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Research Article

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Comparison of old and local apple varieties and seedlings (*Malus domestica* Borkh.) in the variability of some morphological characters of fruits and seeds

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Old and local varieties of cultivated plant species selected from natural populations adapted to long-term cultivation, which represent a rich genetic potential for the development of agroecosystems and agriculture under specific conditions, resources for an environment aestheticization, landscaping and development of cultural traditions. The research focused on determining the economic value of a selected collection of old and local varieties of apple tree (*Malus domestica* Borkh.), widespread in Slovakia for their practical use in organic farming or as genetic resources for breeding new varieties for organic food production. For experimental evaluation, we used two collections: 1) 73 old and local varieties of apple trees concentrated and preserved *ex situ* in a clone repository in the village Bacúch; 2) 77 self-sown seedlings, that spontaneously emerged as a result of free pollination and are growing *in situ* around Nitra, Levice, Nové Zámky, Šaľa, Galanta, Hlohovec, Piešťany, Prievidza, Partizánske, Zlaté Moravce. We determined for all specimens the range for the weight of fruits 53.63–207.40/16.13–197.59 (g), height of fruits 41.47–72.93/29.55–74.04 (mm), diameter of fruits 51.46–84.66/36.85–78.43 (mm), length of core/13.16–27.36/11.24–25.86 (mm), diameter of core 18.26–33.46/13.72–30.86 (mm), weight of 10 seeds 0.38–0.77/0.29–0.98 (g), height of seeds 6.68–9.90/6.16–9.83 (mm), diameter of seeds 3.73–5.71/3.51–5.27 (mm). The results document that in both collections there are genotypes suitable for organic cultivation, and further selective improvement.

Keywords: *Malus domestica*, genetic resources, clone repository, morphometric analysis, variability

Introducion

The native range of apple tree (*Malus domestica* Borkh.) is difficult to determine, as the species is a product of domestication and multiple hybridizations across the world over thousands of years. In Slovakia, fruit growing has a long tradition. Apple trees have a dominant position in fruit growing. *Malus domestica* from the genus *Malus* from the family Rosaceae and the subfamily Pomoideae is an example of the most important, the most widespread and best adapted fruit tree of temperate zone in terms of production. *Malus occupies* a central place in the folklore, culture and art (Robinson et al., 2001; Harris et al., 2002; Juniper and Mabberley, 2006; Velasco et al., 2010).

A local variety is a domesticated, locally adapted, traditional variety of a species of plant that has developed over time, through adaptation to its natural and cultural environment of agriculture, and due to isolation from other populations of the species. Local varieties are generally distinguished from cultivars.

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They have been selected from natural populations and grown for nutritional use or other purposes. Due to their long-term cultivation in different areas, they have adapted to certain specific growing conditions, thus acquiring a high degree of tolerance against adverse environmental factors. Old cultivars and varieties are highly disease resistant to apple scab, powdery mildew, green apple aphid, apple codling moth in general (Militaru et al., 2015; Papp et al., 2015). Cultivation the less susceptible varieties is the most obvious way to reduce problems with pests and diseases; therefore, the choice of apple varieties for organic farming is extremely important. Great effort has been put into developing breeding programmes to create scab resistant varieties. However, older varieties that originated before the appearance of pesticides might be less susceptible than newer varieties and would thereby be a better choice for organic farming (Kühn et al., 2003; Militaru et al., 2015; Papp et al., 2015).

Regarding polyphenols, it is known that old and local apple varieties were characterized by a higher content of polyphenols and stronger antioxidant properties than commercial varieties, which enjoys a high growth rate, but unfortunately, these new varieties are characterized by a very low content of bioactive compounds, including polyphenolic compounds (Kuznetsova et al., 2017; Oszmiański et al., 2019). The consumption of such apple varieties may reduce the polyphenolic compounds in the dietary supply (Iacopini et al., 2010; Donno et al., 2012). Some studies presented the amounts of biologically active substances in old and new varieties were similar (Wojdyło et al., 2008). In the study of Feliciano et al. (2010), both traditional and exotic apple varieties from Portugal showed high amounts of polyphenols. It should be noted that environmental conditions can influence on the polyphenol amounts.

Local varieties represent the means of production for the development of agroecosystems and agriculture in specific conditions, resources for the aestheticization of the environment, landscaping and the development of cultural traditions (Brindza, 2001; Tóth et al., 2004; Ganopoulos et al., 2017). One of the largest collections of old apple varieties is located in a neighbouring Poland and Ukraine and spread over the territory of the then ancient Eastern Galicia in Central Europe (Dovbysh and Borodai, 2011; Żygala et al., 2011).

It is generally known that many local varieties, as well as cultivars, were selected from local self-sown individuals – seedlings (Boček, 2008a, 2008b; Hulin et al., 2012; Posolda et al., 2019). The establishment of clone repositories to save the endangered gene pool of plants has an application in our country for many fruit species such as pear, cherry, plum, chestnut, etc. (Bolvanský and Užík, 2012; Paprstein et al., 2013; Benediková et al., 2016). It is necessary to identify and evaluate genotypes based on the morphometrical and biochemical traits in various conditions, as evidenced by the many authors (Ivanišová et al., 2017; Vinogradova et al., 2017; Grygorieva et al., 2017a,b, 2018a,b; Fatrcová Šramková et al., 2019; Levon and Golubkova, 2019; Vergun et al., 2020).

