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ECONOMIC PRODUCTION, HEALTH BENEFIT, GENETIC VARIABILITY, HERITABILITY AND GENETIC ADVANCE STUDY IN CHAENOMELES GENOTYPES

SUMMARY

The fruits of *Chaenomeles* or Japanese quince contain a number of biologically active substances that have proven medicinal and nutritional properties and are used in medicine, pharmacy and cooking. Due to their phenol concentration and cytotoxic effect, Japanese quince fruit extracts are raw material that promising, in cancer treatment and prevention. Japanese quince fruits contain ascorbic acid, which functions as a biological antioxidant and may help prevent chronic illnesses. The present research study aims to evaluate the unique germplasm collection of 20 chaenomeles genotypes by basic morphological and productivity characteristics, to determine their genetic parameters and identify of promising forms for selection purposes. The results show significant variability with respect to all studied traits, such as height and diameter of the shrub, height and diameter of the fruit, length of the vegetation period, number of blossoms (blossom bud), fruit weight (by shrub), fresh seed weight, seed weight (by fruit), fruit weight, fruit yield (ha). The genotype factor has a significant impact on the

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variation of most of the traits. The environmental factor has a major role to play in variation of the length of the vegetation period, fruit weight and seed weight (by fruit). A high rate of heritability combined with high genetic advance was found for traits, such as height/shrub (89.43%, 819.48), and fruit weight/shrub (89.40%, 50.80), which reveals the opportunity to make a rapid selection progress in improving these traits. The studied *Chaenomeles* accessions are genetically distant from each other to varying degrees and are divided into two main clusters. Genetically distant and highly productive genotypes, such as G3, G2, G27 and G29 can be used for genetic improvement of the culture and further in the selection.

Keywords: *Chaenomeles*, genetic diversity, heritability coefficient, genetic advance, quality components and health benefit

INTRODUCTION

Originating in East Asia, the small shrub known as Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach) was utilised in Chinese medicine three millennia ago (Bieniasz *et al.*, 2017). Japanese quince is the *Chaenomeles* species that is best suited to the climate of North Europe. Seed-based Japanese quince propagation is undesirable and results in significant genetic variation in physical and quality traits. As a viable alternative to chemical preservatives in the food and cosmetic industries, Japanese quince fruits are an important source of bio-compounds that can enhance the diet with potent antioxidants. One of the earliest domesticated plants in the Rosaceae family, a subgenus of Maloideae, is the quince of the *Chaenomeles* genus (Bieniasz *et al.*, 2017; Hummer and Janick 2009). In recent years, much attention has been paid to the problem of rational feeding of the population, based on the creation of balanced foods using plant raw materials high in natural antioxidants, such as vitamins, pectin, polyphenols and minerals, etc. One of the sources of these raw materials can be the fruits of *chaenomeles* or Japanese quince. This is a relatively new crop not only for the fruit growing but also for the processing industry. The value of *chaenomeles* fruits is that they contain a number of biologically active substances (mono- and disaccharides, organic acids, polyphenols, amino acids, essential oils) (Zhang *et al.*, 2019; Turkiewicz *et al.*, 2021), which are with proven medicinal and nutritional qualities. They are used in medicine, pharmacy and cooking (Droficheva *et al.*, 2010; Komar-Tyomnaya *et al.*, 2016; Turkiewicz *et al.*, 2021). Despite more than two hundred years of cultivation history in Europe, Japanese quince is still insufficiently domesticated and used as a fruit crop (Rumpunen, 2001; Komar-Tyomnaya, 2018).

Study of *chaenomeles* biodiversity and the selection of valuable genotypes have been conducted by researchers in many research centers in Europe and Russia, and on a variety of material of different origins and many characteristics. Complementing information on the extent of variability and the emergence of new characteristics resulting from cross-pollination (gene recombination) and the identification of genotypes that are donors of valuable productivity traits are essential for solving various selection problems for this culture (Kumpan, Sukhotskaya, 2010; Mihova *et al.*, 2012).

Numerous agro-ecological parameters, including soil type and regional climate, influence the mineral nutrition of plants and their productivity (Popović *et al.*, 2015; 2024; Milunović *et al.*, 2022; Božović *et al.*, 2020; 2022; Ljubičić *et al.*, 2023; Georgieva *et al.*, 2025a-b). The most important activity in the systematization of the available gene pool of a culture is the assessment of the biological diversity of collected forms and the establishment of a working collection. Collective accessions typically have a wide range of phenotypic and genotypic variability that needs to be assessed (Ryabchun *et al.*, 2012). Genetic heritability, variability, expected genetic advance, correlations and clustering of the cultivars with similarity are important for crop improvement programs (Ghimire and Mandal, 2019). The analysis of the "unique germplasm collection" will enable selection of the most productive and stable genotype and inclusion in the process of further selection. The aim of the study is to assess, unique germplasm collection chaenomeles genotypes by basic morphological and productivity characteristics, to determine genetic parameters and identify promising forms for selection purposes.

