



Study of anthocyanins in several genetic resources from the national eggplant collection of Armenia

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ABSTRACT

The main components of eggplant skin extract are anthocyanins, which play a vital role in determining the skin's color. Cultivated eggplants can potentially increase their anthocyanin content by crossing with various forms that synthesize different types of anthocyanins. This strategy could lead to eggplants with even higher antioxidant properties. Consuming products rich in anthocyanins is beneficial for preventing the development of serious diseases. Therefore, special attention should be paid to the selection of eggplants due to their high content of these biologically active substances.

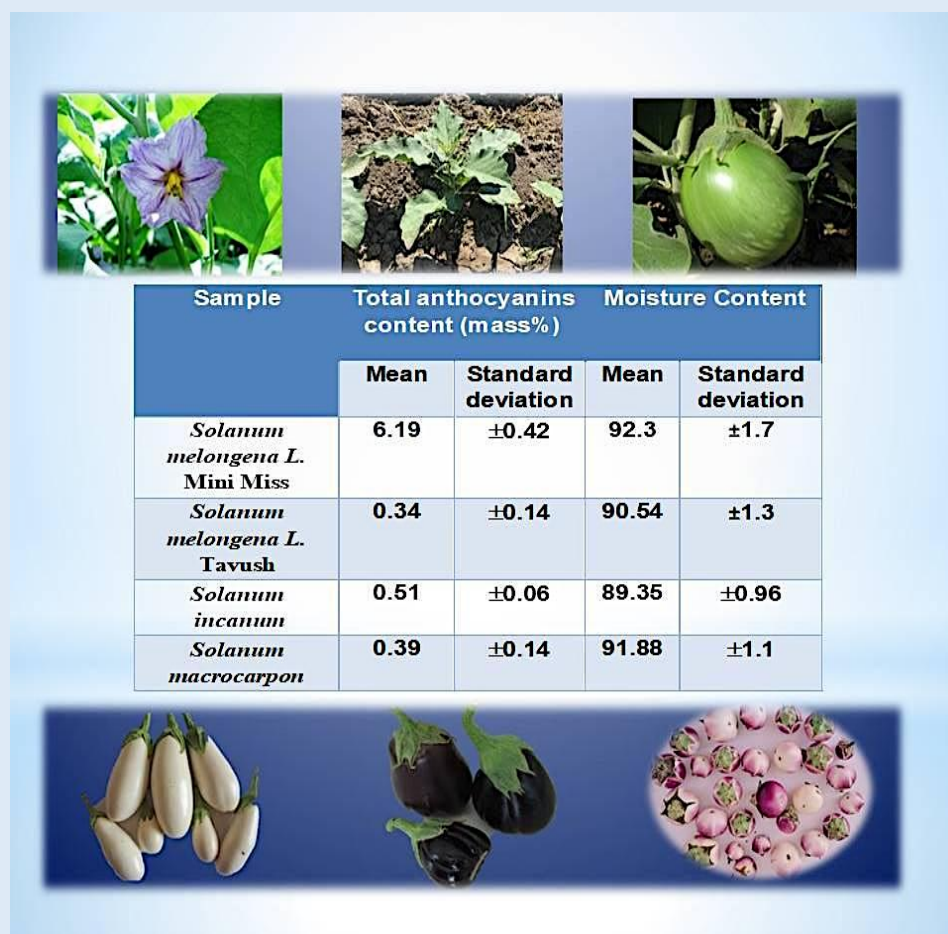
Objective: The study focuses on the effectiveness of various eggplant genetic resources conserved at the National Eggplant Collection in Armenia, specifically examining the anthocyanin content in eggplant fruits.

