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Investigation of the morpho-biochemical composition of fruits of new Armenian tomato breeding lines

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ABSTRACT

Background: Tomato breeding plays a crucial role in meeting the demands of the healthy food market and ensuring food security. Modern agricultural approaches focus on improving the qualitative and functional characteristics of fruits. Additionally, the development of new genotypes adapted to local growing conditions and enriched with biologically active compounds is a key priority in agricultural science. Such plants serve as valuable genetic material for the creation of domestic hybrids, reducing dependence on imported varieties.

Objective: To evaluate the morphological and biochemical characteristics of new Armenian tomato breeding lines – R-Bf-04, R-St-13, R-Zd-02, R-Gr-12, and R-At-23 – under protected cultivation conditions, with the aim of identifying promising genotypes for inclusion in hybridization breeding programs.

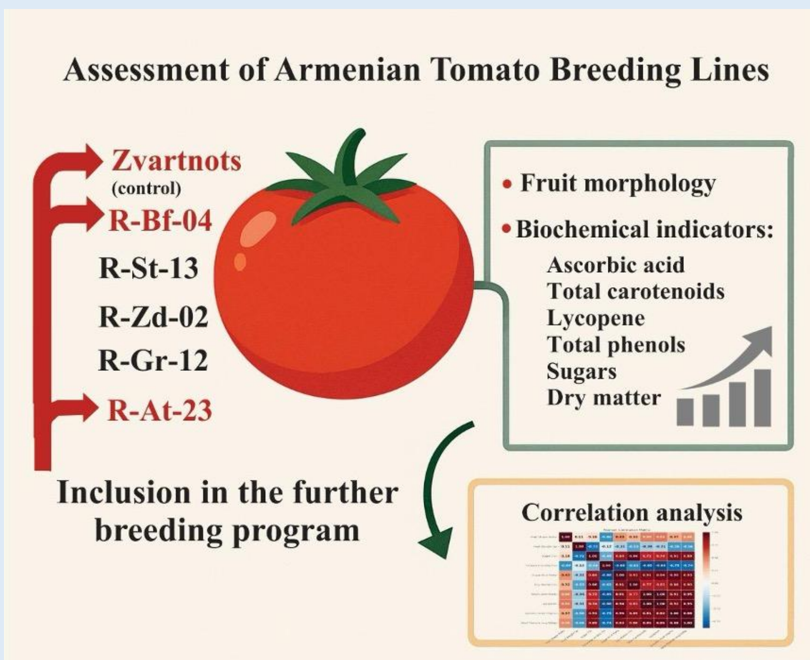
Methods: The research was conducted from 2022–2025 under winter-spring vegetation conditions in a greenhouse. The morphological traits of the breeding lines were assessed in accordance with international descriptors. Biochemical parameters of the fruits, including total sugar, phenolic compound, ascorbic acid, carotenoid, and lycopene content, were determined using spectrophotometric methods. Statistical data analysis was performed using analysis of variance (ANOVA) at a significance level of $p \leq 0.05$, as well as Pearson correlation analysis.

Results: The morphological and biochemical evaluation of the tomato breeding lines revealed a wide variability in the studied traits. Fruit weight ranged from 182.3-255.2g, reflecting differences in morphological type and yield potential. Ascorbic acid and total phenolic content varied from 19.64-22.76mg/% and from 32.4-48.4mg GAE/100g, respectively, indicating diversity in antioxidant activity among the lines. Total carotenoids ranged from 2.56-4.82mg/100g, while lycopene content ranged from 2.36-4.44mg/100g, with lycopene accounting for up to 92% of the total carotenoids. Significant differences were observed in organoleptic parameters, including sugar content, titratable acidity, and sugar–acid ratio, all of which influence fruit flavor quality. Correlation analysis revealed strong positive relationships among sugar content, dry matter, carotenoids, phenolic compounds, and ascorbic acid (vitamin C). The most balanced quality parameters were demonstrated by the R-Bf-04 and R-At-23 lines, along with the control variety, Zvartnots, underscoring their high value for further breeding aimed at improving fruit quality.

Novelty: For the first time, a comprehensive morphological and biochemical characterization of new Armenian tomato breeding lines grown under protected cultivation was conducted. The obtained data reveal a wide range of genetically determined variability in key fruit quality traits. Several lines were identified as having significant potential for inclusion in breeding programs aimed at improving organoleptic properties and enhancing the content of biologically active compounds.

