

Chapter

Genotype and Phenotype Diversity in *Malus domestica* Borkh Species – Perusha Variety

Boryana Stefanova, Petko Minkov and Svetoslav Malchev

Abstract

The study was conducted in the period 2020–2022, in the conditions of the Central Balkan Mountain region (600 to 1200 m above sea level), around the town of Troyan and its adjacent villages and hamlets. Some old local apple forms of Perusha variety were discovered and marked, which are threatened with extinction. The genetic diversity of the local specimens of Perusha variety type was evaluated, and the pomological and reproductive properties of some forms were characterized, to select potential cultivars for further cultivation or selection. It was found that all forms have a green fruit skin color, and the fruit flesh in most cases is juicy, slightly sour, with aroma. All the studied forms of the group have different valuable economic qualities and different directions of use; therefore, it can be confidently stated that there is a perspective for their introduction into practice, in creating new orchards for sustainable fruit production.

Keywords: biodiversity, local genotype, physicochemical, pomological properties, resistance

1. Introduction

With the spread of commercial apple cultivars since the second half of the last century, hundreds of different local cultivars quickly disappeared from orchards. The specific quality characteristics of these fruits are still at risk of being lost today. Old apple cultivars (*Malus domestica* Borkh.) are characterized by good morphological and pomological properties, less need for chemicals in their cultivation, and a higher share of biologically active compounds (BACs). Their sensory acceptability is better compared to commercial cultivars and is of interest for biodiversity conservation [1].

The Republic of North Macedonia studied 13 autochthonous apple cultivars and found that some of them deserve to be grown in traditional orchards in typical rural areas, with reduced use of chemicals and in environmental protection programs. However, the local gene pool is of great socio-cultural importance, and the cultivars should be preserved *in situ* [2].

In two-year study in Serbia on the main biological properties of 10 *ex situ* apple genotypes, such as Bihorka, Budimka, Kraljica, Ovčiji Nos, Petrovača-1, Strekinja, Šimun Viparoš Struga, Šumnjaja, Tip 1, and Zejtinka, the best fruit quality among the

assessed genotypes was found in Ovčiji Nos according to the fruit's chemical composition. All tested genotypes showed field resistance to fire blight and susceptibility to apple scab and mildew [3].

Vrtodusic & Skendrović [4] evaluated the pomological and physicochemical characteristics of 13 traditional Croatian pear varieties, which were studied in the Karlovac region, Central Croatia, to select potential cultivars for further cultivation or selection. The analyzed characteristics showed that the varieties differed significantly in their fruit weight, height and width, fruit index, length and thickness of stalk, fruit firmness, soluble solids content, acidity, ratio of soluble solids to total acidity, and pH. Varieties, such as Krasanka and Dugačka are prominent for the large fruit size and the harmonious ratio of soluble solids and acids. The Tepka variety stood out for its high content of soluble solids, which makes it a valuable raw material for distillation.

Crop Wild Relative (CWR) species are more or less closely related to the cultivated variety, as they include crop ancestors and are a potential source of characteristics beneficial to our crops. Given their importance for agricultural research and development, their conservation is a high priority, particularly their *in situ* conservation, which allows for the continuous evolution of new adaptive characteristics, as well as maintaining the breadth of genetic diversity present in many CWR species [5].

The main parameters related to quality are sugar and acid content, color, firmness, texture, juiciness, taste, nutritional value, absence of diseases or insects, and general appearance [6]. Old cultivars show good resistance to biotic and abiotic stress factors, as they are characterized by different morphological and pomological characteristics compared to commercial apple cultivars.

Old apple varieties offer great potential for the production of functional products due to their antioxidant activity, which neutralizes free radicals, preventing the formation of cellular damage in the fruit. Dietary fiber found in apples is also a plant substance with many benefits for human health, such as reducing fat and cholesterol absorption, normalizing digestion, maintaining gut health, and helping to control diabetes [7].

Consumers are becoming increasingly aware of the importance of a high intake of antioxidants and beneficial elements in the diet; therefore, attention is paid to foods rich in bioactive compounds that are organically grown, without any chemical substances. The restoration of old apple varieties due to their particular composition, taste, resistance to pathogens, and adaptability to climate and soil can meet this requirement [8].