Methods: Experiments were conducted at the experimental station of the Scientific Centre of Vegetable and Industrial Crops in Darakert, Ararat Marz of Armenia during 2022-2023. For these experiments, several genetic resources from the Armenian National Eggplant collection were utilized, including the brinjal (*Solanum melongena* L.) varieties Mini Miss and Tavush, and wild relatives of eggplant, introduced from Taiwan and species from The World Vegetable Center (AVRDC), namely *Solanum macrocarpon* and *Solanum incanum*. The quantitative determination of the total anthocyanins was carried out using pH differential spectrophotometry. The experimental data was subjected to two statistical processing methods. Analysis of Variance (ANOVA) and Hierarchical Cluster Analysis.

Results: The total anthocyanins content in eggplant fruits is 0,34-6,19(mass%), with a standard deviation of ± 0.06 ± 0.42 respectively. The moisture content in the eggplant fruits is 89,35-92,3 (mass%), with a standard deviation $\pm 0,96$ $\pm 1,7$. They have several promising anthocyanin-rich varieties such as *Solanum melongena* L. (Mini Miss and Tavush), and wild relatives from the deceased Tawan and species from the World Vegetable Center (AVRDC), namely *Solanum macrocarpon* and *Solanum incanum*,

Conclusion: Several genetic resources of the national eggplant collection, including Armenian varieties such as *Solanum melongena* L. (Mini Miss and Tavush), as well as wild relatives from the deceased Tawan and species from the World Vegetable Center (AVRDC), namely *Solanum macrocarpon* and *Solanum incanum*, exhibited high anthocyanin content. These valuable resources can play a crucial role in breeding new eggplant varieties and hybrids, as well as in creating innovative forms of functional food.

Keywords: eggplant, anthocyanins, genetic resources



Graphical Abstract: Study of anthocyanins in several genetic resources from the national eggplant collection of Armenia

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INTRODUCTION

In the science of functional food, there have been numerous studies in different countries of the world recently. These studies have contributed to the development of both traditional and modern recipes to develop the use of functional food [1-3]. The mandatory feature that qualifies a food as a functional food is the presence of biologically active substances or compounds that can prevent a disease [4-5]. Biologically active compounds are contained in both prepared and processed products of various fruits, berries and vegetables, including eggplant [6]. Eggplant (*Solanum melongena* L.) is the third most important culture from the nightshade family. With its origins from India where it was introduced to other regions, eggplants most natural form is still found in India. The secondary region where a rich variety of forms of this plant can be found is on the territory of China and other South-East Asian countries. Given its early origins, the history of eggplant culture is very old [7-8].

During the technical maturity phase, eggplant fruits contain a rich array of nutrients, including sugar, starch, pectin, organic acids, vitamins C, B1, B2, B6, PP, carotene, and macro and microelements. Among the amino acids, eggplants mostly contain glutamine and asparagine [8].

The consumption of eggplants in food has shown a reduction of cholesterol in the blood. Similarly, dried eggplant powder can also be used for to provide the same benefits. Typically, eggplants are harvested 25-40 days after the fruit set, when their weight reaches more than 50 g, and the seeds are not yet hardened [9-10].

Fruits of eggplants are used as an independent dish and in the form of preserves. Caviar, stuffed, marinated, fried with slices, commonly known as sauté, are made from fruits. The eggplant fruits are dried, salted. In the southern regions, salted eggplants often replace salted mushrooms, while the peoples of Central Asia and the Caucasus widely use eggplants are very widely used in various national dishes. Their taste is due to the presence of a small amount of sugar and solanine M (melongene -

C31H51NO12), which has a tonic effect on the body and helps reduce the cholesterol content in the blood [11-12].

Modern medicine has established that eggplants have a hypocholesterolemic effect, making them a recommended dietary remedy for the treatment and prevention of atherosclerosis, gallstones, and kidney stones. The high potassium content in eggplants improves heart function and increases diuresis. As a dietary remedy, they are particularly beneficial for cardiovascular diseases, edema of cardiac, renal and hepatic origin, and treatment of gout. Eggplants are especially useful for anemia patients, where including 100-200 g of eggplants in their diet 4 times a day can eliminate their need for supplemental iron, copper and zinc consumption [13-15].

