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Study of anthocyanins and biochemical properties in several genetic resources from the European evaluation network *Capsicum chinese* chili pepper collection in Armenia

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ABSTRACT

Introduction: Pepper crops are gaining increasing interest among vegetables due to their high content of vitamin C and P-active substances. Pepper fruits are also rich in carotene, thiamine, folic acid, proteins, minerals, and antioxidants. These peppers can be classified as dietary products with excellent taste properties, making them suitable for fresh consumption. Considering that chili peppers *C. annuum*, *C. frutescens*, and *C. chinense* share a common phylogenetic prototype, represented by a wild species, we studied *C. chinense* samples to breed new varieties. This study focused on their fruits' vitamin C content and antioxidant properties. *Capsicum chinense* holds great economic importance due to its characteristic pleasant aroma, pungency, and variety of shapes and colors.

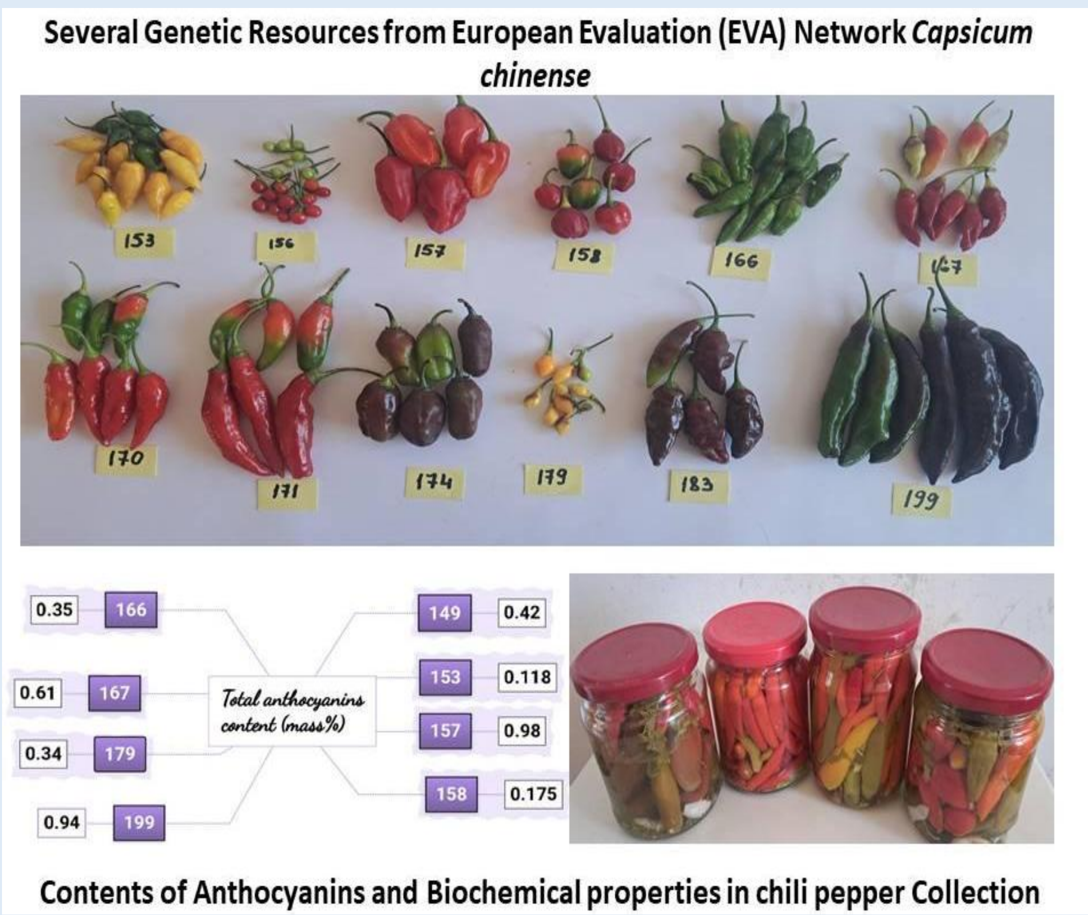
Objective: To study the effectiveness of various *Capsicum chinense* genetic resources conserved within the European Evaluation (EVA) Network, focusing on the anthocyanin content and biochemical properties of pepper fruits cultivated in Armenia.

Methods: The study included *Capsicum chinense* pepper accessions 149, 153, 156, 157, 158, 166, 167, 170, 171, 174, 179, 183, 199, and 200 from the European Evaluation (EVA) Network. Biochemical properties and statistical analysis were conducted using accepted methods.

