

ARAŞTIRMA MAKALESİ

RESEARCH ARTICLE

Genetic Characterization of Aronia Varieties Cultivated *In Vitro* and *In Vivo* Conditions Using iPBS and ISSR Markers

iPBS ve ISSR Markörleri Kullanarak *In Vitro* ve *In Vivo* Koşullarda Yetiştirilen Aronya Çeşitlerinin Genetik Karakterizasyonu

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Abstract

Aronia (*Aronia* spp.) is a minor fruit species within the Rosaceae family that has gained increasing prominence in the food and health sectors in recent years, owing to its high antioxidant capacity. The genus is represented by three primary species: black chokeberry (*Aronia melanocarpa*), red chokeberry (*Aronia arbutifolia*), and their natural hybrid, purple chokeberry (*Aronia prunifolia*). In the present study, eight genotypes representing four commercial cultivars ('Viking', 'Nero', 'Aron', and 'Galicjanka') were evaluated for genetic stability under both *in vivo* and *in vitro* conditions using ISSR and iPBS molecular markers. To separate within-variety variability from between-variety variability, *in vitro* and *in vivo* samples of the same variety were combined into a single population (four populations in total; eight genotypes). ISSR primers generated monomorphic banding patterns and failed to detect polymorphism among the cultivars. In contrast, two of the five iPBS primers produced polymorphic profiles; the primers iPBS2400 and iPBS2378 exhibited polymorphism rates of 57.89% and 62.50%, respectively. These results demonstrate that both primers can detect genetic variation in the analyzed aronia genotypes. However, they differ in the total number of amplified bands and in the levels of polymorphism. For the iPBS2400 primer, the PIC, He, Ne, and Shannon's diversity index (I) values were calculated as 0.426, 0.487, 1.772, and 0.614, respectively. For the iPBS2378 primer, the corresponding values were 0.431, 0.493, 1.809, and 0.617. The PIC values for these primers ranged from 0.426 to 0.431, while He values were between 0.487 and 0.493, and Shannon's information index (I) values ranged from 0.614 to 0.617. Among the examined populations, the 'Aron' population showed the highest genetic diversity, whereas the 'Viking' population showed the lowest. The dendrogram generated using the Jaccard similarity coefficient indicated genetic distances ranging from 0.333 to 1.000. Overall, the findings demonstrate that ISSR markers exhibit limited discriminatory power, whereas iPBS markers are a more robust tool for detecting genetic variability among aronia cultivars. Additionally, the high degree of similarity between *in vivo* and *in vitro* samples supports the genetic stability of tissue culture-derived material.

Keywords: Aronia, *in vitro*, Genetic stability, Molecular markers, ISSR, iPBS

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Öz

Aronya (*Aronia* spp.), yüksek antioksidan kapasitesi nedeniyle son yıllarda gıda ve sağlık sektörlerinde giderek artan bir öneme sahip, Rosaceae familyasına ait küçük bir meyve türüdür. Cins üç ana türle temsil edilir: siyah aronya (*Aronia melanocarpa*), kırmızı aronya (*Aronia arbutifolia*) ve bunların doğal melezi mor aronya (*Aronia prunifolia*). Bu çalışmada, dört ticari çeşidi ("Viking", "Nero", 'Aron' ve "Galicjanka") temsil eden sekiz genotip, ISSR ve iPBS moleküler belirteçleri kullanılarak hem *in vivo* hem de *in vitro* koşullar altında değerlendirilmiştir. Çeşit içi değişkenliği çeşitler arası değişkenlikten ayırma için, aynı çeşide ait *in vitro* ve *in vivo* örnekler tek bir örnekleme birleştirilmiştir (toplamda dört kesinti; sekiz genotip). ISSR primerleri monomorfik bant desenleri oluşturdu ve çeşitler arasındaki polimorfizmi tespit edilememiştir. Buna karşılık, beş iPBS primerinden ikisi polimorfik profiller üretti; iPBS2400 ve iPBS2378 primerleri sırasıyla %57,89 ve %62,50 polimorfizm oranları sergiledi. Bu sonuçlar, her iki primerin de analiz edilen aronya genotiplerindeki genetik varyasyonu tespit edebildiğini göstermektedir. Bununla birlikte, toplam amplifiye bant sayısı ve polimorfizm seviyeleri bakımından farklılık göstermişlerdir. iPBS2400 primeri için PIC, He, Ne ve Shannon çeşitlilik indeksi (I) değerleri sırasıyla 0,426, 0,487, 1,772 ve 0,614 olarak hesaplandı. iPBS2378 primeri için ise karşılık gelen değerler 0,431, 0,493, 1,809 ve 0,617 idi. Bu primerler için PIC değerleri 0,426 ile 0,431 arasında değişirken, He değerleri 0,487 ile 0,493 arasında ve Shannon bilgi indeksi (I) değerleri 0,614 ile 0,617 arasında değişmiştir. İncelenen popülasyonlar arasında en yüksek genetik çeşitlilik 'Aron' popülasyonunda gözlemlenirken, 'Viking' popülasyonu en düşük çeşitliliği göstermiştir. Jaccard benzerlik katsayısı kullanılarak oluşturulan dendrogram, 0,333 ile 1,000 arasında değişen genetik mesafeleri göstermiştir. Genel olarak, bulgular ISSR işaretleyicilerinin sınırlı ayırt edici güce sahip olduğunu, iPBS işaretleyicilerinin ise aronya çeşitleri arasındaki genetik değişkenliği tespit etmek için daha sağlam bir araç olduğunu göstermektedir. Ayrıca *in vivo* ve *in vitro* örnekler arasındaki yüksek benzerlik derecesi, doku kültürü kaynaklı materyalin genetik stabilitesini desteklemektedir.

Anahtar Kelimeler: Aronya, *in vitro*, Genetik stabilite, Moleküler belirteçler, ISSR, iPBS

1. Introduction

Aronia (*Aronia* spp.) is a deciduous shrub species belonging to the Rosaceae family, exhibiting both diploid ($2n = 34$) and tetraploid (hybrid, $2n = 4x = 68$) ploidy. It originates from eastern North America and Canada. The genus is represented by three primary species: black chokeberry (*Aronia melanocarpa*), red chokeberry (*Aronia arbutifolia*), and their natural hybrid, purple chokeberry (*Aronia prunifolia*) (Hardin, 1973; Smolik et al., 2011; Basturk and Ozerson, 2025). In addition, a fourth taxon, *Aronia mitschurinii*, has been described as a large-fruited form derived from intergeneric hybridization between *Sorbus* and *Aronia* genus. Molecular analyses have further demonstrated that the widely cultivated cultivars ‘Viking’ and ‘Nero’ are also classified within black chokeberry species (Obae et al., 2017; Kaya, 2025; Coskun and Dumanoglu, 2025). However, these cultivars exhibit limited genetic variation due to their close phenotypic resemblance and apomictic mode of reproduction (Obae et al., 2017). Black chokeberry (*A. melanocarpa*) is a long-lived shrub species that can reach heights of 2-3 meters. It blooms from May to June and ripens from August to September. The berries are black and range in diameter from 6 to 17 mm. Each plant produces an average of 10 to 15 kg of berries (Sahin and Erdogan, 2022; Ozbucak and Gumus, 2024; Kaya, 2025).

