

CHARACTERIZATION AND IDENTIFICATION OF GENETIC DIVERSITY AMONG ROSE GENOTYPES USING MORPHOLOGICAL AND MOLECULAR MARKERS

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Abstract

The aim of this research was to investigate the genetic variability of *Rosa* species and cultivars using morphological traits and PCR-based start codon targeted (SCoT) molecular markers to determine their degree of relatedness. Morphological analysis revealed significant differences between the genotypes. Thus, the rosehip fruit diameter ranged between 16.4 mm (*R. canina* Baisoara) and 26.5 mm (*R. rugosa* UASVM Cluj-Napoca), whereas the fruits length ranged between 33.7-27.2 mm for *R. canina* and 19.1-20.2 mm for *R. rugosa*. Regarding the number of seeds/fruit, the highest average number was registered in *R. canina* Salina Turda (30.05) and the lowest in *R. rugosa* UASVM Cluj-Napoca (17.15). Analysis of SCoT bands revealed a polymorphism ranging from 62.5% (SCoT 8) to 81.81% (SCoT 2). The PCoA and PCA analysis plots showed a tight grouping of the *R. canina* genotypes, distinct from the others *Rosa* sp. The cluster analysis divided the samples into two groups: the first comprised *R. galica*, *R. rugosa*, and *R. damascena*, while the second consisted of *R. canina*. Significant variations in each subgroup may be attributed to the samples' origins, and to morphological and genetic differences. These results provide new perspectives for exploiting the existing genetic variability among the evaluated species and further using them for ornamental, pharmaceutical, and cosmetic purposes.

Key words: morphological traits, multivariate analysis, *R. canina*, rosehip, SCoT analysis.

INTRODUCTION

There are approximately 200 species in the genus and subgenus *Rosa*, which are categorized and divided into ten sections (Wissemann, 2017).

In the Romanian flora, there are a number of 29 documented spontaneous and subsponaneous species, with an additional of five *Rosa* L. genus hybrids. Out of the 29 species, 16 species have been identified in the northeastern region of Romania (Oprea 2005).

The *Rosa* genus includes a variety of species that have served humanity as an economic resource, food, and medicine throughout history (Mármol et al., 2017). The therapeutic potential of *Rosa* species include anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, antibiotic, analgesic, antidiabetic, neuroprotective, anti-hyperlipidemic, genoprotective, and anti-

obesity properties (Khazaei et al., 2020). These properties are due to the fruits' significant content of bioactive compounds and nutritional properties. The composition of the fruit, and, consequently, its derived products, varies according to the species, agricultural practices, cultivation area, as well as the time of harvest (Mannozi et al., 2020). Furthermore, multiple variables, such as genotype, variety, growth and harvest location, growth conditions, harvesting time, maturity phases, and climatic conditions (i.e., temperature changes, and ultraviolet radiation), may have an impact on the morphological parameters and genetic diversity of rosehips (Benković-Lačić et al., 2022, Medveckienė et al., 2023). This highlights the importance of understanding the genetic variability of *Rosa* species and cultivars using molecular markers, which can help optimize cultivation and harvesting practices to maximize their medicinal potential (Agarwal et

al., 2019). Throughout history, different plant parts of *Rosa* genus have been used for several purposes, including for nutritional, medicinal, and economic resources. *Rosa* species have been used historically since the Hippocratic era in ancient Greece when *Rosa canina* was recommended as a treatment for injuries caused by animals. Traditional Persian medicine records suggested the use of *Rosa canina* as a cure against several ailments, including headaches, gastrointestinal, and neurological disorders (Khazaei et al., 2020).

Currently, traditional remedies enjoy a high degree of trust from public, who often believe that natural products are inherently beneficial to the body and present fewer side effects compared to synthetic medication. Conversely, health professionals are reticent regarding recommending these products, highlighting the lack of standardization of available formulas as well as the absence of rigorous scientific documentation, such as clinical trials, necessary to establish the efficiency, safety profile, and dosage of administration (Ernst, 2000; Fuhrmann et al., 2010; Landis et al., 2014).