In the face of global climate change and the invasion of new pests, the local gene pool is a vital step toward sustainable agriculture. Local varieties and forms are generally considered as better adaptable to the conditions of the region of origin than widespread varieties grown elsewhere [9].

Sadova Perusha variety in a private orchard in the area of Cherni Vrah (Trojan region) was found [10]. The tree has a spherical crown with a height of 9.20 m. The fruits are large over 190 g with a height of 63.20 mm, green color, with a pleasant taste with a balanced sugar-acid ratio. The variety is slightly susceptible to apple scab. Large-sized fruit forms and varieties that found suitable soil and climatic conditions for cultivation, appreciated by the local population and well accepted in the studied areas, such as Winter Green, Troyanka, Tsiganka, Reinette du Canada, Lemonki, Perusha, and several other local forms have been reported [11]. They are the basis of the local processing industry for making dried fruits, marmalades, juices, brandy, pectin, vinegar, etc.

Local varieties are described, in several of our studies, as being very well adapted to the agroecological conditions of the Central Balkan Mountain region, with large fruits, very good taste qualities, attractive appearance, suitable for long-term storage, relatively resistant to diseases and pests. Thus, it is required to search, preserve, and study them. They are suitable for nonconventional fruit plantations, such as family farms, as well as for their inclusion in selection programs, to improve some of their qualities.

The objective of the present research is to evaluate the genetic diversity of the local specimens of Perusha variety, to characterize the pomological and reproductive properties of some forms for the agroecological conditions of the Central Balkan Mountain region, and to select potential varieties for further growing and selection.

2. Material and methods

The study was conducted in the period 2020–2022. During the expeditions in the Central Balkan Mountain, old local apple varieties, threatened with extinction, were discovered, marked, and described in the region of Troyan and its adjacent villages and hamlets. The altitude of the studied areas is from 600 to 1200 m. That factor largely determines the period of ripeness. For forms 1 to 6, which were collected in the region of the village of Balkanec (altitude 600–800 m), the period of ripeness was the end of September 25–30. For forms 7 to 14, which were collected in the region of the town of Apriltsi (800–1100 m above sea level), the fruits ripen later, from the beginning to the middle of October.

The studied trees are over 80 years old, with a large trunk circumference (over 1.5 m), a crown height of over 15 m, with strong vigor. They were engrafted at a height of 30–40 cm from the ground and some at 1.20–2.00 m.

The shape of the crowns varies from globose to freely growing conic. The shape and size of the leaf blade are characteristic of Perusha variety. It has approximately the same length and width, which gives it an ellipsoid shape, large size, and dark green color. The leaf blade margin is entire and approximately uniform-integrated.

The trees bear fruit abundantly, but there is a distinct alternateness. Marked trees are single or in abandoned old orchards, along small hamlets, often overgrown with forest vegetation, without the application of any agrotechnical and pomological measures. Drying is often observed on the trunk and crown. Under these conditions, the fruitfulness and yields are of the order of 120–200 kg per tree. No symptoms of economically important diseases were observed.

Some large-fruited forms F10 and F11, with white flesh, were collected, stored and grafted on seed rootstock. Next year, they will be observed in a new field plantation.

The following indexes are taken into account:

- Reproductive – fruit weight (g), fruit size (mm), (height and average diameter) and fruit stalk length (mm)

Fruit - Observations on the fruit should be made on 10 typical fruits taken from a minimum sample of 20 fruits, at the time of ripeness for eating [12].

- Soluble solids (%)
- Sugars (%) (total, invert, and sucrose) – according to Schoorl and Regenbogen method

- Acids (%) – by titration with 0.1n NaOH
- Pectin (%)
- Glucoacidimetric index, ratio total sugars (%), and organic acids (%)
- Density (firmness) of fruit flesh (kgf/cm²) – determined with a digital penetrometer FHT-15 (3.5 mm), by measuring both sides of 25 randomly selected fruits. The fruit skin of the measured fruits was removed.
- Pomological characteristics:
 - sensory analysis (taste, aroma)
 - color parameters measured with Color meter CM-200S, reported according to the CIELab system of the fruit skin L, a and b;

L – color brightness (L = 0 – black, L = 100 – white); a – the positive values of the indicator display the amount of red, while the negative values signify the intensity of green; b – the positive values are determinant of the yellow hues, while the negative values indicate blue; *H* (hue angle) = b/a. In the evaluation of *H*, we used the most widely accepted international criterion of assigning the angle. The value of the color tone or the dominant wavelength is represented by the a/b ratio [13].

