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Allergen coding genes and miRNA-based DNA fingerprints in revealing differences among *Vaccinium corymbosum* L. and *Vaccinium myrtillus* L.

Profilové charakteristiky alergén kódujúcich génov a miRNA markérov *Vaccinium corymbosum* L. a *Vaccinium myrtillus* L.

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ABSTRACT

DNA-based marker fingerprinting techniques are developed with the aim of their effective use in the identification or comparative studies of plant genomes. In the genus *Vaccinium*, DNA markers help resolve taxonomic relationships among the species included in it. In this study, the allergen coding genes and micro RNAs were used as DNA markers and applied for the characterization of the fingerprints of *Vaccinium corymbosum* L. and *Vaccinium myrtilus* L. Both techniques were found to be effective in distinguishing the analysed *Vaccinium* accessions and in the generating of polymorphism and in the case of Bet v 1 Based Amplicon Polymorphism and Profilin Based Amplicon Polymorphism, separated fingerprints were obtained for *Vaccinium myrtilus* L.

Keywords: blueberries, allergen homologs DNA profiling, miRNA markers

ABSTRAKT

Identifikačné techniky založené na DNA markeroch sú vyvinuté s cieľom ich efektívneho využitia v identifikačných alebo porovnávacích štúdiách rastlinných genómov. V rode *Vaccinium* DNA markery pomáhajú riešiť taxonomické vzťahy medzi druhmi spomenutého rodu. V tejto štúdii, alergény kódujúce gény a mikroRNA boli použité ako DNA markery a aplikované charakterizáciu profilov *Vaccinium corymbosum* L. and *Vaccinium myrtilus* L. Obe techniky sa preukázali ako efektívne pri rozoznávaní analyzovaných druhov *Vaccinium* a tvorbe polymorfizmov a v prípade amplikónového polymorfizmu založeného na Bet v 1 a amplikónového polymorfizmu založeného na profilíne, boli získané rozličné profily pri *Vaccinium myrtilus* L.

Kľúčové slová: čučoriedky, profilovanie homológov alergénov, miRNA markéry

INTRODUCTION

The genus *Vaccinium*, which comprises 450 species, is one of the tribes of the subfamily *Vaccinioideae* (Stevens, 1971). It originated in North America (Rimando et al, 2004) and is valued for its nutritional value mainly because of its abundant antioxidants when compared to other fruits (Nile and Park, 2014; Škrovánková et al. 2015). The importance of investigating this species is also supported by the platform "Genome Database for *Vaccinium*" (GDV; 2023) which provides all available information on the *Vaccinium* genomes. *Vaccinium corymbosum* L. (blueberry) and *Vaccinium myrtillus* L. (bilberry) belong to the main commercial species that are used among the genus *Vaccinium*. The genera were reported as highly polyphyletic (Powell and Kron, 2002; Kron et al., 2002) and molecular and DNA-based markers should resolve taxonomic relationships among the species included in it (Hummer et al., 2019). Breeding strategies rely mainly on phenotypic-based selection of desired traits, but genomic-assisted breeding should be incorporated into breeding programs, as linkage maps that identified some of the loci that regulate fruit quality traits were prepared for species within *Vaccinium* genus (Mengist et al., 2021).

Individual cultivars in the genus *Vaccinium* have traditionally been identified by evaluation of mainly morphological characters which makes distinguishing closely related them difficult (Cho et al., 2017), because of overlapping morphologies and a very high level of interspecific and intersectional level (Ballington, 2001). Further phylogenetic analysis and characterization of genetic resources are needed for *Vaccinium* genus (Zdepski et al., 2011). Different DNA markers were used to characterize the variability of blueberries as well as bilberries. RAPD (Random Amplified Polymorphic DNA) markers were applied in the first studies of genetic polymorphism. The estimation of genetic relationships of visually distinct clones of lowbush blueberries resulted in the amplification of consistent fragments and different polymorphism was reported among 26 diverse clones (53%) and for 26 closely situated clones (87%) (Burgher et al., 2002; Burgher-Maclellan and Mackenzie, 2004). RAPD markers have the potential to produce not only polymorphic banding patterns but the monomorphic profiles of closely related varieties obtained too by this technique (Arce-Johnson et al., 2002). Microsatellite-based techniques, such as ISSR (Inter Simple Sequence Repeats) or SSR (Simple Sequence Repeats) followed RAPD markers with results of distinguishing potential presented very similar to RAPD (Carvalho et al., 2014; Gawroński et al., 2017). Their resolution power is generally more effective in whole genome polymorphism analysis (Carvalho et al., 2018) and the accuracy for the number of cultivars tested (Garriga et al., 2013), due to the highly diverse amplification patterns, that were identified for satellite DNAs in *Vaccinium* species (Sultana et al., 2020). Retrotransposon-based SSAP (Sequence-Specific Amplification Polymorphism) markers indicated a relatively simple genetic background of forty-eight blueberries cultivars analyzed by the polymorphism of *Ty1/Copia* and *Ty3/Gypsy* retrotransposons (Zong et al., 2019). Both types of the above-mentioned retrotransposons were used for RBIP (Retrotransposon Based Insertion Polymorphism) based variability and molecular identity of blueberry cultivars. Obtained RBPI fingerprints reflected individual types of blueberries – north-ern highbush blueberries, southern highbush blueberries, and rabbit-eye blueberries (Wu et al., 2018).

Another approach to assessing genetic variability in plants is to apply markers of coding regions of plant genomes, such as genes for allergens and functional markers based on microRNAs sequences.

Allergen-coding genes are abundant in plant genomes and share high homology among individual species as shown by the massive accumulation of sequence data that are stored in public databases. They act in plants in many basic physiological and cellular processes, such as cytoplasmatic disease resistance, regulation of actin cytoskeleton dynamics, seed storage proteins, plant growth responses by interacting with the plant hormone auxin, etc. (Radauer and Breiteneder, 2017). Pathogenesis-related proteins are a group that contains some of the important allergens. PR-10 subgroup is one

of the most studied because of Bet v 1 allergen, the main allergen of *Betulla verrucosa* L. that is involved in many cross-reactions with its homologs. Bet v 1 is responsible for spring pollen-based allergenic sensitization. High homology in the protein structure of Bet v 1 is due to short amino acid sequences. These key sequences are highly conserved between species, resulting in a very similar to identical protein structure (Seutter von Loetzen et al., 2012), despite the relatively variable remaining parts of the nucleotide sequence of ypr10 genes (genomic similarity 50–>90%) (Fernandes et al., 2013). The involvements of allergen coding genes in basic processes combined with their homology allow their use not only for allergenic plants, what is the case of *V. corrymbosum* L. and *V. myrilis* L., too. Any of these two species has been documented to be allergenic to date, but there may be cross-reactivity, especially with cranberries (AAAI, 2023). High homology of groups of Bet v 1 and profiling families of allergens was a base for developing the DNA markers that can be used universally in the plant kingdom. Bet v 1 Based Amplicon Polymorphism (BBAP) provides polymorphic amplicons that are generated by specific forward and degenerate reverse primers that match homolog sequences of Bet v 1 allergen (Žiarovská and Zeleňáková, 2018). Profilin Based Amplicon Polymorphism (PBAB) is based on the ubiquity of the profilin gene in plant genomes and as in the previous technique, degenerate primers are used to obtain polymorphic fingerprints of profilin homologs (Klongová et al., 2021). Any of these techniques were not used before in the analysis of genetic diversity for *Vaccinium corrymbosum* L. or *Vaccinium myrilis* L.

