FORESTRY IDEAS, 2025, vol. 31, No Special Issue 2 (71): 200–210

Diversity of local apple genetic resources in the Central Balkan Mountain region of Bulgaria

Presented at the Conference 'A century of forest knowledge – education, innovations, challenges (CENFORKNOW 2025)', 7–9 May 2025, Sofia, Bulgaria

Thematic field: Agriculture in mountainous and semi-mountainous regions

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Received: 26 June 2025 / Accepted: 13 December 2025 / Available online: 17 December 2025

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Abstract

In Bulgaria, the apple (*Malus* × *domestica* Borkh.) has a long history of cultivation, and there is a wide diversity of genetic resources in the country. Wild relatives of this fruit species can be found in forests or other habitats. The local genetic resources are valuable due to their good adaptation to the climate and soil conditions of the natural habitat. In addition, they often have a number of valuable characteristics, such as resistance to diseases and pests, which should be conserved and used as donors for breeding programmes. The aim of the current study was to investigate the diversity of seedlings and local genotypes mapped during expedition studies in the Troyan region, Central Balkan Mountains of Bulgaria, in order to assess their characteristics and select the most valuable ones for future breeding purposes. For these purposes, the fruit size, flesh firmness, the total soluble solids content (°Brix), the skin and flesh colour were measured. All the characteristics studied were highly variable in the population. Fruit flesh colouration varied from white to pinkish. Regarding chemical analysis, very high sugar content was recorded in PM 11 (18.08 %), PM 12 (18.32 %), and PM 25 (17.44 %). All analysed samples had higher invert sugar content and lower sucrose content. Of all the studied genotypes, PM 17 and PM 104 had very low acidity. The studied local forms do not comply with modern market demands but are valuable for preserving biodiversity and for breeding purposes because of their good adaptation to the climatic conditions in the region.

Key words: biodiversity, local gene pool, local genotypes, *Malus* × *domestica*.

Introduction

The apple (*Malus* × *domestica* Borkh.) is one of the most widely distributed perennial fruit crops (Gross et al. 2014). This species is relatively old, domesticated initially at least 4000 years ago. Its wild relatives, distributed and grown in diverse habitats, are potentially useful sources of diversity for apple cultivation and breeding (Cornille et al. 2014). Fruit growing developed significantly in the 17th century, and accelerated urbanisation led to a drastic decline in wild and local forms of fruit species growing in nature (Nesheva et al. 2019).

In recent years, interest in the conservation and research of the gene pool of cultivated and wild fruit species has increased (Minkov and Mihova 2022). Plant genetic resources are of fundamental importance for agricultural science, mainly for fruit growing. Germplasm collections serve as important reservoirs of genetic diversity for crop improvement. They contain a great diversity that is essential for the identification of valuable phenotypes that can be used for the development of improved cultivars (Bramel and Volk 2019). Local genotypes are still preserved in many small settlements, especially in mountain areas (Bozhkova 2013). They are well adapted to the climatic and soil conditions of their natural habitat (Vitanov 1977). In addition, some genotypes have unique biological characteristics that should be preserved and used as donors for breeding programmes. The conservation of genetic resources is of regional and global importance. The thorough study of the most valuable characteristics and their description underlines their great importance. In order to collect, study, and preserve old and local fruit and grapevine cultivars and genotypes, a series of expeditions were conducted throughout Bulgaria under the framework of the KP-06-N46/3 project funded by the Bulgarian National Science Fund (BNSF) of the Bulgarian Ministry of Education and Science.

Collecting and evaluating local germplasm is essential for the improvement of cultivars (Mratinic and Fotric-Aksic 2012). The current study aimed to investigate the diversity of seedlings and local genotypes mapped during the expedition studies in the Troyan region in the Central Balkan Mountain region of Bulgaria, in order to assess their qualities and select the most valuable ones for future breeding purposes.

Materials and Methods

In 2021, expedition studies were carried out in Troyan, the Central Balkan Mountain region of Bulgaria, and surrounding areas (Apriltsi, Chiflik, Beli Osam and adjacent villages and neighbourhoods) to document and characterise local genetic resources of fruit species, with a focus on vegetative and reproductive traits of historically marked and newly identified varieties. Over the following two years, 2021 and 2022, fruit quality and diversity were investigated in a population of 50 selected genotypes, many of which persist as single trees aged 85–100+ years (Table 1). These genotypes, often found at altitudes up to 1000 m and preserved due to favourable terrain and microclimatic conditions, reflect the area's rich heritage and potential for conservation and breeding.

