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INTRASPECIFIC VARIATION OF CAROTENOIDS AND VITAMIN C IN FRUITS OF 48 GENOTYPES OF *ELAEAGNUS MULTIFLORA* THUNB

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ABSTRACT

Elaeagnus multiflora Thunb. (cherry silverberry) is an underutilized fruit species with notable phytochemical potential. The study aimed to evaluate the variability of carotenoid and ascorbic acid contents among 48 genotypes of *Elaeagnus multiflora* grown under uniform environmental conditions at the M.M. Gryshko National Botanical Garden, Kyiv, Ukraine, in order to identify promising genotypes for breeding and functional food applications. Carotenoid content ranged from 16.88 to 44.32 mg/100 g fresh weight (FW), while ascorbic acid varied from 15.6 to 33.5 mg/100 g FW, demonstrating significant genotypic variation for both traits. Statistical analysis confirmed significant differences among genotypes for both carotenoid and vitamin C contents ($p < 0.05$). Certain genotypes (EM_03, EM_09, EM_25, EM_27) combined relatively high levels of both compounds and may be considered promising candidates for breeding and functional food applications. Hierarchical cluster analysis identified two main groups of accessions differing in their phytochemical profiles, while principal component analysis (F1: 52.67%; F2: 47.33%) further supported the observed pattern of biochemical differentiation. The results highlight meaningful diversity in nutritionally relevant compounds within the studied germplasm. Overall, *E. multiflora* represents a valuable genetic resource for fruit crop improvement. The identified variation among genotypes provides a basis for selecting elite material for breeding programs and for the development of nutritionally enhanced functional food products.

Keywords: cherry silverberry, carotenoids, ascorbic acid, genotypic variation

INTRODUCTION

The increasing demand for functional foods rich in health-promoting phytochemicals has stimulated scientific interest in underutilized fruit species **(Ochmian *et al.*, 2012; Ivanišová *et al.*, 2017; Zhurba *et al.*, 2021; Bieniek *et al.*, 2022; Lidiková *et al.*, 2024)**. *Elaeagnus multiflora* Thunb., commonly known as cherry silverberry or goumi, is gaining attention due to its resilience, ease of cultivation in temperate climates, and nutritionally rich fruit profile **(Vasyuk and Moroz, 2005; Lee *et al.*, 2007; Bieniek *et al.*, 2017; Grygorieva *et al.*, 2018; Lachowicz *et al.*, 2019; Wi *et al.*, 2024)**. *E. multiflora* is a deciduous shrub native to East Asia, particularly Japan, Korea, and China, but it has been successfully introduced to Europe and North America for its ornamental, ecological, and nutritional attributes **(You *et al.*, 1994; Miao and Lin, 2010; Kim *et al.*, 2020)**. The plant is characterized by its bright red drupes, which are edible and possess a pleasant, slightly acidic flavor. The fruits have been traditionally used in folk medicine for their anti-inflammatory and hepatoprotective properties **(Hong *et al.*, 2007; Lee *et al.*, 2007; Kim *et al.*, 2020; Nazir *et al.*, 2020)**.

Among the phytochemicals present in *Elaeagnus multiflora*, carotenoids and vitamin C are of particular relevance due to their multifaceted roles in plant physiology and human health **(Moroz *et al.*, 2000; Vasyuk and Moroz, 2006; Lachowicz *et al.*, 2019; Bieniek *et al.*, 2022)**. Carotenoids are lipophilic pigments that contribute to the characteristic red-orange coloration of the fruit and play essential roles in photoprotection and intercellular communication **(Saini *et al.*, 2015)**. In human nutrition, they act as potent antioxidants, precursors of vitamin A, and protective agents against various degenerative diseases **(Rodriguez-Concepcion *et al.*, 2018)**.

Vitamin C (ascorbic acid), another key bioactive compound in this species, also contributes significantly to the fruit's health-promoting potential. It functions as a non-enzymatic antioxidant, supports immune responses, and serves as a co-factor in numerous enzymatic reactions **(Alberts *et al.*, 2025)**. Although its concentration may vary depending on genotype and growing conditions, the fruit is widely recognized as a promising natural source of this compound **(Bieniek *et al.*, 2022)**. Carotenoids such as lycopene and β-carotene have been associated with reduced risks of cardiovascular disease, certain cancers, and age-related macular degeneration **(Tufail *et al.*, 2024)**, while vitamin C is known for enhancing immune defense and mitigating oxidative stress **(Carr and Maggini, 2017)**. Their co-occurrence in *E. multiflora* enhances the species' potential as a valuable component of functional diets.