Species of the *Rosa* sect. *Caninae* are primarily odd polyploids, most frequently pentaploids ($2n = 5x = 35$), but their reproduction is sexual. This necessitates meiotic division, which is conventionally complicated by odd polyploid genetic material. To overcome this obstacle, species with an asymmetric polyploid chromosomal formula resort to apomixis: a phenomenon of asexual seed production at the maternal tissue level. *Rosa* sect. *caninae* represents a rare exception to this rule, benefiting from a unique meiotic process known in the specialized literature as "caninae meiosis" (Kovarik et al., 2008).

Prior research has also demonstrated a considerable degree of individual variation within the *Rosa* species. Future investigations on the development of rosehip cultivars may take into account the potential existing ecotypes. Wild edible fruits have distinct gene combinations in addition to a higher degree of gene diversity (Okatan et al., 2019). It is generally known that agricultural genetic diversity is increased by indigenous species (Bozhuyuk et al., 2021). The genetic diversity of wild fruits is projected to expand as a result of frequent propagation of seeds and through

out-crossing. A study exploring *Rosa canina* L. fruits from the Oltenia region revealed a significant degree of variability in morphological characteristics and biochemical composition of fruits from spontaneous flora (Soare et al., 2015).

Due to pronounced polymorphism and interspecific hybridization, the morphological classification of species within the *Rosa* genus has not resulted in globally accepted systematization. This assessment has been supplemented by information obtained through anatomical analysis, micromorphology, and pollen, as well as a series of molecular markers (Schanzer & Kutlunina, 2010). These research tools provide clarity regarding the phylogeny of the *Rosa* genus, but currently, there are no qualitative, coherent, and sufficient data to support an acceptable classification.

The present study aimed to investigate the genetic variability of *Rosa* species and cultivars using morphological traits and namely PCR-based start codon targeted (SCoT) molecular markers, with a view to determining their degree of relatedness.

MATERIALS AND METHODS

Plant material

The samples for the morphological and molecular analysis were collected from different eco-geographic areas in order to assess their morphological and genetic diversity.

Three sources of the *Rosa canina* species were used, one from the Salina Turda area (46.5862°N 23.7861°E), Cluj County (denominated *R. canina* 1), the second from the Stațiunea Muntele Băișorii (46°33'37"N 23°20'53"E) Cluj county, (denominated *R. canina* 2) and one belonging to the 'Can' variety.

From the *Rosa rugosa* species, three provenances were collected. The first from the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca (denominated *R. rugosa* 1), the second from Alexandru Borza Botanical Garden Cluj-Napoca (denominated *R. rugosa* 2) and the third from the Horticultural Research Station Cluj-Napoca (denominated *R. rugosa* 3).

Two other species were also studied, *Rosa gallica* cv. Saint Nicolas and *Rosa damascena*, cv. Tuscany from the Alexandru Borza Botanical Garden Cluj-Napoca.

For morphological analyses the rosehip fruits were collected in the ripening stage V (fruit surface was red) according to Medveckienė et al., 2023. Fruit length and width (mm) were measured using a digital calliper.

DNA extraction

The isolation of DNA from leaves was based on the CTAB (Cetyltrimethylammonium bromide) method using the protocol published by Lodhi et al., 1994, and improved by Pop et al., 2003 and Bodea et al., 2016.

Quantification of extracted DNA was accomplished using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and determined the quantity (ng/μl) and purity (ratio 260/280 nm) of each sample.

PCR amplification with SCoT primers

The molecular markers SCoT (Start Codon Targeted polymorphism) used in this study are based on the amplification of conservative and specific regions that flank the start codon (ATG) of the methionine amino acid on both sides. This codon is located in plant genes. The working protocol used was published by Collard & Mackill (2009), using the SuperCycler Trinity PCR thermocycler (Kyrattec, Australia).

SCoT markers are a type of dominant marker that uses a single primer for PCR amplification. These markers are useful for detecting polymorphisms in plant genomes, due to their ability to target gene-rich regions.

The separation of amplification products was achieved through electrophoresis using a 1.6% agarose gel (Promega, USA) stained with a solution called RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotech, South Korea).

DNA band gels were visualized using the UVP Biospectrum AC Imaging System (UVBiolImaging Systems, Germany). The TotalLab TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) was used to determine the number and size of DNA bands amplified using the SCoT technique.