Phenolic compounds, which are natural heteroaromatic compounds, play an important role in plant biological processes and contribute to the bright colors of flowers and fruits [16-18]. Numerous studies show that flavonoids, a subgroup of plant polyphenolic compounds, are effective antioxidants involved in many oxidative systems that remove of free radicals in the presence of metals [19-20]. Their activity is linked to the presence of OH-groups in their molecular structure [21]. Many clinical and experimental studies have investigated the antitumor effects of flavonoids. It was found that their antiproliferative effect are comparable to modern antitumor agents. Flavonoids can suppress carcinogenesis by affecting the processes of initiation, progressive growth, and tumor metastasis. [21-22]. Eggplants are a significant source of phenolic compounds [22-23].

A study of the content of total phenols in more than 100 varieties of *S. melongena*, including traditional varieties, commercial varieties, wild species, and hybrids between wild species and cultivated varieties, revealed significant variation in phenol levels. Traditional varieties had a higher phenol content than commercial varieties and wild species, highlighting the culture's potential for

accumulating phenolic compounds and emphasizing the importance of selecting varieties with higher phenolic content [24]. Using four different analyzes (ABTS, DPPH, ILP, and SOS) to evaluate the antioxidant activity of 120 types of vegetables, the eggplants were ranked among the top ten vegetables with the highest antioxidant activity [24-25]. Eggplant skin extract, mostly comprised of anthocyanins, has a high ability to trap free radicals and inhibits the synthesis of hydroxyl radicals [25]. Anthocyanins are the most important plant pigments visible to the human eye, making them the main biologically active substance of the eggplant skin that determines its color. They represent a widespread class of phenolic compounds and belong to the group of flavonoids. In higher plants, 6 anthocyanins are most often found - pelargonidin, peonidin, cyanidin, malvidin, petunidin, delphinidin, but according to various literary sources, more than 400 anthocyanins are found in nature [26-27]. Anthocyanins are present in fruits, vegetables, many grains, and wine [28-29]. Many studies have been conducted on the effects of anthocyanins on animals and humans, hinting at the following basic physiological functions: antioxidant activity, antimutagenic effects, anticancer properties that suppress the development of cancer cells and metastases in blood vessels, increased visual acuity, and a reduced risk of atherosclerosis [29-37]. Eating products rich in anthocyanins prevents the

development of many serious diseases, making it necessary to pay special attention to the selection of eggplant on the increased content of these biologically active substances.

The scientific novelty of this research lies in its focus on the antioxidant, biological, morphological and economically valuable properties and characteristics of the varieties bred in Armenia, along with wild relatives from the World Vegetable Center. These studies identify the best parental forms for hybridization, enabling the breeding of new varieties and hybrids with high antioxidant properties. The further use of these varieties in food may contribute to cancer prevention.

MATERIAL AND METHODS

Germplasm material: For these experiments, several genetic resources from the Armenian National Eggplant collection were utilized. These resources included the Mini Miss and Tavish brinjal (*Solanum melongena* L.) varieties, and the wild relatives of eggplant introduced from Taiwan and The World Vegetable Center (AVRDC), namely *Solanum macrocarpon* and *Solanum incanum*. Mini Miss and Tavush were bred in Armenia by the researcher and licensed for the research. Moreover, the Tavush variety was also patented in Russia, but already under the name Snezhni. The researcher has received copyright certificates from both countries.