MATERIAL AND METHODS

The study was conducted in the period 2017-2022 in the experimental field of RIMSA - Troyan. The objective of the present study are 20 chaenomeles genotypes, such as G1', G2', G4', G5', G6', G2, G3, G11, G16, G22, G24, G26, G27, G29, G30, G32, G33, G40, G2D, GTch. The field experiment was established using a Randomized Complete Block Design (RCBD) with three biological replications. Each block/replication contained 5 stabilized plants per genotype (totaling 15 plants per accession to ensure sampling robustness). The inter-row/intra-row spacing in the plantation is 2.5 m/1 m. The shrubs are grown in nonirrigated conditions, naturally grassed between rows, which are mowed several times depending on the rainfall during the vegetation. Pruning and fertilizing were not carried out. The studied morphological traits were reported according to the methodology developed by Rumpunen *et al.*, (2003) for *Chaenomeles* sp., such as height and diameter of shrub (cm), height and diameter of fruit (mm), length of vegetation (days), number of blossoms in the blossom bud, fruit weight/ by shrub (kg), fruit weight (g), seed weight / by fruit (g), fresh seed weight/ (g), fruit yield/ha.

Representative *Japanese quince* fruit samples (500g) were collected annually during the harvesting periods of 2017–2022 at full physiological maturity for the purpose of extracting fresh pulp and performing multi-year analyses of phenols, tannins, and vitamin C. The extraction was performed following official analytical protocols and methods (Helrich, 1990) by using 70% ethanol. Total phenols and tannins were determined using the Folin-Ciocalteu reagent, with absorbance read at $\lambda=730\text{nm}$, expressed in mg quercetin equivalents per gram of fresh weight, according to Nagavani and Raghava Rao (2010); Total tannin content (mg quercetin equivalents per gram of fresh weight) was calculated by subtracting non-tannin phenols from the total phenols; Vitamin C content was determined using the 2,4-dinitrophenylhydrazine (DNP) method, as

described by Al-Ani (Przybylski *et al.*, 1998). A calibration curve was obtained using solutions with varying concentrations of ascorbic acid (vitamin C). The results were expressed as mg Ascorbic acid equivalents per gram of raw fruit. The tested quality features were analyzed using descriptive statistics.

Statistical analysis

Handling the genotype×year (G×Y) interaction, was using a Combined Two-Way ANOVA. Data collected are expressed as mean. The results of three replicates (three plants per genotype) were presented and univariate analysis of variance (ANOVA) was applied. Tukey's HSD (honest significant difference test) was used for multiple mean comparisons. Statistical significant differences were considered at $p < 0.5$ and $p < 0.1$. The statistical analysis was performed using Statistica 10 software (StatSoft, Inc., Tulsa, OK, USA). Annual observation were not analyzed separately before pooling.

Phenotypic variance (S^2_P or δ^2_P):

It is calculated as the sum of genotypic and environmental (mean) variance:

$$S^2_P = S^2_G + S^2_E$$

Genotypic variance (S^2_G or δ^2_G):

Includes additive, dominant effects and gene interactions (epistasis):

$$S^2_G = S^2_A + S^2_D + S^2_I$$

This value is usually obtained from the variance components by analysis of variance – ANOVA.

Inheritance (Heritability) in a broader sense (H^2):

Heritability (H^2), as a measure of heritability, in a broader sense, represents the relationship between genetic and total phenotypic variation.

$$H^2 = (S^2_G / S^2_P) \times 100$$

The following statistical methods were used to process the experimental data: analysis of variance, factor analysis by the principal components method (Vandev, 2009), hierarchical cluster analysis by the Ward method (1963) - to group genotypes based on similarity as a measure of differences (genetic distance). The Euclidean distance between the accessions was calculated (as a measure of divergence), and the data were standardized in advance. Principal component analysis (PCA) was conducted based on the correlation matrix (Bryman and Cramer, 2012). GGE biplot analysis was made, which uses a decomposition of the single values of the first two principal components (Yan and Rajcan, 2002). Genetic advance in absolute unit (GA) and genetic gain (GG) were assessed according to the methods of Johnson *et al.*, (1995). MS Excel (2003) and GENES 2009.7.0 for Windows XP (Cruz, 2009) were used to process the experimental data.

RESULTS AND DISCUSSION

The variation in the values of an indicator is related to the impact of environmental conditions. The stronger the impact, the more the variation of the trait will deviate from the mean. On the other hand, this rule is also related to the adaptability of a genotype to a specific impact. The value of the variance depends on the intensity of the adverse conditions, but also on the stability and plasticity of a genotype and the limitations of the genetic basis. At the same time, the value of the specific trait depends on the other indicators that determine the structure of the yield (Stoyanov, 2020).

Analysis of variance

The study of chaenomeles as a fruit crop was conducted on the main morphological and productive indicators. Significant variability was found with respect to all studied parameters (Table 1).

Rumpunen (2002) and Komar-Tyomnaya (2018) reported a wide range of variation in the basic morphological and biochemical characteristics in the evaluation of chaenomeles accessions.

Komar-Tyomnaya (2018) points out that one of the most variable traits is the length of the vegetation and fruit size, which is also found in the present study. According to Fedulova *et al.*, (2021), of all the traits, the fruit size (length and weight of the fruit), the colour and shape of the corolla are characterized by the highest variability.

The comprehensive two-way ANOVA (Table 1) explicitly demonstrates the partitioning of phenotypic variance components. The Genotype $\times$ Year interaction was mathematically identified as a significant source of variation ($p < 0.05$) for yield-related traits such as fruit weight per shrub, fruit diameter, and total yield per hectare.

This indicates that while genotypes maintain strong genetic determinism over morphological features, their final productive capacity undergoes distinct plastic responses depending on the climatic conditions of the respective environmental year.