Results: The total anthocyanin content in the accessions ranged from 0.11% to 0.98% by mass. Accessions 157, 199, 167, 149, 166, and 179 stood out for their high anthocyanin content, with values of 0.98%, 0.94%, 0.61%, 0.42%, 0.35%, and 0.34%, respectively. Dry matter content in the fruits of chili pepper accessions at the stage of technical ripeness was 3.6% - 5.2%, and at the stage of biological ripeness (before physiological ripeness), it was 4.9% - 6.5%. The sugar content in the fruits at the stage of technical ripeness was 0.8% - 2.8%, and at the stage of biological ripeness, it was 1.5% - 4.5%. The vitamin C content at the stage of technical ripeness was 32.85 - 53.85 mg% (mg/100g), and at biological ripeness, it was 45.35 - 69.55 mg%. Canned pickled peppers were prepared from fruits of different colors and shapes of *Capsicum chinense*.

Conclusion: Several accessions from the EVA Network *Capsicum chinense* pepper collection exhibited high anthocyanin content and favorable biochemical properties in fruits cultivated in Armenia. Plants of different chili pepper accessions demonstrated varying rates of biological traits, phenotypic transitions, and diverse fruit colorations and shapes.

Keywords: chili pepper, anthocyanins, dry matter, sugars, acidity, vitamin C, genetic resources



INTRODUCTION

The science of functional foods has been rapidly advancing across various countries. Functional foods, including vitamins and phenolic compounds, are found in chili pepper products, whether fresh, processed, or prepared as food and fruit powders [1-6]. The high content of such substances in plant fruits is influenced by factors such as climate, varietal composition, agricultural practices, and nutrition. Chemical (Multibar), biological (Ecobiofeed+), and organic (green legume) fertilizers were used. [7-13]. Globally, pepper is widely cultivated and highly regarded as a "record holder" among vegetables due to its high vitamin C and P-active substances, which are 5-10 times greater than in many other crops, such as cucumbers and tomatoes. Additionally, pepper fruits are rich in carotene, thiamine, folic acid, proteins, minerals, and antioxidants, making them a functional food product [13-16]. As a dietary product, vegetable pepper has excellent taste and is suitable for fresh consumption [17-19].

Given the phylogenetic relationship between *C. annuum*, *C. frutescens*, and *C. chinense*—all originating from a common wild prototype—this study focused on *C. chinense* samples to breed new varieties by analyzing their vitamin C content and antioxidant properties [20-24]. *Capsicum chinense* holds significant economic value. The peppers are known for their pleasant aroma, pungency, diverse shapes and colors, and high levels of biologically active compounds.

Capsicum chinense Jacq., commonly known as Chinese pepper, has a distinctive hanging fruit arrangement. It likely originated in the Amazon (possibly Peru), but Dutch physician Nikolaus von Jacquin mistakenly classified it as being from China in 1776. Today, *C. chinense* is widely distributed across tropical regions and the Caribbean, particularly Jamaica. Characteristics like fruit shape, leaf size, fruit size, and flower structure suggest that this species has been cultivated for a long time. The *Capsicum chinense* plant typically bears 2-7 fruits per axil, with round or elongated fruits featuring a wavy surface. These fruits are pungent and aromatic, with their spiciness remaining stable during cooking [25].

The wild type of *Capsicum chinense* is notable for its intense spiciness and its high capsaicin content, reaching up to 400 mg per 100 g of fresh material. Research from various countries has shown that the bioactive substances in the Habanero variety, including high capsaicin levels and vitamin C, as well as P-vitamin active substances like polyphenols (such as anthocyanins), can induce the destruction of malignant cells and reduce tumor sizes [25-27]. Among the accessions, the Habanero variety has the highest capsaicin content. Of the samples studied, 153 had yellow fruits, 157 had red fruits, and 158 and 183 had brown fruits. These samples were imported through the EVA Pepper network program, extensively studied under local conditions, and analyzed for bioactive compounds and anthocyanin content.

Since *Capsicum chinense* originates from tropical climates and thrives best in those regions, it was essential to evaluate these samples' biological, morphological, agronomic, biochemical, and antioxidant properties under local conditions for the first time. The scientific novelty of this research lies in the fact that it marks the first study of wild chili pepper *Capsicum chinense* samples in Armenia, focusing on the antioxidant characteristics of their fruits.

This study evaluated the effectiveness of various *Capsicum chinense* genetic resources, conserved within the EVA Network, by analyzing the anthocyanin and biochemical properties of pepper fruits cultivated in Armenia.

MATERIALS AND METHODS

Germplasm Material: The study utilized *Capsicum chinense* pepper accessions from the EVA Network, specifically the NLD037 resources. The accessions included:

1. EVA_Ca_149 CGN17019
2. EVA_Ca_157 CGN21547
3. EVA_Ca_158 CGN21545
4. EVA_Ca_166 CGN22782
5. EVA_Ca_167 CGN22829
6. EVA_Ca_170 CGN22870
7. EVA_Ca_171 CGN23092
8. EVA_Ca_174 CGN22856
9. EVA_Ca_179 CGN23769
10. EVA_Ca_183 CGN17222
11. EVA_Ca_199 CGN22095
12. EVA_Ca_200 CGN22099
13. EVA_Ca_204 CGN22109
14. EVA_Ca_206 CGN22112.