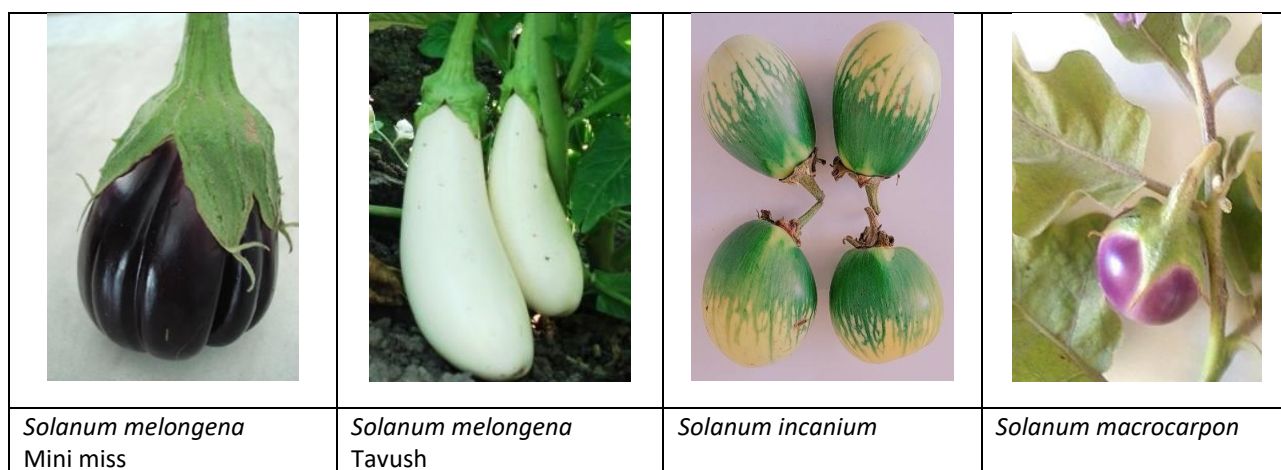


Fig.1. Fruit of eggplants of species *S.melongena*, *S.incanium*, *S.macrocarpon*

Experimental design: Between 2022 and 2023, research was conducted in the village of Darakert, located in Ararat Marz region of Armenia. The experimental setup followed a block-randomized approach design into four replications [38].

Seed germination, transplanting, and plant growth:

Eggplants seeds were sown in mid-May, and the seedlings were transplanted into the open field. The planting layout adhered to a 90x70x30 cm pattern. Throughout the growing season, essential agricultural practices, including irrigation, fertilization, mulching, and pesticide application, were implemented to manage common diseases and pests. Fruits began reaching biological maturity by mid-July, with mass ripening starting in August depending on the variety. At this stage, the ripe eggplants were harvested for subsequent biochemical, the results of which are detailed in this article.

Fruit compositional quality:

Anthocyanins content determination: The quantitative determination of the total anthocyanins was performed using pH differential spectrophotometry.

Preparation of buffers: Buffer 1: A buffer containing 1.86 g of potassium chloride was dissolved in approximately 980 ml of distilled water, concentrated hydrochloric acid was added, and pH was adjusted to 1. The volume of the solution was adjusted to the mark in a 1000-ml volumetric flask.

Buffer 2: 54.43 g of sodium acetate trihydrate was dissolved in 980 ml of distilled water; glacial acetic acid was added until pH = 4.5; and the volume of the solution was adjusted to 1000 ml.

Sample preparation: 25 ml of 70% ethanol was added to a sample weighing 2.5 g. The sample was shaken and heated in a boiling water bath with reflux for 15 minutes. The sample was cooled to room temperature, and its volume was adjusted to the initial (25 ml) volume with 70% ethanol. The solution was filtered.

Optical density measurement: 1 ml of filtrate was placed in six test tubes with a volume of 15-20 ml. 9 ml of buffer 1 was added to test tubes No. 1, 2, and 3, and 9 ml of buffer 2 was added to test tubes No. 4, 5, and 6. The tubes were carefully shaken. After this, the optical density of the resulting solutions was measured at wavelengths of 510 and 700 nm. For statistically significant results, experiments are repeated three times.