Despite growing scientific interest, most phytochemical studies have been limited to a narrow range of cultivars or biotypes, often analyzed under varying environmental conditions **(Vasyuk and Moroz, 2006; Bieniek *et al.*, 2017; Lachowicz *et al.*, 2019)**. Only recently have studies begun to address intraspecific variation, although they remain limited in scope and number of genotypes examined **(Lachowicz-Wiśniewska *et al.*, 2021)**. This underscores the need for broader evaluations involving more genetically diverse collections grown under standardized conditions to identify elite genotypes with enhanced nutritional profiles.

The aim of this study was to evaluate intraspecific variability in carotenoid and ascorbic acid contents among 48 genotypes of Elaeagnus multiflora cultivated under uniform environmental conditions at the M.M. Gryshko National Botanical Garden (Kyiv, Ukraine) and to identify nutritionally superior genotypes with potential value for breeding programs and functional food development.

MATERIAL AND METHODS

Biological material

The study was conducted on 48 genotypes of *Elaeagnus multiflora* (Figure 1, 2) cultivated at the Department of Fruit Plant Acclimatization, M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv, Ukraine; 50°27.280′ N, 30°31.428′ E). The collection is situated at an altitude of approximately 180 meters above sea level and maintained under uniform agronomic and ecological conditions. All genotypes were grown in the same orchard with standardized plant spacing (3 × 2 m), chernozem soil, and consistent cultivation practices, including irrigation and manual weed control, without the use of synthetic fertilizers or pesticides.. Genotypes were designated EM_01 through EM_48. For each genotype, fruits were collected from one plant. Each biochemical determination (ascorbic acid and carotenoid content) was performed in triplicate (analytical replicates), and the results are presented as mean ± standard deviation (SD). The mean values obtained from the analytical replicates were used for statistical analysis.

Fruits were collected at full physiological maturity based on uniform external ripening criteria (typical fruit coloration and softness). All samples were harvested during the same ripening period to minimize seasonal variation. For each genotype, healthy and undamaged fruits of similar size were randomly selected from different parts of the canopy to ensure representative sampling.



Figure 1 Fruit-bearing stage of *Elaeagnus multiflora* Thunb. in the collection of the M.M. Gryshko National Botanical Garden, Kyiv, Ukraine



Figure 2 Morphological diversity of *Elaeagnus multiflora* Thunb. fruits in the *ex situ* collection of the M.M. Gryshko National Botanical Garden

Chemicals and reagents

2,6-Dichlorophenolindophenol (Tillman's reagent) (Sigma-Aldrich, St. Louis, MO, USA), acetone (Chempur, Piekary Śląskie, Poland), and calcium carbonate (Chempur, Piekary Śląskie, Poland) were of analytical grade.

Determination of vitamin C

The ascorbic acid content was determined by a titrimetric method using 2,6-dichlorophenolindophenol (Tilman's reagent) as an oxidizing indicator **(Hrytsajenko *et al.*, 2003)**. In acidic solution, ascorbic acid reduces the blue dye to its leucoform until completely oxidized to dehydroascorbic acid, after which the solution acquires a red coloration characteristic of the oxidized reagent. The results were expressed as mg/100 g fresh matter (FM).

Determination of carotenoids content

Carotenoids were extracted from fruits with 100% acetone and quantified spectrophotometrically **(Musienko *et al.*, 2001)**. Plant material (0.5–1 g) was ground in a porcelain mortar with 5 ml of cooled solvent and 0.2 g calcium carbonate. The extract was centrifuged at 2500 rpm for 10 min, and the supernatant was collected. The residue was repeatedly resuspended until decolorization, after which all extracts were combined and the final volume was adjusted to 50 ml. Optical density was measured at 440 nm using acetone as a blank. The results were expressed as mg/100 g fresh matter (FM). Each sample was analyzed in triplicate, and the mean value was used for statistical analysis.