The biochemical composition and color parameters of the fruits (**Tables 1 and 2**) were analyzed in six forms that are typical because the rest are similar and almost repetitive.

A visual assessment was made of the response of the observed varieties to the economically significant diseases, such as apple scab and powdery mildew on leaves and fruits. Low susceptibility based on single spots or absence of apple scab spots on leaves and fruit was reported and no powdery mildew symptoms were detected.

	Soluble solids (%)	Total sugars (%)	Inverted sugars (%)	Sucrose (%)	Acids (%)	Glucoacidimetric index	Vit. C (mg/%)	Pectin (mg/%)
F1	15.5	9.40	5.00	4.18	0.64	14.69	14.08	1.98
F2	13.6	10.20	6.50	3.52	0.64	15.94	17.60	0.83
F3	15.5	7.35	4.50	2.71	0.64	11.48	10.56	1.40
F4	18.1	10.75	8.55	2.09	0.77	13.96	22.88	0.66
F5	14.7	8.05	7.70	0.33	0.63	12.78	17.60	0.72
F6	17.0	18.40	18.4	—	0.34	54.12	12.32	0.64
<i>av</i>	15.08	10.58	8.54	2.16	0.62		17.60	0.97
<i>St Dev</i>	1.74	3.22	4.02	1.21	0.11		4.69	0.44
CV %	11.54	30.43	47.12	55.97	18.16		26.67	45.81

Table 1.
Biochemical composition of fresh fruit.

	L	a	b	a/b	H = (b/a)	Density (kgf/cm ²)	Brix (%)
F1	53.30	9.16	68.64	0.14	7.50	5.12	17.60
F2	70.49	−4.71	78.97	−0.06	−16.76	7.18	14.00
F3	66.13	−4.21	66.99	−0.06	−15.91	8.74	15.00
F4	35.13	29.89	25.69	1.34	0.86	8.28	13.00
F5	74.68	1.95	119.22	0.02	61.01	9.39	16.80
F6	74.90	−7.37	54.90	−0.13	−7.45	8.59	16.00

Table 2.
Fruit skin color parameters.

3. Results

The Test Guidelines of UPOV gives detailed practical guidelines for studying and identifying, through appropriate characteristics for researching distinctness, uniformity and stability, (DUS), and the development of harmonized descriptions of new varieties.

According to No 24 in the table of characteristics, the corresponding UPOV scale for fruit size is from 1 to 9. The studied forms of the variety are extremely large-fruited and belong to categories 7 – large (Mutsu); 8 – large to very large (Bramley's Seedling); and 9 – very large (Howgate Wonder) (**Table 3**).

F10 has the largest fruit weight of 220 g, followed by F3–192.63 and F1–191.59 g. In contrast, F6 has the smallest fruit weight with 116.80 g (**Figure 1**).