The optical density was calculated using the formula:

$A = (A_{510} - A_{700})_{pH=1} - (A_{510} - A_{700})_{pH=4.5}$, where A_{510} is the optical density of the solution at a wavelength of 510 nm and A_{700} is the optical density of the solution at a wavelength of 700 nm.

pH=1: optical density of the solution in the specific pH,

pH = 4.5: optical density of the solution in the specific pH

The total quantity of anthocyanins was calculated in terms of cyanidin-3-glucoside by the formula

$C = (A \times 449.5 \times F \times V \times 100\%) / (26900 \times l \times m)$, where C- is the total quantity of anthocyanins calculated for cyanidin-3-glucoside at mass%.

A-optical density, 449.5 molecular mass of cyanidin-3-glucoside, g/mol

F-dilution factor,

V-volume of extract, ml; 26900-molar absorption coefficient of cyanidin-3-glucoside;

l-cuvette length, cm;

m-mass of the sample, g.

Determination of the Moisture Content of Fresh Fruits:

Moisture content was assessed using an Ohaus Moisture Analyzer (MB45). Fresh fruits were subjected to heat at

100°C until all water evaporated, and a constant weight was achieved. Each measurement was conducted in triplicate to calculate an average value. All data are represented as mean \pm standard deviation.

The study evaluated bioactive compounds, dry matter, and total sugars in pepper fruits at both technical and biological ripeness across all experimental conditions. Ascorbic acid content was determined using a titration method. Water-soluble vitamins were quantified using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA) was employed at a wavelength of 445 nm in relation to a 0.1N HCl solvent.

Statistical analyses: The experimental data was subjected to two statistical processing methods.

Analysis of Variance (ANOVA): Differences among accessions and within each VG were identified using the Least Significant Difference (LSD) method at a significance level of $\alpha=0.05$. The experimental data were analyzed statistically through Analysis of Variance (ANOVA).

Hierarchical cluster analysis: Fourteen traits related to plant architecture, fruit shape, and quality were analyzed using Ward's coefficient for agglomerative hierarchical clustering in R. The circular dendrogram was then presented using the *circlize* R package for enhanced visualization.

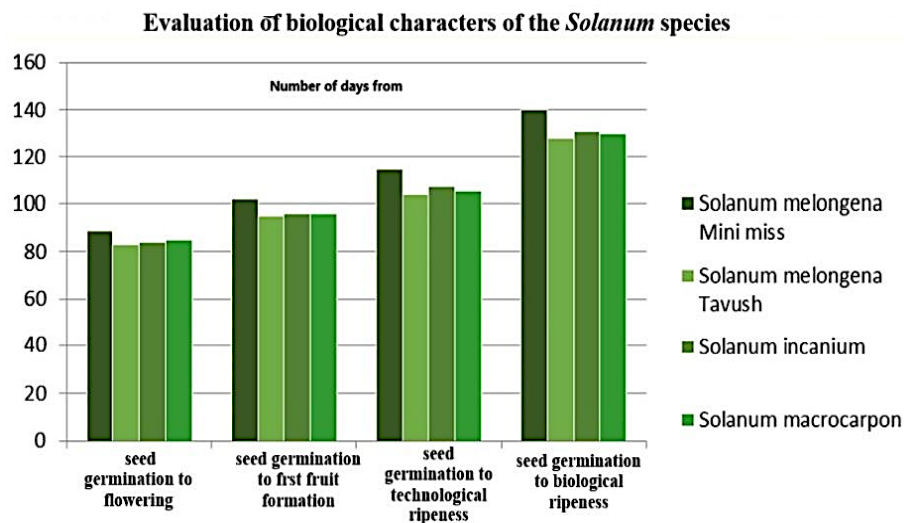


Fig. 2. Evaluation of biological characters of the *Solanum* species.