An average fruit sample was taken from each genotype, and the biometric data, petiole length, fruit height and fruit diameter were measured with a Mitutoyo Digimatic Absolute Calliper 150 mm. Fruit shape was determined according to the

Table 1. List of the genotypes evaluated.

Sample code	Origin of samples	Identification/ Local name	Sample code	Origin of samples	Identification/ Local name
PM 1	Apriltsi region	FSP-1*	PM 102	Chiflik region	ChF-2
PM 2	Apriltsi region	Perusha var.	PM 104	Chiflik region	ChF-4
PM 4	Apriltsi region	FSP-3	PM 105	Chiflik region	ChF-5
PM 6	Apriltsi region	FSP-5	PM 106	Chiflik region	ChF-6
PM 7	Apriltsi region	FSP-6	PM 108	Chiflik region	Yellow Bellflower form
PM 10	Apriltsi region	Tsiganka var.	PM 107	Chiflik region	ChF-7
PM 11	Apriltsi region	Balkanska Ayvaniya	PM 112	Chiflik region	Tsiganka form
PM 12	Apriltsi region	Tsiganka var.	PM 109	Chiflik region	Byala yabalka form
PM 13	Apriltsi region	Cheshka momina	PM 110	Chiflik region	Troyanka-1 form
PM 14	Apriltsi region	FSP-10	PM 111	Chiflik region	Shekerka-1 form
PM 15	Apriltsi region	FSP-11	PM 113	Chiflik region	ChF-8 P.H.
PM 16	Apriltsi region	Jonathan	PM 114	Chiflik region	Limonka-1 form
PM 17	Apriltsi region	Ayvaniya var.	PM 118	Chiflik region	ChF-9
PM 18	Apriltsi region	FSP-12	PM 119	Chiflik region	Shekerka-2 form
PM 19	Apriltsi region	Zimna Petrovka	PM 120	Chiflik region	Zimna zelena
PM 21	Apriltsi region	FSP-13	PM 121	Chiflik region	ChF-10 A.M.
PM 22	Apriltsi region	Troyanka var.	PM 124	Beli Osam region	Zlatista yabalka
PM 25	Apriltsi region	FSP-15	PM 129	Beli Osam region	Reinette du Canada
PM 26	Apriltsi region	FSP-17	PM 1001	Beli Osam region	Belurka-1 form
PM 27	Apriltsi region	Reinette du Canada	PM 1002	Beli Osam region	Belurka-2 form
PM 29	Apriltsi region	VMF-2	PM 1003	Beli Osam region	Shekerka var. form
PM 39	Apriltsi region	FTM-2	PM 1004	Beli Osam region	Koravka var.
PM 43	Apriltsi region	Tsiganka var.	PM 1005	Beli Osam region	Koravka var. form
PM 44	Apriltsi region	FM-1	PM 1006	Beli Osam region	Shekerka var. form
PM 36	Apriltsi region	-	PM 1007	Beli Osam region	Shekerka var. form
PM 101	Chiflik region	ChF-1			

Note: *The local name is given by the abbreviation of the area where the genotype was found.

ECPGR descriptor by calculating the ratio of fruit height to fruit width (Lateur et al. 2022). Fruit weight was measured with a digital scale (Kern, Germany). The apple fruits were subjected to colour evaluation to CIE L*a*b*, including Hue angle (h) and Chroma (c), using a PCE-CSM 2 (PCE Instruments, UK) handheld colourimeter with an aperture of 8 mm. The measurements were done on the equatorial part on both sides of 5 fruits. The firmness of the fruit was measured using a PCE-PTR-200N (PCE Instruments, UK) digital penetrometer after peeling the skin. The total soluble solids content (TSS) was measured using a Kern ORF 85BM (Kern, Germany) digital refractometer. The chemical analyses of the fruit flesh included the determination of total sugars, invert sugars and sucrose according to the method of Schoorl-Regenbogen (1952). The acidity (TA) was determined titrimetrically, using a titrator and the active acidity (pH) – potentiometrically, by a pH meter.