This study aimed to evaluate the genetic resources of apple tree for organic farming in the collection of old and local varieties of *Malus domestica* Borkh. as well as self-sown seedlings widespread in Slovakia.

Material and methodology

Biological material

Two collections of biological material were used as genetic resources for the study:

1. Old and local varieties from different areas of Slovakia which are kept *ex situ* in a clone repository in the village Bacúch – 73 selected genotypes. In the experiments, samples were marked as R and the appropriate number.
2. Wild self-sown individuals – fruit-beraing seedlings from different localities (Nitra, Levice, Nové Zámky, Šaľa, Galanta, Hlohovec, Piešťany, Prievidza, Partizánske, Zlaté Moravce) in the form *in situ* – 77 selected genotypes. In the experiments, samples were marked as S and the appropriate number.

The total number of evaluated genotypes were 150.

Fruits with peduncle were taken from trees in September and October 2010 and analysed in the morphometric laboratory at the Institute of Biodiversity Conservation and Biosafety in Nitra (Slovakia).

Morphometrical analysis

They were evaluated the following characters:

a) fruits – 30 fruits were evaluated from each genotype (n = 30), weight of fruit (g), height of fruit (mm), diameter of fruit (mm), length of core (mm), diameter of core (mm), depth of stalk cavity (mm), depth of eye basin (mm);
b) seeds – 30 seeds were evaluated from each genotype (n = 30), weight of 10 seeds (g), height of seed (mm), diameter of seed (mm).

The weights were determined by digital scale (Kern ADB-A01S05, Germany; KERN DS – type D-72336,

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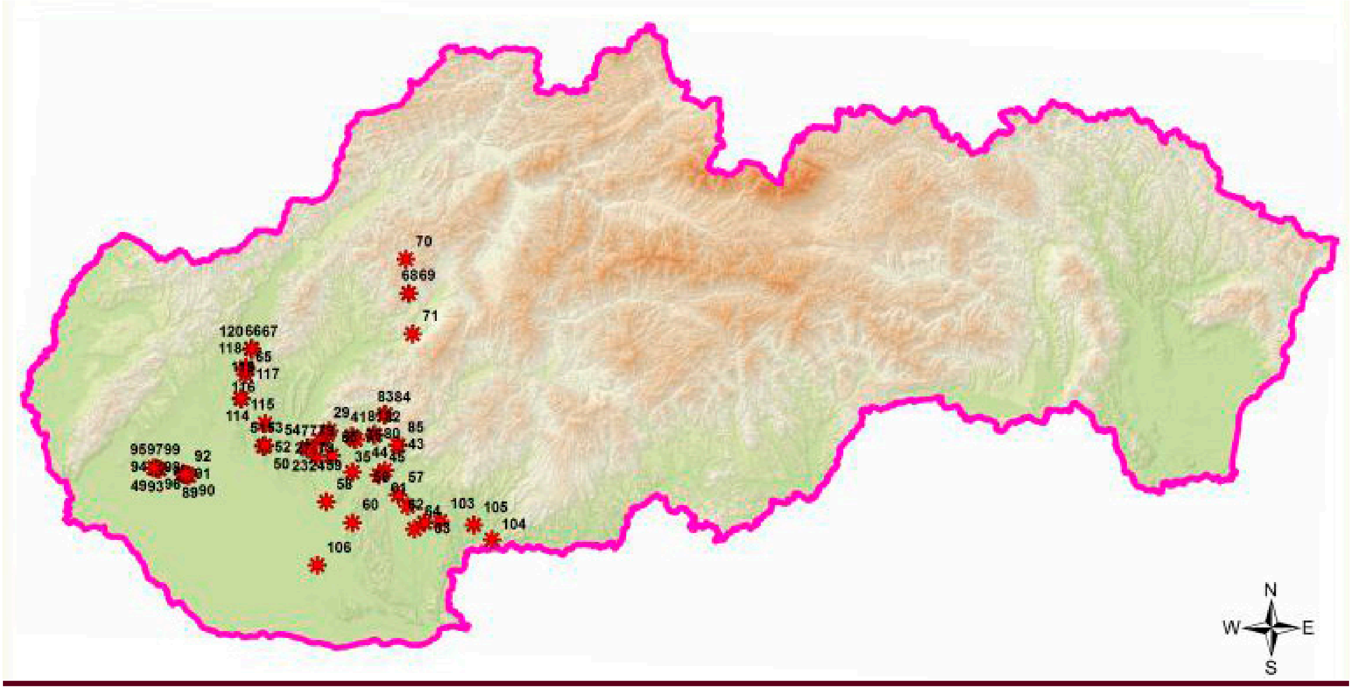

Figure 1 Localization of local varieties and seedlings of *Malus domestica* Borkh. within Slovakia using GPS: the detailed data can be found in Table 1

Table 1 Localities of varieties and seedlings *Malus domestica* Borkh. in Slovakia and their altitude

Genotype	Locality	Region of Slovakia	Altitude, m a.s.l.
R01-R77	Bacúch	central Slovakia	590–630
S01-S10	Nitra	western Slovakia	167
S11-S20	Levice	south-western Slovakia	165
S21-S30	Nové Zámky	south-western Slovakia	114
S31-S40	Šaľa	south-western Slovakia	116
S41-S50	Galanta	south-western Slovakia	119
S51-S60	Hlohovec	western Slovakia	146
S61-S70	Piešťany	western Slovakia	160
S71-S80	Prievidza	western Slovakia	309
S81-S90	Partizánske	western Slovakia	190
S91-S99	Zlaté Moravce	western Slovakia	192

Note: altitude – meters above sea level

Kern and Sohn GmbH, Germany), accurate to 0.01 g. Fruits and seeds were measured by a digital calliper (METRICA 111 – 012, Czech Republic) accurate to 0.02 mm.