Aronia berries contain substantial amounts of vitamins, minerals, and folic acid, as well as exceptionally high levels of anthocyanins and antioxidants (Banach et al., 2020). Owing to this biochemical richness, they exhibit beneficial effects against various health disorders, including diabetes and cardiovascular diseases (Sanlier and Cinar, 2025). They are widely used as natural flavoring and coloring agents in numerous food products, including fruit juices, jams, dried fruits, yogurts, ice cream, and baked goods (Xu et al., 2024). Moreover, their strong antioxidant profile enhances the nutritional value of food products and extends shelf life by delaying oxidative deterioration (Oszmianski and Wojdylo, 2005). The rising health-related and commercial interest in aronia has consequently accelerated the expansion of its cultivation areas (Ozbucak and Gumus, 2024; Metiner and Ersus, 2023; Kaya, 2025; Eskimez and Polat, 2023).

The earliest research on aronia in Türkiye commenced in 2012 with sapling production and initial trial orchards established at the Atatürk Horticultural Central Research Institute. In 2014, researchers established small-scale plantations in Yalova and Kırklareli (TAGEM, 2022). Beginning in 2017, more extensive commercial orchards were developed in Yalova, Tokat, Edirne, Malatya, Kırklareli, and Manisa, during which comprehensive morphological, pomological, and biochemical evaluations were conducted, alongside promotional activities, publications, and the organization of the first harvest festival. During this period, the Institute registered the cultivars ‘Viking’ (2017) and ‘Nero’ (2018), initiated the production (Coskun and Dumanoglu, 2025) of certified planting material, and facilitated the establishment of new orchards across multiple provinces in the subsequent years (Engin and Boz, 2019; Engin and Mert, 2024; Sahin and Erdogan, 2022).

Micropropagation offers substantial advantages for plant production and breeding. It enables the generation of pathogen-free, virus-indexed plant material suitable for certified sapling programs (George et al., 2008). Compared with conventional clonal propagation methods, it allows rapid production of a large number of uniform plants, facilitating international exchange of propagation material (Deberg and Deberg, 1994). Somaclonal variation can occur during tissue culture, and this affects genetic stability. In vitro conditions (hormonal stress, culture time, callus formation) can trigger genetic changes. Therefore, the genetic structure of plants obtained in vitro must be carefully checked. From a breeding and commercial perspective, this matters because poor genetic stability compromises variety purity and can lead to economic losses, especially in fruit varieties (Hasnain et al., 2022). Molecular marker analyses allow rapid, reliable determination of genetic stability in in vitro plants (Rohela et al., 2022). Micropropagation is an integral part of the sustainable and cost-effective cultivation of aronia because it makes it easier to multiply species that are difficult to propagate using traditional vegetative methods and requires less space and labor (Guclu et al., 2010; Eskimez and Polat, 2023).

Molecular markers are powerful tools that enable the construction of genetic maps by amplifying and tracking specific DNA regions (Ozkan and Bilgen, 2019). Their advantages including ease of application, high reproducibility and reliability, rapid analysis, and strong polymorphism have made them among the most widely used approaches in cultivar identification and hybrid breeding studies, alongside morphological, biochemical, and cytological markers (Bilgen et al., 2023; Yin et al., 2025). Beyond elucidating relationships among species, cultivars, and genotypes, these markers are also widely used to assess the genetic stability of plants propagated *in*

vitro (Tikendra et al., 2022). Among these systems, the ISSR (Inter-Simple Sequence Repeat) technique developed by Zietkiewicz et al., (1994) is particularly noteworthy for its high applicability, owing to its PCR-based nature. ISSR markers are widely used to assess genetic diversity owing to their high polymorphism and efficient discriminatory power. They are a quick, cheap, and reliable way to detect genetic differences between species and to distinguish closely related genotypes (Alan et al., 2025; Cekic et al., 2018). Another system that has been effectively applied in numerous plant taxa recently, the iPBS (inter-primer binding site) marker technique, was developed by Kalendar et al., (2010) as a retrotransposon-based amplification method. This approach, which can overcome several limitations of traditional marker systems, introduces a novel dimension to molecular analyses by detecting retrotransposon-derived variation and enabling broad applicability through the use of universal primers (Haliloglu et al., 2022). The universality of iPBS-retrotransposon markers has been well demonstrated, as this system has been successfully utilized in molecular characterization and phylogenetic investigations across a wide range of species, including various berry crops. Nevertheless, the current literature reports no prior use of iPBS markers in aronia cultivars. The iPBS marker system, based on targeting conserved regions of retrotransposons in PCR-based analyses, is a useful tool for detecting genetic polymorphisms in plant genomes and quantifying genetic variation (Kalendar ve Schulman, 2014).

The objective of this study was to evaluate the genetic variation present among four *Aronia melanocarpa* cultivars ('Viking', 'Nero', 'Aron' and 'Galicjanka') propagated under *in vitro* and *in vivo* conditions using molecular markers. Specifically, we aimed to characterize intergenotypic variation using ISSR and iPBS marker systems. This study represents the first application of iPBS markers in aronia, thereby providing previously unknown insights into their utility for molecular characterization. Because somaclonal variations that may arise during *in vitro* micropropagation cannot always be detected phenotypically early on, the genetic accuracy and uniformity of plants should be tested using molecular markers; this approach directly identifies changes occurring at the DNA level, thereby verifying clonal fidelity and ensuring the reliability of the production material. In addition, by comparing *in vitro*-derived plant material with orchard-grown samples, we sought to assess genetic similarities and potential divergence between propagation environments, enabling a comprehensive molecular comparison of the cultivars. This approach enables the genome-level identification of tissue culture-derived somaclonal variations, the isolation of epigenetic changes caused by environmental influences, and the reliable verification of the genetic accuracy of clonal material. Furthermore, it provides a scientific basis for determining whether the resulting plants are stable and homogeneous for breeding programs, commercial production, and international plant material exchange.