Fig.1 Fruit of peppers *Capsicum chinense* species accessions - 149,153, 156, 157, 158, 166, 167, 170, 171, 174, 179, 183, 199, 200.

The experimental design, seed germination, transplanting, and plant growth were carried out using accepted methods [28].

The study was conducted in the Ararat region, 800–1000 meters above sea level. Long-term studies have shown that the average annual air temperature is 11.8°C, with the absolute minimum ranging between -28 °C and -30 °C, and the absolute maximum ranging from 39°C to 41.6°C. The annual precipitation is 272 mm, with 217 frost-free days. The sum of favorable temperatures (above 0°C) is 4500–4800°C, and the sum of active temperatures (above 10°C) is 4000–4200°C. The annual total duration of daylight is 2627 hours, and the region experiences 40 cloudy days per year. Spring weather is highly variable, with strong convection processes developing in the second half of the season. The majority of annual precipitation occurs during spring, particularly in May. Under these climatic conditions, plants native to tropical regions, such as chili peppers, have the opportunity to grow, develop, and bear fruit, which was also observed in our experiments.

The soils at the Darakert experimental farm of the Scientific Center are meadow gray, irrigated, and non-carbonate. The humus content in the arable layer of the soil ranged from 1.80% to 1.95%, total nitrogen was 0.20% to 0.22%, easily hydrolyzed nitrogen was 5.2–5.5 mg, available phosphorus was 31–35 mg, and exchangeable potassium was 40–59 mg per 100 grams of dry soil. The pH of the soil in a water suspension was 7.2–7.3. Thus, the soils of the experimental plot have a relatively high demand for nitrogen, an average demand for phosphorus, and a low demand for potassium.

Chili pepper fruits ripen from July to October. Under our experimental conditions, chili peppers were cultivated with irrigation and fertilizers.

The content of anthocyanins, dry matter, total

sugars, and vitamin C in the fruits was measured spectrophotometrically using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA) [29-32].

The main goal of our study was to determine the content of anthocyanins, dry matter, sugars, and vitamin C in chili pepper fruits. Since anthocyanin coloration is most pronounced in biologically ripe fruits, these fruits were selected for anthocyanin analysis. Vitamin C, dry matter, and sugars were measured twice—once during the technical ripening stage and again at the biological ripening stage. Due to the difficulty in determining capsaicin content, chili pepper samples were primarily selected based on available literature data.

Statistical Analyses: Analysis of Variance (ANOVA) was used to assess the differences between the groups. The data were analyzed to determine if there were statistically significant differences in the content of anthocyanins, dry matter, total sugars, and vitamin C across the different Capsicum chinense accessions. A significance level of $p < 0.05$ was considered statistically significant for all comparisons.

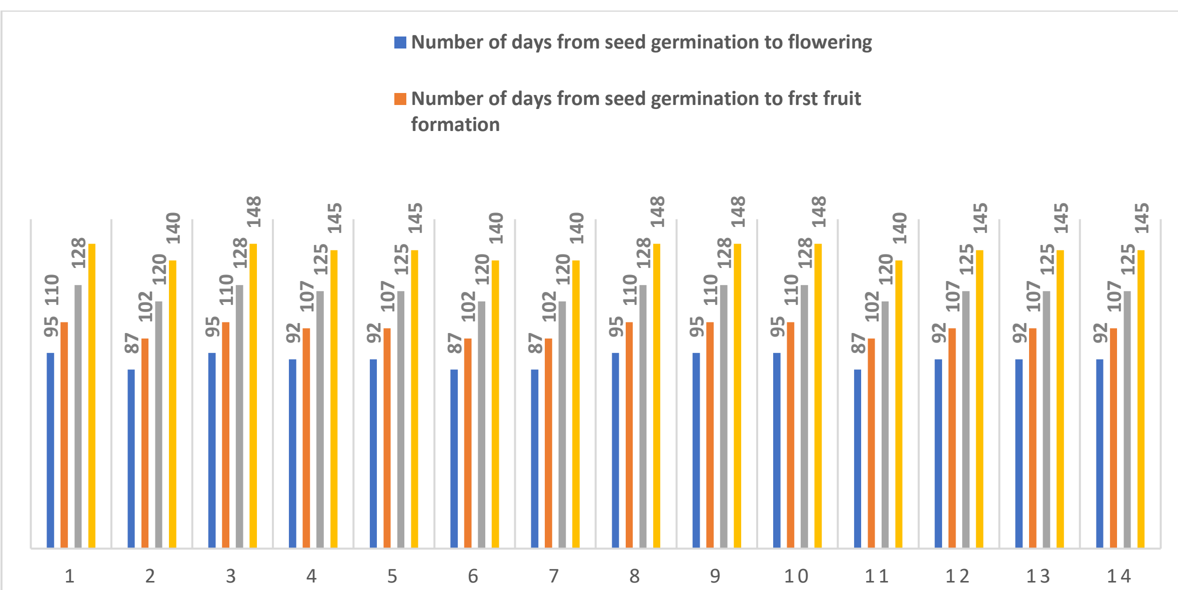


Fig.2 Biological characters of peppers *Capsicum chinense*.