Image analysis

1. Fruit: the shape of the fruit, the shape of the apical part of the fruit (at the stalk), depth of stalk cavity, depth of eye basin, the shape of the basal part of the fruit, basic colour of the skin at the full maturity, the colour of the pulp of ripe fruit.
2. Seeds: the shape of seeds.

Images were obtained using the stereomicroscope ZEISS SteREO Discovery.V20 (MicroImaging GmbH 37081 Göttingen, Germany), and Fuji FinePix S 7000 and Panasonic DMC FZ50 digital cameras.

Statistical analysis

It was evaluated the variability of each character using descriptive statistics. For the characteristics it was used the basic descriptors of variability: average, minimum measured value, maximum measured value, the coefficient of variation (%). The degree of variability was determined by the coefficient of variation values.

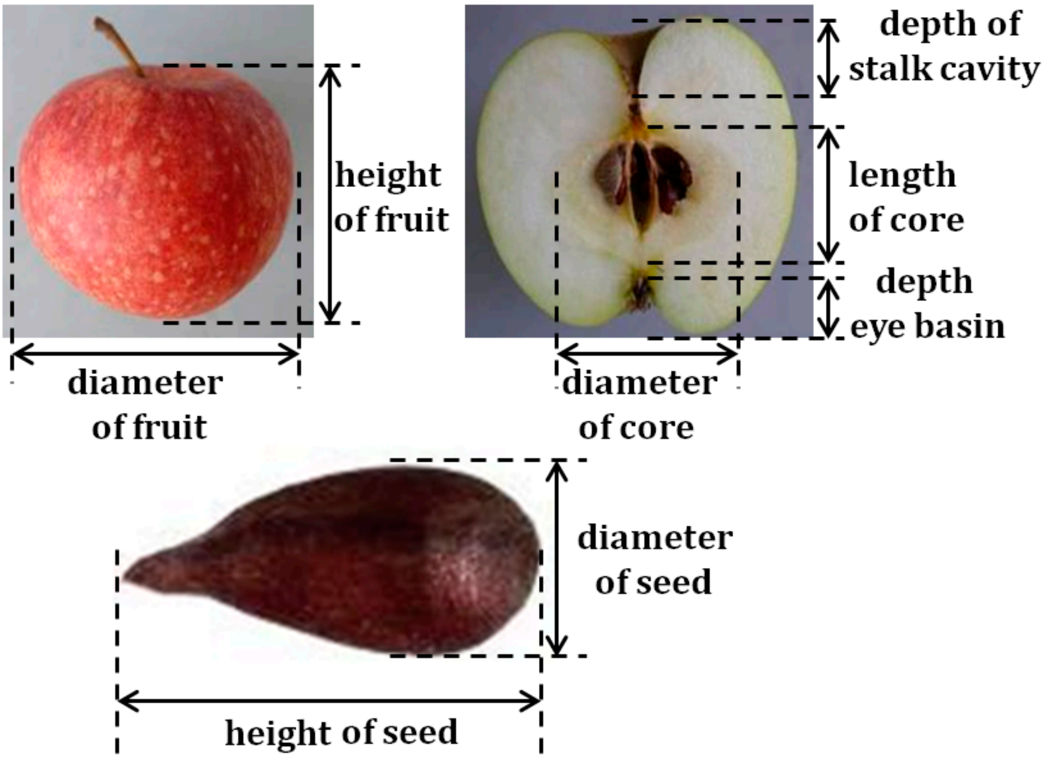

Figure 2 Illustration of measuring process: fruit height and diameter, core length and diameter, depth of stalk cavity and depth of eye basin

The given parameter is independent of the unit of the evaluated character. Theoretically, they can acquire different values (Stehlíková, 1998). We used analysis of variance (ANOVA) in the program STATISTICA 1.10 to determine the dependence between individual characters.

Results and discussion

Evaluation and identification of genotypes based on morphological traits are important for the detection and selection of individuals that are suitable genetic material for hybridization and breeding program of new varieties, which contributes to the global conservation of biological diversity (Monka et al., 2014; Grygorieva et al., 2017a,b, 2018a,b; Motyleva et al., 2017, 2018; Ivanišová et al., 2017; Vinogradova et al., 2017; Brindza et al., 2018, 2019; Fatrcová-Šramková et al., 2019; Horčinová Sedláčková et al., 2020).

Morphometrical analysis of fruits

When evaluating the genotypes under study (Table 2), the average weight of the fruits has been determined in the range of 3.63 g (R18/9) – 207.40 g (R30/7)/16.13 g (S12) – 197.59 g (S03). The coefficients of variation were determined in the range of 4.18 (R33/12) – 17.02 (R18/7) %/11.08 (S38) – 40.61 (S22) %. These data demonstrate that the characters are from the low to very high degree of variability. The above comparisons show that it is possible to search for genotypes with the required fruit size in the populations of wild seedlings.