The two-way ANOVA (Table 1) statistically confirms the highly significant impact of the genotype factor on the majority of the investigated parameters.

For structural and yield traits like fruit diameter ($F=98.84$), height/shrub ($F=51.72$), and fruit weight/shrub ($F=51.42$), the variance is heavily dominated by genetic determinism ($p < 0.001$).

In contrast, the environmental factor (Year) exerted a major and highly significant role in the variation of seed weight/fruit ($F=11.41$, $p < 0.001$) and weight/fruit ($F=3.11$, $p < 0.05$).

The Genotype $\times$ Year interaction was mathematically validated as a significant source of variation ($p < 0.05$) specifically for yield-related traits such as fruit yield/ha ($F=1.70$) and fruit weight/shrub ($F=1.76$), demonstrating that the productive capacity of individual accessions undergoes distinct plastic responses depending on annual climatic fluctuations.

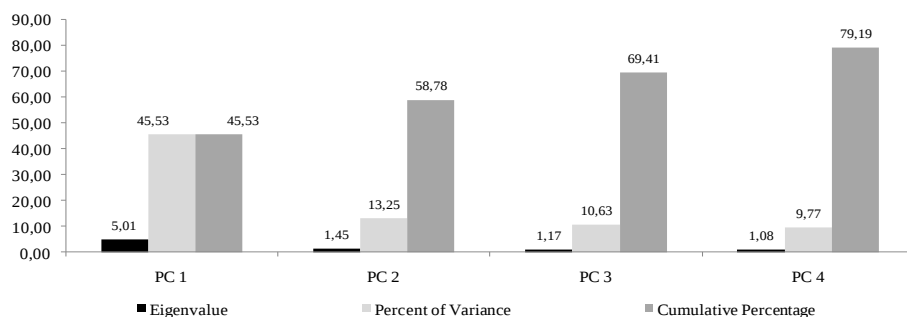
Table 1. Analysis of variance

Traits	Source of variation	Degrees of Freed. (Df)	MS -Mean Square	F-value	Significance (p-value)
Height/shrub	Year - Y	5	6.410	1.39	p = 0.238
	Genotype-G	19	238.420	51.72	p = 0.0001
	G × Y	95	8.520	1.85	p = 0.0045
	Error	240	4.610		
Diameter/shrub	Year - Y	5	62.440	0.53	p = 0.753
	Genotype-G	19	215.956	1.82	p = 0.024
	G × Y	95	142.100	1.20	p = 0.134
	Error	240	118.800		
Vegetation	Year - Y	5	4476.585	0.01	p = 0.999
	Genotype-G	19	653460.100	1.82	p = 0.024
	G × Y	95	412350.000	1.15	p = 0.198
	Error	240	358901.000		
Diameter/fruit	Year - Y	5	208.930	3.16	p = 0.011
	Genotype-G	19	6537.500	98.84	p = 0.0001
	G × Y	95	112.400	1.70	p = 0.014
	Error	240	66.140		
Height/fruit	Year - Y	5	40.600	0.21	p = 0.958
	Genotype-G	19	10334.200	53.51	p = 0.0001
	G × Y	95	285.400	1.48	p = 0.065
	Error	240	193.110		
No of blossoms	Year - Y	5	0.0001	0.50	p = 0.776
	Genotype-G	19	0.0004	2.00	p = 0.011
	G × Y	95	0.0003	1.50	p = 0.054
	Error	240	0.0002		
Fresh weight/seed	Year - Y	5	54.380	2.86	p = 0.019
	Genotype-G	19	189.292	9.96	p = 0.0001
	G × Y	95	28.450	1.50	p = 0.054
	Error	240	19.000		
Seed weight/fruit	Year - Y	5	13.243	11.41	p = 0.0001
	Genotype-G	19	5.421	4.67	p = 0.0001
	G × Y	95	2.150	1.85	p = 0.0045
	Error	240			
Weight/fruit	Year - Y	5	8.261	3.11	p = 0.012
	Genotype-G	19	6.571	2.48	p = 0.0012
	G × Y	95	3.890	1.47	p = 0.069
	Error	240	2.653		
Fruit weight/shrub	Year - Y	5	1.320	1.38	p = 0.241
	Genotype-G	19	49.000	51.42	p = 0.0001
	G × Y	95	1.680	1.76	p = 0.0094
	Error	240	0.953		
Yield fruit/ha	Year - Y	5	36.160	0.96	p = 0.443
	Genotype-G	19	268.297	7.09	p = 0.0001
	G × Y	95	64.500	1.70	p = 0.0140
	Error	240	37.840		

**and * significant at 0.1% and 0.5% probability levels

Fundamental component analysis

During the study period, four main components with a value above one (Figure 1) had an impact on the variation of traits. The first principal component (PC1) has the strongest impact (45.53%) on fruit yield/ha, fruit weight/by shrub, fruit weight, seed weight/ by fruit, fruit diameter and height (Table 2). The second principal component (PC2) has a significantly lower impact, but was important for traits, such as number of blossoms, yield/ha, fruit weight/ by shrub and fruit weight. The third (PC3) and fourth (PC4) principal components have an impact share on traits of about 10%. PC3 affects the diameter and height of shrub, yield/ha and fruit weight/ by shrub, and PC4 mainly affects the yield/ha, fruit weight/ by shrub and seed weight/ by fruit.