RESULTS AND DISCUSSION

Biological and Phenological character evaluation: Figure 2 shows that the period from seed germination to flowering in chili pepper accessions ranged from 87 to 95 days. The time until the first fruits formed was 102 to 110 days, with technical maturity reaching 120 to 128 days and biological maturity at 140 to 148 days. Chili pepper varieties 153, 166, 167, and 179 were notable for their early maturity, with the entire period lasting 120 days and biological maturity reached in 140 days. The remaining accessions were mid-ripening (Fig. 2).

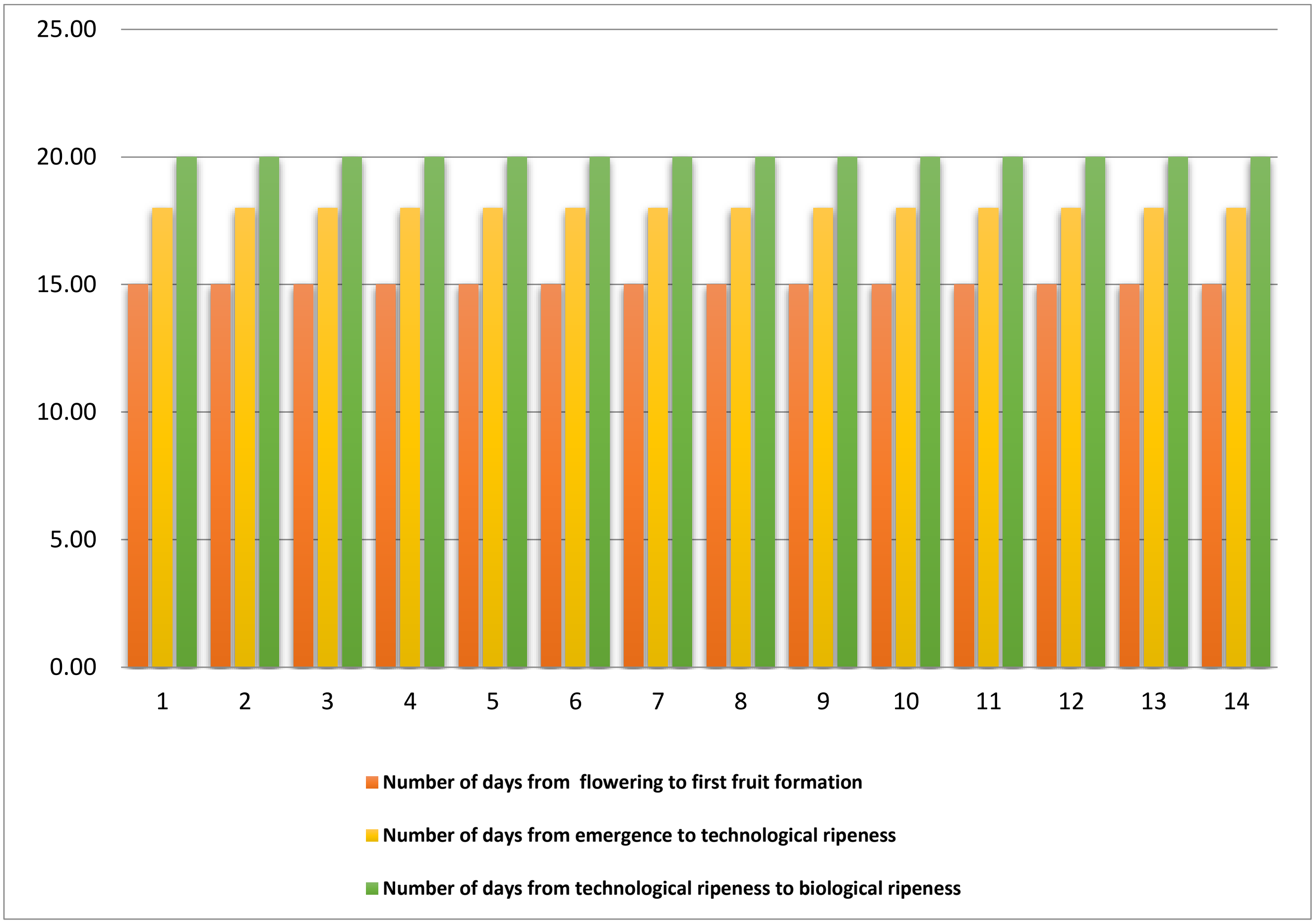


Fig. 3 Phenological character of peppers *Capsicum chinense*.