2. Materials and Methods

2.1. Plant material

This study included four *Aronia melanocarpa* cultivars: 'Viking', 'Nero', 'Aron' and 'Galicjanka'. For each cultivar, both *in vivo* and *in vitro* forms were assessed, yielding a total of 8 genotypes (Table 1). Plant materials were obtained from orchard-grown specimens at the Tekirdağ Namık Kemal University Agricultural Biotechnology Research and Production Unit (ZIRAATBIYOTEK), along with leaf tissues collected from healthy plants maintained in the tissue culture laboratory. *In vitro* micropropagation was performed according to the protocol of Celebi-Toprak and Alan (2020) using Murashige and Skoog (1962) basal medium. The *in vitro* plantlets were propagated in the tissue culture laboratory at ZIRAATBIYOTEK, and young, aseptically obtained leaf tissues were used for DNA extraction. All leaf samples were preserved at -80 °C prior to isolation.

Table 1. Populations and samples of aronia genotypes in the study

No	Population	Sample name
1	1	Viking <i>in vivo</i>
2	1	Viking <i>in vitro</i>
3	2	Nero <i>in vivo</i>
4	2	Nero <i>in vitro</i>
5	3	Aron <i>in vivo</i>
6	3	Aron <i>in vitro</i>
7	4	Galicjanka <i>in vivo</i>
8	4	Galicjanka <i>in vitro</i>

2.2. DNA extraction

Genomic DNA was extracted following a modified CTAB protocol as described by Doyle and Doyle (1987; 1990) and Kafkas et al., (2005). Before DNA isolation, fresh leaf sample was ground using a ball mill (Retsch MM400) (Yılmaz et al., 2023). The isolated DNA samples were stored at -20°C until polymerase chain reaction (PCR) analysis. DNA concentration and purity were assessed using an LTK INNO Microplate Spectrophotometer, after which all samples were diluted to 5 ng μL^{-1} and stored at -20°C for downstream PCR analysis.

2.3. iPBS-PCR

For the iPBS analysis, five primers (Table 2) were selected based on the literature (Kalendar et al., 2010). PCR optimization was performed according to the protocol described by Pour et al. (2019). Each reaction was performed in a final volume of 10 μL and consisted of 10 $\times$ Green Buffer, 10 mM dNTPs, 110 μM primer, 5 U/ μL Taq DNA polymerase, and 5 ng/ μL genomic DNA. The amplification program included an initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at each primer's specific melting temperature (T_m) for 1.30 min, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. PCR amplicons were resolved on 2% agarose gels prepared with 1 $\times$ TBE buffer, and the resulting banding profiles were visualized under UV illumination and documented using the Vilber Lourmat Quantum ST5 Gel Imaging System.

Table 2. iPBS primer information

Primer no	Primer name	Primer Sequence (5'-3')	T_m (°C)
1	iPBS2400	CCCCTCCTTCTAGCGCCA	56
2	iPBS2387	GCGCAATACCCA	50
3	iPBS2237	CCCCTACCTGGCGTGCCA	54
4	iPBS2392	TAGATGGTGCCA	50
5	iPBS2077	CTCACGATGCCA	50

2.4. ISSR-PCR

ISSR analyses in this study were conducted using 31 ISSR primers (Table 3). The ISSR primers used in this study were selected from the University of British Columbia (UBC) primer set, based on their high reproducibility

Table 3. ISSR primer information

Primer No	Primer Name	Sequence*	T_m (°C)	Primer No	Primer name	Sequence*	T_m (°C)
1	ISSR 808	(AG) ₈ C	52	17	ISSR 850	(GT) ₈ YC	56
2	ISSR 809	(AG) ₈ G	52	18	ISSR 851	(GT) ₈ YG	54
3	ISSR 811	(GA) ₈ C	52	19	ISSR 852	(TC) ₈ RA	52
4	ISSR 812	(GA) ₈ AA	50	20	ISSR 853	(TC) ₈ RT	52
5	ISSR 814	(CT) ₈ TG	50	21	ISSR 855	(AC) ₈ YT	52
6	ISSR 815	(CT) ₈ G	52	22	ISSR 856	(AC) ₈ YA	52
7	ISSR 818	(CA) ₈ G	52	23	ISSR 858	(TG) ₈ RT	52
8	ISSR 820	(GT) ₈ A	52	24	ISSR 862	(AGC) ₆	56
9	ISSR 825	(AC) ₈ T	50	25	ISSR 813	(CTC) ₆ TT	50
10	ISSR 830	(TG) ₈ G	52	26	ISSR 823	(TCT) ₆ CC	52
11	ISSR 840	(GA) ₈ YT	52	27	ISSR 827	(AC) ₈ G	52
12	ISSR 841	(GA) ₈ YC	54	28	ISSR 829	(TG) ₈ C	52
13	ISSR 842	(GA) ₈ YG	54	29	ISSR 835	(AG) ₈ YC	54
14	ISSR 843	(CT) ₈ RA	52	30	ISSR 836	(AG) ₈ YA	52
15	ISSR 844	(CT) ₈ RC	54	31	ISSR 849	(GT) ₈ YA	52
16	ISSR 846	(CA) ₈ RT	54				

*Note: Degenerate bases follow the IUPAC nucleotide code: R = A/G, Y = C/T

and polymorphism, following the ISSR methodology described by Zietkiewicz et al. (1994), which is extensively referenced in the literature, with minor modifications to optimize its application in the present study. PCR amplifications were performed in 12.5 µL reaction volumes, each containing 1× PCR buffer (10× Green Buffer with MgCl₂), 2.5 mM dNTP mix, 5 pmol of ISSR primer, 5 U/µL Taq DNA polymerase, and approximately 5 ng/µL genomic DNA. The thermocycling protocol was as follows: an initial denaturation at 95°C for 5 min, followed by 40 cycles consisting of denaturation at 94°C for 1.5 min, annealing at primer-specific optimized temperatures (T_m) for 60 s, and extension at 72°C for 2 min. The last step of amplification was an extension at 72°C for 7 minutes. PCR products were separated on 2.5% agarose gels prepared with 1× TBE buffer, and the resulting banding patterns were visualized under UV illumination and documented using a Gel Imaging System (Vilber Lourmat Quantum ST5).

2.5. Statistical analysis

The binary (1-0; presence-absence) scoring data obtained in this study were subjected to a comprehensive set of statistical analyses. Following marker scoring, the polymorphic information content (PIC) of each primer was calculated using the formula proposed for dominant marker systems by Roldán-Ruiz et al. (2000). To further assess allelic variation, the frequencies of band presence (p) and absence (q = 1 - p) were computed using the GenAlEx v6.5 software (Peakall and Smouse, 2012). In addition, molecular variance analysis (AMOVA) was performed based on the outputs generated in GenAlEx. Genetic distances among genotypes were estimated using the Jaccard distance coefficient (Jaccard, 1901), and all computations were carried out in the Python programming environment, primarily utilizing the Scikit-learn library (Pedregosa et al., 2011).