The differences in the weight of tested varieties were significant, and that is in full compliance with the studies assortment of old apple varieties from Denmark 77–205 g (Kühn et al., 2003), Montenegro 62.23–182.34 g (Božović et al., 2013), Croatia 26–325 g (Jakobek et al., 2020), Romania 117.0–186.5 g (Mitre et al., 2015), Bosnia and Herzegovina 63.77–208.97 g (Stanivuković et al., 2017).

Dvořák et al. (1976) classified fruits according to 3-years-old average weight as extremely small (below 15 g); very small (16–48 g); small (49–70 g); smaller (71–110 g); medium (111–150 g); larger (151–200 g); large (201–250 g); very large (251–350 g) and extremely large (above 351 g). Michálek (2003) divides apple varieties according to the size of the fruit while declaring the size of the fruit according to the dimensions – height and diameter of the fruit at the place of the largest diameter. According to the given descriptor, it recognizes smaller fruits – the average transverse diameter is up to 55 mm, medium-sized (55–70 mm), large fruits (71–85 mm) and very large fruits (more than 85 mm). According to the above

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Table 2 Variability of fruits of old and local varieties of *Malus domestica* Borkh.

Weight of fruits (g)													
	Seedlings						**Genotypes from repository Bacúch**						
	n	**min**	**max**	**x̄**	**V**	**H**		**n**	**min**	**max**	**x̄**	**V**	**H**
Genotypes with low values													
S12	30	12.20	20.50	16.13	18.83	k	**R18/9**	30	47.7	59.7	53.63	7.59	g
S69	30	30.00	45.00	36.45	11.95	j	**R35/10**	30	50.7	70.1	59.36	11.13	f
Genotypes with high values													
S03	30	142.90	331.00	197.59	28.25	a	**R30/7**	30	170.5	241.5	207.40	11.76	a
S20	30	86.90	215.50	185.28	20.75	a	**R16/12**	30	168.4	205.4	187.39	6.53	ab
Height of fruits (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S12	30	27.40	32.30	29.55	5.60	e	**R33/12**	30	38.70	43.70	41.47	3.33	d
S69	30	33.60	39.30	36.38	4.70	ed	**R1/4**	30	41.5	44.6	42.86	1.86	d
Genotypes with high values													
S22	30	59.30	87.90	74.04	14.08	a	**R30/7**	30	66.70	78.50	72.93	5.70	a
S03	30	58.60	83.40	67.91	12.03	a	**R30/8**	30	62.20	80.50	71.32	9.03	a
Diameter of fruits (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S12	30	33.50	40.00	36.85	6.03	f	**R18/9**	30	48.90	54.20	51.46	3.71	e
V69	30	43.10	50.10	45.67	5.57	e	**R22/3**	30	49.00	53.80	51.58	2.94	e
Genotypes with high values													
S03	30	72.60	95.10	78.43	8.12	a	**R30/8**	30	76.30	90.90	84.66	6.56	a
S20	30	59.30	83.50	77.86	8.86	a	**R16/12**	30	80.20	84.30	82.41	1.75	a
Depth of stalk cavity (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S24	30	0.00	5.30	2.04	83.00	cd	**R22/3**	30	0.00	3.60	1.67	67.63	e
S12	30	1.00	4.40	2.96	34.28	cd	**R1/12**	30	4.20	6.40	5.36	16.29	d
Genotypes with high values													
S20	30	11.30	18.80	16.11	12.66	a	**R14/11**	30	13.20	16.30	14.82	6.83	a
S30	30	8.90	17.80	14.44	17.50	a	**R27/11**	30	9.20	16.60	13.77	18.17	b
Depth of eye basin (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S38	30	0.70	2.60	1.22	52.24	d	**R18/9**	30	1.20	3.20	2.10	33.14	c
S08	30	0.50	2.90	1.23	56.33	d	**R33/12**	30	1.10	3.10	2.17	26.16	c
Genotypes with high values													
S22	30	8.10	18.10	11.69	29.09	a	**R3/16**	30	7.20	10.00	8.86	9.77	a
S20	30	4.80	19.20	9.58	39.18	b	**R16/14**	30	7.60	9.50	8.60	6.71	a

Table 2 continued

Length of core (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S10	30	10.10	12.70	11.24	9.24	d	**R31/2**	30	10.90	15.70	13.16	14.61	dc
S30	30	10.80	13.70	12.26	8.41	d	**R33/12**	30	11.40	14.50	13.42	8.84	dc
Genotypes with high values													
S03	30	20.00	29.50	25.86	14.11	a	**R3/2**	30	26.40	28.50	27.36	3.31	a
S47	30	18.50	25.60	22.06	11.67	a	**R41/4**	30	21.70	33.60	26.80	18.47	a
Diameter of core (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S22	30	0.94	1.08	1.01	4.35	c	**R1/4**	30	17.50	19.00	18.26	3.60	d
S76	30	0.98	1.03	1.00	1.54	c	**R3/2**	30	17.10	20.10	18.52	6.89	d
Genotypes with high values													
S75	30	12.70	14.40	13.72	4.79	a	**R20/8**	30	30.90	35.80	33.46	6.49	a
S47	30	13.20	16.30	14.98	7.70	a	**R18/7**	30	26.20	30.30	28.42	5.46	b
Index of fruit shape													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S02	30	0.69	0.79	0.74	4.48	ab	**R1/4**	30	0.65	0.74	0.69	4.17	b
S67	30	0.69	0.78	0.76	3.56	ab	**R7/7**	30	0.68	0.72	0.70	1.49	b
Genotypes with high values													
S22	30	0.94	1.08	1.01	4.35	a	**R41/4**	30	1.15	1.35	1.26	4.21	a
S76	30	0.98	1.03	1.00	1.54	a	**R5/4**	30	0.97	1.25	1.09	6.60	ab

Note: n – the number of measurements; min, max – minimal and maximal measured values; $\bar{x}$ – arithmetic mean; V – coefficient of variation (%); H – LSD homogeneity test at $P_{0.05}$

descriptor, the fruits of seedlings can be characterized as small to large.