The data were processed by the Excel Data Analysis tool pack and IBM SPSS Statistics 26 software. Hierarchical cluster analysis using between-groups linkage was used to group the studied genotypes.

Results and Discussion

The fruit characteristics of the different cultivars and genotypes were highly variable. Red colour, low acidity, larger fruits, and firmness seem to have been under selection during the domestication and improvement of apple (Ma et al. 2015, Migicovsky et al. 2021). Fruit size is genetically determined, but is influenced by many agrotechnical and environmental factors. All genotypes studied developed under the same agro-environmental conditions and a diversity of biometric fruit data was observed (Table 2).

The highest variation was observed in fruit weight. The lowest apple fruit weight was 48.8 g (PM 19), and the highest was 156 g (PM 29). However, the analysis of the mean values of all measured parameters indicates a wide range of diversity between the local genotypes and does not meet the requirements of the market. For example, the fruits of the cultivar 'Florina' sold in Romanian supermarkets have a diameter of 65.6 mm and an average weight of 173.33 g (Iordănescu et al. 2021). The lowest deviation was observed in the fruit petiole length, which varied from 6.48 mm (PM 12) to 18.36 mm (PM 18).

The dimensions of the fruit determine its shape. According to the ECPGR descriptor (2022), fruit shape is determined by the fruit height/fruit width ratio. According to this, five groups were formed. Ratios smaller than 0.75 determine the flat shape, 0.76–0.85 determine a slightly flat shape, 0.86–0.99 – intermediate, 1–1.1 – slightly elongated and >1 – elongated. Three different shapes were observed in the group of apple genotypes studied. The intermediate shape was observed with the highest frequency (25 %), while the elongated and slightly elongated shape of the fruit was not observed in the analysed population (Fig. 1).

Due to their high nutritional value, apples are among the most widely consumed fruits worldwide. Ceci et al. (2021), comparing the chemical composition of old forms and commonly grown (commercial) cultivars, noted that the old forms were distinguished by the content of polyphenolic compounds and higher antioxidant capacity. Native wild, old and local apple genotypes are an important source of phytochemical compounds and are characterised by higher contents of bioactive compounds, which can be used as genetic material for apple breeding programmes (Balta et al. 2022). The quality of apples depends on the interaction of genetic and environmental factors (Begić-Akagić et al. 2014). Size, sweetness, and crispness are still the key traits and quality characteristics of apples, which are most important to consumers (Carrillo-Rodriguez et al. 2013). Sugars and organic acids are essential components that determine taste qualities (Wu et al. 2007, Bordonaba and Terry 2010). Sugars not only determine the sweetness of the fruit, but also serve as a carbon source for the synthesis of other components such as pigments, amino acids, vitamins, and aromatic compounds (Qin et al. 2025). Wild

Table 2. Diversity of the biometric data.

Characteristic	Minimum	Maximum	Mean	Std. Deviation
Fruit weight, g	48.80	156.00	95.73	±28.85
Fruit height, mm	39.52	65.08	50.68	±5.43
Fruit diameter, mm	48.33	73.33	59.99	±6.15
Petiole length, mm	6.48	18.36	12.63	±2.68

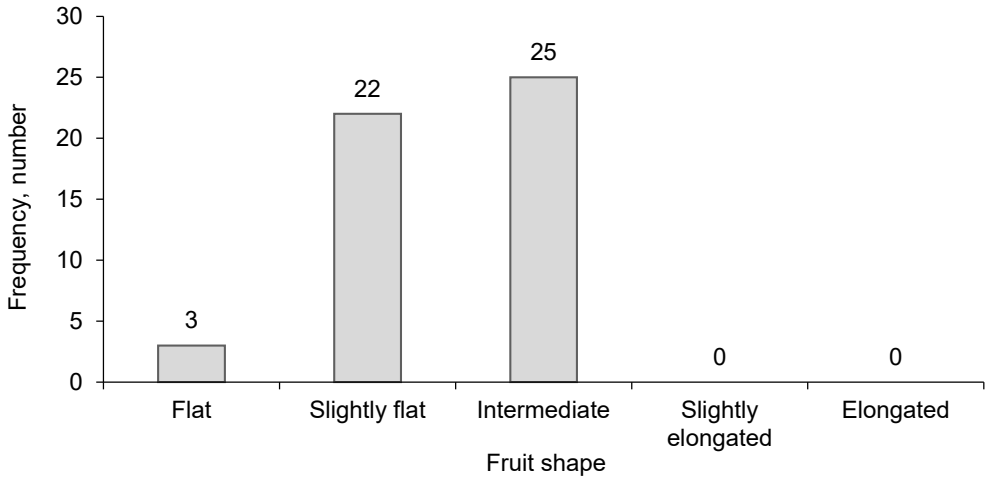


Fig. 1. Distribution of the accessions according to fruit shape.