The study of phenological characteristics revealed that all the studied accessions exhibited similar indicators for the following periods: from flowering to emergence to fruit ripeness and from technical ripeness to biological ripeness. The duration of the transitions between phenological stages was approximately the same in all chili pepper samples, with these periods being 15, 18, and 20 days, respectively (Fig. 3).

Fruit shapes were categorized as follows:

- **Morron Type**: Accessions 149, 153, and 157
- **Tomato Type**: Accessions 156 and 158
- **Horned/Triangular**: Accessions 166, 167, 170, 171, 199, and 200
- **Square**: Accessions 174 and 183
- **Oval**: Accession 179 (See Fig. 4)

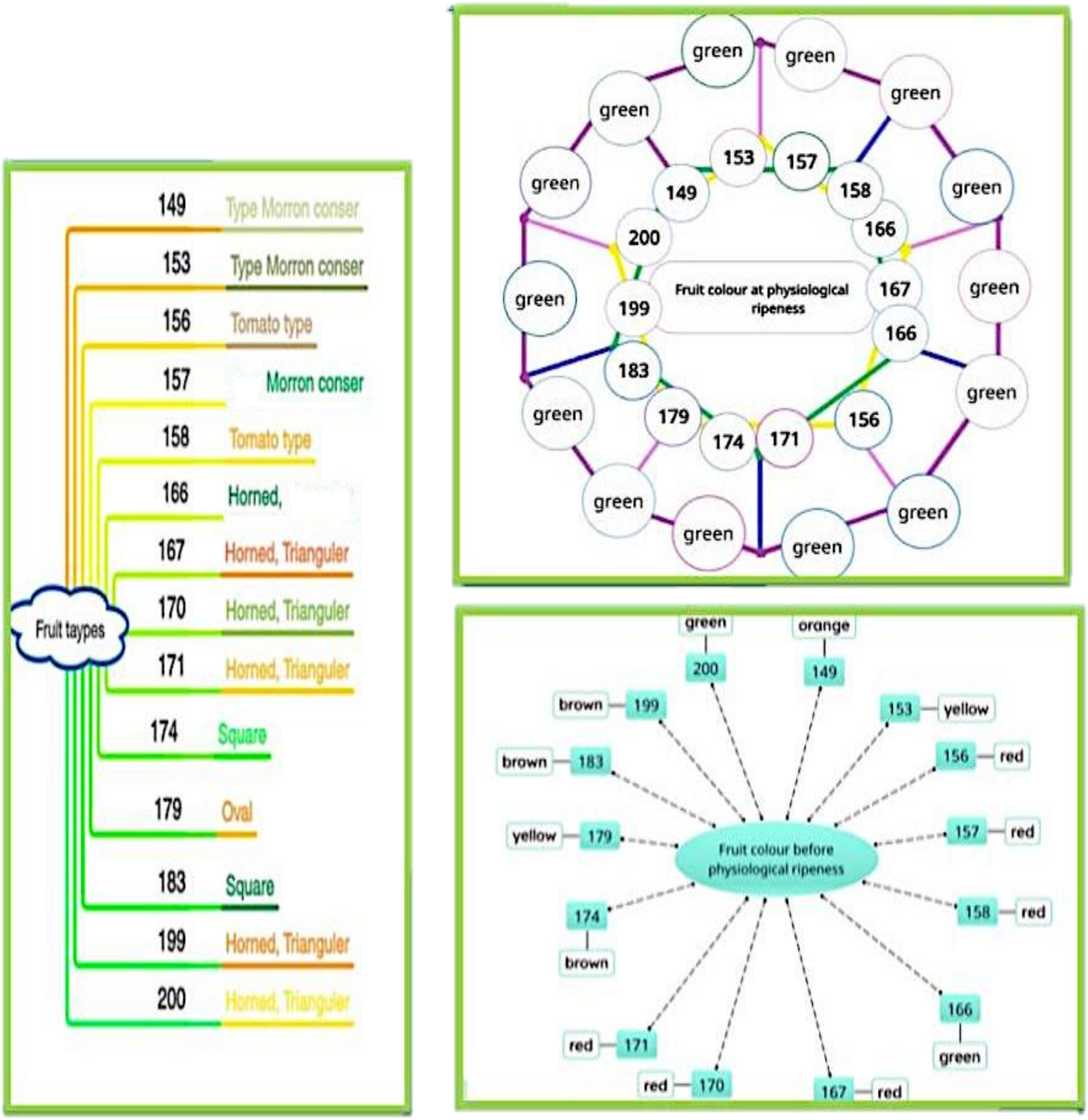


Fig.4 Characters of peppers *Capsicum chinense*.

The study of fruit morphological characteristics revealed that all the studied accessions produced green fruits at technical ripeness, while fruit colors at biological ripeness varied. Specifically, accession 149 had orange fruits; accessions 153 and 179 had yellow fruits; accessions 156, 157, 158, 167, 170, and 171 produced red fruits; accessions 174, 183, and 199 had brown fruits; and accessions 166 and 200 remained green.