3. Results and Discussion

In the present study, four commercial aronia cultivars ('Viking', 'Aron', 'Galicjanka' and 'Nero') were assessed using ISSR and iPBS molecular marker systems across eight genotypes representing both *in vitro* and *in vivo* forms. To enhance analytical power and to discriminate intra-varietal from inter-varietal variability, *in vitro* and *in vivo* samples belonging to the same cultivar were merged into a single population (four populations in total; eight genotypes). This approach enabled a more accurate estimation of allele frequencies for dominant marker systems (ISSR, iPBS). A total of 31 ISSR primers were used in the study, and all primers produced similar band profiles in aronia cultivars and did not provide significant polymorphism among cultivars. While none of the 31 ISSR primers screened generated polymorphic loci, two of the five iPBS primers (40%) yielded polymorphic banding patterns, thereby providing a more robust resolution of both inter-population and intra-population genetic structure (iPBS2378 and iPBS2400). The gel images of all tested iPBS primers are provided in *Figure 1* and *Figure 2*.

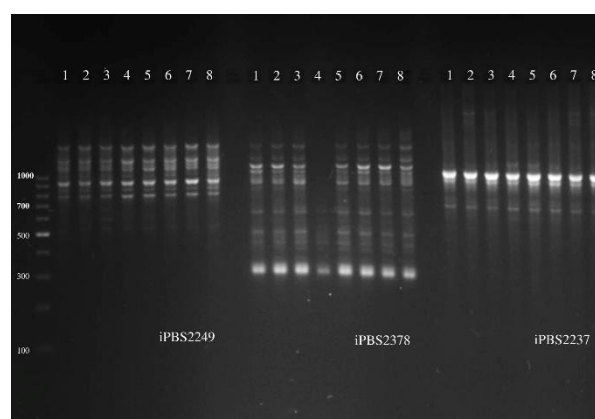


Figure 1. Gel electrophoresis images of the iPBS2249, iPBS2378, and iPBS2237 primers (1: Viking *in vivo*, 2: Viking *in vitro*, 3: Nero *in vivo*, 4: Nero *in vitro*, 5: Aron *in vivo*, 6: Aron *in vitro*, 7: Galicjanka *in vivo*, 8: Galicjanka *in vitro*)



Figure 2. Gel electrophoresis images of the iPBS2390 and iPBS2400 primers (1: Viking in vivo, 2: Viking in vitro, 3: Nero in vivo, 4: Nero in vitro, 5: Aron in vivo, 6: Aron in vitro, 7: Galicjanka in vivo, 8: Galicjanka in vitro)

3.1. iPBS band profile and genetic diversity parameters

Results of the examination of the banding patterns generated by the iPBS2400 and iPBS2378 primers revealed that the iPBS2400 primer amplified a total of 19 fragments ranging from 320 to 1580 bp, 11 of which were polymorphic. Accordingly, the iPBS2400 primer exhibited a polymorphism rate of 57.89%. The iPBS2378 primer amplified 8 fragments ranging from 350 to 800 bp. Of these, 5 were polymorphic, indicating a polymorphism rate of 62.50%. These results demonstrate that both primers can detect genetic variation in the analyzed aronia genotypes. However, they differ in the total number of amplified bands and in the levels of polymorphism. For the iPBS2400 primer, the PIC, He, Ne, and Shannon's diversity index (I) values were calculated as 0.426, 0.487, 1.772, and 0.614, respectively. For the iPBS2378 primer, the corresponding values were 0.431, 0.493, 1.809, and 0.617 (Table 4). The results show that both primers revealed moderate genetic diversity within the studied cultivars.

Table 4. Band profile and genetic diversity parameters of the iPBS primer

Primer name	Band length (baz çifti) (bp)	Total Band number (TBS)	Polymorphic Band number	Polymorphism Rate (PO) (%)	PIC	He	Ne	I
iPBS2400	320/1580	19	11	57.895	0.426	0.487	1.772	0.614
iPBS2378	350/800	8	5	62.5	0.431	0.493	1.809	0.617

3.2. Genetic diversity parameters in aronia populations

In the analysis, the in vitro and in vivo samples of each aronia cultivar were treated as a single population. Evaluation of genetic diversity parameters among the aronia populations revealed notable variation across cultivars. The 'Viking' population exhibited the lowest polymorphic information content (PIC = 0.156), whereas the 'Aron' population displayed the highest PIC value (0.281). A similar trend was observed for expected heterozygosity (He),

Table 5. Genetic diversity parameters of aronia populations

Aronia Population	PIC	He	Ne	I
Viking	0.156	0.313	1.313	0.217
Nero	0.250	0.500	1.500	0.347
Aron	0.281	0.563	1.563	0.390
Galicjanka	0.219	0.438	1.438	0.303

which ranged from 0.313 in 'Viking' to 0.563 in 'Aron', indicating substantially greater allelic diversity in the latter. The effective number of alleles (Ne) varied between 1.313 and 1.563, again reaching its maximum in the 'Aron' population and its minimum in 'Viking'. Likewise, the Shannon information index (I) ranged from 0.217

to 0.390, with ‘Aron’ demonstrating the greatest genetic diversity and ‘Viking’ the least. In contrast, the ‘Galicjanka’ population exhibited intermediate values for all parameters, reflecting moderate levels of genetic variability relative to the other cultivars (Table 5).

3.3. Genetic diversity parameters in aronia populations

AMOVA results demonstrated that 92% of the total molecular variance was partitioned within populations, whereas only 8% was attributed to variation among populations (Table 6; Figure 3). These findings indicate that genetic differentiation among the four aronia cultivars analyzed is minimal, with the majority of genetic diversity being retained within populations. The limited inter-population variation is likely due to the narrow genetic background of commercial cultivars and their predominant propagation through vegetative means. Conversely, the substantial intra-population variance suggests the presence of minor banding differences between genotypes derived from *in vitro* and *in vivo* conditions.

Table 6. Molecular variance analysis of aronia populations

Source	df	SS	MS	Est. Var.	%
Among Pops	3	12.875	4.292	0.333	8%
Within Pops	4	14.500	3.625	3.625	92%
Total	7	27.375		3.958	100%