and old local forms typically contain significantly higher contents of acids (malic acid) and a lower level of sucrose (Li et al. 2021) which corresponds well with our results, as most of the apple fruits studied exhibited a high total acidity together with high pH values. In the 50 apple samples examined, the TSS values ranged from 15.11 (PM 104) to 23.20 °Brix (PM 44) and are relatively high, given that TSS in commercial dessert apples generally falls between 12 and 18 °Brix (Bordonaba and Terry 2010) (Table 3). In the group studied, the average fruit firmness was 9.36 kgf·cm^{-2}. A substantial difference was observed in total sugar content, with values ranging from 6.88 % (PM 1001) to 14.90 % (PM 102). Invert sugars predominate in apple fruits. The sucrose content varied from 0.23 % (PM 1005) to 5.49 % (PM 107), which is 3 to 5 times lower than that of the invert sugar (from 4.50 % (PM 109) to 11.36 % (PM 1005)). Most of the apple fruits studied exhibited a high total acidity and high pH values. The deviation of both was low across the entire studied group.

The TSS content is an important fruit characteristic that is associated with the sugar content and the level of ripeness. It may vary within the same variety as a result of climatic factors (Gómez-Devia and Nevo 2024). There is a relationship between the TSS content and fruit firm-

Table 3. Diversity of the physical and chemical components.

Characteristic	Minimum	Maximum	Mean	Std. Deviation
TSS content, °Brix	15.11	23.20	19.11	1.96
Fruit firmness, kgf·c^{m-2}	4.90	12.81	9.36	1.78
Total sugars, %	6.88	14.90	10.50	2.20
Invert sugar, %	4.50	11.36	8.28	1.70
Sucrose, %	0.23	5.49	2.10	1.40
Acids (TA), %	0.26	1.31	0.76	0.22
pH	3.42	6.47	3.99	0.51

ness – the higher the TSS content, the riper and softer the fruits tend to be. Fruit firmness is strongly associated with consumers' preference for certain cultivars and is, therefore, a key target for breeders (Szczesniak 2002, Nybom et al. 2013). The firmness of the fruit flesh is relevant to an assessment of the fruit quality, the shelf life, and consumer acceptance (Hend et al. 2009). According to Harker et al. (2008) apple firmness is the dominant factor for consumer acceptance, but TSS and TA content also play a role in defining quality for specific cultivars. The largest were the groups of apple genotypes with a high TSS content. The group with TSS in the range of 17–19 ºBrix and the group with TSS in the range of 19–21 ºBrix, had a frequency of 32 % each (Fig. 2). A high percentage of the population also had a very high TSS over 21 ºBrix. Sixty percent of the examined genotypes had an average fruit firmness in the range of 5–10 kgf·cm^{-2} (Fig. 3).

A high deviation was found in the colour parameters describing the skin ground colour (Fig. 4). The hue angle was measured from around 20 to 105, showing the variation from red to green skin ground colour. The L parameter reflects the variation in the brightness of the colouration. It is mainly between 40 and 80, indicating a lighter skin colouration. Genotypes with lower L values were identified in the studied population, indicating darker colouration. The green-red parameter a* was mostly around 0, suggesting a neutral balance between green and red tones. A higher a* value showed domination of red colouration, but it is not common in the studied population. Blue-yellow axis b* indicated a strong shift towards the yellow colouration of the skin ground colour. The chroma index (c*) measures the vividness of the skin colour and was around 50.