	Fruit weight (g)	Fruit height (mm)	Fruit diameter average (mm)	Fruit stalk length (mm)
	X ± StDev	X ± StDev	X ± StDev	X ± StDev
F1	191.59 ± 10.73	64.83 ± 4.15	77.73 ± 2.33	22.21 ± 5.41
F2	176.75 ± 10.43	61.43 ± 4.02	76.29 ± 1.21	8.87 ± 0.20
F3	192.63 ± 27.26	65.33 ± 4.84	77.70 ± 2.84	8.68 ± 1.15
F4	156.50 ± 27.72	62.97 ± 1.87	74.97 ± 2.10	11.04 ± 1.95
F5	187.08 ± 37.61	62.83 ± 4.38	80.08 ± 5.70	9.10 ± 1.19
F6	116.80 ± 12.70	49.79 ± 3.54	64.00 ± 4.17	13.54 ± 3.65
F7	132.60 ± 16.43	55.02 ± 1.92	67.50 ± 4.56	10.78 ± 1.55
F8	146.50 ± 5.92	60.67 ± 5.61	75.54 ± 1.65	11.06 ± 1.39
F9	154.60 ± 10.78	55.33 ± 1.32	76.15 ± 2.38	10.15 ± 1.58
F10	202.40 ± 29.80	78.00 ± 2.35	65.50 ± 5.93	11.80 ± 2.28
F11	189.00 ± 38.20	68.60 ± 6.19	81.10 ± 4.15	10.60 ± 1.14
F12	147.60 ± 17.07	54.40 ± 2.07	71.90 ± 5.93	17.40 ± 1.52
F13	127.80 ± 18.95	58.00 ± 3.39	67.30 ± 3.08	19.20 ± 3.35
F14	119.78 ± 9.26	54.63 ± 3.14	67.46 ± 3.43	8.56 ± 0.88

Average values indicate the property according to the LDS test with $p \leq 0.05$.

Table 3.
Morphometric indices.

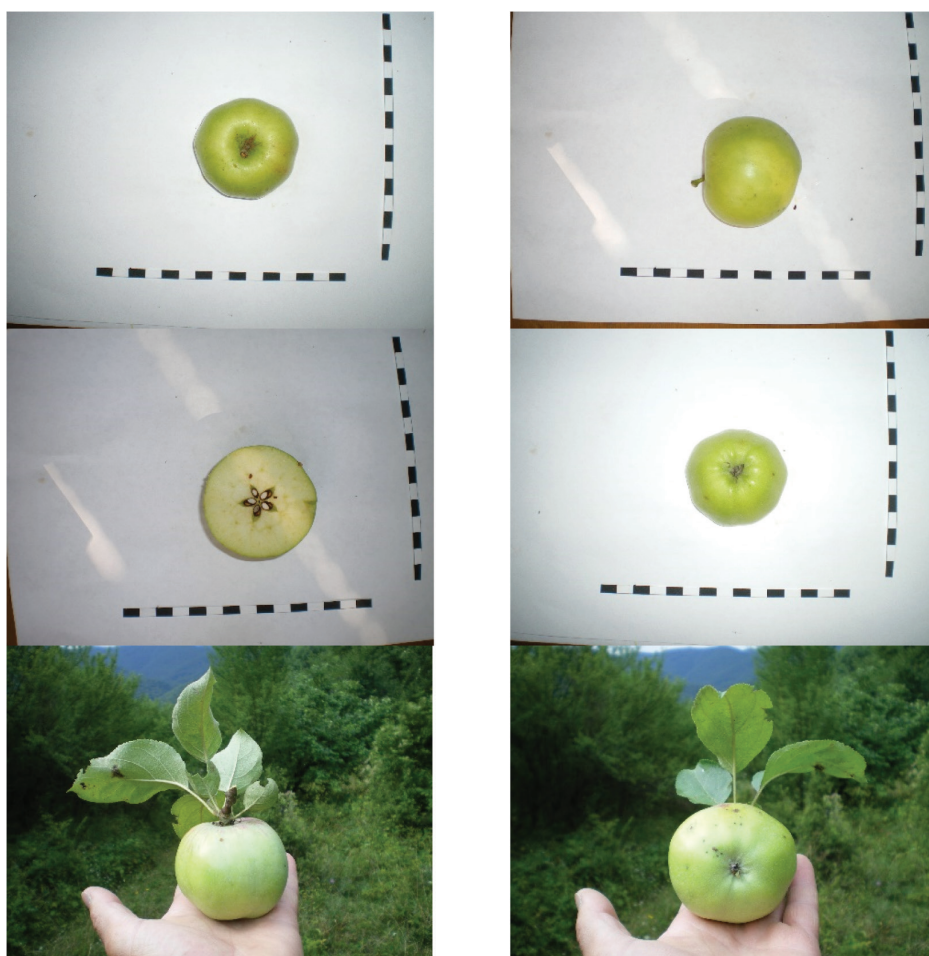


Figure 1.
The apple forms F6.