Table 1. The SCoT primers used for the assessment of genetic relationships between *Rosa* sp. and cultivars

No.	Primer	Nucleotide sequence 5'-3' of the primer
1	SCoT 1	CAACAATGGCTACCACCA
2	SCoT 2	CAACAATGGCTACCACCC
3	SCoT 3	CAACAATGGCTACCACCG
4	SCoT 5	CAACAATGGCTACCACGA
5	SCoT 6	CAACAATGGCTACCACGC
6	SCoT 7	CAACAATGGCTACCACGG
7	SCoT 8	CAACAATGGCTACCACGT
8	SCoT 9	CAACAATGGCTACCACGA
9	SCoT 10	CAACAATGGCTACCAGCC
10	SCoT 11	AAGCAATGGCTACCACCA
11	SCoT 12	ACGACATGGCGACCAACG
12	SCoT 16	ACCATGGCTACCACCGAC
12	SCoT 16	ACCATGGCTACCACCGAC
13	SCoT 18	ACCATGGCTACCACCGCC
14	SCoT 19	ACCATGGCTACCACCGGC
15	SCoT 21	ACGACATGGCGACCCACA
16	SCoT 22	AACCATGGCTACCACCAC
17	SCoT 25	ACGACATGGCGACCCGCA
18	SCoT 26	ACGACATGGCGACCCACGT

Statistical analysis

The morphological collected data were statistically analysed using IBM SPSS Statistics 19 software. Using the Shapiro-Wilk test, the groups' normality was confirmed. The medians and percentiles (25-50-75) for non-normally distributed data were established in combination with the mean values and standard deviations for the regularly distributed data for the descriptive statistics. To do multiple comparisons, one-way ANOVA was utilized together with a post hoc Duncan test. Multivariate analysis of the data was performed using the Paleontological Statistics software (PAST) 4.11 analysis program (Hammer et al. 2001).

RESULTS AND DISCUSSION

Morphological analysis

Previous studies in *Rosa* genus (Tomljenović et al., 2022; Ercisli and Esitken, 2004) indicate the existence of variability in morphological characteristics. This provides a suitable basis for the selection process for high-quality genotypes between species of this genus.

In the present study three important morphological characteristics for eight genotypes were analyzed.

Regarding the diameter of the rosehip fruit, the lowest average value was recorded at *R. canina* Baisoara (16.46 mm), while at *R. rugosa* UASVM Cluj-Napoca was recorded the highest value (26.51 mm) with statistically significant differences (Figure 1).

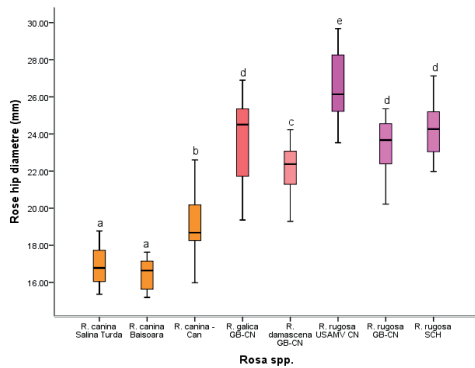


Figure 1. Rosehip fruits diameter of the genotypes analysed in the four species of *Rosa*. Each boxplot represents maximum, upper quartile, median, lower quartile and minimum values. Different letters between boxplots indicate statistically significant differences for the analysed trait, at a significance level of $p < 0.05$ (Duncan test)

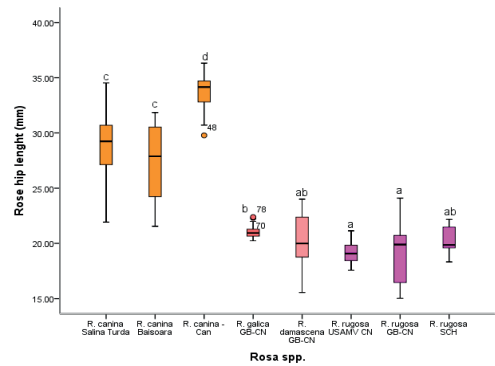


Figure 2. Rosehip fruits length of the genotypes analysed in the four species of *Rosa*. Each boxplot represents maximum, upper quartile, median, lower quartile and minimum values. Different letters between boxplots indicate statistically significant differences for the analysed trait, at a significance level of $p < 0.05$ (Duncan test)

Within the *R. canina* species, the 'Can' variety had the largest average diameter of the fruit (19.22 mm).