The average height of fruits of the genotypes under the study was in the range 41.43 mm (R33/12) – 72.93 mm (R30/7)/29.55 mm (S12) – 74.04 mm (S22) and the diameter of fruits was in the intervals of 51.46 mm (R18/9) – 84.66 mm (R30/8)/36.85 mm (S12) – 78.43 mm (S03). The collection of self-sown seedlings showed a significantly higher variation range in both evaluated traits. The coefficients of variation confirm the low or the medium degree of variability of the characters. Parameters are shown in table 2.

Jakobek et al. (2020) recorded heights (34–79 mm) and diameters (41–89 mm) of old varieties. The average height and diameter of old apple varieties cultivated in Bosnia and Herzegovina (Stanivuković et al., 2017) were recorded in the interval 50.08–67.21 mm and 53.52–80.23 mm, respectively. Results showed by Božović et al. (2013) in Montenegro, where the intervals of evaluated traits were 42.29–64.70 mm and 54.08–78.27 mm respectively, are similar to the data shown. Michálek (2003) states that from the market point of view, mainly varieties with medium to large fruits are in demand. Small or too large fruits are commercially unattractive. This customer requirement must be taken into account at assessing genotypes as a potential gene pool in breeding programs, as there are 19.5 % of samples with small fruits (below 55 mm) in our research collection.

An important diagnostic feature is the depth of stalk cavity and depth of eye basin, because the measured features may have a specific range for each variety and genotype. We determined the average depth of the stalk cavity in the collection of old and local varieties/wild seedlings in the range of 1.67 mm (R22/3) – 14.82 mm (R14/11)/2.04 mm (S24) – 16.11 mm (S20). The results show that some fruits did not have stalk cavity (Table 2). We determined the average depth of eye basin in the collection of old and local varieties/wild seedlings in the range of 2.10 mm (R18/9) – 8.86 mm (R3/16)/1.22 mm (S38) – 11.69 mm (S22). We

Figure 3 Variability in the shape and the colour of fruits of evaluated genotypes of seedlings of *Malus domestica* Borkh.

did not find any significant differences between the collections. The values of the coefficients of variation confirm the low or extremely high degree of variability of the traits.

Michálek (2003) distinguishes the shapes of the stalk cavity as narrow and shallow, wide and shallow, wide and deep, narrow and deep. In some varieties, a characteristic swollen formation is formed, which often overgrows and tilts the stalk to one side. We recorded a relatively large variability of the pomological feature (Figure 3). In the calyx part of the fruit, the shape, size and eye basin are important features. The depth of eye basin and its shape can be important because they are a little variable (Figure 4). According to Michálek (2003), we know the following eye basin: the small eye basin, the spacious eye basin, the short eye basin, the funnel eye basin, plumpness eye basin.

The average length of core of the genotypes under the study was in the range of 13.16 mm (R31/2) – 27.36 mm (R3/2)/11.24 mm (S10) – 25.86 mm (S03), and diameter of core was in the range of 18.26 mm (R1/4) – 33.46 mm (R20/8)/13.72 mm (S75) – 30.86 mm (S3). We did not find any significant differences between the collections in the length and diameter of core, but diameters were relatively lower in the collection of wild seedlings. Coefficients of variation confirm the low or medium degree of variability of both characters.

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Figure 4 Variability in the characters of the depth of stalk cavity and depth of eye basin of the evaluated genotypes of the apple tree (*Malus domestica* Borkh.)

We determined the average value of the fruit shape index in the collection of old and local varieties and in the collection of wild seedlings. It is in the range from 0.69 (R1/4) to 1.26 (R41/4) and from 0.74 (S02) to 1.01 (S22). The comparison of genotypes with low and high values of the trait and variation ranges of the evaluated trait shows that genotypes with different values of the fruit shape index were determined in both collections. We did not find any significant differences between the collections. The coefficients of variation confirm the low degree of variability of the trait in both collections. Jakobek et al. (2020) recorded fruit shape index values (0.7–1.2) of old varieties.

The results from the analysis of variance of the evaluated traits (Table 3, Table 4) confirm the statistically significant differences between the evaluated genotypes.