PC1, PC2, PC3, PC4 = principal component 1, 2, 3 and 4, respectively

Figure 1. Effect of four main components of chaenomeles tested traits

Table 2. Impact of main components on tested traits of chaenomeles genotypes

Traits	PC 1	PC 2	PC 3	PC 4
Height/shrub	0.326	-0.399	0.342	-0.202
Diameter/shrub	0.272	-0.314	0.571	-0.154
Vegetation period	-0.092	-0.616	-0.102	0.183
Diameter/fruit	0.333	0.026	-0.337	-0.31
Height/fruit	0.319	0.068	-0.379	-0.436
Number of blossoms/blossom bud	0.181	0.264	0.255	-0.424
Fresh weight/seed	0.242	-0.349	-0.334	0.037
Weight of seed/fruit	0.343	-0.175	-0.254	0.326
Weight/fruit	0.354	0.142	-0.079	0.091
Fruit weight/shrub	0.364	0.24	0.146	0.43
Yield fruit/ha	0.364	0.24	0.146	0.40

*PC1, PC2, PC3, PC4 = principal component 1, 2, 3 and 4, respectively

This impact is usually associated with the contrasting manifestations during the different periods of the study, which gives preference to different indicators. Various studies on the variation of the elements of the yield and on the yield itself show that in a large number of cases the main components are two or three. The presence of a larger number of components is associated with a very complex impact of the environment, genotype and the interaction between them.

Table 3 presents the complex impact of the four main principal components on each of the chaenomeles genotypes. The impact of the first principal component is decisive for the manifestations of traits in accessions, such as G2, G3, G29, G27 and G22, and of the second component - in G1', G5', G26, G40, G2D and GTch. Genotypes, such as G2', G4', G6', G30 and G33 are influenced by the impact of the third principal component, and in G11, G16 and G24 the impact of the principal component 4 is predominant. Figure 2A shows the location of the vectors of the traits of the coordinate system, using the impact of the first two main components - PC1 and PC2 on the overall variation. The angles between the vectors of traits, such fruit weight/shrub and fruit yield/ha, and height/shrub and diameter/shrub are sharp, indicating a positive correlation between them.

Table 3. Impact of main components in chaenomeles genotypes

Genotypes	PC 1	PC 2	PC 3	PC 4
G1'	-0.73	1.56	1.42	-1.03
G2'	-1.83	0.357	0.933	-0.64
G4'	-1.05	0.055	0.725	0.65
G5'	-0.41	0.924	0.682	-0.157
G6'	-0.16	0.578	0.974	0.028
G2	4.62	0.641	-0.175	0.331
G3	2.26	-1.35	0.728	0.746
G11	-0.654	-0.335	-1.793	0.586
G16	-1.13	-1.453	-0.538	0.254
G22	1.90	0.095	-0.944	0.025
G24	-4.49	0.529	0.575	1.488
G26	-2.99	0.785	-0.125	0.431
G27	2.81	-1.601	0.147	-1.808
G29	4.22	1.27	0.56	2.296
G30	0.346	-0.305	0.348	0.221
G32	-140	-2.92	-0.711	0.731
G33	-0.35	-1.58	1.725	-1.277
G40	0.22	1.246	-0.608	-1.864
G2D	-0.258	1.273	-2.128	-0.865
GTch	-0.923	0.238	-1.799	-0.142

*PC1; PC2; PC3, PC4 = principal component 1, 2, 3 and 4, respectively

A positive correlation is established between diameter/fruit and fruit height, as well as between the vegetation and the number of blossoms in the blossom bud. There are negative correlations between traits, such as height/shrub, diameter/shrub and height/fruit; fruit weight/ by shrub, yield/ha and height/fruit.

Figure 2B shows the location of the Chaenomeles accessions according to their values for the main components PC1 and PC2. The genotypes are positioned

in two quadrants. The first group is relatively smaller and includes 8 accessions with a positive value for PC1 and a negative value for PC2. Genotypes G32 and G33 are further from the abscissa than others, which shows that besides similarities in most of the studied traits there are some differences between them, especially in diameter and height of the fruit, seed weight/ by fruit and number of blossoms.

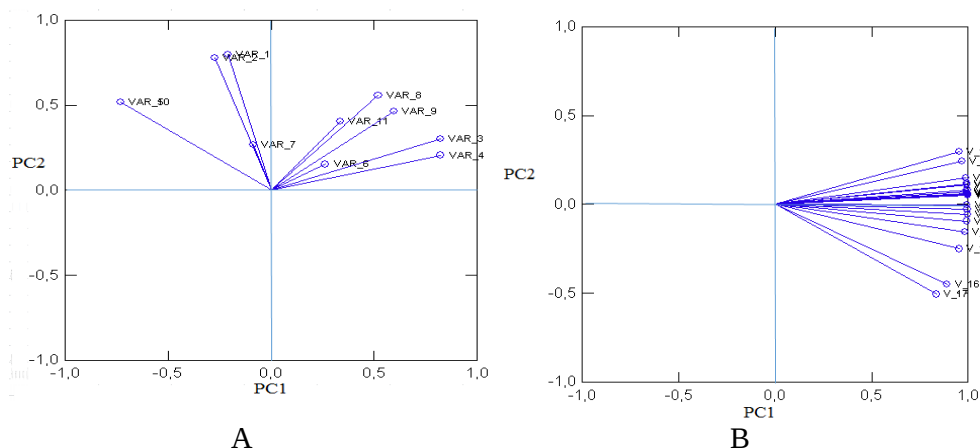


Figure 2. Projection of the traits *Chaenomeles* sp. genotypes (A) on a vector plane (B)

Genotypes G24 and G26 are also distant from the others, although this is less pronounced. The second group of accessions is located in the quadrant bounded by the positive values of PC1 and PC2. Accessions G2 and G29 have higher PC2 values. This is probably because of the higher values of height and diameter of the shrub, vegetation and fresh seed weight. The main parameters that dominate the second group of accessions are diameter/fruit, height/fruit, vegetation, number of blossoms in the blossom bud.