MicroRNA-based markers represent a functional type of markers that have been used in different plants (Fu et al., 2013; Mondal and Ganie, 2014; Yadav et al., 2014; Ražná et al., 2016). DNA markers corresponding to miRNA genomic sequences represents highly efficient, stable, reproducible, low-cost and protocol-transferable genotyping procedure within the field of marker-based techniques (Fu et al., 2013; Yadav et al., 2014). MicroRNAs play an important role in plant responses to abiotic stresses of various natures, including low temperature, drought, salinity, oxidative stress, UV-B radiation, heavy metals, etc. (Yu et al., 2016). They are particularly important in plant growth and development, hormone regulation, organ differentiation, alternative assembly, and metabolite accumulation (Xu et al., 2020). The looped regions of miRNA precursors are relatively highly conserved among closely related species, resulting in good marker transferability. MiRNA-based primers can bind to different positions within the loop structure of miRNA molecules and thus can generate fragments of different lengths or can amplify regions between adjacent miRNAs. The observed polymorphism indicates changes in the sequences of miRNA loci, which may result in changes in target genes regulation and as a consequence of the genome's response to a certain factor (Fu et al., 2013; Yadav et al., 2014). In the genome of blueberries, fruit ripening-related miRNAs and miRNAs with potential functions in abiotic stress were identified (Hou et al., 2017; Li et al., 2022).

The aim of this study was to apply two novel fingerprint techniques based on allergen coding genes and micro RNAs for *Vaccinium* polymorphism study and to characterize them for analysis of *Vaccinium corymbosum* L. and *Vaccinium myrtilus* L.

MATERIAL AND METHODS

A total of 13 blueberry genotypes (Table 1) were used in the analysis. They were obtained in local stores, immediately transported to laboratory and kept frozen until individual analysis were performed

Allergen coding assays have been conducted according to the protocol of Žiarovská and Urbanová (2022). Primers of BBAP assay match the coding region of ypr-10 gene (Žiarovská and Zeleňáková, 2016) and are combined with four degenerated reverse primers. PCR products were amplified by MasterMix Robust HS Elizyme (Elizabeth Pharmacon) with 2–10 ng DNA and 400 nM of each primer. PCR profile was as follows: 95 °C for 5 min; 40x (at 95 °C for 45 s; 54 °C for 45 s; 72 °C for 35 s); by final elongation at 72 °C for 10 min. Generated amplicons were loaded on 1.5% agar gels, separated by electrophoresis and stained by GelRed™ (Biotium). The 100 bp DNA Ladder (ThermoScientific) was used

as a size marker. Profiles of BBAP were analysed by the free available software GelAnalyzer (GelAnalyzer 19.1, 2023) and binary matrices of amplicon presence were prepared. Nei-Li coefficient of genetic distance was used to calculate basic relationships among individual species and a dendrogram was constructed by UPGMA method.

The miRNA-based assay has been conducted according to the protocol of Ražná et al. (2020).

Table 1. The list of analysed blueberry genotypes

Sample Order	Cultivar	Species	Origin
1	Emerald	*V. corrymbosum*	Argentina
2	JE2	*V. corrymbosum*	Spain
3	Biloxi	*V. corrymbosum*	Peru
4	Duke	*V. corrymbosum*	Italy
5	-	*V. corrymbosum*	Argentina
6	-	*V. corrymbosum*	Poland
7	Elliott	*V. corrymbosum*	Poland
8	Suzi Blue	*V. corrymbosum*	Chile
9	-	*V. corrymbosum*	South Africa
10	Bluecrop	*V. corrymbosum*	Poland
11	-	*V. corrymbosum*	Slovakia
12	-	*V. corrymbosum*	Peru
13	V. myrtillus	*V. myrtillus*	-

Primers were designed according to the study Barvkar et al. (2013) using the database miRBase (mirbase). Type of microRNA molecules which are part of the regulation of antioxidant stresses were selected based on Bej and Basak (2014), Bavkar et al. (2013) and Sunkar (2010). as follows in sequences 5 - 3´:

miR168_F	CAC GCA TCG CTT GGT GCA GGT
miR168_R	CCA GTG CAG GGT CCG AGG TA
miR408a_F	GGC TGG GAA CAG ACA GAG CAT GGA
miR408a_R	GGG AAA AAG GCC AGG GAA GAG G

Amplification products are separated on 15% TBE-urea polyacrylamide (PAGE) gels, running in 1× TBE running buffer at constant power 180 V, 30 mA for 90 min. The 10 bp DNA Ladder (Invitrogen) was used as a size marker. The gels are stained with PAGE GelRed™ Nucleic Acid Gel stain and are visualized on G-Box Syngene electrophoresis documentation system. For the recording of loci number and their position, as well as the identification of unique fragments, the gels are analysed by GeneTools software (Syngene). Presence (1) or absence (0) of the individual as well as the position of individual miRNA loci were documented and utilized for binary matrix construction followed by the UPGMA (Unweighted Pair Group Method using arithmetic Averages) hierarchical cluster analysis (ref.).

The percentage of polymorphism was based on directly counting polymorphic and total loci. Marker index (MI) was calculated based on polymorphic information index and effective multiplex ratio: MI = PIC × (np (np/n)), where np is the number of polymorphic loci, and n is the total loci number.

RESULTS

Allergen coding assays

A total of 69 alleles were amplified by degenerate BBAP primer pairs and its non-degenerated variants in the set of analyzed *Vaccinium* accessions. They were distributed among 14 different loci, from which the most abundant profile was amplified for *Vaccinium myrtillus* L. genotype. The range of the amplicon length was from 65 bp up to 1550 bp (Figure 1). Individual positively matched length levels varied for analyzed genotypes of blueberries from two up to ten. A unique amplicon was identified in the Duke variety of *Vaccinium corymbosum* L. with a length of 183 bp when the degenerate primer was used.

The dendrogram constructed on the resulting binary matrix of BBAP amplicons distribution showed the separation of all blueberry accessions except for one genotype of *V. corrymbosum* from Peru and variety JE2. A total of four main subbranches were separated (Figure 2) with a distinguishing of the only *Vaccinium myrtillus* L. accession used in the analysis.