Statistical analysis

Statistical analyses were performed using XLSTAT. Data normality was verified prior to analysis. One-way analysis of variance (ANOVA) was used to assess differences among genotypes for carotenoid and ascorbic acid contents. When significant effects were detected, means were separated using the Tukey–Kramer post hoc test ($p < 0.05$). Results are presented as mean ± standard deviation (SD), and statistically significant differences among means are indicated by different letters in Figures 3 and 4. Cluster analysis and dendrogram construction were performed using Euclidean distance and the average linkage method. Principal component analysis (PCA) was conducted and visualized as a biplot.

RESULTS AND DISCUSSION

Carotenoid content in fruits of *Elaeagnus multiflora* genotypes

The present study revealed a substantial variation in total carotenoid content among the 48 genotypes of *Elaeagnus multiflora*, ranging from 16.883 (EM_14) to 44.318 mg/100 g FW (EM_25). Several genotypes, including EM_25 (44.32 mg/100 g FW), EM_09 (43.21 mg/100 g FW), EM_07 (41.11 mg/100 g FW), and EM_21 (39.13 mg/100 g FW), exhibited particularly high carotenoid concentrations, confirming the strong influence of genetic factors on carotenoid biosynthesis in *Elaeagnus multiflora* fruit. These genotypes may be considered superior in terms of nutritional value and could be prioritized in breeding programs aimed at enhancing the functional food potential of this species. In contrast, genotypes such as EM_14 (16.88 mg/100 g FW), EM_44 (17.44 mg/100 g FW), and EM_15 (18.52 mg/100 g FW) demonstrated the lowest carotenoid content, suggesting limited value from a phytochemical perspective.

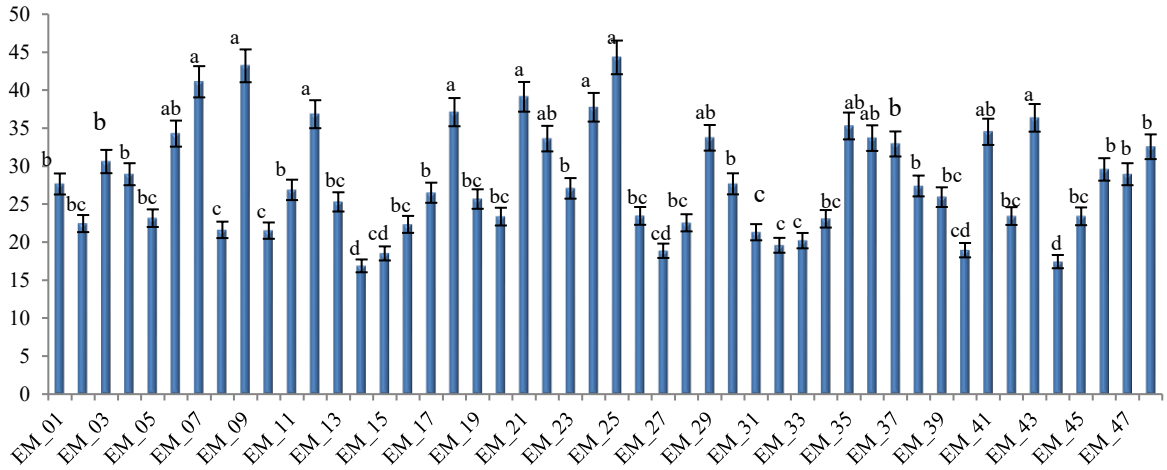


Figure 3 Variation in total carotenoid content among 48 *Elaeagnus multiflora* Thunb. genotypes, mg/100 g FW (means in columns followed by different letters are different at $p \leq 0.05$. Each value represents the mean of three independent experiments (±SD))

The observed differences may result from inherent genotypic capacity for carotenoid biosynthesis, which is regulated by genetic factors controlling key enzymes in the isoprenoid pathway **(Rodríguez-Concepción *et al.*, 2018)**. Environmental conditions were uniform for all accessions, supporting the conclusion that genotype was the primary source of variation.