An analysis of chili pepper plant yields showed that in 2022, the yield per plant ranged from 0.104 to 0.617 kg, while in 2023, it ranged from 0.100 to 0.620 kg. The yield per hectare varied between 4,576 and 27,148 kg in 2022 and 4,400 and 27,280 kg in 2023. The accessions that stood out for their high yield were 171, 199, 174, 157, 170, and 200 (Table 1).

Table 1. Productivity characteristics of the *Capsicum chinense* species.

Accessions	Productivity per plant (kg)	Productivity per hectare(kg)	Productivity per plant (kg)	Productivity per hectare(kg)
	2022		2023	
149	0,138	6072	0,135	5940
153	0,375	16500	0,372	16368
156	0,112	4928	0,114	5016
157	0,523	23012	0,525	23100
158	0,379	16676	0,377	16588
166	0,300	13200	0,297	13068
167	0,294	12936	0,300	13200
170	0,498	21912	0,504	22176
171	0,617	27148	0,620	27280
174	0,531	23364	0,528	23232
179	0,104	4576	0,100	4400
183	0,595	26180	0,588	25872
199	0,610	26840	0,608	26752
200	0,473	20812	0,475	20900
P-Value				0.572653671
$F_{critical}$				2.576927084
$LSD_{0.05}$				0.17
S_x				0.12

The total anthocyanin content in the accessions ranged from 0.11% to 0.98% by mass. Accessions 157, 199, 167, 149, 166, and 179 stood out for their high anthocyanin levels, with values of 0.98%, 0.94%, 0.61%, 0.42%, 0.35%, and 0.34% by mass, respectively (Table 2).

Table 2. Content of anthocyanins in fresh pepper fruits *Capsicum chinense* species in mass%.

Sample	Total anthocyanins content (mass%)	
	Mean	Standard deviation
1.EVA_Ca_149 CGN17019	0.42	±0.003
2.EVA_Ca_153 CGN17036	0.11	±0.02
4.EVA_Ca_157 CGN21547	0.98	±0.074
5.EVA_Ca_158 CGN21545	0.17	±0.090
6.EVA_Ca_166 CGN22782	0.35	±0.035
7.EVA_Ca_167 CGN22829	0.61	±0.068
11.EVA_Ca_179 CGN23769	0.34	±0.05
13.EVA_Ca_199 CGN22095	0.94	±0.004

Several studies have shown that bioactive compounds in vegetable fruits tend to increase significantly during the stage leading up to physiological ripeness [33-37], and our experiments yielded similar results.

In recent years, functional food science has made significant progress in understanding the complex relationships between nutrition, chronic diseases, and overall health. Functional foods, rich in bioactive compounds, are potentially beneficial in preventing and managing chronic diseases [38].

Food bioactive compounds (FBCs) are naturally occurring nutritional and non-nutritional substances in foods that benefit the human body, promoting health. Although present in small amounts, FBCs in functional foods have been linked to a reduced risk of chronic conditions such as cardiovascular disease, cancer, metabolic syndrome, type II diabetes, and obesity [39-42].

Figure 5 shows that the dry matter content in chili pepper fruits ranged from 3.6% to 5.2% at physiological ripeness, and from 4.9% to 6.5% before physiological ripeness. The highest values were observed in accessions 174, 172, 170, 200, 153, and 156, with dry matter content of 5.2%, 5.2%, 5.1%, 4.9%, 4.8%, and 4.6% at physiological ripeness, respectively, and 6.5%, 6.4%, 6.2%, 6.1%, 5.6%, and 5.4% before physiological ripeness ($P < 0.05$).