Conclusion: The conducted evaluation revealed a significant genetic potential of the tomato breeding lines, each exhibiting a unique combination of valuable traits that contribute to improved fruit quality. These lines may serve as promising donors in hybridization programs aimed at developing tomatoes with enhanced functional value and the ability to successfully compete with imported hybrids.

Keywords: tomato, breeding lines, morphological traits, biochemical composition, ascorbic acid, phenolic compounds, carotenoids, lycopene, organoleptic qualities, sugars



Graphical Abstracts: Investigation of the morpho-biochemical composition of fruits of new Armenian tomato breeding lines.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the leading crops in global agriculture, ranking first in cultivation area and production volume. Due to its high nutritional value and versatile use, tomatoes are in high demand both in fresh form and as processed products. According to FAO data (2022), global tomato production reached 189.1 million tons in 2021, representing a 2% increase compared to 2020 (184.8 million tons) and a 4% increase relative to 2019 levels. The largest producers are China, India, Turkey, the United States, and Egypt [1].

Tomatoes remain the most consumed vegetable worldwide, with an average per capita consumption of 18.18kg per year [2]. Turkey is in first place, with 98.62kg of tomatoes consumed per capita recorded in 2012–2013, followed by Egypt (90.06kg), Greece (85.78kg), Armenia (84.12kg), and Tunisia (83.91kg). In the United States, per capita consumption stands at 37.74kg, while the average European consumption is 22.58kg. In Mediterranean regions, the average can reach up to 44.50kg [2].

Armenian tomato production has shown a steadily increasing trend. This growth is supported by favorable climatic conditions, the development of greenhouse farming, the adoption of modern agrotechnologies, and the diversification of varieties and hybrids. The main cultivation areas are concentrated in the Armavir and Ararat regions. According to preliminary estimates, in 2023, tomato yields in the country reached record levels, enabling Armenia to rank 28th among 151 tomato-exporting countries worldwide [3].

This progress has also been driven by the implementation of the Government Strategy for Agricultural Development of the Republic of Armenia for 2020–2030, which focuses on improving the efficiency of agricultural production, enhancing infrastructure, and promoting innovative approaches [4].

Despite the positive production trends, the range of Armenian tomato varieties and hybrids, particularly for protected cultivation, remains limited. Greenhouse production is dominated by imported hybrids – primarily of Dutch origin [5] – which are not always optimally adapted to local soil and climatic conditions. This situation reduces plant resilience to local stress factors and increases growers' dependence on external seed markets. Therefore, a pressing challenge is the development of competitive domestic tomato breeding lines and hybrids that are well adapted to Armenian growing conditions and meet current quality standards.

In recent years, tomato breeding has increasingly focused not only on improving agronomic traits but on enhancing the nutritional and functional value of fruits [5]. Recent advances in the field of functional nutrition highlight its significant role in disease prevention and health promotion, as demonstrated in numerous scientific studies [6-8]. Functional nutrition involves the use of products enriched with biologically active compounds that, when consumed regularly, can have a beneficial impact on human health [9-13]. At the same time, modern consumers are paying more attention to product quality, including taste, structural characteristics, nutritional content, and antioxidant activity [14–18].

Genotypes combining high levels of biologically active compounds with adaptability to local growing conditions are especially important in breeding efforts. The study of such breeding lines enables the development of valuable genetic material for creating hybrids tailored to the demands of the modern agro-industrial complex and the functional food market.

The objective of the present study is to evaluate the biochemical parameters of new Armenian tomato breeding lines to identify genotypes with high nutritional value for their subsequent inclusion in the breeding program.

MATERIAL AND METHODS

Research material: The study was conducted at the SCVIC MEofRA, located in the village of Darakert, Ararat region, Armenia (40.115018° N, 44.417768° E), in a glazed greenhouse during the winter-spring growing cycles from 2022 to 2025. The research objects included the Zvartnots variety as a control, and the breeding lines R-Bf-04, R-St-13, R-Zd-02, R-Gr-12, and R-At-23, all of which were developed at the Scientific Center.

Greenhouse conditions: The temperature regime in the greenhouse was maintained at 25–28°C during the day and 16–20°C at night. The relative humidity ranged from 75% to 85%. Forty-five-day old seedlings were transplanted into the greenhouse soil following a spacing scheme of (90+60) × 50 cm. The plant nutrition system was developed based on agrochemical soil analysis, accounting for the crop's growth stages and physiological needs. Agrochemical analyses were performed using a colorimetric method with the Gallery Aqua Master Discrete Analyzer (Thermo Fisher Scientific) in accordance with ISO 14001:2015 standard.