Phenotypic and genotypic variance

The size of the genotypic variant is higher than the phenotypic variant for traits, such as height and diameter of the shrub, height and diameter of fruit, fruit weight/ by shrub, fruit yield/ha, fresh seed weight (Table 4). This shows that the genotypic component of variation is a major factor contributing to the overall phenotypic variation of these indicators. Regarding the length of the vegetation, fruit weight/ by shrub, seed weight/ by fruit and number of blossoms in the blossom bud, the values of the phenotypic variant prevail, which means that the environment makes a major contribution to their entire phenotypic variation.

Regarding the components of variation (Table 4), the highest phenotypic variance (V_p) among the fruit dimensions was recorded for fruit height ($V_p=362.12$) and fruit diameter ($V_p=173.99$), followed by the length of the vegetation period ($V_p=118.80$). The lowest phenotypic variation was observed for the number of blossoms ($V_p=0.0003$). Regarding the genotypic variance (V_g), robust genetic components were reported for fruit height ($V_g=169.01$) and fruit diameter ($V_g=107.85$), confirming that selection based on fruit biometric dimensions would be highly stable and effective due to the massive genetic variance component available in this germplasm collection.

According to Rumpunen *et al.*, (2003) the high phenotypic and genetic diversity of *Chaenomeles* species in terms of basic morphological characteristics is conducive to crop improvement through selection. The efforts of breeders should be focused on the creation of cultivars that are locally adapted, with increased productivity and cold tolerance, as well as universal cultivars used as a high-vitamin fruit crop and for decorative purposes.

Heritability and genetic advance

Heritability is an indicator of phenotypic variation due to genetic causes and has a predictive function in plant selection. The higher the heritability rate, the simpler is the selection procedures. Heritability in a broad sense only shows whether there is enough genetic variation in the population and how the population will respond to selection pressure (Taneva, 2019). In the conditions of the present study, the highest heritability coefficient in the broad sense (H^2) was found for height (89.43%), fruit weight/ by shrub (89.40%) and fruit yield/ha (50.37%), (Table 4).

The vegetation period (44.49%) and weight/fruit (45.08%) are characterized by the lowest heritability coefficient. In assessing the heritability of some major traits in apple genotypes, Koohneshin *et al.*, (2018) establish the highest and the lowest heritability coefficient for yield and period up to blossoming, respectively, with values of 64.10 and 5.75%.

In another study, which examined 40 apple cultivars, Rasool *et al.*, (2019) indicate the highest heritability in the fruit parameters, such as weight/fruit (99.5%) and fruit length (98.5%). Traits with higher values of heritability in a broad sense are less affected by the environment, thus the selection to improve them can be useful. In traits with low heritability, genetic improvement through selection would be difficult (Ghimire and Mandal, 2019).

Genetic advance (GA) is an important parameter for a selection combined with heritability, which can predict the degree of improvement of the desired trait. High genetic advance was estimated for height/shrub (819.48), diameter/shrub (751.64), fruit yield/ha (112.28) and fresh seed weight (99.05). For all other traits lower values were found, from 0.002 for fruit weight / by shrub to 50.80 fruit weight/by shrub.

High heritability combined with high genetic advances indicates the presence of additive genetic effects, while high heritability combined with low genetic advances is an indicator of the presence of non-additive genetic effects involved in trait control.

Table 4. Genetic component of variation tested genotypes for quantitative traits

Genetic Parameters	Min	Max	Vg	Vp	H ² (%)	GCV (%)	PCV (%)
Height/shrub	120.00	175.00	39.01	43.62	89.43	4.31	4.56
Diameter/shrub	150.00	230.00	16.19	134.99	11.99	3.51	10.15
Vegetation period	168.00	185.00	53.45	118.80	45.00	1.82	2.71
Diameter/fruit	35.20	58.40	107.85	173.99	61.99	9.40	11.95
Height/fruit	32.10	54.60	169.01	362.12	46.67	8.21	12.02
Number of blossoms	3.00	5.00	0.0001	0.0003	33.33	15.11	26.17
Fresh weight/seed	0.25	0.48	28.38	47.38	59.89	14.50	18.73
Seed weight/fruit	1.10	3.80	0.71	1.87	37.97	11.20	18.17
Weight/fruit	32.50	78.40	16.17	35.89	45.05	12.50	18.62
Fruit weight/shrub	1.20	5.80	8.01	8.96	89.40	33.06	35.15
Yield fruit/ha	6.40	23.50	38.41	76.25	50.37	33.16	46.73
Total phenols	2.10	4.85	1.14	1.52	75.00	33.17	38.30
Total tannins	1.50	3.60	0.88	1.22	72.13	37.38	44.02
Ascorbic acid-Vit. C	4.10	7.80	1.94	2.94	65.99	24.03	29.56

Vg-genotypic variance, *Vp*-phenotypic variance, *GA*-genetic advance, *GG*-genetic gain, *H²* (%) -broad-sense heritability, *GCV* (%) -genotypic coefficient of variation, *CVP* (%) -phenotypic coefficient of variation

In the present study, additive genetic effects were found for shrub height and diameter, and fruit yield/ha. The genetic effects of fruit weight/ by shrub (*GG*=23.52) and seed weight/ by fruit (*GG*=83.21) are expected to be of the same nature, despite the lower heritability coefficient of the latter trait.