Similar results in rosehip fruit diameter were obtained by Ghiorghita et al. (2012) in a study of genotypes from local populations of *R. canina* (10.92-16.40 mm). Ercisli and Esitken (2004) following a study conducted in the province of Erzum in Turkey found values (15.04-19.69 mm) close to those recorded in our country, in the case of varieties of *R. canina*.

Within the *R. rugosa* genotypes, the highest value, statistically ensured, was obtained in the UASVM Cluj-Napoca genotype, while in the other two genotypes there were no significant differences (Figure 1).

The second morphological character analyzed was the length of rosehip fruit. In this case between *R. canina* genotypes, 'Can' cultivars be significantly different from other *R. canina* genotypes (Figure 2). The amplitude of the variation of rosehips fruits length ranged between 33.73 - 27.28 mm for *R. canina* and 19.15-20.24 mm for *R. rugosa*. The results of Duncan's multiple range tests showed that the genotype means of *R. rugosa*, *R. gallica*, and *R. damascena* are substantially lower than the mean genotypes of *R. canina*. Furthermore, it is evident that the *R. canina* 'Can' genotype's mean fruit length is decreased by a number that the boxplot identifies as an outlier (Figure 2).

According to other studies (Ghiorghita et al., 2012) the rosehip fruits length in *R. canina* genotypes varied between 16.49-25.83 mm and between 25.44-33.36 mm according to the study conducted by Ercisli and Esitken (2004). In the study conducted by Soare et al. (2015), the fruit length significantly varied among evaluated *R. canina* varieties collected from different regions of Dolj and Olt with altitudes ranging from 148 and 191 m (Soare et al., 2015). Thus, the highest fruit length has been noticed in the samples collected from the regions of Dolj, namely Şimnic and Carcea. Conversely, low values have been noticed in the regions of Olt. Although the fruits collected from Dolj regions presented the highest values in fruits length, significantly lower values have been observed in the fruits collected from the Argetoaia Dolj County. Significant differences have been observed in selected rosehip species and cultivars based on their morphological parameters. It has been revealed that *R. canina* fruits presented moderate values in length (21.5 mm) and width (16.0 mm). Similar values have been observed in *R. rugosa* fruits in the case of length and width with values of with 15.9 mm and 15.9 mm, respectively (Cunja et al., 2016). In a different study, the rosehips of *R. canina* and *R. corymbifera* presented an elongated spheroid and/or oval form. *R. sempervirens* exhibited the lowest values in width (11.8 mm), whereas *R. canina*, *R. corymbifera*, and *R. micrantha* were revealed to have

significantly higher values with an average of 18.8 mm. The hips of *R. corymbifera*, *R. micrantha*, and *R. sempervirens* had an average shape index of 1.26, suggesting a spherical shape; however, the hips of *R. canina* pseudo-fruits had an index value of 1.64, which corresponds to a higher elongation (Fascella et al., 2019). The pseudofruits collected from the Northeastern region of Romania revealed significant differences in fruit shape and dimensions. Thus, the fruits' morphological parameters varied between 16.2 mm and 8.8 mm regarding width in the case of *R. corymbifera* and *R. micrantha*, both collected from the Suceava county with an altitude of ~ 500 m. Regarding the fruits' length an average value between 24.0 and 12.6 has been observed in the samples of *R. canina* collected from the Iași region (400 m altitude) and in the sample of *R. caryophyllacea* collected from Suceava county (614 m altitude) (Rosu et al., 2011).

Rosehip fruits collected in the full maturity stage from the Eastern region of Poland over the course of three years revealed significant differences in morphological parameters. Thus, the study revealed that not only there are differences among species and their geographical regions, but also between the years of collection. The highest shape indexes were recorded in 2019 and 2021 with values between 2.09 and 1.94, as revealed by the highest value in fruit length (27.9-26.9 mm) and width (13.9-13.4 mm), indicating a rounder shape. Conversely, lower values in fruit width and length have been recorded in samples collected in 2020. These differences may be influenced by several factors, including genotype, variety, environmental conditions (*i.e.*, fluctuations in temperature, precipitations, sunlight duration and intensity), region, growth conditions, harvesting period, and maturation stages (Szmagara et al., 2023).

Another morphological character analysed in this research was the number of seeds/rosehip fruit.