Integrated pest and disease management methods were applied, including preventive treatments with biopreparations.

The experiment was established using a randomized block design with three replications.

Morphological evaluation of tomato fruits: Morphological evaluation was performed according to the international standard "Descriptors for Tomato (*Lycopersicon* spp.)" [19]. Fruit shape index was calculated as the ratio of longitudinal to transverse diameter, measured using a caliper with 0.1 mm accuracy [20].

Determination of ascorbic acid: Biochemical analyses were carried out at the Laboratory of Plant Biotechnology, Phytopathology, and Biochemistry of the SCVIC. Ascorbic acid content was measured using a spectrophotometric method with a Carry 60 UV-Vis spectrophotometer (Agilent Technologies, USA). The analysis followed a standard protocol involving 2,4-dinitrophenylhydrazine and measuring absorbance at a wavelength of 520nm. Calibration curves were constructed using L-ascorbic acid solutions [5].

Determination of total phenol: The total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu reagent, measuring absorbance at a wavelength of 765nm. Calibration solutions were prepared with gallic acid (GA), and the results were expressed as mg GAE/100g FW [21].

Determination of total carotenoids and lycopene: Pigments were extracted using a hexane/ethanol mixture (3:1, v/v) at a ratio of 10 mL per 1g of homogenized sample. Extraction was carried out at room temperature for 30 minutes. After centrifugation, the upper phase containing the pigments was collected. Total carotenoids were measured spectrophotometrically at 450nm and expressed as mg of β-carotene equivalents per 100g fresh weight (FW) [22]. Lycopene content was determined by measuring absorbance at 470nm and calculating concentrations using an extinction coefficient $\varepsilon = 3{,}450\ (\text{mg/mL})^{-1}\cdot\text{cm}^{-1}$ Results were expressed as mg of lycopene per 100g FW [22].

Determination of total sugar content: Total sugar content was determined using spectrophotometry by measuring the optical density at 490nm. Calibration solutions were prepared based on L-glucose [23].

Titratable acidity (TA): TA was determined by acid-base titration using a 0.1N NaOH solution and phenolphthalein as an indicator. The endpoint of the reaction was identified by the appearance of a stable pink coloration. Results were expressed as a percentage of citric acid equivalents, applying a conversion factor of 0.0064 [23].

Sugar/Acid ratio: Calculated as the ratio of total sugar content to titratable acidity, the sugar/acid ratio provided an assessment of the fruit's taste quality.

Determination of dry matter content: Dry matter content was measured using a refractometer.

Correlation Analysis: To identify relationships among the studied parameters, Pearson correlation analysis was performed using Python software. The results were visualized as a heatmap [24].

Statistical Data Analysis: Analysis of variance (ANOVA) was applied for statistical processing of the data at a significance level of $p \leq 0.05$. Values are presented as means ± standard deviation (±SD).

RESULTS AND DISCUSSION

Morphological characteristics of fruits: Analysis of the tomato breeding lines revealed that all samples produced fruits with round or slightly flattened-round shapes, with a shape index ranging from 0.95 to 1.03. All genotypes exhibited bright red or deep red fruit coloration. The number of seed locules varied from 4 to 7. Fruit weight ranged from 182.3 to 255.2 g (Table 1).

Table 1. Morphological characteristics of fruits of the studied tomato lines

N	Breeding line	Shape	Color	Number of locules	Fruit shape index	Weight, g ±SD
1	Zvartnots (Control)	Round	Red	4–6	0.98	184.5 ± 3.8
2	R-Bf-04	Round, slightly flattened	Intense red	5–6	1.01	248.2 ± 5.1
3	R-St-13	Round	Red	4–5	0.97	211.4 ± 4.7
4	R-Zd-02	Round	Bright red	6–7	0.95	215.3 ± 5.0
5	R-Gr-12	Round, flat	Bright red	6–7	1.01	255.2 ± 5.6
6	R-At-23	Round, flat	Bright red	5–6	1.03	182.3 ± 4.0

Ascorbic acid and total phenolic compounds: The analysis of ascorbic acid and total phenolic content in tomato breeding lines revealed significant genotypic differences (Table 2). The highest ascorbic acid level was found in the control variety Zvartnots (22.76 mg/%), closely followed by R-At-23 (21.86 mg/%) and R-Bf-04 (21.62 mg/%). Moderate values were observed in R-St-13 (20.03 mg/%), and lower levels in R-Zd-02 (19.75 mg/%) and R-Gr-12 (19.64 mg/%).