RESULTS AND DISCUSSION

Biological and Phenological characters evaluation:

In this research work, the study of the biological features of eggplant and the duration of the phenophase transition is highlighted by the fact that it is necessary to find out in which months the edible fruits ripen, which provide useful antioxidant properties in the form of anthocyanins. The results of the studies (Fig. 2) showed that in the plants of the mentioned types and varieties of eggplant, it takes 83-89 days from seed germination to flowering, 95-102 days from seed germination to the ripening of the first fruit, 105-115 days from seed

germination to the technical ripening of fruits, and 128-140 days from seed germination to biological ripening of fruits. In Armenia, people typically consume only the ripened fruits of eggplants, starting from July 1st. Regular harvesting provided quality fruits from July to October 10th. Tavush variety from *Solanum melongena* stood out for mid-ripening for 104 days, followed by *Solanum macrocarpon* for 105 days, *Solanum incanium* for 107 days, and Mini miss variety from *Solanum melongena* for 115 days. The first harvest of the fruits of this variety was done 15 days after the Tavush variety.

Evaluation of Phenological characters of the Solanum species

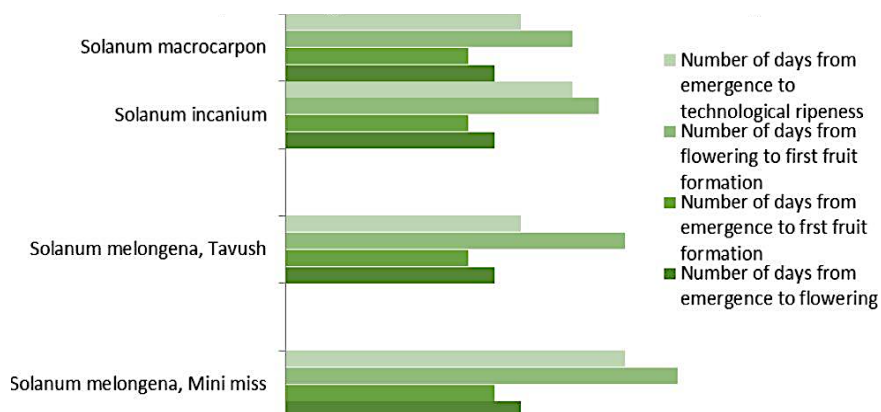


Fig. 3. Evaluation of phenological characters of the Solanum species

Studies of the duration of transition of phenological stages have shown that all varieties have passed through these stages in a very short period. Several authors believe that such a duration of biological characteristics and phenotypic transitions in mulberry vegetable crops

contributes to the synthesis of the highest amount of biologically active substances in the fruits obtained under open field conditions in mid-summer and early autumn under solar energy and day length, which improves biochemical and antioxidant properties [39-41].

Table 1. Evaluation of the morphological characters of the Solanum species.

Morphological characters	Solanum melongena Mini miss	Solanum melongena Tavush	Solanum incanum	Solanum macrocarpon
Bush color	green	green	green	Green
Presence of spikes on the bush	absent	few	absent	Absent
Plant height(cm)	60,4	75,5	45,6	50,3
Leaf length(cm)	18,6	20,5	21,3	15,4
Leaf width(cm)	11,7	14,5	15,8	9,6
Leaf colour	green	green	green	Green
Petal colour	violet	violet	violet	Violet
Flower diameter(cm)	6,5	6,8	6,3	6,1
Colour of fruits at technological ripeness	violet	white	green	White-violet
Presence of spikes on the fruit calyx	absent	few	absent	Absent
Fruit length(cm)	15,8	28,6	16,5	6,5
Fruit diameter(cm)	14,6	5,8	15,3	4,8
Fruit shape	oval	cylindrical	oval	Oval
Fruit flesh colour	white	white	green	Whiate
Change of fruit flesh colour at cutting(min)	3	3	4	4
Seed weight per fruit(g)	3,5	3	3,5	2
Seed colour	brown	brown	brovsn	Brovsn
1000-seed weight(g)	5,3	5	5,2	5,7

Table 1. shows the morphological data of the studied species and varieties.