Fruit shape (No. 28 according to UPOV) is specified as Obloid 7 (symmetrical in diameter, wider than tall). The data on the height and average fruit diameter show that (Table 3). The greatest height is registered for F10 (78 mm), whereas the largest diameter is registered for F11 (81.10 mm).

Bozovic, et al. [14] indicate old cultivars that are of interest for production on a larger scale, due to their biological and economic properties. In the apple varieties, they observed ripening occurs from mid-July to mid-October for the Northern Montenegro region. The largest fruit size was found in Ilinjača (167.50 g), Dunjka (170.15 g), and Moračka krstovača krupna (182.34 g). Most cultivars have a round-flat and round-conical shape. The main skin colors of fruits in these varieties are green, greenish-yellow, and yellow, and an additional color (red or pink) is present in varying percentages. This is largely consistent with the present results.

The fruit stalk length of the apple forms (No. 46 according to UPOV) is determined as very short 1 (F2; F3; and F14); short 3 (F5; F7; F9; and F11); very long 9 (F1; F13; and F12).

Table 4 shows the following.

Form Perusha	Coloring of fruit, taste qualities
F1	Fruit skin – green covered with russet and a slight blush, thick, firm. Fruit flesh – whitish, tender, soft, juicy with crystal structure, slightly sour, with aroma
F2	Fruit skin – green with gray spots and a little russet at the stalk area, thick and tough. Fruit flesh – greenish, firm, but not very firm, dense, juicy, slightly sour, with aroma.
F3	Fruit skin – green yellowish with gray spots, a little russet and blush from eye basin, thick, tough, dense, tough. Fruit flesh – whitish, dense, crispy, with aroma.
F4	Fruit skin – green with golden over color at the stalk area, covered with gray spots, thick, greasy, with an abundant wax coating. Fruit flesh – light green, tender, juicy, sweet, with a slight aroma.
F5	Fruit skin – yellowish green, sprinkled with brown spots and russet at the stalk areas. Fruit flesh – white, soft, slightly floury, dry, sweet, without aroma.
F6	Fruit skin – green, thick, covered with an abundant wax coating. Fruit flesh - white, dense with granular structure, sour, crispy with aroma
F7	Fruit skin – yellow to intensively yellow, thick, firm with a wax coating. Fruit flesh – white with floury texture, dry, astringent, without aroma.
F8	Fruit skin – light green with blush on the sunny side, thick, tough, dense. Fruit flesh – white, tender, juicy, sweet, without aroma.
F9	Fruit skin – green with intensive yellow over color, tough, dense, with an abundant wax coating. Fruit flesh – white, very soft, juicy, slightly sour with aroma.
F10	Fruit skin – greenish with a wax coating, thick, dense, tough. Fruit flesh – white, slightly sour to sour, tender, average sour, with a slight aroma.
F11	Fruit skin – light green, smooth, dense, tough with wax coating. Fruit flesh – white, sweet to slightly sour, juicy with aroma.
F12	Fruit skin – green with russet at the stalk areas, thick, dense, tough. Fruit flesh – white, soft, average sour, aroma.
F13	Fruit skin – light yellow with predominant blush, thick, dense, tough, covered with a wax coating. Fruit flesh – yellowish, comparatively tender, sweet, and juicy, without aroma.
F14	Fruit skin – green with yellowish over color and blush, tough, dense. Fruit flesh – white, soft, sweet to slightly sour, with aroma.

Table 4.
Pomological description of the forms of Perusha variety type (2021).

Fruit skin: It is mainly green to yellow-green for the group of PERUSHA variety, often with russet at the stalk area, thick, thin, rough, or tough.

Fruit: ground color (No. 35 according to UPOV).

The color of the fruit skin is a characteristic trait because most forms F1; F2; F3; F4; and F6 (**Figure 1**); F9; F10; and F14 are green (6 green Granny Smith according to UPOV) to light green (5 yellow-green Cox's Orange Pippin According to UPOV) F8; F10; F11.

Forms F5; F7; and F13 are in the yellow range of the color scale (No. 3 is yellow with standard varieties Delorgue, Gala, Transparent de Croncels according to UPOV). The geographical location and the position of the fruits, concerning the sunlight, favoring the formation of the color, probably have an impact.