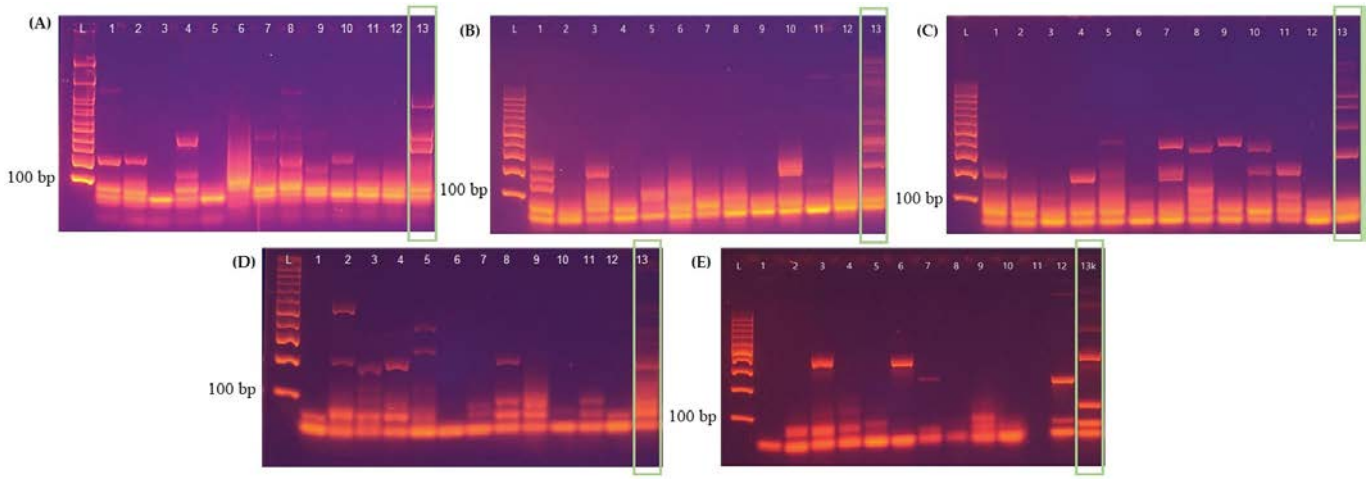

Figure 1. Obtained Bet v 1 profiles for individual primer combinations. (A) – degenerate reverse primer; (B)-(E) – variants of reverse non-degenerated primers L - 100 bp DNA Ladder

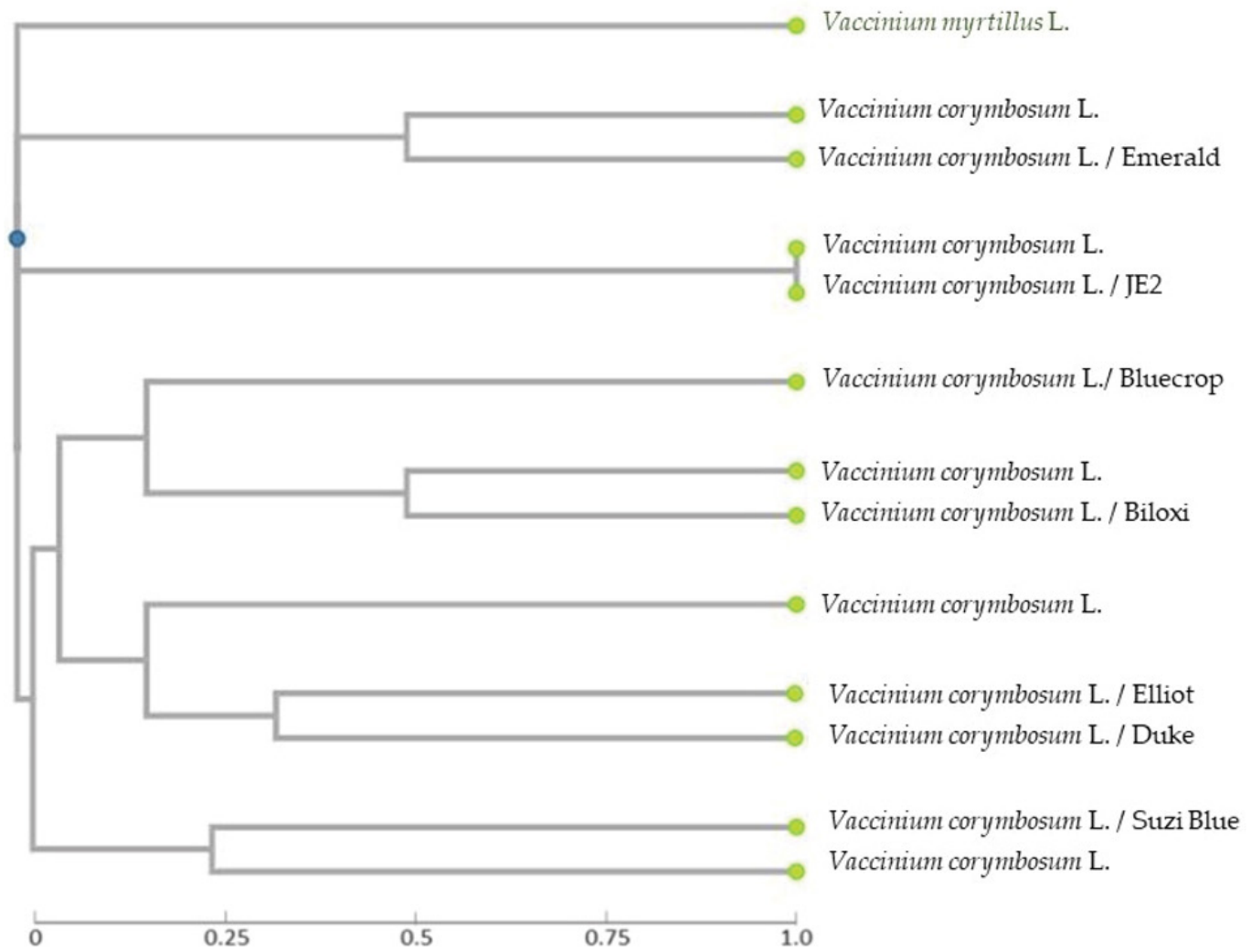

Figure 2. Dendrogram of Jaccard genetic similarity values among analyzed blueberry genotypes for BBAP-generated fingerprints

Two very distinctive PBAP fingerprints were obtained for the analyzed accessions, where no amplicon variability was observed for *Vaccinium corymbosum* L. genotypes and a profile with three different amplicons was amplified in the case of *Vaccinium myrtillus* L. (Figure 3).

miRNA-based assays

The loci of both types of stress-sensitive miRNA-based markers reflected an almost equal representation (miR168 – 50.8% and miR408 – 49.2%) but despite this, it was possible to observe genotype-specific patterns in the profiles of the amplified miRNA-based loci (Figures 4 and 5). In 13 analyzed accessions was detected the amplification of 66 loci by marker miR168, which represents approximately 5.1 locus per genotype. The polymorphism level reached 98.5%. Compared to the second marker, miR408 applied, the parameters were as follows: 64 amplified loci, representing 4.9 locus per genotype and polymorphism level 98.4%. For both used miRNA markers, *Vaccinium myrtillus* L. was not separated by its obtained fingerprint from *Vaccinium corymbosum* L. genotypes.