Less variation was observed in the fruit flesh colour parameters (Fig. 5). The hue angle of the population was around 90°, showing that the fruit flesh is in the yellow tones of the colour scale. One of the genotypes was interesting and different from the whole group, with a fruit flesh in the pink shade. In most of the studied genotypes, the colour of the fruit flesh is around 70°, suggesting that the flesh is quite light, nearly white or creamy in colour. The a*

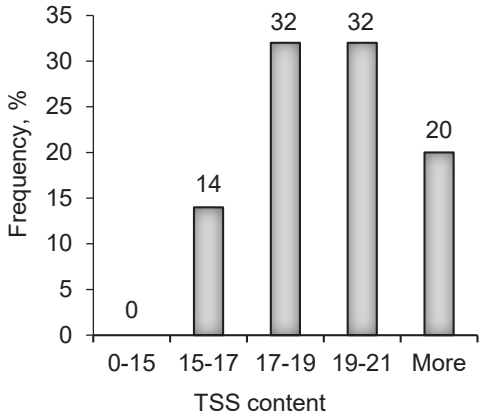


Fig. 2. Distribution of the accessions according to TSS content, °Brix

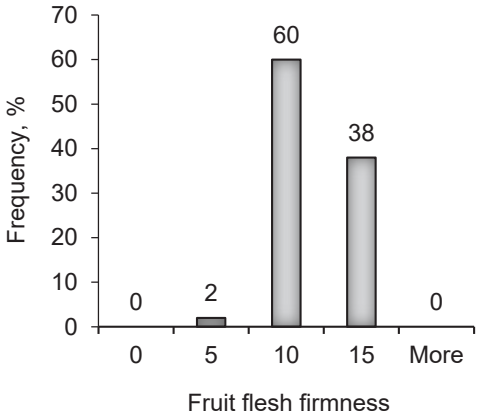


Fig. 3. Distribution of the accessions according to the fruit firmness, kgf·cm^{-2}

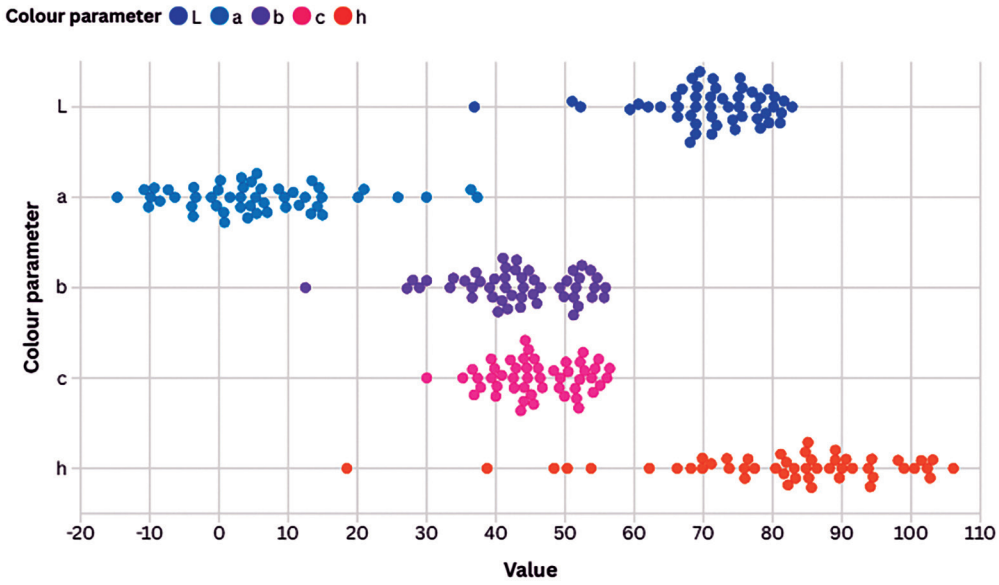


Fig. 4. Distribution of the colour parameters of the skin ground colour in the studied group.