In this case, according to the statistical analysis it can be observed that the highest average number of seeds was registered in *R. canina* Salina Turda genotype (30.05) and the lowest average number of seeds was found in

R. rugosa UASVM Cluj-Napoca genotype (17.15). Also, in this case, according to Figure 3, genotype *R. rugosa* UASVM Cluj-Napoca the average mean of number of seeds/rosehip fruit decreased by two numbers, identified in the boxplot as an 'outlier'.

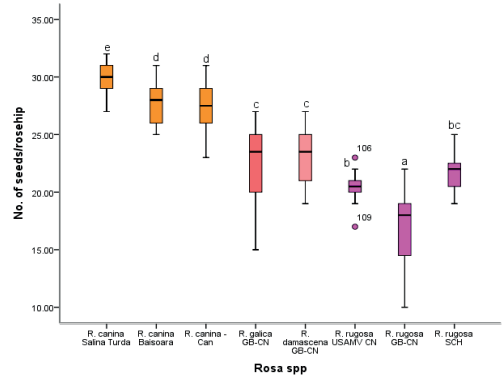


Figure 3. Number of seeds/rosehip fruit of the genotypes analysed in the four species of *Rosa*. Each boxplot displayed maximum, upper quartile, median, lower quartile and minimum values. Different letters between boxplots indicate statistically significant differences for the analysed trait, at a significance level of $p < 0.05$ (Duncan's test)

Gunes (2010) revealed that the average number of seeds/rosehip fruit was between 12.8 and 35.6 in a natural population of *Rosa* in northern Anatolia, Turkey. In a different study, significant differences have been noticed in seed number among and between *Rosa* sp. Thus, the highest number of seeds have been recorded in *R. gallica* genotypes (27-35), followed by *R. dumalis* (21-28). Regarding the *R. canina* genotypes, high variations in seed number (17-32) have been seen which may be due to genotype, cultivar, harvesting region, soil characteristics, altitude, harvesting time and maturation stage (Ercişli & Eşitken, 2004; Ipek & Balta, 2020).

Multivariate analysis (hierarchical clustering using paired group UPGMA, Euclidean similarity index) performed with the mean values of all morphological parameters highlights the relationships both for the *Rosa* spp. (column dendrogram) and for the distance of the three analysed traits (row dendrogram), which is also reflected in the heatmap in Figure 4.

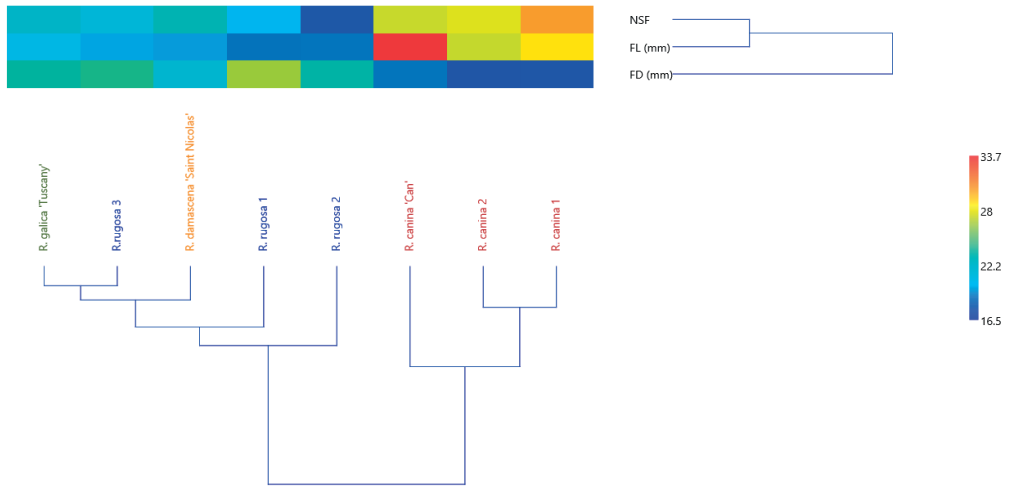


Figure 4. Two-way UPGMA dendrogram based on morphological traits, showing the relationships between the *Rosa* spp. genotypes and based on the Euclidean distance index