Previous studies have reported comparable or even higher carotenoid concentrations in *E. multiflora* fruit. For instance, **Lachowicz-Wiśniewska *et al.***

(2021) found total carotenoid contents in fruits of six genotypes ranging from 95.69 to 170 mg/100 g dry weight (DW), while in the same study, the carotenoid content in seeds ranged from 12.48 to 15.30 mg/100 g DW, and in leaves from 56.75 to 96.28 mg/100 g DW. Another study by **Lachowicz *et al.* (2020)** reported 40.09 to 97.15 mg/100 g DW in the fruits of cultivars Sweet Scarlet and Jahidka, with markedly higher contents in the leaves (236.64 to 241.45 mg/100 g DW).

Vasyuk and Moroz (2006) recorded carotenoid levels between 13.2 and 19.6 mg/100 g DW in six genotypes of *E. multiflora*. However, the variability across studies may be due to differences in analytical methods, ripening stages, growing conditions, and units of measurement (fresh vs. dry weight). Unlike previous studies that evaluated only a limited number of E. multiflora genotypes, the inclusion of 48 genotypes in the present study enabled a more comprehensive assessment of intraspecific variability in carotenoid accumulation. The broader genetic representation allowed the identification of genotypes with contrasting phytochemical profiles, thereby providing a stronger basis for germplasm characterization and the selection of promising breeding material.

Other species within the genus *Elaeagnus* L. also show promising carotenoid levels. In *E. umbellata* Thunb., **Wang and Fordham (2007)** reported 43.4 to 59.3 mg/100 g FW in six genotypes, while **Khattak (2012)** found 19.9 ± 3.2 mg/g DW. In contrast, *E. latifolia* L. had significantly lower carotenoid content, with **Dasila and Singh (2021)** reporting only 0.83 mg/100 g FW.

Comparisons with *Hippophae rhamnoides*, a related species from the same family (Elaeagnaceae), further highlight the carotenoid-rich nature of *E. multiflora*. **Andersson *et al.* (2009)** found 10 to 120 mg/100 g FW in sea buckthorn fruits, while **Gao *et al.* (2000)** reported 1.0 to 13.3 mg/100 g FW. Extremely high concentrations were recorded in juice (314–2139 mg/100 g, **Zhang *et al.* (1989a)** and seed oil (up to 120 mg/100 g, **Yang, 2001**).

Importantly, *E. multiflora* is not only rich in total carotenoids but also exhibits a complex and valuable carotenoid profile. It should be noted that in the present study only total carotenoid content was quantified; therefore, information on individual carotenoid compounds is based on previously published literature. Direct profiling of individual carotenoids was not performed in this study and represents a direction for future research. According to **Lachowicz *et al.* (2019)** and **Lachowicz-Wiśniewska *et al.* (2021)**, 16 carotenoid compounds were identified in the fruit, including lycopene (the predominant compound), α- and β-carotene, lutein, violaxanthins, neoxanthin, and the rare colorless carotenoid phytoene. Lycopene alone accounts for approximately 80% of total carotenoids and is recognized for its strong antioxidant and anticancer potential **(Eggersdorfer and Wyss, 2018)**. The presence of phytoene, which has high bioavailability and protective effects on the skin, adds to the functional value of the fruit **(Meléndez-Martínez and Stinco, 2019)**.

Overall, the wide genotypic variation in carotenoid content observed in this study confirms the importance of *E. multiflora* as a promising target for nutritional breeding.

Ascorbic acid content in fruits of *Elaeagnus multiflora* genotypes

The ascorbic acid content in the fruits of 48 genotypes of *E. multiflora* exhibited considerable variation, ranging from 15.6 to 33.5 mg/100 g fresh weight (FW). The highest concentration was recorded in genotype EM_03 (33.5 mg/100 g), followed closely by EM_27 (32.3 mg/100 g), EM_10 (31.7 mg/100 g), EM_40 (31.0 mg/100 g), and EM_22 (30.8 mg/100 g). Conversely, the lowest contents were found in genotypes EM_06 (15.6 mg/100 g) and EM_41 (15.9 mg/100 g).