Iqbal et al. (2011) described analytical methods tested in a laboratory for estimation of volume of axi-symmetric fruits like apples based on single view fruit images and the shape-based analytical models. The fruits are categorized into spherical, ellipsoid and paraboloid shapes with appropriate analytical models for their volume estimation. In both our collections of genotypes, spherical, elliptical and parabolic fruits are

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Table 3 Analysis of variance of evaluated fruit traits of genotypes of old and local varieties of *Malus domestica* Borkh. from the repository Bacúch

Factors	f	S	MS	F	H	LSD	
Weight of fruit (g)							
Between genotypes	9	124512.500	13 834.720	137.226	0.000	0.05	14.447
Within genotypes	90	9073.531	100.817			0.01	17.018
Total	99	133586.031					
Height of fruit (mm)							
Between genotypes	9	4463.000	495.888	70.057	0.000	0.05	3.828
Within genotypes	90	637.052	7.078			0.01	4.509
Total	99	5100.052					
Diameter of fruit (mm)							
Between genotypes	9	5470.781	607.864	73.086	0.000	0.05	4.149
Within genotypes	90	748 535	8.317			0.01	4.888
Total	99	6219.316					
Depth of stalk cavity (mm)							
Between genotypes	9	652.568	72.507	81.934	0.000	0.05	1.353
Within genotypes	90	79.645	0.884			0.01	1.594
Total	99	732.213					
Depth of eye basin (mm)							
Between genotypes	9	321.236	35.693	54.705	0.000	0.05	1.162
Within genotypes	90	58.721	0.652			0.01	1.369
Total	99	379.958					
Length of core (mm)							
Between genotypes	9	494.136	54.904	81.520	0.000	0.05	1.180
Within genotypes	90	60.615	0.673			0.01	1.391
Total	99	554.752					
Diameter of core (mm)							
Between genotypes	9	502.640	55.849	29.630	0.000	0.05	1.975
Within genotypes	90	169.636	1.884			0.01	2.327
Total	99	672.277					

Note: f – number of degrees of freedom; S – the sum of squares; MS – average square; F – Fischer test value; P – statistical significance by Fischer test; H – homogeneity; LSD – a least significant difference

Table 4 Analysis of variance of evaluated fruit traits of seedlings of *Malus domestica* Borkh.

Factors	f	S	MS	F	H	LSD	
Weight of fruit (g)							
Between genotypes	9	247224.800	27469.420	50.756	0.000	0.05	33.472
Within genotypes	90	48708.177	541.202			0.01	39.431
Total	99	295932.990					
Height of fruit (mm)							
Between genotypes	9	11953.730	1328.193	65.014	0.000	0.05	6.503
Within genotypes	90	1838.614	20.429			0.01	7.661
Total	99	13792.348					
Diameter of fruit (mm)							
Between genotypes	9	12673.470	1408.163	83.695	-0.000	0.05	5.901
Within genotypes	90	1514.241	16.824			0.01	6.952
Total	99	14187.709					
Depth of stalk cavity (mm)							
Between genotypes	9	827.314	91.923	33.774	-0.000	0.05	2.373
Within genotypes	90	244.951	2.721			0.01	2.796
Total	99	1072.266					
Depth of eye basin (mm)							
Between genotypes	9	575.351	63.928	46.523	-0.000	0.05	1.686
Within genotypes	90	123.668	1.374			0.01	1.986
Total	99	699.019					
Length of core (mm)							
Between genotypes	9	1722.531	191.392	39.174	0.000	0.05	3.180
Within genotypes	90	439.705	4.885			0.01	3.746
Total	99	2162.237					
Diameter of core (mm)							
Between genotypes	9	1096.727	121.858	34.799	-0.000	0.05	2.692
Within genotypes	90	315 156	3.501			0.01	3.171
Total	99	1411.883					

Note: f – number of degrees of freedom; S – the sum of squares; MS – average square; F – Fischer test value; P – statistical significance by Fischer test; H – homogeneity; LSD – a least significant difference

most represented (Figure 3), which is in accordance with the literature data.

Apples may vary in colour, from uniformly dark-red, red, reddish, green, orange, yellow, white, or bi-coloured, such as striped or blushed red on a yellow or green background. Results have shown high variability of shapes and colours in both collections of *Malus domestica*.

The core of the fruit usually consists of five seed carpels pockets or carpels. Sometimes some fruits have only four or three carpels. Each pocket contains seeds. The number of seeds per carpel is determined by the vigour and health of the plant. Different varieties of apples will have a different number of seeds. Each carpel generally contains two seeds. Seeds are smooth, shiny, and chestnut brown (Jackson, 2003; Huff, 2012–2013).

The individual varieties are characterized not only by the shape of the core but also by its size and its location (Michálek, 2003). Figure 5 documents some differences in the shape of the core. On the cross-section, we can see 10 vascular bundles in a circle around the core. They seem like darker or lighter dots. In total, we can observe 10 vascular bundles, of which 5 are located directly opposite the tops of the carpels, the other 5 are between them. The vascular bundles determine the angularity of the fruit. If they are in a circle and evenly

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Figure 5 Comparison of selected genotypes from the evaluated collection of seedlings of *Malus domestica* Borkh. in the number of vascular bundles in the longitudinal and cross section

developed, the fruit is uniformly rotund in cross-section. If they are in two circles, the outer ones tend to be more developed and the fruit is thus become slightly angular (Kohout, 1960; Dvořák et al., 1976; Michálek, 2003). The examples on the presented photo (Figure 5) document that in the evaluated collection of genotypes has a relatively large variability of this pomological feature.