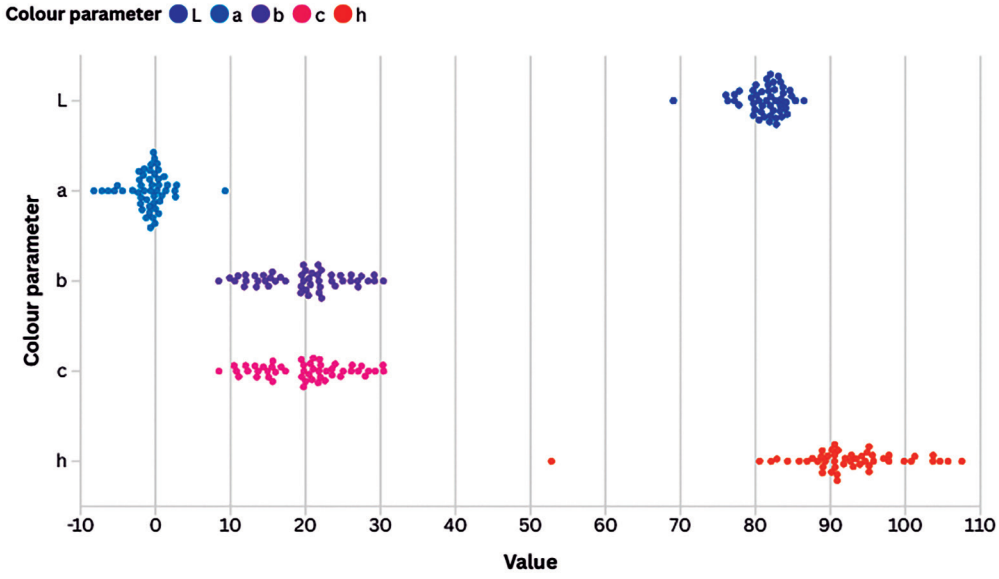


Fig. 5. Distribution of the colour parameters of the fruit flesh in the studied group.

value was tightly grouped around 0, which means the flesh does not lean distinctly green or red. The genotype with the pink colouration of the flesh had the highest value of a*. For most of the genotypes, the b* parameter was around 20, indicating a

mild yellow colouration of the fruit flesh. Chroma index was also close to 20, so that yellow hue is present but not a highly saturated colour.

A cluster analysis was performed to group the studied accessions based on their similarities in the biometric and chemical characteristics. According to the biometric data, this statistical method revealed two main groups (Fig. 6). Cluster one includes accessions with very small, small, and small to medium fruits (Lateur et al. 2022) and with longer petioles – from PM 15, with an average fruit weight of 86.7 g, FH – 49.76 mm, FW – 58.9 mm, and PL – 13.85 mm, to PM 19, with an average fruit weight of 48.8 g, FH – 43.77 mm, FW – 43.32 mm, and PL – 15.04 mm. Cluster two includes the accessions with larger fruits and short petioles, from PM 1 to PM 120, with sizes ranging from small to medium, medium, or medi-

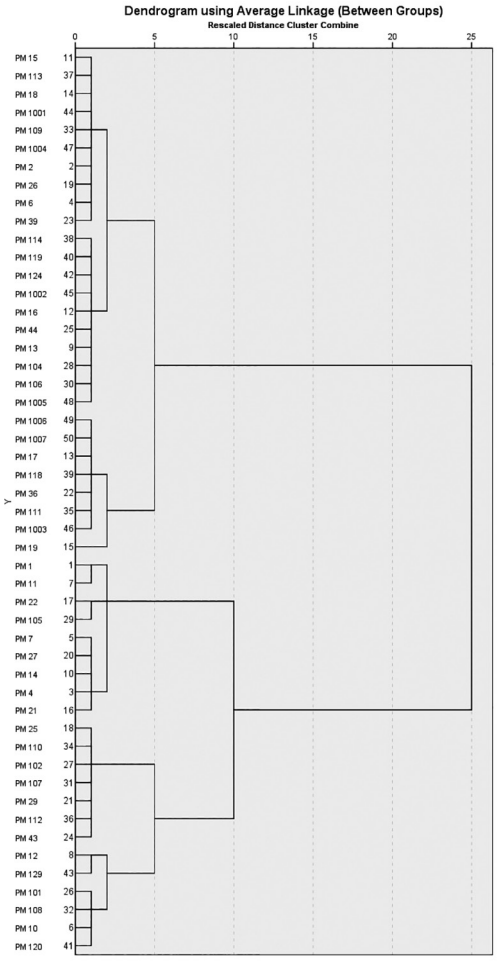


Fig. 6. Dendrogram of grouping of the genetic resources by their biometrical data.