Fruit: Color of flesh (No. 53 according to UPOV). The fruit flesh varies from soft to firm, but not very firm, in most cases with a juicy, slightly sour, aroma. In some

samples, it is dense. The flesh is white to whitish in all forms (F5; 6; 7; 8; 9; 10; 11; 12; and 14), except for F2 and F4, which are greenish. Forms F1; F2; F4; F8; F9; F10; and F11 are distinguished by juicy fruit flesh, whereas F5 and F7 are dry.

Most of the presented forms have aromatic flesh (F1; 2; 3; 6; 9; 11; 12; and 14), and the rest are without aroma. Sweet fruit flesh is found in forms 4; 5; 8; and 13; sweet and sour in 9; 10; 11; and 14; and sour in 1; 2; and 6. According to this indicator, the taste requirements of a large part of consumers are covered – sweet and sour.

In some representatives of the variety, after storage of the fruits, the pulp acquires a floury structure (F5).

The European consumer prefers sour flavors, white fruit flesh, juicy, and aromatic (Forms 1 and 2). In the Balkan countries, the tastes of sweet, sweet-sour, and juicy fruits are preferred (F9; 10; and 11) (**Table 4**).

In our research, the soluble solids in the fruits were from 13.60% (F2) to 18.10% (F4). The coefficient of variation is 11.54%. Higher values of soluble solids are F4, F6, which makes them valuable raw material for processing – drying and distillation (**Table 1**).

Total sugars in these forms are also higher, compared to the whole group, 18.40% and 17.00%, respectively. In general, they vary from 7.35% (F3) to 18.40% (F6), with a coefficient of variation of 30.43%.

Similar results were reported by Kulina et al. [15] for soluble solids content (SSC,%) in apple fruits from 12.93% for the cultivar Bjeličnik to 16.15% for Ljepocvjetka in Bosna and Hercegovina cultivars. According to the soluble solids (14–16%), most of the varieties in their research are classified as having medium-high and high content of SSC. The content of total acids in the studied varieties varied from 0.43% for Petrovača variety to 0.71% for Šampanjka variety.

Bozovic et al. [14] indicated that the SSC in autochthonous varieties in Northern Montenegro ranged from 9.6% to 15.2%, as both authors gave lower levels. Varieties with a high content of soluble solids, such as Aleksandrija (16.0%), Rebrača (15.5%), Jolovača (14.6%), and Dunjka (14.5%) can be recommended as good material for the processing industry. Chemical analyses show that apple varieties differ significantly according to the studied habitats. The fruit chemical composition varies greatly depending on variety, location, and multiple abiotic factors, such as environmental conditions and agrochemical properties of the soil.

The content of soluble solids (17.0%) and total sugars (18.40%) in F6 is high, represented only by inverted sugar in the absence of sucrose, as acids are at least 0.34%. This makes it extremely unbalanced in taste.

The most favorable ratio between sugars and acids is reported at F2 (glucoacidimetric index 15.94) when a harmonious and refreshing taste is felt, which is an important criterion for evaluation and consumption.

Sucrose content is important for the dietary and nutritional properties of fruits. In Form 6, there is no sucrose, which makes it a suitable food for diabetics (**Table 1**).

Vitamin C ranged from 12 to 22 mg/% in the whole group, CV: 26.67%.

Apple pectin improves bowel functions, keeps the feeling of repletion for a long time, prevents obesity, and removes toxins and heavy metals from the body.

Stoyanova et al. [10] reported the highest amount of pectin in Sadova Perusha variety (1.98%), which completely coincides with F1 (1.98 mg/%) from the present study (**Table 1**). The pectin is below 1 mg/% in the variants studied, except for F1 (1.98 mg/%) and F3 (1.40 mg/%).

The color coordinates L – color brightness; +a – red color; –a – green color; +b – yellow color; –b – blue color; the qualitative indicator color tone a/b (dominant wavelength) and hue angle H = (b/a) of six forms were measured (**Table 2**).

The highest value for skin brightness was recorded for F6 ($L = 74.90$), as F5 had the most dominant yellow color ($b = 119.22$).