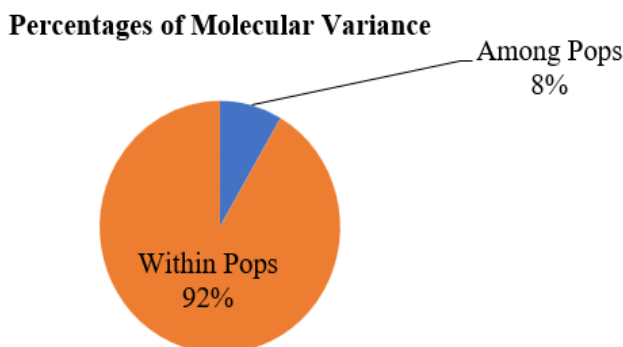


Figure 3. Inter-and intra-population genetic variation in aronia

3.3. Aronia genetic distance matrix

Based on the dendrogram generated using the Jaccard similarity coefficient and the Complete Linkage clustering method, genetic distances among the aronia samples were found to range from 0.333 to 1. The lowest genetic distance (0.333) was detected between the ‘Viking’ *in vitro* and ‘Aron’ *in vivo* samples, indicating a comparatively higher similarity in their banding patterns than in other sample combinations. Conversely, the highest genetic distance (1.0) was observed between ‘Viking’ *in vivo* and ‘Nero’ *in vitro*, ‘Viking’ *in vivo* and ‘Galicjanka’ *in vivo* and ‘Viking’ *in vivo* and ‘Galicjanka’ *in vitro*, demonstrating that these pairs exhibited completely distinct band profiles across all loci. When the *in vitro* and *in vivo* forms of each cultivar were compared, the genetic distances between these pairs were generally moderate. Overall, the analysis revealed the presence of measurable genetic divergence both among cultivars and between the *in vitro* and *in vivo* forms of the same cultivar under different growth conditions (Table 7).

In the molecular characterization of aronia cultivars, ISSR markers generated entirely monomorphic banding patterns, whereas iPBS markers revealed only a limited degree of polymorphism. This outcome reflects the exceptionally close genetic relatedness among the cultivars examined (‘Nero’, ‘Viking’, ‘Aron’, and ‘Galicjanka’). Indeed, previous research has demonstrated that these cultivars show minimal differentiation when assessed with molecular markers, with only a few unique bands detectable. In particular, earlier RAPD and ISSR studies reported that ‘Nero’, ‘Viking’, and ‘Galicjanka’ possess nearly indistinguishable band profiles, highlighting the substantial difficulty of discriminating these cultivars using conventional DNA fingerprinting approaches (Smolik et al., 2011). The primary factors underlying this situation include the reliance of European aronia cultivation on the clonal

propagation of a very limited number of genotypes originally introduced from North America, as well as the fact that currently cultivated varieties were selected from an inherently narrow genetic base (Jeppsson, 2000). Furthermore, relying solely on vegetative propagation across all cultivars preserves their clonal integrity, resulting in very low genetic variation within each variety. Consistent with this, earlier research indicates that genetic diversity among commercially grown aronia genotypes is very limited, with cultivars such as ‘Nero’, ‘Viking’ and ‘Galicjanka’ having very similar genetics that make them nearly impossible to tell apart using standard molecular profiling methods (Shahin et al., 2019). The low polymorphism rates observed in this study are consistent with previous reports and clearly reflect the high genetic uniformity of the aronia germplasm used in commercial production. Moreover, the application of iPBS markers to aronia for the first time highlights the novelty and scientific contribution of this research. The fact that two out of the five primers employed generated polymorphic banding patterns indicates that the iPBS system possesses a greater discriminatory capacity than ISSR markers. This outcome suggests that retrotransposon-derived genomic regions harbor detectable levels of variation among genotypes and that iPBS markers may serve as a valuable alternative tool for assessing genetic diversity in aronia.

Table 7. Distance matrix created according to the Jaccard Complete Linkage method in aronia

	Viking <i>in vivo</i>	Viking <i>in vitro</i>	Nero <i>in vivo</i>	Nero <i>in vitro</i>	Aron <i>in vivo</i>	Aron <i>in vitro</i>	Galicjanka <i>in vivo</i>	Galicjanka <i>in vitro</i>
Viking <i>in vivo</i>	0	0.625	0.750	1	0.571	0.875	1	1
Viking <i>in vitro</i>	0.625	0	0.500	0.750	0.333	0.833	0.833	0.846
Nero <i>in vivo</i>	0.75	0.500	0	0.727	0.600	0.818	0.818	0.833
Nero <i>in vitro</i>	1	0.750	0.727	0	0.833	0.818	0.700	0.600
Aron <i>in vivo</i>	0.571	0.333	0.600	0.833	0	0.818	0.700	0.727
Aron <i>in vitro</i>	0.875	0.833	0.818	0.818	0.818	0	0.800	0.700
Galicjanka <i>in vivo</i>	1	0.833	0.818	0.700	0.700	0.800	0	0.700
Galicjanka <i>in vitro</i>	1	0.846	0.833	0.600	0.727	0.700	0.700	0

Parab et al. (2021), in *Ficus carica* var. Black Jack, genetic stability between plants regenerated under *in vitro* conditions and the donor (parent) plant was assessed using ISSR markers, yielding similar banding patterns across all samples. The monomorphic profiles observed in this study indicated the absence of genetic variation between *in vitro*-derived plants and the original parent material. Likewise, in our study, ISSR markers produced monomorphic patterns in both *in vivo* and *in vitro* samples, suggesting that the employed methodologies effectively preserved genetic stability and that tissue culture conditions did not introduce additional genetic variability. Therefore, it can be concluded that ISSR markers represent a reliable and effective tool for verifying the genetic integrity of tissue-cultured plants. Saha et al. (2016) also reported consistent findings in *Morus alba* L. var. S-1, where RAPD and ISSR analyses demonstrated 100% genetic similarity between micropropagated plantlets and the donor plant. In their study using ISSR primers in grapes, Çilesiz (2025) obtained 88 polymorphic bands. UPGMA and PCA analyses successfully separated the genotypes. ISSR has shown high efficacy in grape genetic characterization. Aljuaid et al. (2022) used a combination of three polymerase chain reaction (PCR)-based molecular marker methods to verify the genetic stability of micropropagated grapes: CDDP (conserved DNA-derived polymorphism), ISSR (inter-simple sequence repeats), and DNA barcoding. ISSR analyses showed very low genetic difference ($\approx 1-7\%$) between micropropagated plants and the parent plant. Somaclonal variation was detected by ISSR analyses. These results demonstrate that *in vitro* culture conditions maintain genetic integrity and reduce the likelihood of somaclonal variation. (Bednarek et al., 2020). Similarly, in our study, ISSR markers produced monomorphic banding patterns in both *in vivo* and *in vitro* samples, with no detectable differences