Morphometrical analysis of seeds

On the seeds, we evaluated the characteristics of the weight of 10 seeds (g), the height of seeds (mm) and the diameter of seeds (mm). We determined the average weight of seeds in the genotypes under the study in the range from 0.38 g (R15/5) to 0.77 g (R41/1) and from 0.29 g (S28) to 0.98 g (S92). In the collection of wild seedlings, we recorded a higher range of variation in the evaluated trait. The coefficients of variation confirm the low or medium degree of variability of the trait. The average height of seeds for the collection of old and local varieties was in the range from 6.67 mm (R18/9) to 9.89 mm (R16/14) and for the collection of wild seedlings from 6.16 mm (S40) to 9.83 mm (S67). We did not find any significant differences between the collections. The coefficients of variation confirm the low degree of variability of the trait. We determined the

Table 5 Variability of seeds of old and local varieties and of wild seedlings of *Malus domestica* Borkh.

Weight of seeds (g)													
	Seedlings						**Genotypes from repository Bacúch**						
	n	**min**	**max**	**x̄**	**V**	**TH**		**n**	**min**	**max**	**x̄**	**V**	**TH**
Genotypes with low values													
S28	30	0.24	0.40	0.29	15.38	c	**R15/5**	30	0.34	0.41	0.38	5.79	b
S12	30	0.25	0.36	0.30	13.20	c	**R23/3**	30	0.36	0.42	0.39	5.17	b
Genotypes with high values													
S92	30	0.88	1.00	0.98	5.18	a	**R41/1**	30	0.74	0.81	0.77	2.99	a
S89	30	0.85	1.00	0.93	7.14	a	**R29/5**	30	0.72	0.76	0.74	1.78	a
Height of seeds (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S40	30	5.81	6.72	6.16	4.66	c	**R18/9**	30	6.19	7.05	6.67	4.19	b
S62	30	5.44	6.71	6.25	6.07	c	**R31/10**	30	6.15	7.42	6.68	5.24	b
Genotypes with high values													
S67	30	9.06	10.63	9.83	5.58	a	**R16/14**	30	9.24	10.56	9.89	4.85	a
S38	30	8.10	10.22	9.34	6.30	a	**R27/11**	30	9.35	10.05	9.66	2.44	a
Diameter of seeds (mm)													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S82	30	3.02	3.96	3.51	7.88	c	**R23/3**	30	3.23	4.05	3.73	7.55	c
S07	30	3.19	3.99	3.57	6.77	c	**R41/4**	30	3.24	4.14	3.75	6.94	c
Genotypes with high values													
S72	30	4.85	5.48	5.26	3.64	a	**R5/4**	30	4.48	9.87	5.71	27.05	a
S89	30	4.66	5.47	5.08	4.37	a	**R19/12**	30	4.51	7.79	5.63	20.93	a
Index of seed shape													
	Seedlings						**Genotypes from repository Bacúch**						
Genotypes with low values													
S62	30	1.17	1.41	1.35	5.26	bc	**R19/12**	30	0.66	1.62	1.36	25.59	b
S40	30	1.44	1.68	1.54	4.69	b	**R7/6**	30	0.55	1.88	1.45	32.58	b
Genotypes with high values													
S49	30	2.07	2.84	2.45	10.60	a	**R27/11**	30	2.32	2.83	2.52	7.06	a
S19	30	1.88	2.62	2.28	10.57	a	**R16/12**	30	2.11	2.71	2.43	7.77	a

Note: n – the number of measurements; min, max – minimal and maximal measured values; x̄ – arithmetic mean; V – coefficient of variation (%); H – LSD homogeneity test at $P_{0.05}$

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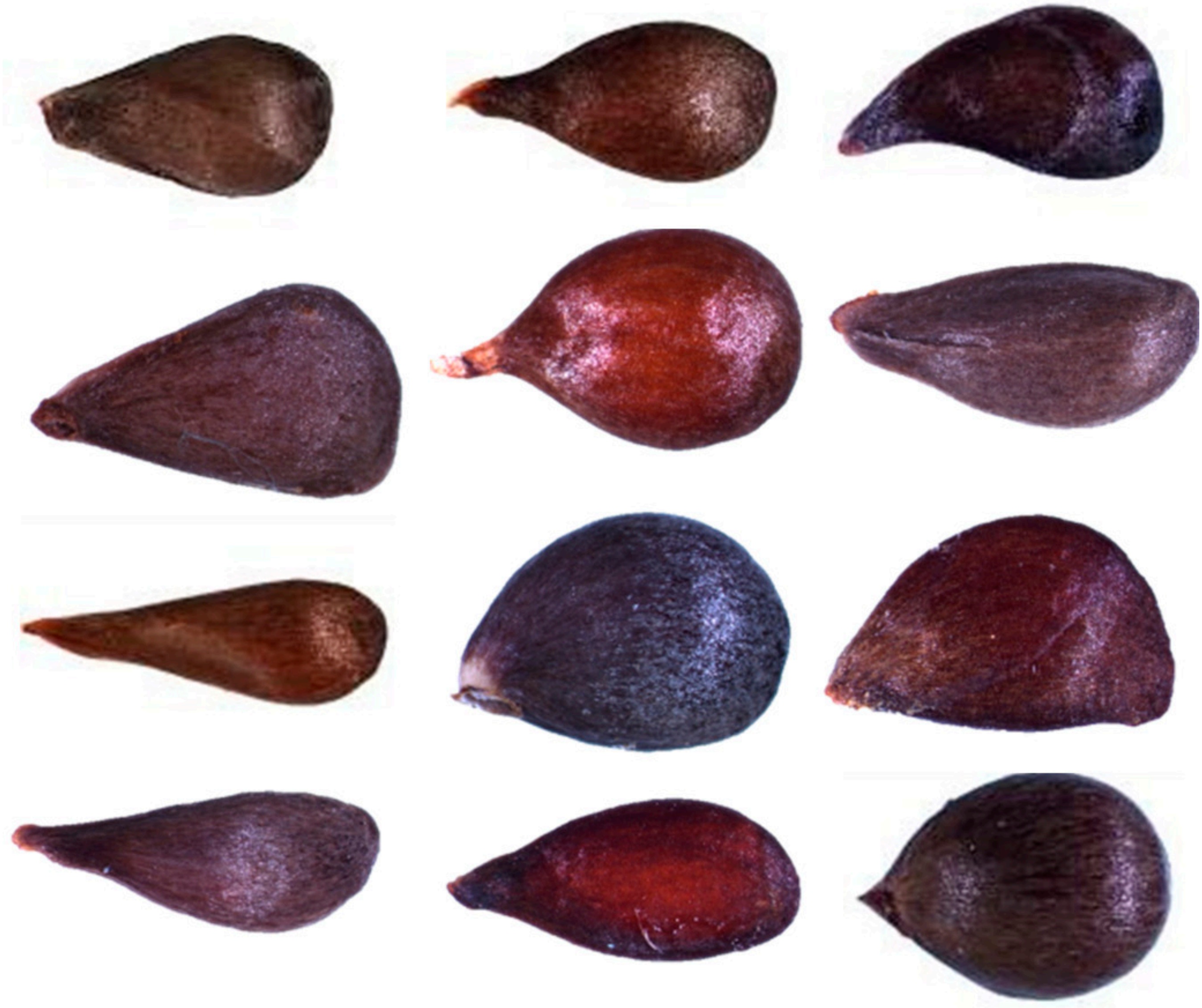