There are also high values for brightness in forms F2 (70.49) and F5 (74.68). Form 4 has the lowest brightness values ($L = 35.13$), where the red color is most pronounced ($a = 29.89$).

Minkov [1] studied heirloom local apple varieties threatened with extinction. According to that study, Sadova Perusha has high values for brightness ($L = 71.74$) and stands out with the largest negative value ($-a = 6.09$), which defines its green color. The qualitative indicator color tone a/b (dominant wavelength) has the highest value for the fruits of Sadova Perusha (7.92).

The most pronounced are correlation with L to a ($r = -0.9399$) and L to a/b ($r = -0.9128$). The highest positive correlation is between (a) and (a/b) $r = 0.961941$. There is no statistically proven relationship between hue (hue angle (b/a) and color tone (a/b) . Color parameters are independent of Density and Brix (Table 5).

With regression coefficient $R^2 = 1$, the equation $y = -1.0491x + 66.758$ gives us reason to predict the brightness of the color (L), by the value of the parameter (a) (Figure 2).

	L	a	b	a/b	H = (b/a)	Density (kgf/cm ²)	Brix (%)
L	1						
a	-0.93,994	1					
b	0.711,146	-0.53,113	1				
a/b	-0.91,286	0.961,941	-0.64,779	1			
H = (b/a)	0.162,686	0.144,813	0.671,304	0.003153	1		
Density (kgf/cm ²)	0.32,754	-0.14,952	0.173,232	0.017086	0.288,632	1	
Brix (%)	0.424,268	-0.37,186	0.571,115	-0.58,478	0.496,633	-0.28,956	1

Table 5.
Matrix of Pearson correlation coefficients for CIELAB values and color indexes considered.

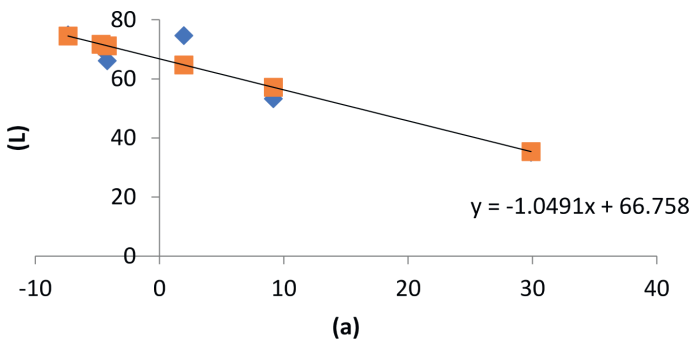


Figure 2.
Regression dependence of (L) against (a), $R^2 = 1$.

4. Conclusions

The study on PERUSHA variety (*Malus Domestica* BORKH) showed the following conclusions:

The fruit skin of all studied Perusha forms is mainly green to yellow-green, dense, thin or rough, firm and tough, giving greater transportability and a long storage period.

The fruit flesh varies from soft to firm, but not very firm, in most cases is juicy, slightly sour, with aroma. These qualities closely resemble standard commercial varieties and satisfy the requirements of all users.

The smallest amounts of sucrose are found in F5 (0.33%) and F4 (2.09%), except for F6 where it is completely absent, which defines them as suitable food for diabetics. The glucoacidimetric index of these forms is, respectively, 17.60 and 22.88, which gives them a pleasant and balanced taste.

All the studied forms of the group have different valuable economic qualities and different directions of use. Therefore, there is a huge perspective for their introduction into practice in the establishment of new orchards for sustainable fruit production.

Considering the diversity of pomological traits, their potential as sources of genetic variation, and their possible tolerance to abiotic and biotic stress factors, it is important to preserve traditional apple varieties as a source of quality fruit for consumption or processing, protecting these valuable genetic resources along with the area's biodiversity.

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Author details

Boryana Stefanova^{1*}, Petko Minkov¹ and Svetoslav Malchev²

1 Agricultural Academy Bulgaria, Research Institute of Mountain Stockbreeding and Agriculture of Troyan, Bulgaria

2 Agricultural Academy Bulgaria, Fruit Growing Institute Plovdiv, Bulgaria

*Address all correspondence to: stefanova_b@abv.bg

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