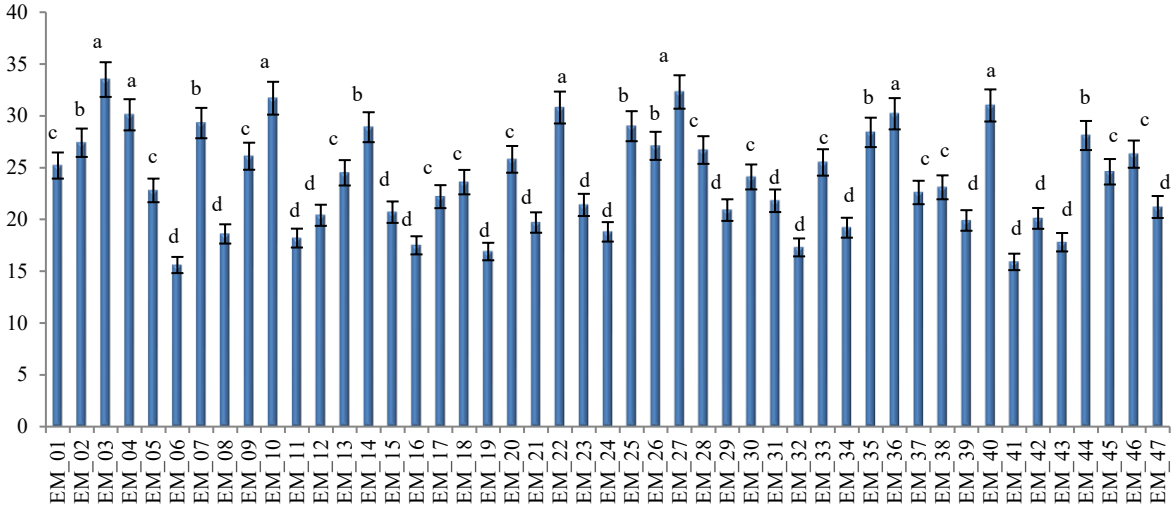


Figure 4 Variation in ascorbic acid content among 48 *Elaeagnus multiflora* Thunb. genotypes, mg/100 g FW (means in columns followed by different letters are different at $p \leq 0.05$. Each value represents the mean of three independent experiments (±SD))

These findings are consistent with earlier work by **Moroz *et al.* (2000)**, who reported a range of 15.8 to 33.1 mg/100 g FW in six genotypes of *E. multiflora*, suggesting that the vitamin C content is predominantly genotype-dependent. Our results also align with **Khattak (2012)**, who measured 22 mg/100 g FW in *E. multiflora* fruits.

Compared to our results, **Lachowicz *et al.* (2019)** reported lower ascorbic acid contents in six genotypes of *E. multiflora*, ranging from 14.1 to 17.6 mg/100 g FW, possibly due to differences in environmental conditions, genetic background, or ripeness at harvest. Even lower values were found by **Bieniek *et al.* (2017)** (4.22–7.70 mg/100 g) and **Szałkiewicz and Kawecki (2003)** (2.35–4.34 mg/100 g), reflecting significant variability across studies.

Interspecific comparisons further demonstrate the relatively high vitamin C content of *E. multiflora* fruits. For instance, *E. umbellata* genotypes grown in Pakistan contained only 14.1–14.3 mg/100 g FW **(Ahmad *et al.*, 2005)**, while Romanian biotypes ranged from 10.85 to 17.31 mg/100 g FW **(Petrescu and Paraschiv, 2022)**. In contrast, *E. latifolia* exhibited an exceptionally high value of 662.85 mg/100 g FW, as reported by **Dasila and Singh (2022)**, although this is likely an outlier among *Elaeagnus* species.

A comparison with the fruits of other genera further emphasizes the nutritional value of *E. multiflora*. According to **Morales *et al.* (2013)**, *Arbutus unedo* L. contains 141.99 mg/100 g FW, while *Prunus spinosa* L., *Crataegus monogyna* Jacq., and *Rubus ulmifolius* Schott. contain only 0.91, 1.51, and 7.41 mg/100 g FW, respectively. Meanwhile, Hippophae rhamnoides (sea buckthorn), another member of the Elaeagnaceae family, is a known vitamin C powerhouse, with reported contents between 207.8 and 436.2 mg/100 g FW **(Gutzeit *et al.*, 2008)**.