A similar pattern was observed for total phenolic compounds. Zvartnots (48.4 mg GAE/100g), R-At-23 (47.6 mg GAE/100g), and R-Bf-04 (45.3 mg GAE/100g) exhibited the highest levels. R-St-13 contained 36.7 mg GAE/100g, while R-Zd-02 and R-Gr-12 showed lower concentrations of 33.2 and 32.4 mg GAE/100g, respectively.

Table 2. Ascorbic acid and total phenolic content in the fruits of tomato breeding lines ($p \leq 0.05$)

N	Breeding line	Ascorbic acid, mg/% ±SD (FW)	Total phenols, mg GAE/100g (FW) ±SD
1	Zvartnots (Control)	22.76 ± 0.10	48.4 ± 1.04
2	R-Bf-04	21.62 ± 0.12	45.3 ± 1.05
3	R-St-13	20.03 ± 0.10	36.7 ± 0.89
4	R-Zd-02	19.75 ± 0.13	33.2 ± 0.94
5	R-Gr-12	19.64 ± 0.09	32.4 ±.1.02
6	R-At-23	21.86 ± 0.15	47.6 ± 0.87

Overall, the highest total antioxidant activity was observed in lines R-At-23 and R-Bf-04, as well as in the control variety, Zvartnots, highlighting their potential value for breeding programs focused on improving nutritional quality.

According to literature, the accumulation of biologically active compounds such as ascorbic acid (vitamin C) and phenolics is influenced by genotype and agroecological factors, including season, light intensity, and cultivation practices [25]. Reported vitamin C levels in fresh tomatoes typically range from 14 to 27 mg/100g [26], while total phenolics vary between 20 and 50 mg/100g [27]. Notably, the highest concentrations of phenolics are located in the fruit skin [28]. Significantly lower amounts are found in the pulp and seeds, emphasizing the role of anatomical structures in determining antioxidant potential [28].

Our findings are consistent with these data: under winter–spring conditions, ascorbic acid content ranged from 19.64 to 22.76 mg/% FW, and total phenolics from 32.4 to 48.4 mg/100 g FW. These results confirm considerable genetic variety and the impact of environmental conditions on antioxidant levels in tomato fruits.

Total carotenoids and lycopene: Carotenoids are key natural pigments responsible for tomato fruit coloration and possess strong antioxidant properties. Their content level depends on many factors, with genotype playing a decisive role [29]. Among carotenoids, lycopene is the dominant pigment tomatoes, contributing to their red color and light-absorbing properties. It is synthesized through a series of desaturation and isomerization steps, resulting in a structure with 11 conjugated double bonds [30].

In this study, total carotenoid and lycopene contents were analyzed in several tomato breeding lines (Figure 1).