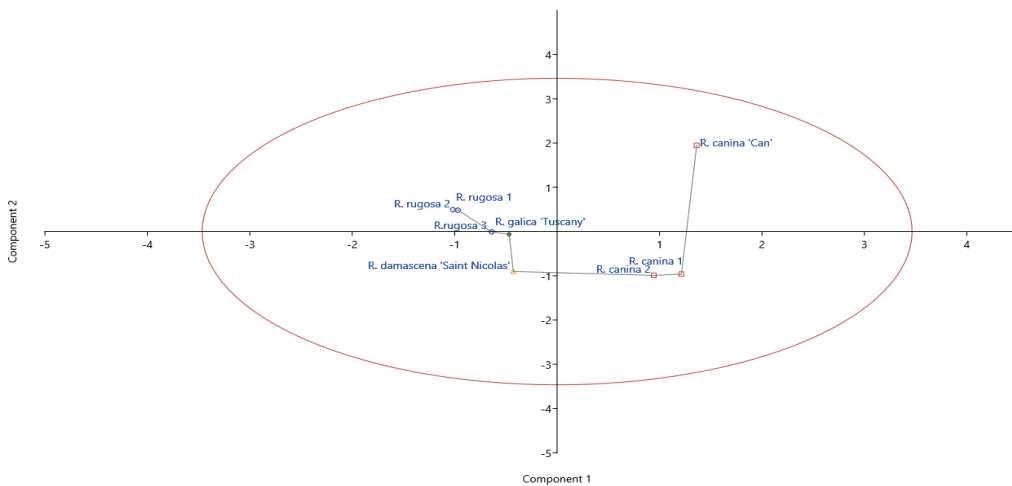


Figure 5. Principal component analysis (PCA) based on the analysed morphological traits of the genotypes of *Rosa* spp.

Thus, the grouping pattern of the eight genotypes revealed two major clusters marked as A and B (Figure 5). The first cluster of two-way dendrogram (marked as A) grouped *R. gallica* cv. 'Tuscany', *R. damascena* cv. 'Saint Nicolas' and *R. rugosa* with different provenances (*R. rugosa* UASMV-CN; *R. rugosa* GB-CN; *R. rugosa* SCH) which were discriminated mainly based on the number of seeds/rosehip fruits (NSF) and rosehip fruits diameter (FD) as shown in Figure 5. The

second main cluster (B) was composed of two subgroups: one included *R. canina* cv. 'Can' and the other one with *R. canina* from two different provenances, Băișoara and Salina Turda, respectively. These subgroups were discriminated according to the mean values of rosehip fruit length (FL) and diameter (FD). The results of this study are in agreement with those reported by Singh et al. (2017) who stated that cluster analysis can be used as a valuable tool for genetic discrimination of wild roses.

Moreover, wild roses are important sources of valuable germplasm for creating variability and improving roses for future economic needs such as food, cosmetics, and pharmaceuticals (Uggla & Martinsson, 2005; Singh et al., 2020). In this study, the results of PCA of the analysed morphological traits indicated that the first three principal components (PCs) accounted for 95.91% of the total variation (data not shown). According to Reim et al. (2012), such results indicate a high level of morphological variation, suggesting that in terms of the genetic characterization of wild *Rosa* spp., evaluation of different morphological traits is still necessary. It is worth noting that, the PCA results of this study confirmed in a high proportion the grouping of *Rosa* genotypes based on UPGMA cluster analysis (Figure 4 and Figure 5).

Molecular analysis

Some previous scientific reports highlight that morphological traits have been used for germplasm characterization (Veasey et al., 2001; Rakonjac et al., 2010), but morphological traits alone may not be sufficient to determine the relationship between species (Llyod et al., 1992). Combining morphological descriptors with molecular markers could enable a more precise and efficient characterization of rose genotypes. Among DNA-based molecular markers, start codon targeted markers (SCoT) have been used to study genetic diversity in *Rosa* genotypes (Agarwal et al., 2019). The results of our study showed that these molecular markers have demonstrated their ability to reveal the genetic relationships between the analysed *Rosa* genotypes.

Of the 18 tested primers, only 12 generated amplification products for all eight samples analysed (Table 2). Six primers (SCoT 3, SCoT 7, SCoT 9, SCoT 11, SCoT 21, SCoT 22) did not generate scorable bands for all 8 samples and were therefore not included in this study. PCR amplifications with the 12 used primers were performed in duplicate to ensure the validity of the results. As an example, Figure 6 shows the electrophoretic profile obtained after PCR amplification with primer SCoT 2.