In conclusion, the results of this study confirm that *E. multiflora* fruits are a valuable source of vitamin C, with several genotypes exhibiting contents comparable to or exceeding those found in related or commonly consumed fruits.

To assess intraspecific variation in the accumulation of bioactive compounds among *E. multiflora* genotypes, hierarchical clustering was performed based on total carotenoid and ascorbic acid contents. This multivariate statistical approach enables the grouping of genotypes according to similarities in their phytochemical profiles and has been successfully applied in previous studies involving other plant species to reveal patterns of biochemical diversity and support genotype selection for breeding and functional **use (Hossain *et al.*, 2011; Oszmiański *et al.*, 2018; Hussain *et al.*, 2023; Zargar *et al.*, 2023; Liang *et al.*, 2024)**. The dendrogram (Figure 5) revealed two distinct clusters, reflecting differences in phytochemical composition.

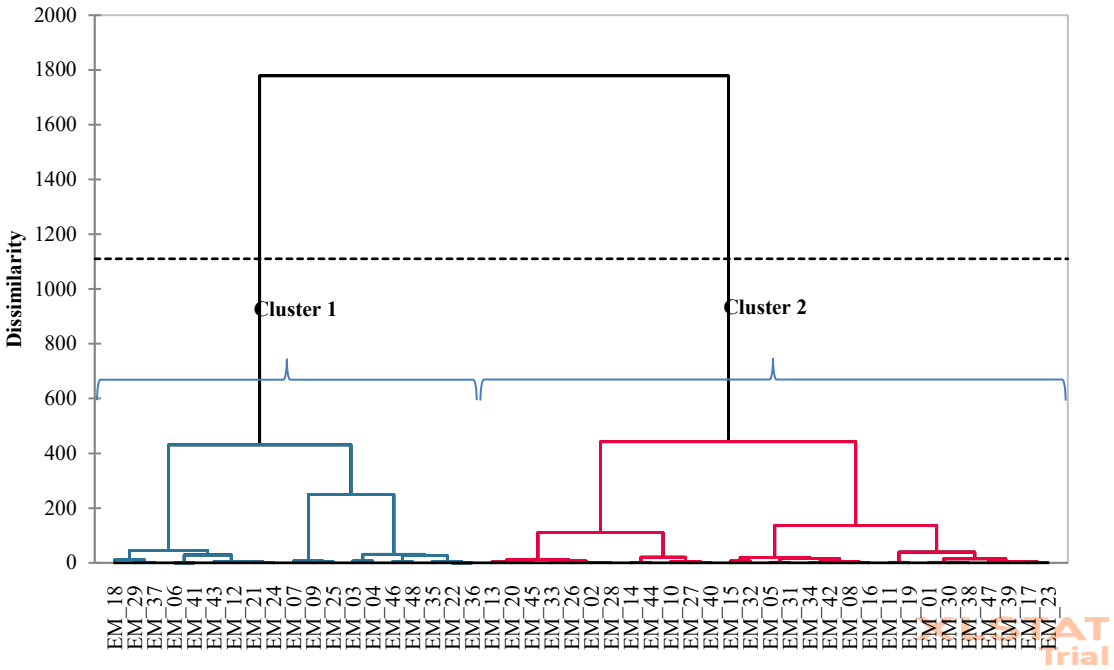


Figure 5 Hierarchical cluster analysis of *Elaeagnus multiflora* Thunb. 48 genotypes based on total carotenoid and ascorbic acid contents

Cluster analysis based on total carotenoid and ascorbic acid contents separated the 48 genotypes into two main groups (Figure 5). Cluster 1 comprised 29 genotypes (EM_01, EM_02, EM_05, EM_08, EM_10, EM_11, EM_13, EM_14, EM_15, EM_16, EM_17, EM_19, EM_20, EM_23, EM_26, EM_27, EM_28, EM_30, EM_31, EM_32, EM_33, EM_34, EM_38, EM_39, EM_40, EM_42, EM_44, EM_45, EM_47), while the remaining genotypes formed Cluster 2. Cluster 1 was generally characterized by lower to moderate carotenoid content (mostly 16.9–28.9 mg/100 g) and a relatively wide range of ascorbic acid levels (15.6–32.3 mg/100 g), indicating variability in vitamin C accumulation among genotypes with similar carotenoid profiles. Overall, the clustering pattern reflects variation in carotenoid and ascorbic acid contents among the studied genotypes. Although the analysis was based on only two biochemical traits, it provides a useful visualization of phytochemical similarity and may serve as a preliminary tool for identifying genotypes with contrasting nutritional profiles. Further studies including additional biochemical, agronomic, and genetic traits are required to determine the biological significance of the identified clusters.