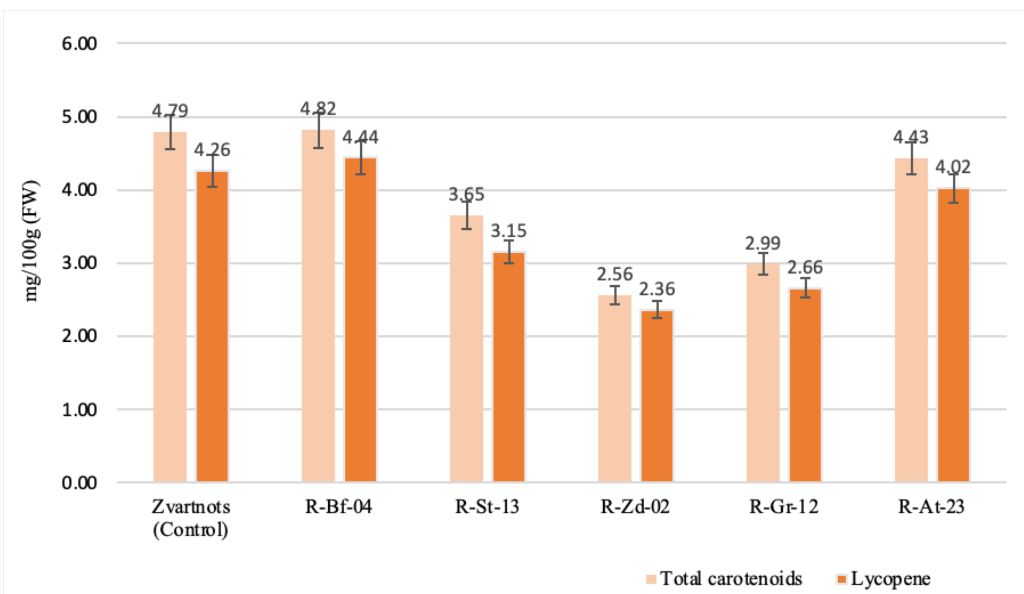

Figure 1. Quantitative content of total carotenoids and lycopene in the fruits of tomato breeding lines ($p \leq 0.05$).

The highest concentrations were found in R-Bf-04 (4.82 and 4.44 mg/100g, respectively), followed by the control variety Zvartnots (4.79 and 4.26 mg/100g) and R-At-23 (4.43 and 4.02 mg/100g), indicating their high potential as sources of bioactive pigments.

Moderate levels were recorded in R-St-13 (3.65 and 3.15 mg/100g), while lower values were found in R-Gr-12 (2.99 and 2.66 mg/100g) and R-Zd-02 (2.56 and 2.36 mg/100g).

In all samples, lycopene accounted for 86.3-92.2% of the total carotenoids, typical for fully ripe fruits. Of particular interest is R-Zd-02. This line showed the highest lycopene proportion (92.2%), despite lower overall carotenoid content, indicating a genotype-specific accumulation of trans-lycopene [18].

The spectral curves (Figure 2) obtained with the spectrophotometer clearly show characteristic absorption peaks at 445nm and 470nm, corresponding to the maximum light absorption of carotenoids. The 445nm peak was used to estimate total carotenoids, while the 470nm peak was employed for lycopene quantification. Variations in absorption intensity among samples reflect differences in pigment concentrations, supporting both the reliability of the biochemical measurements and the genetic basis of these traits.

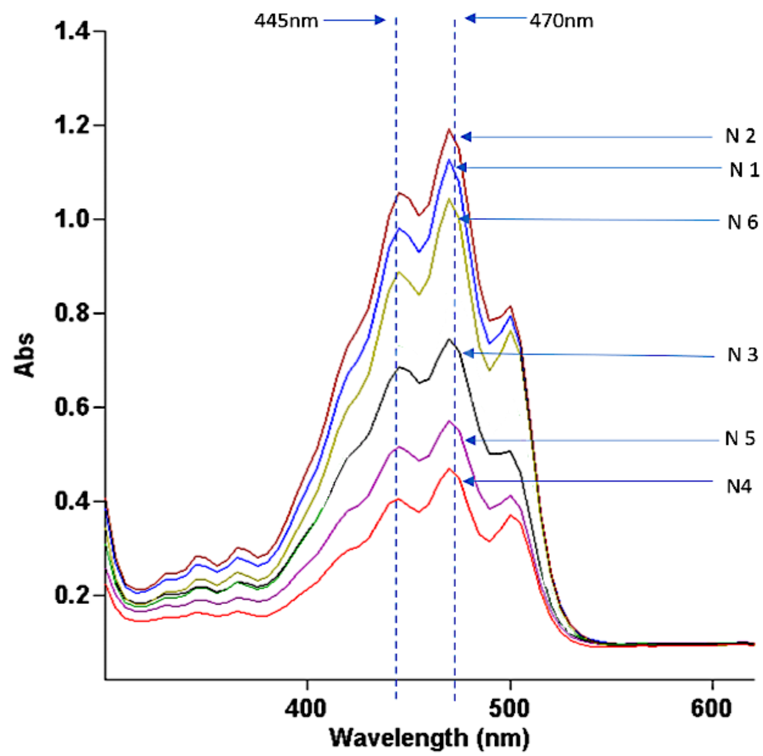

Figure 2. Absorption spectra of extracts at 445 and 470 nm: 1. Zvartnots (Control); 2. R-Bf-04; 3. R-St-13; 4. R-Zd-02; 5. R-Gr-12; 6. R-At-23

The results of the present study are comparable with those reported in the literature. Lycopene content in tomato fruits is largely determined by genetic factors and varies depending on the cultivar and growing conditions [31]. According to various authors, lycopene concentration can range from 4.9-12.7mg per 100g FW or from 3.5-6.9mg per 100g FW, depending on measurement conditions [32].