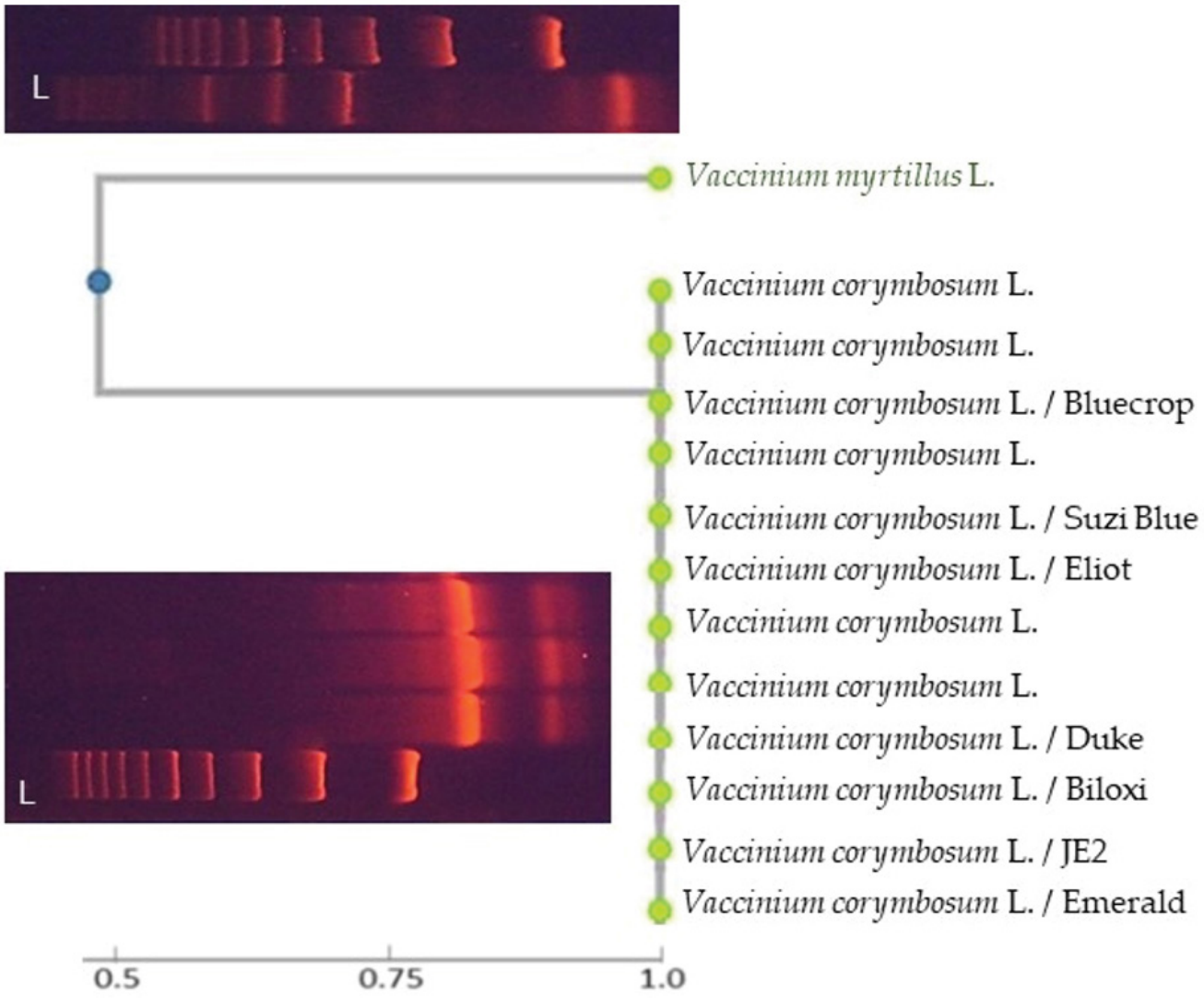

Figure 3. Obtained PBAP fingerprints and dendrogram of Jaccard genetic similarity values among analyzed blueberry genotypes. L - 100 bp DNA Ladder

