-field and in polytunnel production both years. The GGE analysis did not reveal high differences between the data points, indicating that location has less influence on the measured compounds and parameters than seasonality. These cultivars yield fruits with high amounts of bioactive compounds in both systems. Máriapócs data points were the closest to the “ideal variety” in both years, which reveals its valuable genetic background as a generalist among locally adapted landraces.

5. Conclusions

The selected phytonutrient parameters of seven indeterminate-type Hungarian tomato PGRs investigated in the open field and in polytunnels in an organic production system for two years revealed that the seasonality of years has a higher impact on the investigated parameters than the location of the production. GGE analysis identified the ideal environments of the investigated PGRs in terms of bioactive and fruit quality parameters; in the cases of “Balatonboglár”, “Mátrafüred” and “Fadd”, phytonutrient content is expected to be higher when grown in protected cultivation, while “Tarnaméra” is suggested to be cultivated in the open field. The analysis revealed that the influence of location is lower than that of the environmental conditions in different years in the cases of “Máriapócs” PGR and the “San Marzano” variety. These findings are directly applicable in tomato PGR cultivation practice and they support and provide a baseline for the detailed genetic analysis of promising PGRs, with the aim of utilizing favorable genetic characteristics in future tomato breeding.


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