In the study by Ayuso-Yuste et al. (2022), it was established that traditional cultivars adapted to local conditions show a significant increase in lycopene levels during ripening, reaching up to 132.64 mg/kg fresh weight. At later stages of maturity, traditional cultivars contained more lycopene than commercial hybrids [33]. Furthermore, Kang et al. (2022) emphasized the crucial role of genotype in the ability of tomatoes to efficiently accumulate lycopene, which fully aligns with our findings [34].

Total sugars, titratable acidity, and sugar-acid balance: The organoleptic quality of tomatoes is largely determined by the balance between sugar and acid content, which shapes the fruit's flavor perception. According to the results obtained, the studied tomato breeding lines exhibited significant variability in these parameters. Sugar content among the breeding lines ranged from 2.74% to 3.34%. The highest value was recorded in the control variety, Zvartnots, at 3.41%. Among the breeding lines, the highest sugar levels were noted in R-At-23 and R-Bf-04, at 3.34% and 3.15%, respectively, highlighting their potential value from an organoleptic standpoint (Table 3).

The study showed that TA ranged from 0.31% to 0.41%, with relatively low values recorded in the control, R-Bf-04, and R-At-23 lines. A decrease in acidity combined with elevated sugar content resulted in high sugar-acid ratio values for these genotypes: 7.75, 7.68, and 7.11, respectively. These ratio values indicated fruits with a sweeter taste.

Table 3. Total sugar content, titratable acidity, and their ratio in the fruits of tomato breeding lines ($p \leq 0.05$)

N	Breeding line	Total sugars, % ±SD	Titratable acidity, % ±SD	Sugar/Acid Ratio
1	Zvartnots (Control)	3.41 ± 0.08	0.44 ± 0.01	7.75
2	R-Bf-04	3.15 ± 0.07	0.41 ± 0.01	7.68
3	R-St-13	2.86 ± 0.05	0.51 ± 0.02	5.61
4	R-Zd-02	3.05 ± 0.06	0.53 ± 0.02	5.75
5	R-Gr-12	2.74 ± 0.05	0.48 ± 0.01	5.71
6	R-At-23	3.34 ± 0.07	0.47 ± 0.01	7.11

Sugar and acid content are largely determined by the genetic background of the cultivar, which explains the differences in flavor profiles. Non-volatile compounds, such as sugars, acids, and soluble solids, are critical for evaluating flavor and are widely used as indicators of organoleptic quality [35].

All studied greenhouse-grown lines showed notable variation in flavor balance. Genotypes R-Bf-04 and R-At-23, along with the control, Zvartnots, had more favorable sugar–acid ratios, bringing them closer to dessert-type tomatoes. In contrast, R-St-13, R-Zd-02, and R-Gr-12 had lower ratios (5.61–5.75), resulting in a sourer taste.

Dry matter content: Dry matter is a key trait of table tomato cultivars, influencing fruit firmness, flavor, and market suitability.

Significant variation in dry matter content among the breeding lines in this study highlights the genetic basis of this trait. The highest dry matter levels were recorded in lines R-At-23 (6.12%), R-Bf-04 (6.03%), and the control (6.08%), indicating their potential for consumption in their fresh forms due to firmer fruit texture (Figure 3). Lower dry matter values were observed in R-Zd-02 (5.79%), R-St-13 (5.57%), and R-Gr-12 (5.50%), suggesting higher water content. These lines had a juicier texture, which could be favored by consumers seeking tender, juicy fruits.

Dry matter is a widely accepted indicator of both eating quality and technological suitability. According to the literature, dry matter in cultivated tomato varieties generally ranges from 3.72% to 8.88%, while wild types may reach 9.62–11.33% [36]. High dry matter content is typically considered a positive trait, as it is associated with increased levels of sugars, organic acids, and other soluble solids, all of which enhance organoleptic quality. Additionally, higher dry matter is linked to improved taste, processing efficiency, transportability, and shelf life.

In this study, tomato breeding lines adapted to greenhouse conditions showed dry matter content between 5.50% and 6.12%. It is important to note that environmental factors such as light intensity, humidity, and nutrient regimes significantly affect dry matter accumulation. Excessive irrigation and high nitrogen input can reduce dry matter levels [37], which should be considered in greenhouse management.