between tissue-cultured plants and the original parent material. These comparable outcomes further support the reliability of ISSR markers for assessing genetic stability and confirm that tissue culture techniques can be used effectively to achieve healthy clonal propagation under appropriate culture conditions while maintaining genomic fidelity. Tissue culture has been reported to activate the transposition of plant transposable elements (Hirochika et al., 1996; Courtial et al., 2001), and their integration into new genomic loci can lead to somaclonal variation (Wessler et al., 1995). Accordingly, the application of retrotransposon-based molecular markers such as S-SAP, IRAP, and REMAP represents a promising approach for the analysis of genetic changes in in vitro-derived plants. Siringam and Vanijajiva (2023) reported that plants of *Melientha suavis* regenerated *in vitro* exhibited no genetic variation compared to the parent plant when assessed using iPBS and SRAP markers, demonstrating the reliability of iPBS markers for evaluating genetic stability. In contrast, in our study, the primers iPBS2400 and iPBS2378 revealed detectable-though limited-polymorphism among aronia cultivars. The iPBS2400 primer generated a higher number of bands with lower polymorphism, whereas iPBS2378 produced fewer bands but yielded a higher polymorphism rate, along with comparable or greater values for diversity parameters (PIC, He, Ne, I). This distinction indicates that iPBS markers not only serve as reliable tools for confirming genetic stability but can also be effectively employed to assess genetic diversity. Asif and Othman (2005) successfully used inter-retrotransposon Amplified Polymorphism (IRAP) markers for the analysis of banana somaclones. Two DNA fingerprinting approaches, randomly amplified polymorphic DNA (RAPD) and IRAP, were employed to characterize somaclonal variants of Musa. IRAP primers were designed from conserved, genome-wide dispersed long terminal repeat (LTR) retrotransposon families to evaluate somaclonal variation in two Musa clones differing in resistance to *Fusarium oxysporum* f. sp. *cubense* race 4. Using either single primers or primer combinations oriented according to LTR sequences, IRAP markers efficiently amplified diverse retrotransposon loci across the Musa genome and enabled the detection of novel insertion events. The observed somaclonal variation is likely attributable to widespread insertion-deletion events and the activation of retroelements induced by stress associated with micropropagation. These findings suggest that the use of multiple DNA marker systems is essential for comprehensive detection of genome-wide variation and accurate characterization of somaclonal variants. Therefore, our findings demonstrate that the iPBS system, a retrotransposon, is a powerful approach capable of detecting intervariety differences even in species with a narrow genetic base, such as aronia, thus adding a diversity-focused dimension to the stability-focused studies in the current literature. The results indicate that genetic differentiation among aronia cultivars is generally low to moderate, likely reflecting the narrow genetic base of commercially cultivated varieties. The genetic discrepancies observed between the *in vitro* and *in vivo* forms of the same cultivars suggest the potential occurrence of somaclonal variation during tissue culture, consistent with earlier reports (Larkin and Scowcroft, 1981; Miguel and Marum, 2011). According to our analysis results, for some sample pairs the Jaccard distance reached 1.00, indicating the complete absence of common bands and thus completely different band profiles. The ‘Viking’ *in vitro* sample differed completely from ‘Nero’ *in vitro* and from ‘Galicjanka’ and its *in vitro* form, indicating that there is no overlap in the specific band combinations among these cultivars.

In our study, AMOVA results revealed that most of the genetic variation (92%) originated from within-population differences, specifically between *in vivo* and *in vitro* samples within each cultivar. In contrast, only a small proportion (8%) was attributed to differences among cultivars. This outcome may reflect the influence of minor discrepancies between clonal pairs on the overall variance when both *in vivo* and *in vitro* forms of the same cultivar are analyzed together. In contrast, Petrova et al. (2023) reported no detectable polymorphism between orchard grown plants and *in vitro* shoots of *Crocus sativus* using iPBS markers, thereby confirming the genetic homogeneity and stability of the *in vitro* material. Thus, while completely monomorphic banding patterns have been documented in particular species, our findings demonstrate that *in vitro* culture conditions may contribute to genetic variation, albeit to a limited extent.

Leonard et al. (2013) investigated the origin of *A. mitschurinii* using AFLP markers and demonstrated that this species is distinct from the wild Aronia taxa (*A. melanocarpa*, *A. arbutifolia*, and *A. prunifolia*), yet shows close genetic affinity to *×Sorbaronia fallax* and *×Sorbaronia* ‘Ivan’s Beauty.’ The authors also emphasized that *A. mitschurinii*, regarded as a breeding-derived species in Europe, is commonly referred to as *A. melanocarpa* cultivars in North America, a group that includes widely cultivated genotypes such as ‘Viking,’ ‘Nero’ and ‘Aron’. These findings highlight the complex evolutionary background underlying the origin of *A. mitschurinii*. In our

study, only limited genetic variation was detected between the *in vivo* and *in vitro* forms of the different aronia cultivars. When considered together with the AFLP-based results reported in the literature, these findings further corroborate the genetically complex structure of aronia genotypes and point out the need for more comprehensive molecular approaches to elucidate interspecific relationships.

4. Conclusions

In the present study, four commercial aronia cultivars ('Viking', 'Aron', 'Galicjanka' and 'Nero') were assessed using ISSR and iPBS molecular marker systems across eight genotypes representing both *in vitro* and *in vivo* forms. To enhance analytical power and to discriminate intra-varietal from inter-varietal variability, *in vitro* and *in vivo* samples belonging to the same cultivar were merged into a single population (four populations in total; eight genotypes). This approach enabled a more accurate estimation of allele frequencies for dominant marker systems (ISSR, iPBS). While none of the 31 ISSR primers screened generated polymorphic loci, two of the five iPBS primers (40%) yielded polymorphic banding patterns, thereby providing a more robust resolution of both inter-population and intra-population genetic structure. Furthermore, it is recommended that somaclonal variations and wild relatives be used in future breeding studies related to aronia, given the narrow genetic base of these four varieties. It has also been shown that this marker system is not useful for this species, as the ISSR markers used could not detect variation in aronia.

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Authorship Contribution Statement

Concept: Ayaz, S., Ayçiçek, Ö. E.; Design: Ayaz, S., Topçu, H.; Bilgen, B. B.; Data Collection or Processing: Ayaz, S., Ayçiçek, Ö. E.; Statistical Analyses: Ayaz, S., Ayçiçek, Ö. E.; Literature Search: Ayaz, S., Ayçiçek, Ö. E., Topçu, H.; Writing, Review and Editing: Topçu, H., Bilgen, B. B.