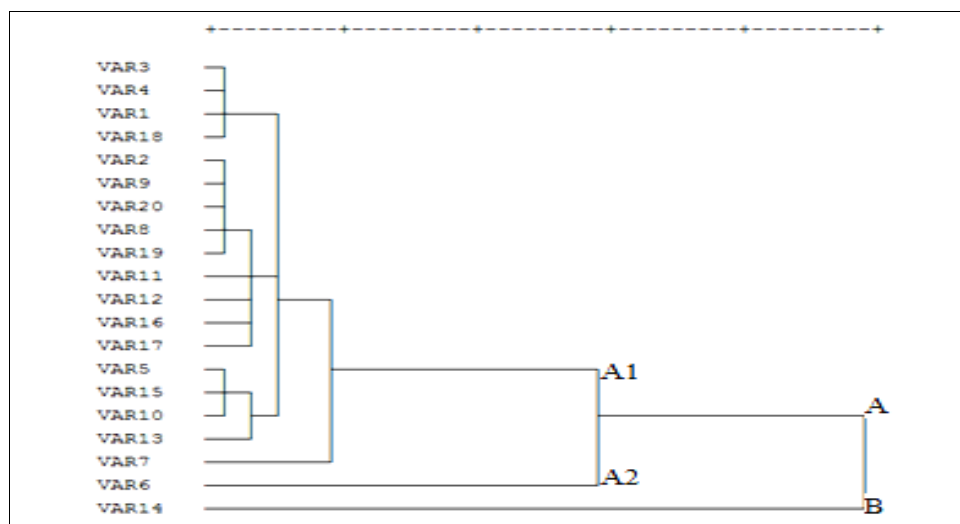
This shows the opportunity to conduct an effective selection of genotypes by phenotype in the early generations and to achieve rapid selection progress on these important indicators in chaenomeles species. The lowest heritability coefficient combined with the lowest genetic advancement was found in vegetation period and fruit weight/ by shrub. Therefore, in the heritability of these traits, non-additive genetic actions predominate, and effective selection of genotype by phenotype in early generations would not be appropriate.

Phenotype and genotypic coefficient of variation

As mathematically expected, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) across all investigated traits (Table 4), reflecting the additional contribution of environmental fluctuations to the total phenotypic expression. The closest proximity between PCV and GCV was observed for shrub height (4.56% and 4.31%, respectively) and fruit weight/shrub (35.06% and 33.15%, respectively), indicating that these specific traits are highly stable and primarily governed by genetic factors rather than environmental modifications. Conversely, the largest gaps between PCV and GCV were identified for the number of blossoms (26.17% vs. 15.11%) and seed weight/fruit (18.17% vs. 11.20%), which underscores a more pronounced environmental influence on these components.

Hierarchical cluster analysis

The use of multivariate methods such as cluster analysis and PC analysis is an effective way to assess genetic diversity. In the presence of data from different environmental conditions, it is preferable to apply cluster analysis, which includes the genotype-environment interaction in the grouping, and is more important for selection (Valkova, 2017). The results of the hierarchical cluster analysis are presented in Figure 3.



V1-1'; V2-2'; V3-4'; V4-5'; V5-6'; V6-2; V7-3; V8-11; V9-16; V10-22; V11-24; V12-26; V13-27; V14-29; V15-30; V16-32; V17-33; V18-40; V19-2D; V20-Tch

Figure 3. Dendrogram of chaenomeles genotypes

Chaenomeles genotypes are divided into two main clusters. Cluster "A", which includes almost all genotypes, consists of two main subclusters ("A1", "A2"). Only genotype G2 is located in subcluster A2, which shows that it is genetically different from other accessions in the same cluster in many respects. G2 plants are tall, with a wide medium diameter, relatively small-sized fruits and high productivity, expressed by fruit weight/ by shrub. In terms of fruit yield, it ranks second, after genotype G29.

Ljubičić *et al.*, (2023) is recommended the application of GGE biplot analysis for better visualization of results in yield and stability evaluation. According to Božović *et al.*, (2020; 2022), Rakašćan *et al.*, (2021), Popović *et al.*, (2024), Simić *et al.*, (2025) and Kosev *et al.*, (2026) the GGE biplot is a good statistical tool for analyzing data from testing genotypes in multiple environments.

The second subcluster "A1" is the most numerous. The cluster from the upper to the lower end of the dendrogram includes genotypes that are divided into separate groups. The first group includes G4'; G5', G1 and G40, showing similarity in fruit weight/ by shrub, length of vegetation and seed weight/ by fruit.

They are also characterized by insignificant differences in terms of fruit yield/ha. The next group consists of accessions G2', GTch, G11, G2D and G16, which are similar in fruit diameter. They are characterized by the lowest fruit weight/ by shrub, length of the vegetation period around the average for the group and low fruit yield/ha.