These findings support the selection of tomato breeding lines with optimal dry matter content for fresh-market greenhouse production.

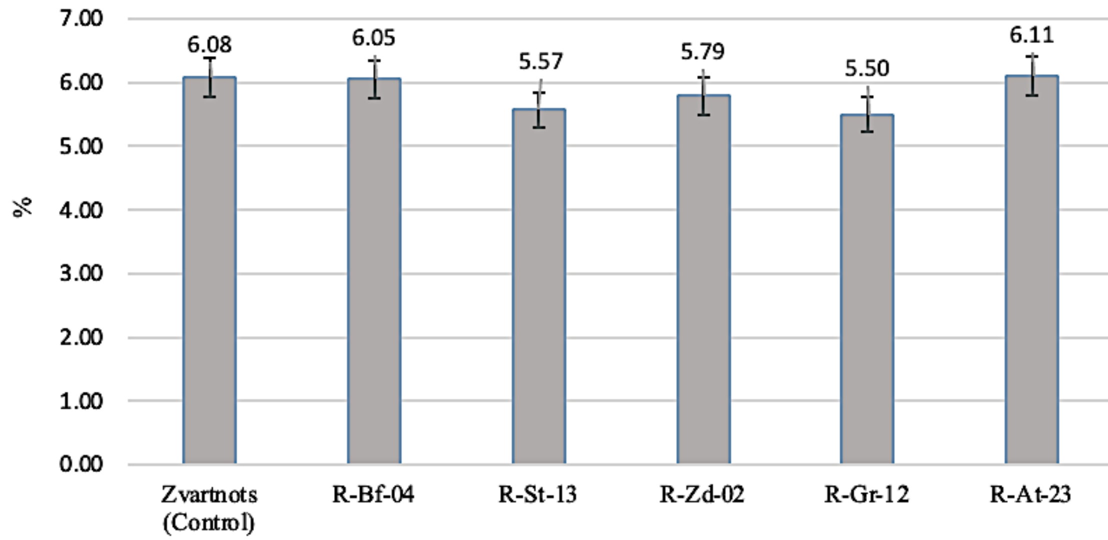

Figure 3. Dry matter content in the fruits of tomato breeding lines ($p \leq 0.05$)

Correlation analysis: To identify relationships between morphological and biochemical traits in tomato breeding lines, Pearson correlation analysis was performed (Figure 4). The analysis revealed statistically significant associations reflecting the complex nature of fruit quality.

A moderate negative correlation was found between fruit weight and dry matter content (r = −0.53), suggesting dilution of dry matter in larger fruits. Weak negative correlations were also observed between fruit weight and lycopene (r = −0.31) and sugar content (r = −0.12), indicating that larger fruit size does not necessarily improve taste or nutritional quality.

Conversely, a strong positive correlation existed between sugar content and sugar/acid ratio (r = 0.84), as sugars largely determine fruit flavor balance. An extremely high correlation between sugar content and dry matter (r = 0.96) confirms dry matter as a reliable indirect indicator of taste maturity, consistent with literature [38].

Dry matter content also correlated strongly with carotenoids (r = 0.72) and lycopene (r = 0.74), reflecting pigment accumulation during fruit ripening. Notably, total carotenoids and lycopene showed perfect correlation (r = 1.00), confirming lycopene as the dominant carotenoid in tomatoes [34].

Strong positive associations were also found between dry matter and phenolic compounds (r = 0.90) and ascorbic acid (r = 0.89), highlighting their role as antioxidant indicators. According to Kang et al. (2022), genotypes with high sugar and dry matter generally have elevated phenolics (r = 0.89) and vitamin C (r = 0.91) [34], supporting the universality of these relationships for tomato quality assessment.

Particular attention should be given to the near-perfect correlation between ascorbic acid and phenolics (r = 0.98) and the strong association between lycopene and vitamin C (r = 0.92), possibly indicating a synergistic accumulation in genotypes with high antioxidant potential.

Thus, biochemical traits in tomato fruits are closely interrelated and largely genetically determined.