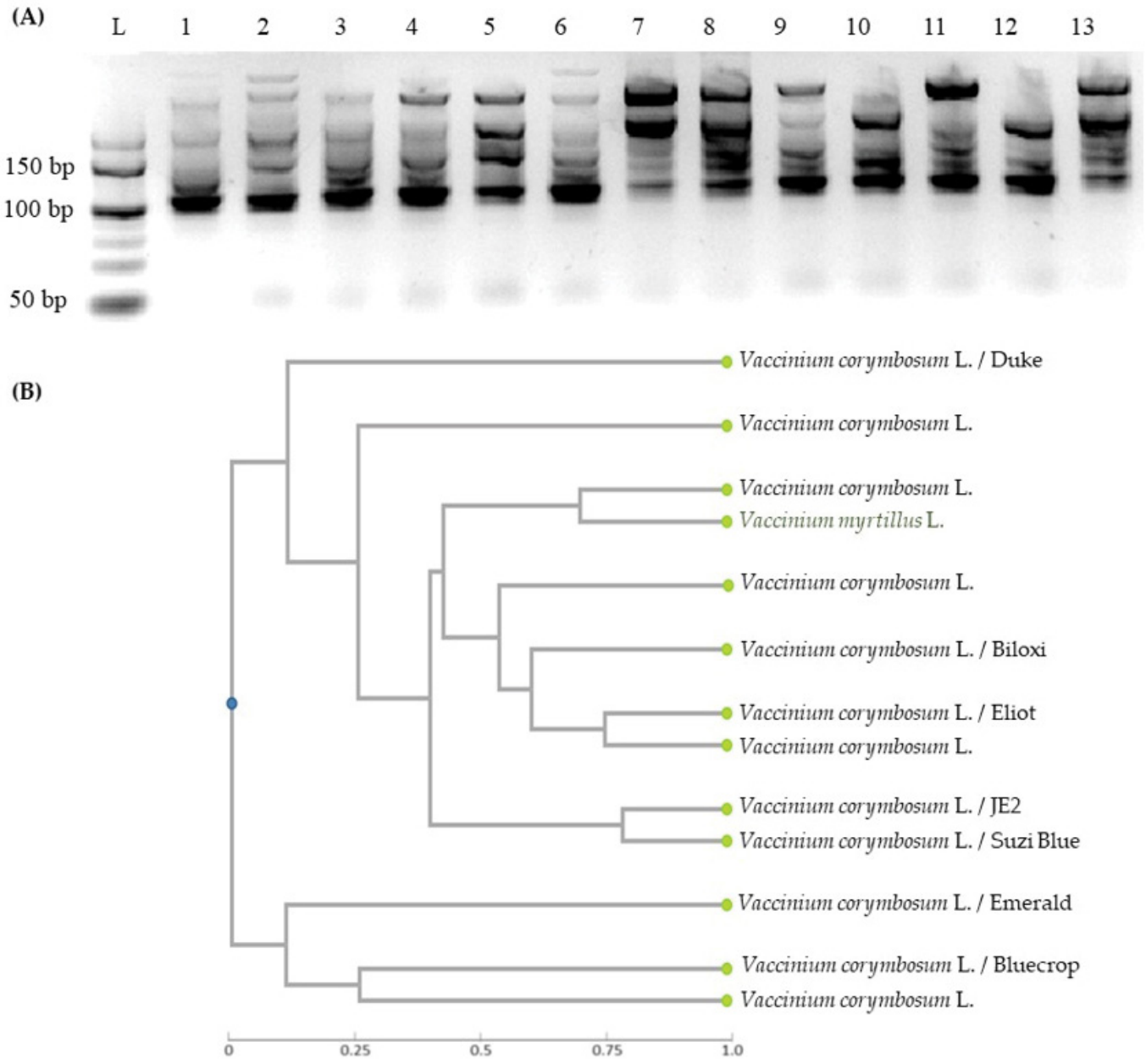

Figure 4. Obtained fingerprints (A) and constructed dendrogram (B) based on the amplification profile of miR408 loci in analyzed blueberry genotypes. L - 10 bp DNA Ladder

The most pronounced amplification of miR168 loci, seven per genotype, was observed in genotypes from South Africa, Poland (Bluecrop) and Peru and in the case of miR408 marker, in genotype from Chile (Suzi Blue). Cluster analysis showed the similarity of the loci profile between the *Vaccinium myrtillus* L. genotype and the genotype from Argentina and at the same time showed the linkage at the level of miR168 and miR408 loci representation between the sample of Slovak origin (11) and genotypes from Peru (Biloxi) and South Africa.

Comparing the techniques used in the study they were similar in the effectiveness of distinguishing the analyzed blueberry genotypes (Table 2). For BBAP and miR168, two of the analyzed genotypes provided the same fingerprinting pattern and were grouped together in constructed dendrograms. The ability of the used markers to detect polymorphisms was comparable with the calculated values of PIC, which ranged from 0.17 up to 0.37, but for all of the used techniques, the values were in the range expected for dominant marker

systems. Allergen-based homologs markers provide less effectiveness for the polymorphism-based analysis of blueberry genomic variability description, as their marker index value was lower when compared to miRNA markers-based techniques, but the higher discrimination power index was calculated for them, which corresponds to more different fingerprints of individual accessions, that were obtained.

ANOVA with posthoc Tukey HSD Test was performed finally for *Vaccinium co-rymbosum* L. genotypes only to compare the marker techniques used in this study (table 3 and 4) and exclude the effect of *Vaccinium myrtillus* L., where very different fingerprints were generated for BBAP and miRNA assays. Allergen coding-based assays and miRNA-based assays generated polymorphic patterns that were significantly different at $P < 0.01$ when compared to themselves.

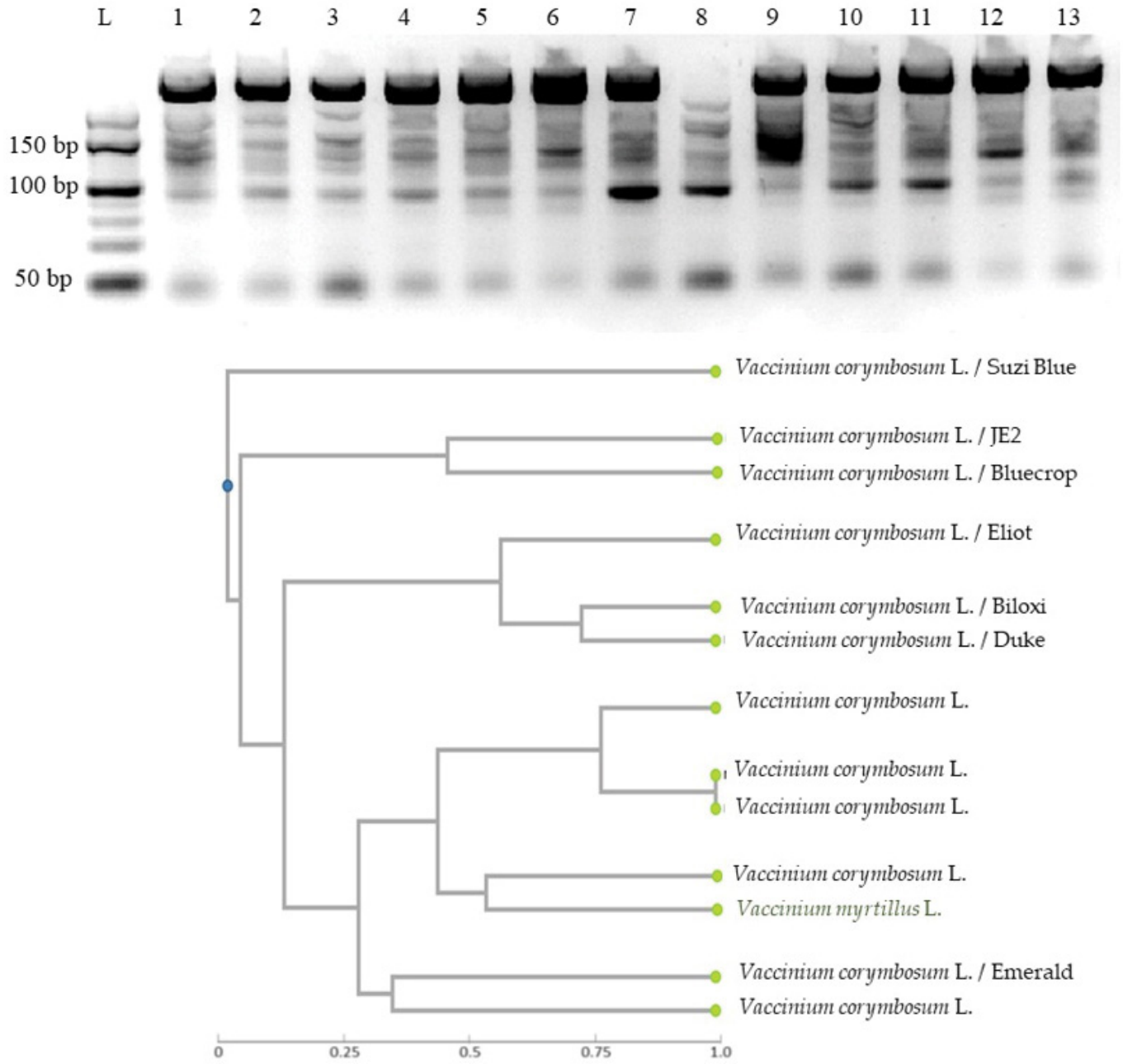

Figure 5. Obtained fingerprints (A) and constructed dendrogram (B) based on the amplification profile of miR168 loci in analyzed blueberry genotypes. L - 10 bp DNA Ladder