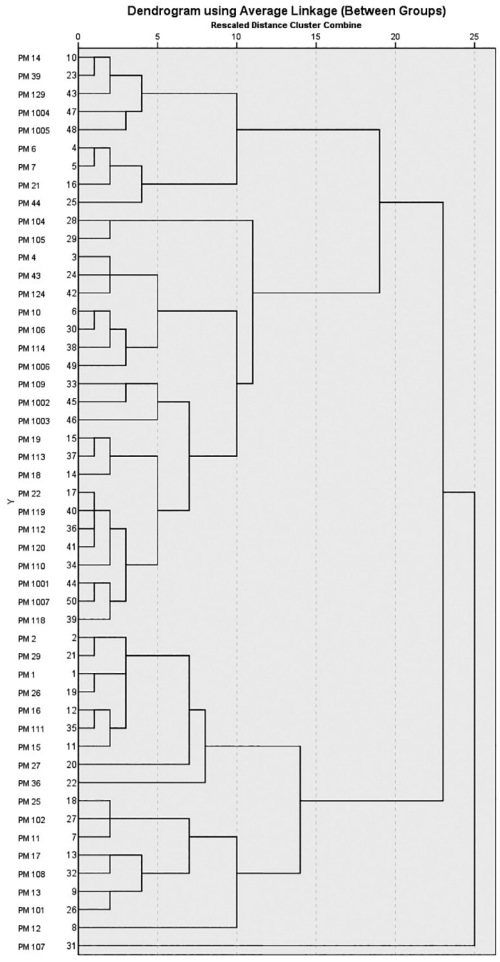


Fig. 7. Dendrogram of grouping the genetic resources by their chemical components.

um to large. In the last group with medium to large fruits, only PM 12 (fruit weight 156 g, FH – 52.51 mm, FW – 73.33 mm, PL – 6.48 mm) and PM 129 (fruit weight 151.4 g, FH – 56.82 mm, FW – 71.29 mm, PL – 10.12 mm) are included. The genotypes in the second cluster comprise 44 % of the studied group and are more valuable due to their greater fruit size.

The apple genotypes analysed could also be divided into 2 main groups according to the chemical components. Forty-nine accessions form one large group with similar chemical content. PM 107 was identified as the most different from the others (Fig. 7). This accession formed a separate cluster and was the only one with a high sucrose content compared to the others (5.49 %). The remaining 49 apple genotypes form four different clusters. Cluster 2, formed from PM 14 to PM 44, includes 9 genotypes with very high TSS content (above 20 ºBrix), high invert sugars and low sucrose content. Cluster 3, formed by PM 104 to PM 118, combines genotypes with average values of the examined chemical characteristics. Cluster 4 – PM 2 to PM 36 – is represented by 9 accessions with low total acidity levels (from 0.49 % for PM 111 to 1.02 % for PM 27). Cluster 5 contains PM 25 to PM 12, which have a high total sugars content from 11.95 % for PM 101 to 14.89 % for PM 102, high invert sugars from 7.72 % (PM 17) to 11.04 % (PM 11), high sucrose from 3.08 % (PM 101) to 5.12 % (PM 12) and low total acidity from 0.25 % (PM 17) to 1 % (PM 12).

Conclusions

The fruit quality of local genetic apple resources was investigated using standard methodologies and techniques. The genetic resources analysed are not oriented towards today's quality standards for apple fruit. However, a rich diversity of different and interesting, old and local genotypes is a prerequisite for the inclusion of new genitors in breeding programmes.

The genotypes with larger fruits and higher sugar content, such as PM 12 and PM 129, are particularly valuable for breeding programmes aimed at improving fruit quality. A natural genotype (PM 18) with pink fruit flesh was found, which can be used as a donor to introduce desirable traits into new cultivars.

The local apple genotypes are well adapted to the climatic and soil conditions of the Central Balkan region. This adaptation makes them resilient to local environmental stresses and valuable for the development of cultivars that can thrive under similar conditions.

The study underlines the importance of ongoing research and development efforts to utilise the genetic resources of local genotypes. Continued research and characterisation of these resources will support the development of breeding programmes.

Acknowledgment

This research was supported by the Bulgarian National Science Fund (BNSF), under project KP-06-H46/3 from 2020, 'Innovations and traditions in the conservation and use of old local genetic resources in fruit growing and viticulture'.

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