References

- Alan, F., Uzun, A. and Pinar, H. (2025). Genetic diversity and population structure of some blackberry genotypes from Türkiye using ISSR markers. *Genetic Resources and Crop Evolution*, 72: 9001-9019.
- Aljuaid, B. S., Ismail, I.A., Attia, A. O. and Dessoky, E. D. S. (2022). Genetic stability of *in vitro* propagated grapevine (*Vitis vinifera* L.) cv. AlBayadi. *Journal of Agriculture and Crops*, 8(1): 12-19. <https://doi.org/10.32861/jac.81.12.19>
- Asif, J. M. and Othman, R. Y. (2005). Characterization of Fusarium wilt-resistant and Fusarium wilt-susceptible somaclones of banana cultivar Rastali (Musa AAB) by random amplified polymorphic DNA and retrotransposon markers. *Plant Molecular Biology Reporter*, 23: 241-249.
- Banach, M., Wiloch, M., Zawada, K., Cyplik, W. and Kujawski, W. (2020). Evaluation of antioxidant and anti-inflammatory activity of anthocyanin-rich water-soluble aronia dry extracts. *Molecules*, 25(18):4055. <https://doi.org/10.3390/molecules25184055>
- Basturk, B. and Ozerson, Z. (2025). The role of functional foods in health policies: An evaluation through the case of Aronia. *Journal of Original Studies*, 6: e2832. <https://doi.org/10.47243/jos.2832>
- Bednarek, P. T., Pachota, K. A., Dynkowska, W. M., Machczyńska, J. and Orłowska, R. (2021). Understanding *in vitro* tissue culture-induced variation phenomenon in microspore system. *International Journal of Molecular Sciences*, 14: 22 (14):7546. <https://doi.org/10.3390/ijms22147546>
- Bilgen, B. B., Yuruk, B. and Nasirian, H. (2023). Brown rust resistance screening and molecular characterization of wheat cultivars by molecular markers. *Turkish Journal of Agriculture and Forestry*, 47(5): 787-800.
- Cekic, C., Calis, O. and Erdem, O. (2018). Genetic diversity of wild raspberry genotypes (*Rubus idaeus* L.) in North Anatolia based on ISSR markers. *Applied Ecology and Environmental Research*, 16(5): 6835-6843.
- Celebi-Toprak, F. and Alan, A. R. (2020). A Successful Micropropagation Protocol for Three Aronia (*Aronia melanocarpa*) cultivars. ISHS Acta Horticulturae 1285: XXX. International Horticultural Congress IHC2018: II International Symposium on Micropropagation and In Vitro Techniques. <https://doi.org/10.17660/ActaHortic.2020.1285.27>
- Cilesiz, Y. (2025). Genetic relationship among grape (*Vitis vinifera* L.) genotypes grown in inner Anatolia region Turkey analyzed by ISSR and RAPD markers. *Scientific Reports*, 15: 32842. <https://doi.org/10.1038/s41598-025-18463-3>
- Coskun, S. and Dumanoglu, H. (2025). Current knowledge on aronia systematics and cultivation. *Fruit Science*, 12(1): 1-6. <https://doi.org/10.51532/meyve.1550561>
- Courtial, B., Feuerbach, F., Eberhard, S., Rohmer, L., Chiapello, H., Camilleri, C. and Lucas, H. (2001). Tnt1 transposition events are induced by *in vitro* transformation of *Arabidopsis thaliana*, and transposed copies integrate into genes. *Molecular Genetics and Genomics*, 265: 32-42.
- Debergh, G. F. and Debergh, J. (1994). Micropropagation and the production of high-quality plants. *Plant Cell, Tissue and Organ Culture*, 37: 1-13.
- Doyle, J. J. and Doyle, J. L. (1987). A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, 19: 11-15.
- Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12: 13-15.
- Engin, S. P. and Boz, Y. (2019). The latest developments at berry cultivation of Turkey. *International Journal of Anatolia Agricultural Engineering*, 1(5): 108-115.
- Engin, S. P. and Mert, C. (2024). Determination of phenological and morphological properties of some aronia cultivars in Yalova Conditions. *Bahçe*, 53(1): 27-33.
- Eskimez, I. and Polat, M. (2023). Effects of plant growth regulators on micropropagation of aronia (*Aronia melanocarpa* (Michx.)). *Fruit Science*, 10: 92-99. <https://doi.org/10.51532/meyve.1189817>
- George, E. F., Hall, M. A. and De Klerk, G. J. (2008). Plant Propagation by Tissue Culture. Springer Nature. <https://doi.org/10.1007/978-1-4020-5005-3>
- Guclu, F., Koyuncu, F. and San, B. (2010). The *in vitro* micropropagation of some clonal cherry rootstocks. *Suleyman Demirel University Journal of Natural and Applied Sciences* 14(2): 144-147.
- Haliloglu, K., Turkoglu, A., Ozturk, H. I., Ozkan, G., Elkoca, E. and Poczai, P. (2022). iPBS-Retrotransposon markers in the analysis of genetic diversity among common bean (*Phaseolus vulgaris* L.) germplasm from Türkiye. *Genes*, 13(7): 1147.
- Hardin, J. W. (1973). The enigmatic chokeberries (*Aronia*, *Rosaceae*). *Bulletin of the Torrey Botanical Club*, 100(3): 178-184.
- Hasnain, A., Naqvi, S. A. H., Ayesha, S. I., Khalid, F., Ellahi, M., Iqbal, S., Hassan, M. Z., Abbas, A., Adamski, R., Markowska, D., Baazeem, A., Mustafa, G., Moustafa, M., Hasan, M. E. and Abdelhamid, M. M. A. (2022) Plants *in vitro* propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. *Frontiers in Plant Science*, 13:1009395. <https://doi.org/10.3389/fpls.2022.1009395>
- Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H. and Kanda, M. (1996) Retrotransposons of Rice Involved in Mutations Induced by Tissue Culture. *Proceedings of the National Academy of Sciences*, 7783-7788, U. S. A.
- Jaccard, P. (1901). Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bulletin de la Société vaudoise des sciences naturelles*, 37: 547-579.