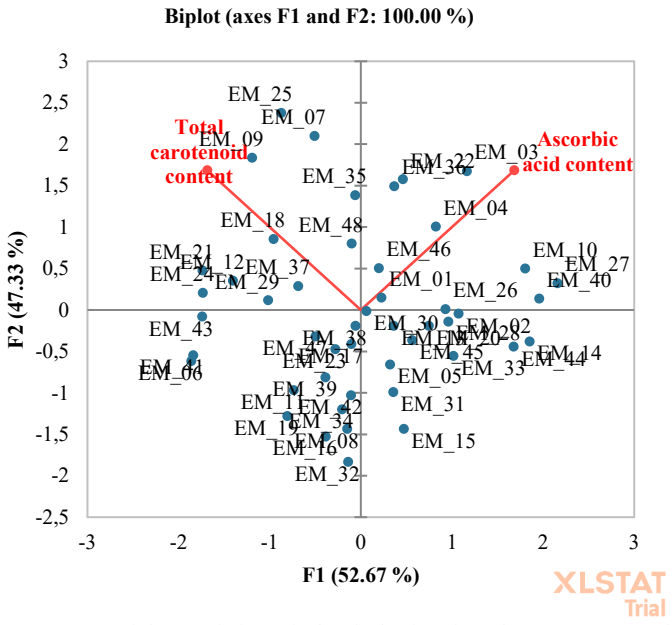


Figure 6 PCA Biplot Depicting Biochemical Diversity of 48 Genotypes of *Elaeagnus multiflora* Thunb. Based on Carotenoid and Vitamin C Content

The principal component analysis (PCA) was performed based on two variables, carotenoid and ascorbic acid contents. The first two principal components together accounted for 100% of the total variance (F1: 52.67%; F2: 47.33%), which reflects the two-variable structure of the dataset. The PCA biplot (Figure 6) shows the distribution of genotypes according to these two biochemical traits, with F1 and F2 representing gradients of carotenoid and ascorbic acid contents, respectively. This visualization provides a simplified graphical representation of the relationships among genotypes based on the measured phytochemical parameters. This biplot clearly distinguished two major genotype groups corresponding to the clusters identified by hierarchical analysis. Cluster 1 genotypes generally occupied the region associated with moderate carotenoid and variable ascorbic acid levels, whereas Cluster 2 genotypes were positioned toward higher carotenoid and more consistent ascorbic acid contents.

The complementary contribution of both components indicates that total carotenoid and ascorbic acid contents jointly define the biochemical diversity among the genotypes. Such multivariate analysis is useful for identifying promising genotypes combining favorable antioxidant traits, which is essential for breeding programs targeting improved nutritional quality.

CONCLUSION

This study demonstrates substantial genotypic variability in carotenoid and ascorbic acid contents among 48 *Elaeagnus multiflora* genotypes grown under uniform environmental conditions. Multivariate analyses (PCA and cluster analysis) confirmed the existence of distinct phytochemical groups and highlighted the strong contribution of both compounds to the overall biochemical diversity of the species. Several genotypes exhibiting consistently high levels of both carotenoids and vitamin C were identified, indicating their potential value as promising candidates for breeding programs aimed at improving the nutritional quality of fruit crops. The observed biochemical diversity also underscores the importance of conserving and characterizing germplasm resources of *E. multiflora* for future utilization. Overall, the results emphasize the value of *E. multiflora* as a functional fruit crop with significant potential for the development of nutrient-rich food products and its integration into sustainable horticultural systems. The findings provide a scientific basis for the selection of elite genotypes and support further research on the species' role in enhancing dietary diversity and food security.

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