Table 2. The level of polymorphism detected with SCoT primers in *Rosa* spp.

Primer name	Total number of bands generated/ primer	No. of polymorphic bands generated/ primer	Percentage of polymorphism (%)	Size range (bp) of the generated bands
SCoT 1	7	5	71.42	435-1765
SCoT 2	11	9	81.81	460-1890
SCoT 5	10	8	80.00	345-1660
SCoT 6	12	9	75.00	385-2680
SCoT 8	8	5	62.50	330-1595
SCoT 10	11	8	72.72	360-1700
SCoT 12	12	9	75.00	245-1580
SCoT 16	9	6	66.66	320-1600
SCoT 18	13	10	76.92	285-2150
SCoT 19	9	6	66.66	315-2250
SCoT 25	15	12	80.00	245-3050
SCoT 26	13	10	76.92	435-2050
Total	130	97	-	-

In this study cluster analysis was used to assess the genetic relationships between eight *Rosa* genotypes. The built UPGMA dendrogram is based on the clustering of the recorded SCoT data and uses Euclidean genetic distances with the bootstrapping value set to 10000. The value of the cophenetic coefficient was 0.9359, which indicates a very good correlation between the original binary matrix generated from the analysis of agarose gels and the values obtained in the genetic distance matrix used for the construction of the dendrogram that expresses the genetic relationships between the four *Rosa* species analyzed with SCoT molecular markers.

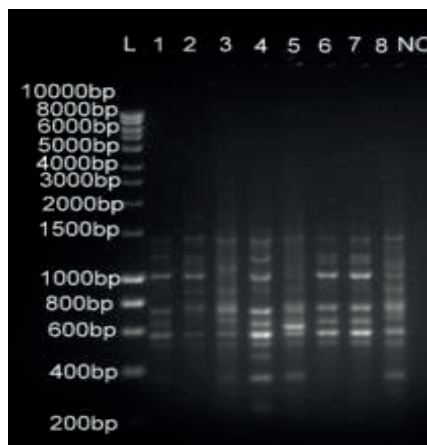


Figure 6. The electrophoretic profile obtained after PCR amplification with the primer SCoT 2. L-1Kb ladder (Bioline); 1 - *Rosa canina* 1; 2 - *Rosa canina* 2; 3 - *Rosa canina* cv. 'Can'; 4 - *Rosa damascena* cv. 'Saint Nicolas'; 5 - *Rosa gallica* cv. 'Tuscany'; 6 - *Rosa rugosa* 1; 7 - *Rosa rugosa* 2; 8 - *Rosa rugosa* 3

Cluster Analysis

In Figure 7 it can be observed that the dendrogram obtained from the cluster analysis groups the eight analysed samples into two main groups (clusters): the first cluster is composed of three species, respectively *R. gallica*, *R. rugosa* and *R. damascena*, while the second group is made up of *R. canina*. It should be noted that there are differences between the samples from each subgroup, due to the different provenances of the samples within the same species, but also to the morphological and genetic differences between the species grouped in the same subgroup. These differences provide interesting data regarding the possibility of exploiting the existing genetic variability and the use of this biological material for decorative, medicinal and cosmetic purposes.

Principal coordinate analysis (PCoA) was used to validate the veracity of the results obtained from the cluster analysis. Thus, the plot generated by the PAST 4.11 program and based

on the Euclidean similarity index using $c = 2$ as transformation exponent, shows the fact that the eight samples analysed and coming from four different species of *Rosa* are grouped axially based on 2 main coordinates that have the highest Eigen values recorded after ranking of the main 7 components, respectively axis 1 with a value of 47.123 (37.362%) and axis 2 with a value of 22.036 (17.472%).

In Figure 8, it can be seen that the three analysed species, respectively *R. damascena*, *R. rugosa* and *R. gallica*, are grouped in the 1st and 2nd Cartesian quadrants, while the samples from the species *R. canina* are grouped close, in the 3rd Cartesian quadrant. As a conclusion, the results obtained regarding the cluster analysis are also confirmed by the analysis of the main coordinates, highlighting the grouping of the data in the PCoA plot in a similar way to that in the UPGMA dendrogram, in both cases the Euclidean index of genetic dissimilarity was used.