Accessions G24, G26, G32 and G33 are located below them and occupy the middle part of the cluster analysis figure. They are characterized by a lower value of fruit weight / by shrub and the lowest fruit yield/ha. The other three genotypes G22, G27 and G30 are united in one subgroup due to the lower fruit weight/ by shrub, the average fruit yield and the average number of blossoms in the blossom bud. At the bottom of the dendrogram is designed genotype G3, whose fruits have higher height, high fruit yield/ha and one of the highest fruit weight/ by shrub.

Genotype G29 occupies an independent place and forms a cluster "B". Its specific location is mainly due to the wide diameter of the shrub, the highest fruit weight/ by shrub and the highest fruit yield/ha.

The results of the cluster analysis and the formation of the separate groups show that the genotypes of close origin do not always fall into the same cluster. This is most often observed when clustering is performed at the values of important traits for selection. To make faster progress in the selection process while improving this crop, it is recommended to use genetically distant and highly productive genotypes such as G3, G2, G27 and G29 to be included in future selection programs.

The multivariate PCA and hierarchical clustering analyses clearly differentiate genotypes G2, G3, G27, and G29 due to their superior morpho-productive characteristics. Genotypes G2 and G29 exhibit high common adaptive potential due to their strong vegetative architecture, whereas G3 and G27 are distinguished by stable individual fruit weight. The genetic distance among these four distinct genotypes indicates that their inclusion in future cross-breeding programs will maximize heterosis and deliver offspring with optimal stability and biochemical value.

Quality composition in fresh Japanese quince and health benefit

The results show that in Japanese quince fruits, in 70% ethanol as a solvent, the total phenol content was on average 8.49 mg quercetin equivalents per gram, the total tannins content 6.14 mg and total Ascorbic acid (Vitamin C) 5.79 mg (Table 5, Figure 4).

Table 5. Average polyphenolic composition (mg) and Ascorbic acid (mg) in fresh JQ fruits, 2017-2022

Traits	2017	2018	2019	2020	2021	2022	Average	Std. Dev.
Total phenols ¹	8.30	8.50	8.7	8.40	8.45	8.61	8.49	0.11
Total tannins ¹	6.10	6.05	6.20	6.27	6.08	6.16	6.14	0.08
Ascorbic acid, vit.C	5.80	5.70	5.89	5.68	5.75	5.95	5.79	0.11

¹Expressed as mg quercetin equivalents per gram of fresh weight

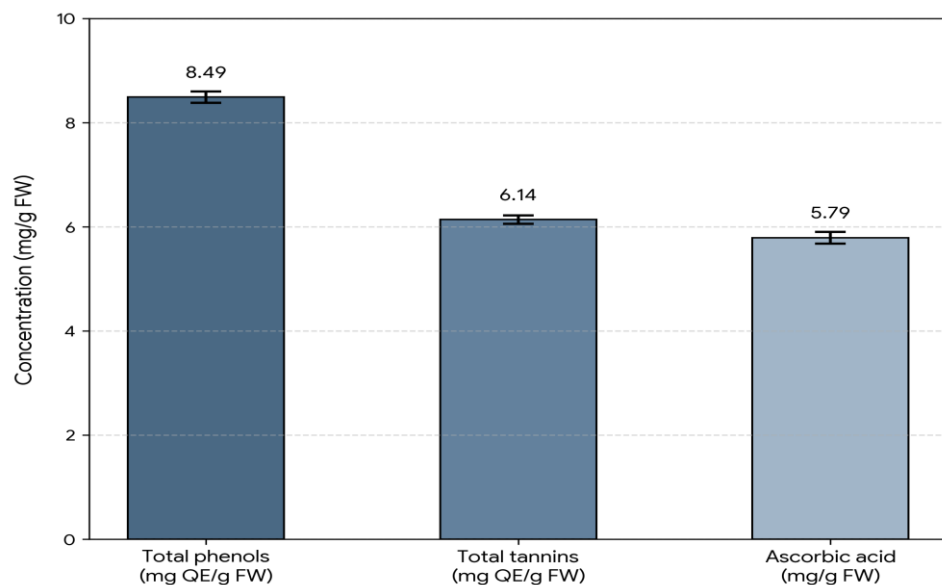


Figure 4. Average polyphenolic composition and Ascorbic acid in fresh JQ fruits in 70% ethanol. Error bars represent the standard deviation ($\pm$ SD) across the 2017–2022 investigation period.

Our empirical findings (Table 5) demonstrate that the investigated *Chaenomeles* genotypes exhibit an exceptionally high accumulation of bioactive compounds. The average multi-year total phenol content reached 8.49 mg quercetin equivalents per gram of fresh weight, while the total tannin fraction stabilized at an average of 6.14 mg/g. These concentrations are significantly higher than the baseline reports in earlier literature for standard genotypes, which often range around 3.22 mg/g for phenols and 2.51 mg/g for tannins. Furthermore, the average ascorbic acid (Vitamin C) concentration remained highly robust across the evaluation years, averaging 5.79 mg/g. This massive abundance of structural polyphenols and natural antioxidants significantly augments the mechanical and physiological health-promoting value of these fruits, cementing their role as an elite functional food ingredient capable of reducing systemic oxidative stress and serving as premium raw materials for the pharmaceutical industry.