In particular, the height of the plants, the size of the leaves, the shape of the fruits, the time of the color change after cutting the fruit, the color of the seeds and the weight of 1000 seeds were noted. All these are undoubtedly important data for the purpose of determining the heart shape of the fruits suitable for food and purchasing power and other characteristics of the plants of the studied species and varieties [42]. In this regard, our research aligns with the views of several authors who emphasize the importance of studying fruits in relation to agricultural practices (such as fertilizers, pesticides, and weed management) and environmental factors. Consequently, the high antioxidant content observed during mass harvesting is more pronounced in quality, healthy fruits compared to smaller, unmarketable ones [43-44].

Phenolic substances, including anthocyanins, have an important place in the antioxidant properties of eggplant. Anthocyanins affect the color of plants and fruits, which can be seen by the purple color of the fruit

peel. This indicated that the higher the amounts of anthocyanins in the fruit, the darker the eggplant fruits will be [45]. Among the varieties we studied, the technically ripe fruits of the *Solanum melongena* Mini Miss variety are purple, the *Solanum melongena* Tavush variety are white, *Solanum incanum* are green, and *Solanum macrocarpon* are white with a purple shade. However, the flowers of the Tavush variety are purple, which indicates the content of anthocyanin in them. In Russia, several scientists have studied the antioxidant properties of eggplant varieties with purple and white fruits. Moreover, the Snezhni variety bred by the researchers were selected from the white eggplant varieties. Through multi-faceted studies, it was found that this variety has a greater potential of containing other substances and genes characteristic the exhibit antioxidant properties. Since the fruits of that variety have a yellow-orange color in the stage of biological ripening and are not bitter, it is likely carotene content providing antioxidant properties to the fruits [45].

Table 2. Evaluation of the productivity characters of the *Solanum* species

Species/sorts	Fruit number per plant	Productivity per plant (kg)	Productivity per hectare(kg)	Fruit number per plant	Productivity per plant (kg)	Productivity per hectare(kg)
	2022			2023		
<i>Solanum melongena</i> Mini miss	12	3360	147 840 000	11	2970	130 680 000
<i>Solanum melongena</i> Tavush	24	4800	211 200 000	22	4180	183 920 000
<i>Solanum incanum</i>	8	2320	102 080 000	7	1960	86 240 000
<i>Solanum macrocarpon</i>	28	1400	61 600 000	25	1250	55 000 000
P-Value		0.000587109			0.028924313	
F critical		9.276628153			10.12796449	
LSD _{0.05}		2.30			2.40	
S _x %		1.26			1.28	

The analysis of yield data showed that *Solanum melongena* Tavush and *Solanum melongena* Mini miss varieties provided high yields in both years, with the yield

per plant being 4800 and 3360 kg in 2022, respectively, and 4180 and 2970 kg in 2023. All this contributed to their high yield per hectare.

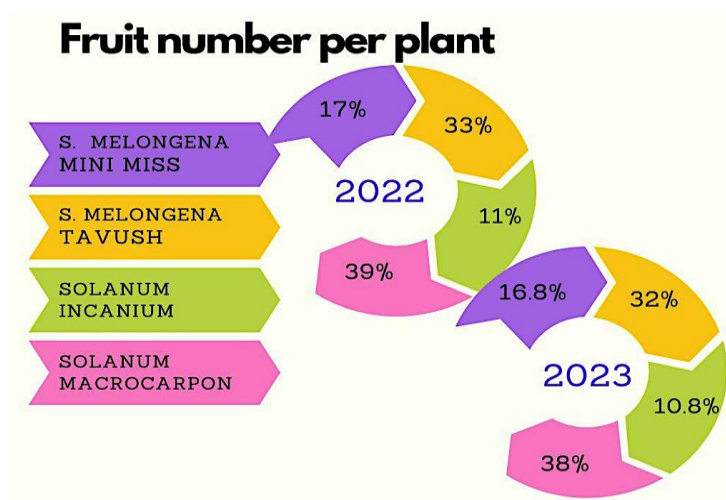


Fig. 4. Fruit number per plant of the *Solanum* species.