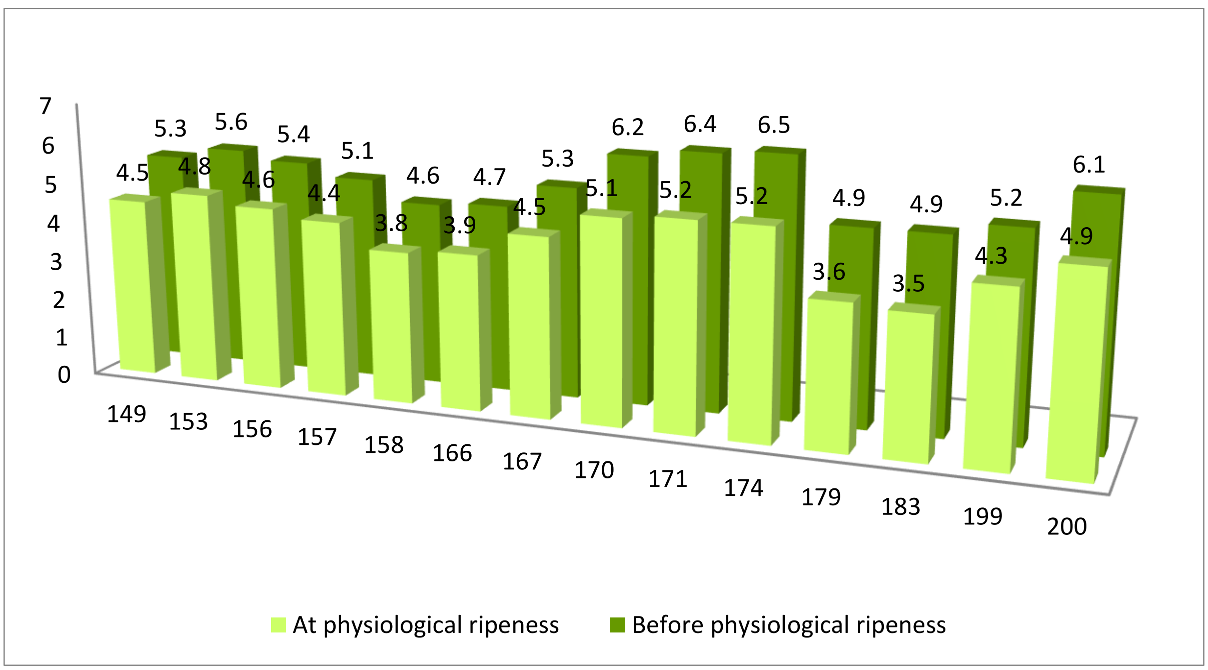


Fig. 5 Dry matter content % of peppers *Capsicum chinense* species.

Figure 6 shows that the sugar content in chili pepper fruits ranged from 0.8% to 2.8% at physiological ripeness, and from 1.5% to 4.5% before physiological ripeness. The following accessions exhibited the highest sugar content: 200, 171, 174, 170, 167, 199, and 157, with values of 2.8%, 2.6%, 2.2%, 2.2%, 2.1%, 1.9%, and 1.8% at physiological ripeness, respectively, and 4.5%, 4.5%, 4.1%, 3.4%, 3.1%, 3.4%, and 2.8% before physiological ripeness ($P < 0.05$).

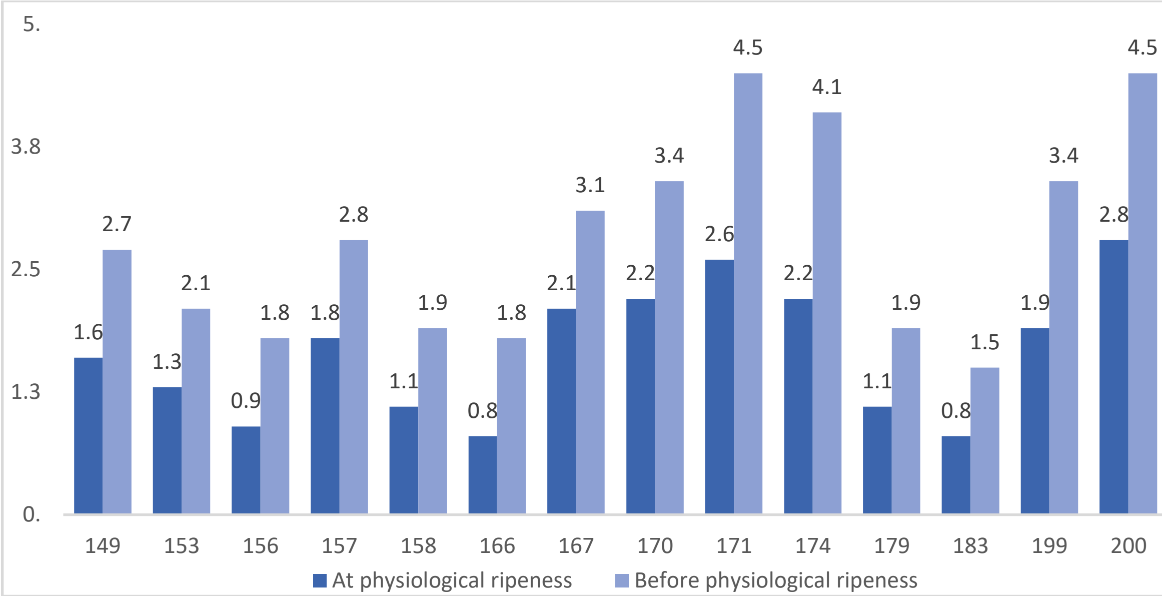


Fig. 6 Total sugar content % of peppers *Capsicum chinense* species.

Vitamin C is crucial for our overall health and well-being. It should be considered a functional food ingredient because it is an important bioactive compound with antioxidant properties [43-44]. Figure 7 shows that the vitamin C content in chili pepper fruits ranged from 32.85 to 53.85 mg% at physiological ripeness, and from 45.35 to 69.55 mg% before physiological ripeness.