Figure 6 Comparison of selected genotypes from the evaluated collection of seedlings of *Malus domestica* Borkh. in the shape of seeds

average diameter of seeds in the collection of old and local varieties in the range 3.73 mm (R23/2) – 5.71 mm (R5/4) and for the collection of wild seedlings 3.51 mm (S82) – 5.26 mm (S72). We did not find any significant differences between the collections. The coefficients of variation show that the degree of variability of this trait within both collections varies from low to high (Table 5). Our results do not diverge from the data of Jacobek et al. (2020), who estimated the weight of seeds in fruits from 0.07 to 0.53 g, and the weight of a single seed from 0.03 to 0.08 g.

We determined the average value of the seed shape index in the collection of old and local varieties in the range 1.36 (R19/12) – 2.52 (R27/11) and in the collection of wild seedlings 1.35 (S62) – 2.45 (S49), respectively. A comparison of genotypes shows that genotypes with different seed shape indices were identified in both collections. We did not find any significant differences between the collections. The coefficients of variation show that the degree of variability of this trait within both collections varies from low to high.

Figure 6 shows a comparison of selected genotypes from the evaluated collection of the natural seedlings of the apple tree (*Malus domestica*) in seed shapes.

The analysis of variance of the evaluated traits (Table 6) confirmed the statistically significant differences between the evaluated genotypes.

Table 6 Analysis of variance of evaluated seed traits of genotypes from two collections of *Malus domestica* Borkh.

Factors	f	S	MS	F	H	LSD	
Genotypes from repository Bacúch							
Weight of 10 seeds (g)							
Between genotypes	9	0.888	0.098	42.074	0.000	0.05	0.069
Within genotypes	90	0.211	0.002			0.01	0.082
Total	99	1.099					
Height of seeds (mm)							
Between genotypes	9	78.918	8.768	24.898	0.000	0.05	0.853
Within genotypes	90	31.696	0.352			0.01	1.005
Total	99	110.614					
Diameter of seeds (mm)							
Between genotypes	9	16.238	1.804	10.330	0.000	0.05	0.601
Within genotypes	90	15.718	0.174			0.01	0.708
Total	99	31.957					
Seedlings							
Weight of 10 seeds (g)							
Between genotypes	9	0.724	0.080	268.465	0.000	0.05	0.024
Within genotypes	90	0.027	0.000			0.01	0.029
Total	99	0.751					
Height of seeds (mm)							
Between genotypes	9	43.955	4.884	25.377	0.000	0.05	0.024
Within genotypes	90	17.320	0.192			0.01	0.743
Total	99	61.276					
Diameter of seeds (mm)							
Between genotypes	9	10.109	1.123	15.295	0.000	0.05	0.389
Within genotypes	90	6.609	0.073			0.01	0.459
Total	99	16.719					

Note: f – number of degrees of freedom; S – the sum of squares; MS – average square; F – Fischer test value; P – statistical significance by Fischer test; H – homogeneity; LSD – a least significant difference

Conclusions

Based on morphometric analysis of fruits and seeds of both collection:

1. of old and local varieties,
2. of spontaneous seedlings from free pollination, we determined the range of phenotypic variability for all traits and combinations of traits in both groups of evaluated genotypes.

When comparing the ranges of variability for all evaluated traits, we found a significant degree of agreement. The results confirm that some individuals that grow wild and represent spontaneous seedlings from free pollination have a set of economically important traits and are ready to be used as potential genetic resources for a breeding program. Future efforts focused on "wild forms" should focus on preserving all unique genotypes to maintain both cultural heritage and biological genetic diversity.

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