Solanum macrocarpon stood out with the number of fruits per plant in both years, which had 28 fruits in 2022 and 25 fruits in 2023. (Fig.3) After that, this number was 24 and 22 pieces, respectively, for the *Solanum melongena* Tavush variety. Although *Solanum macrocarpon* has the highest number of fruits, the weight of one fruit is very small, so the yield is low. One fruit of *Solanum melongena* Tavush variety weighs 3

times more than one fruit of *Solanum macrocarpon*. A high yield results in obtaining a large number of biochemical substances characterizing antioxidant properties, enabling people to consume these fruits for several months and increase their endurance in case of the most dangerous diseases. In this sense, our obtained results coincide with the opinions of a number of authors [45].

Table 3. Total anthocyanins content(mass%) of the *Solanum* species.

Sample	Total anthocyanins content (mass%)		Moisture Content	
	Mean	Standard deviation	Mean	Standard deviation
<i>Solanum melongena</i> L. Mini Miss	6.19	±0.42	92.3	±1.7
<i>Solanum melongena</i> L. Tavush	0.34	±0.14	90.54	±1.3
<i>Solanum incanum</i>	0.51	±0.06	89.35	±0.96
<i>Solanum macrocarpon</i>	0.39	±0.14	91.88	±1.1

The total content of anthocyanins in the studied types and varieties of eggplant is 0.34-6.19 mass %, and the moisture content is 89.35-92.3%. The highest results were recorded with *Solanum melongena* Mini miss

variety. This variety is valuable both for the breeding of new varieties and hybrids with high antioxidant properties, use as a donor, and processing in production.

CONCLUSION

The results of the studies showed that, in the plants of the mentioned types and varieties of eggplant, it takes 83-89 days from seed germination to flowering, 95-102 days from seed germination to the ripening of the first fruit, 105-115 days from seed germination to the technical ripening of fruits, and 128-140 days from seed germination to biological ripening of fruits.

Phenolic substances, including anthocyanins, significantly contribute to the antioxidant properties of eggplant. Anthocyanins not only enhance antioxidant activity, but also influence the coloration of plants and fruits. Among the varieties we studied, the technically ripe fruits of the *Solanum melongena* Mini Miss variety are purple, *Solanum melongena* Tavush are white, *Solanum incanum* are green, and *Solanum macrocarpon* are white with a purple shade. However, the flowers of the Tavush variety are purple, which indicates the content of anthocyanin in them.

The analysis of yield data showed that *Solanum melongena* Tavush and *Solanum melongena* Mini miss varieties provided high yields in both years: the yield per plant was 4800 and 3360 kg in 2022 and 4180 and 2970 kg in 2023. All this contributed to their high yield per hectare. Although *Solanum macrocarpon* has the highest number of fruits, the weight of one fruit is very small, resulting in lower overall yields. For example, one fruit of *Solanum melongena* Tavush variety weighs 3 times more than one fruit of small-fruited *Solanum macrocarpon*. Higher yields are directly correlated with increased availability of biochemical substances with antioxidant properties, which can extend the consumption period of these fruits and potentially improve their endurance against dangerous diseases.

The total content of anthocyanins in the studied eggplant types and varieties ranged from 0.34% to 6.19% by mass, while the moisture content ranged from 89.35% to 92.3%. The highest anthocyanin concentrations were

recorded in the *Solanum melongena* Mini miss variety, highlighting its value for the breeding of new varieties and hybrids with high antioxidant properties, use as a donor, and processing in production. These valuable resources can play a crucial role in breeding new eggplant varieties and hybrids, as well as creating innovative forms of functional food.

Author contributions: K.S. designed the research. K.S., M.G. provided a variety of Mini Miss, Tavush of *S. melongena*, *S. incanum*, *S. macrocarpon* for research. A.Zh., G.S., D.Gh. performed biochemical analysis. G.K., V.V. performed statistical analyses. K.S. and G.K. wrote the manuscript. K.S. edit the article. All authors read and approved the final version of the manuscript.

List of Abbreviations: S. - *Solanum*

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

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