Table 2. Characteristics of fingerprints for *Vaccinium corrymbosum* L. and *Vaccinium myrtilus* L. obtained by allergen coding and miRNA-based markers

Technique	polymorphism	PIC	MI	DI	R
BBAP	100%	0.17	0.003	0.98	3.69
PBAP	66.67%	0.25	0.004	0.97	1.84
miR408	100%	0.37	0.04	0.66	10.25
miR168	100%	0.37	0.03	0.64	9.78

PIC – Polymorphic information content; MI – Marker index; DI - Diversity index; D – Discrimination power

Table 3. Results of ANOVA for individual marker techniques used in this study to reveal polymorphism in the set of *Vaccinium corrymbosum* L. accessions

Source	Sum of squares SS	degrees of freedom v	mean square MS	*F* statistic	*P*-value
Treatment	12.48	1	12.48	500.07	1.1102e-16
Error	6.36	262	0.0256		
Total	19.49	263			

Table 4. Results of Tukey HSD for individual marker techniques used in this study to reveal polymorphism in the set of *Vaccinium corrymbosum* L. accessions

Treatments pair	Tukey HSD *Q* statistic	Tukey HSD *P*-value	Tukey HSD inferfence
Allergen based markers vs miRNA- based markers	31.62	0.0010053	$P < 0.01$

DISCUSSION

The genus *Vaccinium* has a circumpolar distribution, and its species can be found in Europe, North America, Asia and Africa (Cho et al., 2017). In the field of DNA-based marker fingerprinting, many comparative studies have been reported with the aim of developing a reliable technique for individual species or cultivars identification. RAPD markers were the first to be applied and were proven to be able to differentiate *Vaccinium corymbosum* L. and *Vaccinium angustifolium* Ait. cultivars by different authors (Cho et al., 2017; Burgher-Maclellan and Mackenzie, 2004; Arce-Johnson et al., 2007). Microsatellites present in the genome of *Vaccinium* types and varieties were applied in the studies of assessing genetic similarity and indexing of the genetic resources or in the studies of identification of cultivated and wild *Vaccinium* species (Carvalho et al., 2014; Gawroński et al., 2017; Carvalho et al., 2018). Retrotransposon-based marker techniques provided another well-established source of *Vaccinium* fingerprinting (Wu et al., 2018) and using SSAP markers, southern highbush blueberry, northern highbush blueberry, and rabbit-eye blueberry were distinguished (Zong et al., 2019).

Here, two other marker techniques were used to analyze the specific fingerprints of *Vaccinium corrymbosum* L. when compared to *Vaccinium myrtilus* L. – allergen coding gene-based variability and microRNA coding-based variability.

Allergen coding gene variability is well-studied across various plant species (Radauer and Breiteneder, 2007; Sinha et al., 2014; Führer et al., 2022) and provide a good source of marker variability detected by PCR. In

the current study, using both allergen coding marker-based techniques, a total of 72 alleles were amplified among 13 analyzed *Vaccinium* accessions. This supports the previous studies, which summarized allergen coding genes to have many homologous genes that may result in many isoforms. *In silico* comparison of the amino acid sequences of Bet v 1 allergen shows a very variable identity among plant species (Breiteneder and Ebner, 2000) which enables the use of these regions as DNA-based markers for fingerprinting the variability of plant genomes. The homology of amino acid sequences in the region of forward primer for BBAP strategy is relatively high and includes the confirmed epitope for IgE [39]. Reverse primers amplify a relatively variable region of the *ypr-10* gene compared to Bet v 1 and match the amino acid variability at position 119 of Bet v 1 protein (P15494) (Breiteneder and Ebner, 2000). In this study, both, BBAP and PBAP fingerprints resulted in the separation of *Vaccinium myrtillus* in the constructed dendrograms.

The miRNA-based markers combine the advantages of relatively high polymorphism, reproducibility, and ease of use with predicted functionality (Fu et al., 2013; Mondal and Ganie, 2014; Yadav et al., 2014). As these markers are derived from conserved miRNA sequences, a high degree of species transferability is expected. As a result, miRNA-based markers provide an effective tool for comparative genome mapping and understanding phylogenetics among different crop species (Yadav et al., 2014). miRNA-based primers combined with different sites of occurrence at the same position in a stem-loop structure can produce fragments of useful size for genotyping.

Plant miRNAs play vital roles in cell growth and development. In addition, they are also major regulators of plants' genome responses to stress factors (Zhou et al., 2020).

CONCLUSION

Evaluating genetic diversity and understanding the genetic relationships within the germplasms of *Vaccinium* can provide crucial information to address their characteristics and breeding problems. In the current study, the allergen coding genes and microRNA-based techniques were used to characterize the fingerprints of *Vaccinium corymbosum* L. and *Vaccinium myrtilus* L. Both technique types were found to be effective in distinguishing the analyzed *Vaccinium* accessions and in the generating of polymorphism and in the case of BBAP and PBAP techniques, separated fingerprints were obtained for *Vaccinium myrtilus* L. The results obtained from this study will provide valuable data for the analysis of the *Vaccinium* germplasm variability.

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REFERENCES

AAAI (2023) American Academy of Allergy, Asthma & Immunology. Available at: https://www.aaaai.org/ [Accessed 20 March 2023].

Arce-Johnson, P., Ríos, M., Zúñiga, M., Vergara, E. (2007) Identification of Blueberry Varieties using Random Amplified Polymorphic DNA Markers. Acta Horticulturae, 574 (3), 154–196. DOI: https://doi.org/10.21273/HORTSCI12105-17

Ballington, J.R. (2001) Collection, utilization, and preservation of genetic resources in *Vaccinium*. Horticulture Science, 36, 213–220. DOI: https://doi.org/10.21273/HORTSCI.36.2.206

Barvkar, V.T., Pardeshi, V.C., Kale, S.M., Qiu, S., Rollins, M., Datla, R., Gupta, V.S., Kadoo, N.Y. (2013) Genome-wide identification and characterization of microRNA genes and their targets in flax (*Linum usitatissimum*): Characterization of flax miRNA genes. Planta, 237, 1149-1161. DOI: https://doi.org/10.1007/s00425-012-1833-5

Bej, S., Basak, J. (2014) MicroRNAs: The Potential Biomarkers in Plant Stress Response. American Journal of Plant Sciences, 5, 748–759. DOI: https://doi.org/10.4236/ajps.2014.55089

Breiteneder, H., Ebner, CH.M.B. (2000) Molecular and biochemical classification of plant-derived food allergens. Journal of Allergy and Clinical Immunology, 106, 27-36. DOI: https://doi.org/10.1067/mai.2000.106929

Burgher, K.L., Jamieson, A.R., Lu. X. (2002) Genetic relationships among lowbush blueberry genotypes as determined by randomly amplified polymorphic DNA analysis. Journal of the American Society for Horticultural Science, 127, 98-103. DOI: https://doi.org/10.21273/JASHS.127.1.98