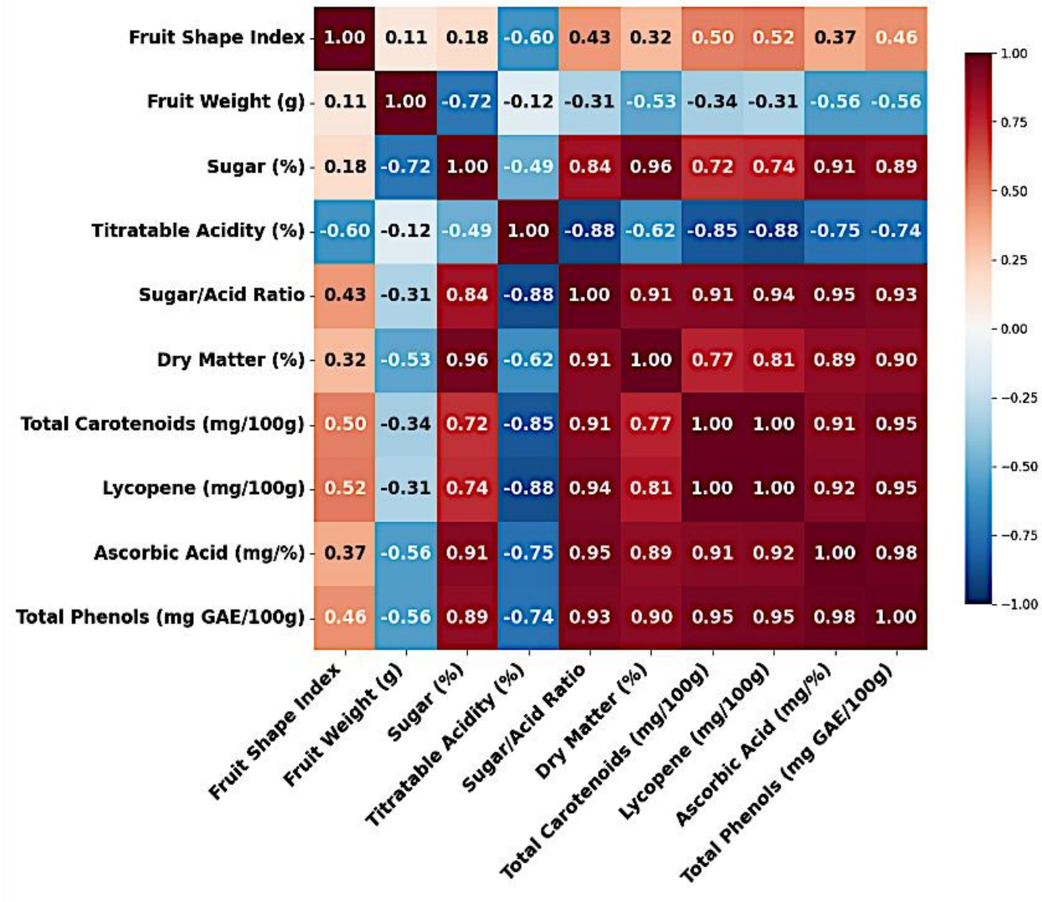

Figure 4. Heatmap of Pearson correlation coefficients for the tomato breeding lines

CONCLUSION

The results of this study demonstrate substantial variability among newly developed Armenian tomato breeding lines in terms of morphological and biochemical traits. This research highlights the distinctive characteristics of region-specific genetic material, which have not been previously described in scientific literature. The comprehensive evaluation allowed for the identification of the most promising genotypes for future breeding efforts. Lines R-At-23 and R-Bf-04, along with the control variety, Zvartnots, showed high values in key fruit quality parameters, including sugar, dry matter, lycopene, phenolic compound, and ascorbic acid content. The combination of elevated nutritional and antioxidant content with balanced organoleptic characteristics makes these genotypes particularly valuable for inclusion in hybridization programs. Their use as parental lines will contribute to the targeted development of new tomato hybrids with improved flavor profiles and increased concentrations of bioactive compounds.

List of abbreviations: SCVIC, Scientific Centre of Vegetable and Industrial Crops; MeofRA, Ministry Economy of the Republic of Armenia; SD, Standard deviation; FW, Fresh weight; TA, titratable acidity; ANOVA, analysis of variance; GAE, Gallic acid equivalent.

Competing interests: The authors declare no financial, professional, or personal competing interests that could have appeared to influence the work reported in this manuscript.

Authors' contributions: Authors' contributions: GS and AH designed the study. IV, ZH, HM and AH conducted the biochemical analyses. AH conducted the morphological analyses and interpreted all statistical results. AH, GM

and AA were responsible for the preparation of tables, graphical materials, and references. GS and LT edited the manuscript. All authors reviewed and approved the final version of the manuscript.

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