In addition to giving the fruit its colour, these pigments are linked to a number of health advantages, such as defence against oxidative stress and a lower chance of developing chronic illnesses like diabetes and cardiovascular disease (Petrov *et al.*, 2025). The average vitamin C content varies by genotype from 2.0 to 24.2 mg/g (Bieniasz *et al.*, 2017; Hellin *et al.*, 2003; Ros *et al.*, 2004).

Summarizing previously published findings indicates the great health benefits of *Chaenomeles*. Because of their unique biochemical makeup and biocompound richness, Japanese quince fruit extracts exhibit high biological activity (Du *et al.*, 2013; Strugała *et al.*, 2016). Through its direct impact on

hepatic gene expression and metabolism, Japanese quince polyphenolic extract has been shown in numerous studies to have a possible role in regulating glucose metabolism in the human liver HepG2 cell line. Japanese quince reduces the generation of glucose in the liver and activates AMPK, which is implicated in the regulation of gluconeogenesis. Japanese quince's capacity to boost glucose absorption and glycogen production is linked to its ability to lower blood glucose levels. The results for Japanese quince polyphenols show potential as a phytomedicine or dietary component suitable for preventing prediabetes, type 2 diabetes, and metabolic syndrome because non-diabetic but with elevated blood glucose levels are strongly predictive of a high risk of developing type 2 diabetes (Zakłos-Szyda *et al.*, 2015; Boucher *et al.*, 2014; Zakłos-Szyda and Pawlik, 2018). Japanese quince polyphenols are helpful in treating prediabetes because they increase glucose absorption and enhance hepatic insulin sensitivity by lowering PTP1B expression syndrome (Zakłos-Szyda and Pawlik, 2018).

Biological activity of Japanese quince (JQ) fruits has shown that they have a great deal of potential for human health, including growth promotion of the beneficial intestinal bacteria *Lactocaseibacillus casei* and *Lactiplantibacillus plantarum*, protection against free radicals on the lipid membrane, and inhibition of cyclooxygenase involved in inflammatory reactions (Strugała *et al.*, 2016). Because of their phenol content and cytotoxic action, Japanese quince fruit extracts have been demonstrated by other researchers to be a promising raw material for cancer treatment and prevention (Gorlach *et al.*, 2011; Lewandowska *et al.*, 2013; Owczarek *et al.*, 2017; Strek *et al.*, 2007).

In addition, ascorbic acid, the primary physiologically active form of vitamin C, is abundant in Japanese quince fruits and their juice. Ascorbic acid functions as a biological antioxidant and may help prevent chronic diseases (Bieniasz *et al.*, 2017; Frei, *et al.*, 2012; Ros *et al.*, 2004). Some dietary fibres and pectin have been identified (Thomas *et al.*, 2000; 2003), which are helpful in controlling body weight and may stop the development of heart disease and type 2 diabetes (Kendall *et al.*, 2010).

The multivariate PCA and hierarchical clustering analyses clearly differentiate genotypes G2, G3, G27, and G29 due to specific agronomic syndromes that are highly valuable for future breeding programs. Genotype G29 represents an ideal morphotype for maximum biomass and productivity, distinguished by the widest shrub diameter, maximum fruit weight per shrub, and the highest overall fruit yield per hectare. Genotype G2 complements this ideotype by partitioning its resources into high vegetative vigor and absolute shrub architecture stability. On the other hand, genotypes G3 and G27 possess unique breeding value due to their high individual fruit weight and strict biometric consistency under multi-year evaluations. Utilizing these genetically divergent yet highly productive accessions in planned cross-breeding schemes will maximize heterotic effects and facilitate the combination of optimal vegetative architecture with high biochemical value (phenols and Vitamin C) in the offspring."

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CONCLUSIONS

The fruits, leaves, and seeds Japanese quince (*Chaenomeles japonica*) are great raw materials for the development of functional foods because of their high level of bio-compounds, including phenols, vitamin C, triterpenes, fibres, essential amino acids, and microelements. Their biochemical makeup and antibacterial, anti-inflammatory, and anti-cancer characteristics increased their use in the pharmaceutical industry. It was shown that quince waste, even after industrial processing, is still useful and appropriate for remanufacturing and creating cutting-edge, high-value goods that can benefit the economic and ecological benefits.

The evaluation of the unique germplasm collection of 20 chaenomeles genotypes shows significant variability in the studied morphological and economic qualities, such as height and diameter of the shrub, height and diameter of fruit, length of the vegetation period, number of blossoms/blossom bud, fruit weight/shrub, fresh seed weight, seed weight/fruit, weight/fruit, fruit yield/ha. The genotype factor has a significant impact on the variation of most of the traits. The environmental factor has a major role to play in variation of length of the vegetation period, weight/fruit and seed weight/ by fruit.

A high heritability coefficient and a high genetic advance were found for height/shrub (89.43%, 819.48) and fruit weight/shrub (89.40%, 50.80), which reveals the opportunity to make a rapid selection progress in improving these traits. Conversely, for the length of the vegetation period (45.00%, 10.98) and single fruit weight (Weight/fruit, 45.05%, 0.002), lower values of the heritability coefficient and genetic advancement were reported, which suggests that a phenotypic selection based on these specific indicators in the early generations would be less effective due to a more pronounced non-additive genetic variance component.

The studied *Chaenomeles* accessions are genetically distant from each other to varying degrees and are divided into two main clusters. Genetically distant and highly productive genotypes, such as G3, G2, G27 and G29 can be used for genetic improvement in *Chaenomeles* breeding.

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