Burgher-Maclellan, K., Mackenzie, K. (2004) An Overview of RAPD Analysis to Estimate Genetic Relationships in Lowbush Blueberry. Small Fruits Reviews, 3, 295-305. DOI: https://doi.org/10.1300/J301v03n03_06

Carvalho, M., Matos, M., Carnide, V. (2014) Fingerprinting of *Vaccinium corymbosum* cultivars using DNA of fruits. Horticulture Science (Prague), 41, 175–184. DOI: https://doi.org/10.17221/21/2014-HORTSCI

Carvalho, M., Matos, M., Carnide, V. (2018) Identification of cultivated and wild *Vaccinium* species grown in Portugal. Spanish Journal of Agricultural Research, 16, e07SC01. DOI: https://doi.org/10.5424/sjar/2018163-12502

Cho, K.H., Park, S.J., Kim, S.J., Kim, S.H., Lee, H.CH., Kim, M.J., Chun, J.A. (2017) Blueberry Cultivar Identification Using Random Amplified Polymorphic DNA and Sequence-characterized Amplified Region Markers. Horticulture Science, 52, 1483–1489. DOI: https://doi.org/10.21273/HORTSCI12105-17

Fernandez, H., Michalska, K., Sikorski, M., Jaskolskie, M. (2013) Structural and functional aspects of PR-10 proteins. The FEBS Journal, 280, 5, 1169-1199.

Fu, D., Ma, B., Mason, A.S., Xiao, M., Wei, L., An, Z. (2013) MicroRNA-based molecular markers: a novel PCR-based genotyping technique in Brassica species. Plant Breeding, 132, 375–381. DOI: https://doi.org/10.1111/pbr.12069

Führer, S., Unterhauser, J., Zeindl, R., Eidelpes, R., Fernández-Quintero, M.L., Liedl, K.R., Tollinger, M. (2022) The Structural Flexibility of PR-10 Food Allergens. International Journal of Molecular Sciences, 23, 8252. DOI: https://doi.org/10.3390/ijms23158252

Garriga, M., Parra, P.A., Caligari, P.D.S., Retamales, J.B., Carrasco, B.A., Lobos, G.A., García-Gonzáles, R. (2013) Application of inter-simple sequence repeats relative to simple sequence repeats as a molecular marker system for indexing blueberry cultivars. Canadian Journal of Plant Sciences, 93, 913-921. DOI: https://doi.org/10.4141/cjps2013-057

Gawroński, J., Kaczmarska, E., Dyduch-Siemińska, M. (2017) Assessment of genetic diversity between *Vaccinium corymbosum* L. cultivars using RAPD and ISSR markers. Acta Scientiarum Polonorum, Hortorum Cultus, 16, 129–140.

GDV (2023) Genome Database for *Vaccinium*. Available at: GDV (vaccinium.org) [Accessed 12 May 2023].

GelAnalyzer 19.1 - Free desktop app for 1D gel electrophoresis evaluation (by Istvan Lazar Jr., PhD and Istvan Lazar Sr., PhD, CSc.). Available at: http://www.gelanalyzer.com/?i=1 [Accessed 10 February 2023].

Hajyzadeh, M., Turktas, M., Khawar, K.M., Unver, T. (2015) miR408 overexpression causes increased drought tolerance in chickpea. Gene, 555, 186–193. DOI: https://doi.org/10.1016/j.gene.2014.11.002

Hou, Y., Zhai, L., Li, X., Xue, Y., Wang, J., Yang, P., Cao, C., Li, H., Cui, Y., Bian, S. (2017) Comparative Analysis of Fruit Ripening-Related miRNAs and Their Targets in Blueberry Using Small RNA and Degradome Sequencing. International Journal of Molecular Sciences, 18, 2767. DOI: https://doi.org/10.3390/ijms18122767

Hummer, K.E., Williams, K.A., Bushakra, J.M. (2019) North American Crop Wild Relatives of Temperate Berries (*Fragaria* L., *Ribes* L., *Rubus* L., and *Vaccinium* L.). In: Greene, S.L., Williams, K.A., Khoury, C.K., Kantar, M.B., Marek, L.F., eds. North American Crop Wild Relatives, Volume 2: Important Species. New York, New York, USA: Springer International Publishing, 283–327.

Klongová, L., Kováčik, A., Urbanová, L., Kyseľ, M., Ivanišová, E., Žiarovská, J. (2021) Utilization of specific primers in legume allergens based polymorphism screening. Science, Technology and Innovation, 13, DOI: https://doi.org/10.5604/01.3001.0015.5431

Kron, K.A., Judd, W.S., Stevens, P., Kron, K. A., Judd, W.S., Stevens, P.F., Crayn, D.M., Anderberg, A.A., Gadek, P.A., Quinn, C.J., Luteyn, J.L. (2002) Phylogenetic classification of Ericaceae: molecular and morphological evidence. Botanical Reviews, 68 (3), 335–423.

Kruszka, K., Pacak, A., Swida-Barteczka, A., Nuc, P., Alaba, S., Wroblewska, Z., Karlowski, W., Jarmolowski, A., Szweykowska-Kulinska, Z. (2014) Transcriptionally and post-transcriptionally regulated microRNAs in heat stress response in barley. Journal of Experimental Botany, 65, 6123–6135. DOI: https://doi.org/10.1093/jxb/eru353

Li, Y., Wang, X., Guo, Q., Zhang, X., Zhou, L., Zhang, Y., Zhang, C. (2022) Conservation and Diversity of miR166 Family Members From Highbush Blueberry (*Vaccinium corymbosum*) and Their Potential Functions in Abiotic Stress. Frontiers of Genetics, 13, 919856. DOI: https://doi.org/10.3389/fgene.2022.919856

Melnikova, N.V., Dmitriev, A.A., Belenikin, M.S., Koroban, N.V., Speranskaya, A.S., Krinitsina, A.A., Krasnov, G.S., Lakunina, V.A., Snezhkina, A.V., Sadritdinova, A.F., Kishlyan, N.V., Rozhmina, T.A., Klimina, K.M., Amosova, A.V., Zelenin, A.V., Muravenko, O.V., Bolsheva, N.L., Kudryavtseva, A.V. (2016) Identification, expression analysis, and target prediction of flax genotroph microRNAs under normal and nutrient stress conditions. *In Fron Plant Sci*, 7, 399. DOI: https://doi.org/10.3389/fpls.2016.00399

Mengist, M.F., Bostan, H., Young, E., Kay, K.L., Gillitt, N., Ballington, J., Kay, C.D., Ferruzzi, M.G., Ashrafi, H.A., Lila, M.A., Iorizzo, M. (2021) High-density linkage map construction and identification of loci regulating fruit quality traits in blueberry. Horticulturte Research, 8, 169. DOI: https://doi.org/10.1038/s41438-021-00605-z

Mondal, T.K.; Ganie, S.A. (2014) Identification and characterization of salt responsive miRNA-SSR markers in rice (*Oryza sativa*). In Gene, 535, 204–209. DOI: https://doi.org/10.1016/j.gene.2013.11.033.