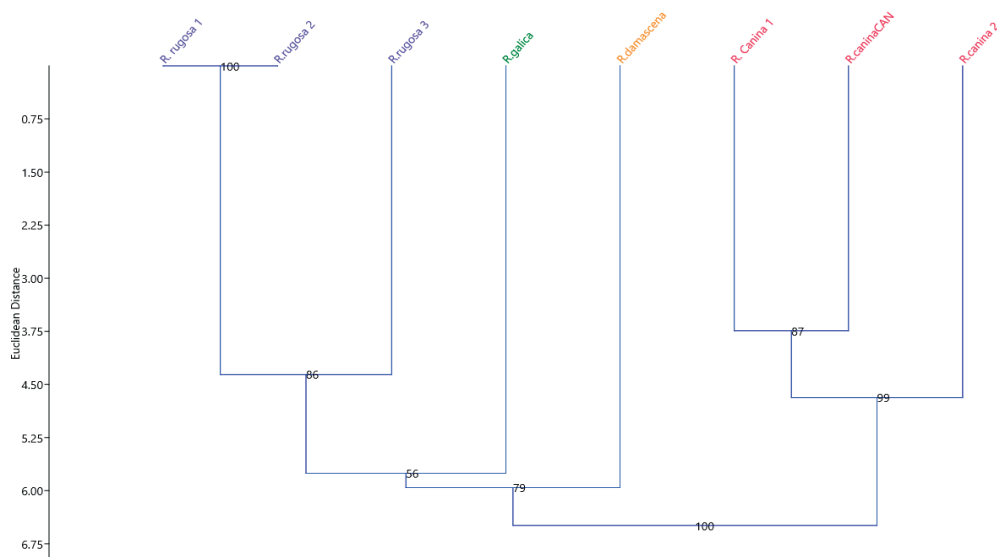


Figure 7. UPGMA dendrogram based on SCoT molecular markers analysis, showing the relationships between the *Rosa* spp. genotypes and based on the Euclidean distance index

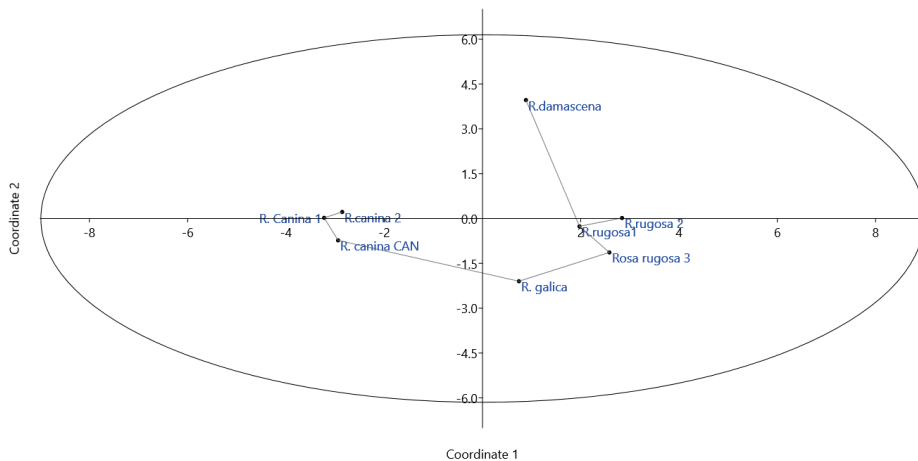


Figure 8. Principal coordinate analysis (PCoA) based on SCoT molecular markers analysis of *Rosa* spp.

CONCLUSIONS

The findings of this study revealed that some *Rosa* genotypes demonstrate the complex nature of the inheritance of some morphological traits, as seen by their significant differences in fruits length and diameter, but also the number of seeds per fruit. The assessment of genetic diversity using SCoT molecular markers provided valuable information for the genetic characterization of *Rosa* genotypes with different provenances.

The cluster analysis divided the analyzed samples into two main clusters: the first cluster comprised three species, namely *R. galica*, *R. rugosa*, and *R. damascena*, while the second group consisted of *R. canina*. It is evident that there are significant variations among the samples in each subgroup that may be attributed to the samples various origins within the same species as well as to both morphological and genetic differences between the species comprised in the same subgroup.

These results can be exploited in the near future to use the genetic potential of these genotypes for the improvement of traits required for adaptation to different environmental conditions.

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