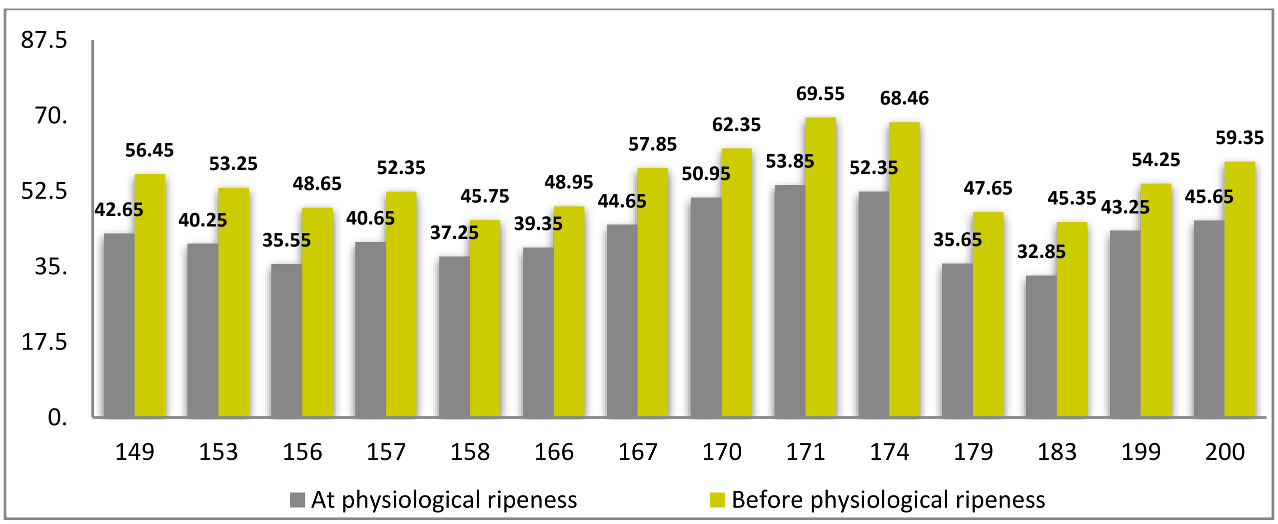


Fig. 7 Ascorbic acid content (mg%) of peppers *Capsicum chinense* species.

The following accessions exhibited the highest levels of vitamin C: 171, 174, 170, 200, 167, 199, and 149, with values of 53.85, 52.35, 50.95, 45.65, 44.65, 42.65, and 41.55 mg% at physiological ripeness, respectively, and 69.55, 68.46, 62.35, 59.35, 57.85, 56.45, and 53.45 mg% before physiological ripeness ($P < 0.05$).

CONCLUSION

Several genetic resources from the EVA Network *Capsicum chinense* pepper collection, conserved in Armenia, exhibited high anthocyanin levels and favorable biochemical properties in their fruits. The plants of different chili pepper accessions demonstrated varying rates of biological traits, phenotypic transitions, as well as diverse coloration and morphological shapes.

The total anthocyanin content in the accessions ranged from 0.11% to 0.98% by mass. Accessions 157, 199, 167, 149, 166, and 179 were particularly notable for their high anthocyanin content, with values of 0.98%, 0.94%, 0.61%, 0.42%, 0.35%, and 0.34% by mass, respectively. Furthermore, high dry matter content was observed in accessions 174, 172, 170, 200, 153, and 156, with values of 5.2%, 5.2%, 5.1%, 4.9%, 4.8%, and 4.6% at physiological ripeness, respectively, and 6.5%, 6.4%, 6.2%, 6.1%, 5.6%, and 5.4% before physiological ripeness. The following chili pepper accessions stood out with high sugar content: 200, 171, 174, 170, 167, 199, and 157, with values of 2.8%, 2.6%, 2.2%, 2.2%, 2.1%, 1.9%, and 1.8% at physiological ripeness, respectively, and 4.5%, 4.5%, 4.1%, 3.4%, 3.1%, 3.4%, and 2.8% before physiological ripeness.

The following chili pepper accessions were particularly notable for their high vitamin C content: 171, 174, 170, 200, 167, 199, and 149, with values of 53.85, 52.35, 50.95, 45.65, 44.65, and 42.65 mg% at physiological ripeness, respectively, and 69.55, 68.46, 62.35, 59.35, 57.85, and 56.45 mg% before physiological ripeness.

These accessions offer significant potential for breeding new chili pepper varieties and hybrids, as well as for the development of functional foods.

Author contributions K.S. and G.M. designed the research. K.S., M.G. provided variety *Capsicum chinense* research. A.Zh., G.S., D.Gh. performed biochemical analysis. G.K., V.V. G.Sh. performed statistical analyses. K.S., I.Ts., G.K. wrote the manuscript. K.S. and I.Ts. edit the article. All authors read and approved the final version of the manuscript.

List of Abbreviations: C. – Capsicum, EVA. - European Evaluation Network, ANOVA - Analysis of Variance.

Competing Interests: There are no conflicts of interest to declare.

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