Nile, S.H., Park, S.W. (2014) Edible berries: bioactive components and their effect on human health. Nutrition, 30, 134-144. DOI: https://doi.org/10.1016/j.nut.2013.04.007

Pan, J., Huang, D., Guo, Z., Kuang, Z., Zhang, H., Xie, X., Ma, Z., Gao, S., Lerdau, M.T., Chu, C., Li, L. (2018) Overexpression of microRNA408 enhances photosynthesis, growth, and seed yield in diverse plants. Journal of Integrative Plant Biology, 60, 323–340. DOI: https://doi.org/10.1111/jipb.12634

Powell, A.E., Kron, K.A. (2002) Hawaiian blueberries and their relatives a phylogenetic analysis of *Vaccinium* sections Macropelma. Myrtillus, and Hemimyrtillus (Ericaceae). Systematic Botany, 27, 768–779. DOI: https://doi.org/10.1043/0363-6445-27.4.768

Radauer, Ch., Breiteneder, H. (2007) Evolutionary biology of plant food allergens. Journal of Allergy and Clinical Immunology, 120, 518-525. DOI: https://doi.org/10.1016/j.jaci.2007.07.024

Ražná, K., Bežo, M., Hlavačková, L., Žiarovská, J., Miko, M., Gažo, J., Habán, M. (2016) MicroRNA (miRNA) in food resources and medicinal plant. Potravinárstvo, 1, 188–194. DOI: https://doi.org/10.5219/583

Ražná, K., Žiarovská, J., Gálová, Z. (2020) MicroRNA-based markers in plant genome response to abiotic stress and their application in plant genotyping. In: Non-Coding RNAs. London: Intech Open (1st ed.), 71-81. ISBN 978-1-78985-655-2

Rimando, A.M., Kalt, W., Magee, J.B., Dewey, J., Ballington, J.R. (2004) Resveratrol, Pterostilbene, and Piceatannol in *Vaccinium* berries. Journal of Agricultural and Food Chemistry, 52, 4713-4719. DOI: https://doi.org/10.1021/jf040095e

Seutter Von Loetzen, CH., Hoffmann, T., Hartl, M.J., Schweimer, K., Schwab, W., Rösch, P., Hartl-Spiegelhauer, O. (2012) Secret of the major birch pollen allergen Bet v 1: identification of the physiological ligand. Biochemical Journal, 457, 3, 379-390.

Sinha, M., Singh, R.P., Kushwaha, G.S., Iqbal, N., Singh, A., Kaushik, S., Kaur, P., Sharma, S., Singh, T.P. (2014) Current Overview of Allergens of Plant Pathogenesis Related Protein Families. The Scientific World Journal, ID 543195. DOI: https://doi.org/10.1155/2014/543195

Stevens, P.F. (1971) A classification of the Ericaceae: subfamilies and tribes. Botanical Journal of the Linnean Society, 64, 1–53.

Sultana, N., Menzel, G., Heitkam, T., Kojima, K.K., Bao, W., Serçe, S. (2020) Bioinformatic and Molecular Analysis of Satellite Repeat Diversity in *Vaccinium* Genomes. Genes (Basel), 11, 527. DOI: https://doi.org/10.3390/genes11050527

Sunkar, R. (2010) MicroRNAs with macro-effects on plant stress responses. Seminars in Cell & Developmental Biology, 21, 805-811. DOI: https://doi.org/10.1016/j.semcdb.2010.04.001

Škrovánková, S., Sumczynski, D., Mlček, J., Juríková, T., Sochor, J. (2015) Bioactive Compounds and Antioxidant Activity in Different Types of Berries. International Journal of Molecular Sciences, 16, 24673-24706. DOI: https://doi.org/10.3390/ijms161024673

Uehara, M., Sato, K., Abe, Y., Katagiri, M. (2001) Sequential IgE epitope analysis of a birch pollen allergen (Bet v 1) and an apple allergen (Mal d 1). Allergology International, 50, 57-62. DOI: https://doi.org/10.1046/j.1440-1592.2001.00201.x

Wu, Z., Fang, Q., Li, Y., Chen, W., Zong, Y., Guo, W. (2018) Development of retrotransposon-based insertion polymorphism molecular marker and cultivar identification of blueberry. Acta Horticulturae Sinica, 45, 753-763.

Xu, X., Chen, X., Chen, Y., Zhang, Q., Su, L., Chen, X., Chen, Y., Zhang, Z., Lin, Y., Lai, Z. (2020) Genome-wide identification of miRNAs and their targets during early somatic embryogenesis in *Dimocarpus longan* Lour. Scientific Reports, 10. DOI: https://doi.org/10.1038/s41598-020-60946-y

Yadav, C.B.Y., Muthamilarasan, M., Pandey, G., Prasad, M. (2014) Development of novel microRNA-based genetic markers in foxtail millet for genotyping applications in related grass species. Molecular Breeding, 34, 2219–2224. DOI: https://doi.org/10.1007/s11032-014-0137-9

Yu, Y., Wu, G., Yuan, H., Cheng, L., Zhao, D., Huang, W., Zhang, S., Zhang, L., Chen, H., Zhang, J., Guan, F. (2016) Identification and characterization of miRNAs and targets in flax (*Linum usitatissimum*) under saline, alkaline, and saline-alkaline stresses. BMC Plant Biology, 16, 124. DOI: https://doi.org/10.1186/s12870-016-0808-2

Zdepski, A., Debnath, S.C., Howell, A., Polashock, J., Oudemans, P., Vorsa, N., Michael, T.P. (2011) Cranberry. In: Folta, K., Kole, C., eds. Genetics, Genomics and Breeding of Berries. Boca Raton, FL, USA: CRC Press, 200. ISBN 9781578087075.

Zhou, X., Khare, T., Kumar, V. (2020) Recent trends and advances in identification and functional characterization of plant miRNAs. Acta Physiologica Plantarum, 42. DOI: https://doi.org/10.1007/s11738-020-3013-8

Zong, Y., Kang, H., Fang, Q., Chen, X., Zhou, M., Ni, J., Zhang, Y., Wang, L., Zhu, Y., Guo, W. (2019) Phylogenetic relationship and genetic background of blueberry (*Vaccinium* spp.) based on retrotransposon-based SSAP molecular markers. Scientia Horticulturae, 247, 116–122. DOI: https://doi.org/10.1016/j.scienta.2018.11.017

Žiarovská, J., Urbanová, L. (2022) Utilization of Bet v 1 homologs based amplified profile (BBAP) variability in allergenic plants fingerprinting. Biologia, 77, 517-52. DOI: https://doi.org/10.1007/s11756-021-00943-2

Žiarovská, J., Zeleňáková, L. (2018) Application of Genomic Data for PCR Screening of Bet v 1 Conserved Sequence. In: Vlachakis, D., ed. Clinically Relevant Plant Species, Systems Biology. IntechOpen.