- Jeppsson, N. (2000). The effect of cultivar and cracking on fruit quality in black chokeberry (*Aronia melanocarpa*) and hybrids between chokeberry and rowan (*Sorbus*). *Gartenbauwissenschaft*, 65(2): 93-98.
- Kafkas, S., Ozkan, H. and Sutyemez, M. (2005). DNA polymorphism and assessment of genetic relationships in walnut genotypes based on AFLP and SAMPL markers. *Journal of the American Society for Horticultural Science*, 130(4): 585-590.
- Kalendar, R. and Schulman, A. H. (2014). Transposon-Based Tagging: IRAP, REMAP, and iPBS. In: *Molecular Plant Taxonomy: Methods and Protocols* (Vol. 1115), Ed(s): Besse, P., pp. 233-255. Totowa, NJ: Humana Press, U. S. A.
- Kalendar, R., Antonius, K., Smýkal, P. and Schulman, A. H. (2010). iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. *Theoretical and Applied Genetics*, 121(8): 1419-1430.
- Kaya, Ç. (2025). The biochemical composition, health benefits, and potential use of the aronia (*Aronia melanocarpa* L.) in the food industry. *Nevşehir Journal of Science and Technology*, 14(1): 1-15.
- Larkin, P. J. and Scowcroft, W. R. (1981). Somaclonal variation A novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, 60(4): 197-214.
- Leonard, P. J., Brand, M. H., Connolly, B. A. and Obae, S. G. (2013). Investigation of the origin of *Aronia mitschurinii* using amplified fragment length polymorphism analysis. *Horticulture Science*, 48(5): 520-524.
- Metiner, E. E. and Ersus, S. (2023). The effects of different drying methods on the quality of Aronia (*Aronia melanocarpa*) fruit and its dried powder. *Journal of Agriculture Faculty of Ege University*, 60(2): 353. <https://doi.org/10.17100/nevbittek.1626095>
- Miguel, C. and Marum, L. (2011). An epigenetic view of plant cells cultured *in vitro*: Somaclonal variation and beyond. *Journal of Experimental Botany*, 62(11): 3713-3725.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15(3): 473-497.
- Obae, S. G., Brand, M. H., Connolly, B. A., Beasley, R. R. and Lance, S. L. (2017). Microsatellite markers for *Aronia melanocarpa* (Black Chokeberry) and their transferability to other aronia species. *Horticulture science*, 52(1): 20-23.
- Oszmianski, J. and Wojdylo, A. (2005). *Aronia melanocarpa* phenolics and their antioxidant activity. *European Food Research and Technology*, 221: 809–813. <https://doi.org/10.1007/s00217-005-0002-5>
- Ozbucak, T. and Gumus, A. F. (2024). Determination of ecological and phytochemical variations of aronia fruit. *Turkish Journal of Agricultural and Natural Sciences*, 11(4): 1035-1045. <https://doi.org/10.30910/turkjans.1499475>
- Ozkan, S. and Bilgen, B. B. (2019). Genetic characterization of sainfoin (*Onobrychis viciifolia*) varieties and populations using microsatellite markers. *Journal of Tekirdag Agricultural Faculty*, 16(1): 45-54.
- Parab, A. R., Lynn, C. B. and Subramaniam, S. (2021). Assessment of genetic stability on *in vitro* and *ex vitro* plants of *Ficus carica* var. black jack using ISSR and DAMD markers. *Molecular Biology Reports*, 48(11): 7223-7231.
- Peakall, R. and Smouse, P. E. (2012). GenA1Ex 6.5: Genetic analysis in Excel. Population genetic software for teaching and research an update. *Bioinformatics*, 28(19): 2537-2539.
- Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M. and Duchesnay, É. (2011). Scikit-learn: Machine learning in Python. *The Journal of Machine Learning Research*, 12: 2825-2830.
- Petrova, M., Bonchev, G., Dimitrova, L., Dimitrova, M. and Vassilevska-Ivanova, R. (2023). *In vitro* cultivation of saffron (*Crocus sativus* L.) and assessment of genetic homogeneity using iPBS markers. *Comptes rendus de l'Académie bulgare des Sciences*, 76(11): 1670–1678. <https://doi.org/10.7546/CRABS.2023.11.04>
- Pour, A. H., Ozkan, G., Nalci, O. and Haliloglu, K. (2019). Estimation of genomic instability and DNA methylation due to aluminum (Al) stress in wheat (*Triticum aestivum* L.) using iPBS and CRED-iPBS analyses. *Turkish Journal of Botany*, 43(1): 27-37.
- Rohela, G. K., Jogam, P., Saini, P., Sandhya, D., Peddaboina, V. and Shekhawat, M. S. (2022). Assessing the Genetic Stability of *In Vitro* Raised Plants. In: *Commercial Scale Tissue Culture for Horticulture and Plantation Crops*. Ed(s): Gupta, S. and Chaturvedi, P. Springer, Singapore. https://doi.org/10.1007/978-981-19-0055-6_11
- Roldán-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A. and De Loose, M. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Molecular Breeding*, 6(2): 125-134.
- Saha, S., Adhikari, S., Dey, T. and Ghosh, P. (2016). RAPD and ISSR based evaluation of genetic stability of micropropagated plantlets of *Morus alba* L. variety S-1. *Meta Gene*, 7:7-15.
- Sahin, A. and Erdogan, Ü. (2022). Aronia (*Aronia melanocarpa* Michx Elliot) production and evaluation methods in the world and Turkey. *Turkish Journal of Agriculture - Food Science and Technology*, 10(1): 81-85.
- Sanlier, N. and Cinar, E. N. (2025). Aronia berry (*Aronia melanocarpa* L.) as a bioactive treasure: Insights into its health-promoting effects. *Journal of Functional Foods*, 135: 107116. <https://doi.org/10.1016/j.jff.2025.107116>

- Shahin, L., Mohamed, M. H. and El-Mahrouk, M. E. (2019). Aronia (Chokeberry): an underutilized, highly nutraceutical plant. *Journal of Applied Botany and Food Quality*, 92: 204-213.
- Siringam, T. and Vanijajiva, O. (2023). The effect of plant growth regulators on micropropagation of *Melientha suavis* Pierre. and assessment of genetic fidelity of regenerants based on iPBS and SRAP markers. *Biodiversitas Journal of Biological Diversity*, 24(9): 5325-5334.
- Smolik, M., Ochmian, I. D. and Smolik, B. (2011). RAPD and ISSR methods used for fingerprinting selected, closely related cultivars of *Aronia melanocarpa*. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 39(2): 276-284.
- TAGEM (2022). T.C. Ministry of Agriculture and Forestry, General Directorate of Plant Production, Aronia Feasibility Report and Investor Guide, Education and Publication Department, Ed: Toprak, E., Türkteşmel, E. Ankara, Türkiye. (In Turkish)
- Tikendra, L., Dey, A., Jamir, I., Sahoo, M. R. and Nongdam, P. (2022). Cytokinin influence on *in vitro* shoot induction and genetic stability assessment of *Dendrocalamus latiflorus* Munro: A commercially important bamboo in Manipur, North-East India. *Vegetos*, 35(4): 1085-1095.
- Wessler, S.R., Bureau, T.E. and White, S.E. (1995) LTR-retrotransposons and MITES important players in the evolution of plant genomes. *Current Opinion in Genetics and Development*, 5: 814-821
- Xu, J., Li, F., Zheng, M., Sheng, L., Shi, D. and Song, K. A. (2024). Comprehensive Review of the Functional Potential and Sustainable Applications of *Aronia melanocarpa* in the Food Industry. *Plants (Basel)*, 13(24):3557. <https://doi.org/10.3390/plants13243557>
- Yilmaz, E. C., Bilgen, B. B. and Tuna, M. (2023). Evaluation of the genetic structure of some accessions belonging to *Onobrychis* spp. using microsatellite DNA markers. *Journal of Tekirdag Agricultural Faculty*, 20(2): 399-409
- Yin, W. Y., Cui, X. Q., Zhao, Q. J., Deng, J. L., Huang, C. Y., Zhang, Z. B. and Li, J. W. (2025). Genetic relationship analysis and fingerprint construction of some species in the genus *Vanda* (*Orchidaceae*) by interprimer binding site (iPBS) markers. *Genetic Resources and Crop Evolution*, 72(1): 935-946.
- Zietkiewicz, E., Rafalski, A. and Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20(2): 176-183.