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Investigation of genetic diversity and relationships of blue-berried honeysuckle (*Lonicera caerulea* L.) cultivars, genetic lines and populations at the Vilnius University Botanical Garden

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Abstract

Blue-berried honeysuckle (*Lonicera caerulea* L.) as a fruit crop is relatively rare in Lithuania and neighboring countries. Since 1975, the Vilnius University Botanical Garden has collected and investigated blue-berried honeysuckle cultivars. We studied cultivars, genetic lines, and representatives of some populations using molecular marker techniques (RAPD, ISSR, cpDNA). We evaluated the genetic variability and relationships of the studied accessions. Molecular studies indicate that *L. caerulea* retains high genetic diversity at the intraspecific level.

Key words: blue-berried honeysuckle, molecular markers, genetic diversity, relationships

Introduction

Honeysuckle (*Lonicera* L.) is a frequent element of forest undergrowth and represents a group of species. *Lonicera xylosteum* L. is the only spontaneous *Lonicera* L. species in Lithuanian forests. In neighbouring Latvian forests, there is one more species, blue-berried honeysuckle (*Lonicera caerulea* L.). This species is native to northern boreal forests in Asia, Europe, and North America and produces edible blueberries in some regions. Due to its fruit quality (berries contain high levels of phenols, flavonoids, and anthocyanins that account for their antibacterial, antioxidant, and anti-inflammatory properties) (Chaovanalikit et al., 2004; Kithma et al., 2020) and biological properties (early ripening, frost hardiness, and pest resistance), this species is becoming popular in horticulture in countries with a temperate climate. Now blue-berried honeysuckle is being intensively investigated to use them more widely for human needs. Blue-berried honeysuckle as a fruit crop is relatively rare in Lithuania and neighbouring countries. Since 1975, a of collection blue-berried honeysuckle has been accumulated and studied at the Vilnius University Botanical Garden. For more effective management of this genetic material and its use in germplasm enhancement, detailed molecular studies are necessary.

Collection of blue-berried honeysuckle

Vilnius University Botanical Garden (VUBG) was

founded in 1781, and since 1814 the blue-berried honeysuckle has been a dendrology object in the collection (Žilinskaitė, 2003). The breeding of blue-berried honeysuckle as a berry plant started in 1975, when the Department of Pomology was established. For 15 years, starting in 1994, researchers of the department participated in the Lithuanian plants' genetic resources programme. Morphological characterization of accessions (weight, size, and chemical composition of berries, shrub, and leaves characteristics), identification of plant disease agents, and plant resistance were carried out (Žilinskaitė et al., 2007). In 2007 work started on genetic variation and phylogenetic analysis of species, genetic lines and cultivars using molecular markers techniques (RAPD, ISSR, cpDNA). Later, it became possible to study representatives of some populations as well.

Now the collection of blue-berried honeysuckle contains 34 cultivars and 32 genetic lines. The number of species under discussion depends on the database referenced: one species and five subspecies (*Catalogue of Life*) or five species and four subspecies (*The Plant List*). A major portion of the cultivars was brought as green cuttings from Saint Petersburg's Plant Industry Institute (VIR), Russia. Plants of the genetic lines were previously grown from seeds of wild populations collected in the forests of the Altay region, Russia. They were selected for their superior fruit flavour, size, and productivity. They were propagated by green cuttings.

Investigation of cultivars and genetic lines

Fifty-one accessions, including 19 elite cultivars of Russian origin and 32 genetic lines, were investigated using Random Amplified Polymorphic DNA (RAPD) analysis (Naužemys et al., 2011). Using the TREECON software package, a dendrogram was created from the Unweighted Pair-Group Method of Arithmetic Averages (UPGMA) using the Nei and Li (1979) genetic distance matrix. Bootstrap values were obtained after 1000 iterations. The UPGMA analysis grouped all studied accessions in two main clusters (Fig. 1).

a source of additional diversity in breeding programs.

Investigation of blue-berried honeysuckle taxonomy

The taxonomy is quite complicated due to the phenotypic plasticity, ability to hybridize, and distribution across different ecological zones (Plekhanova and Rostova, 1994). We used RAPD markers and sequencing of seven non-coding chloroplast DNA (cpDNA) regions to assess the phylogenetic relationships among the taxa within the polymorphic complex *L. caerulea* (*altaica*, *edulis*, *emphylocalyx*, *kamtschatica*, *pallasii*, *stenantha*) and to determine the

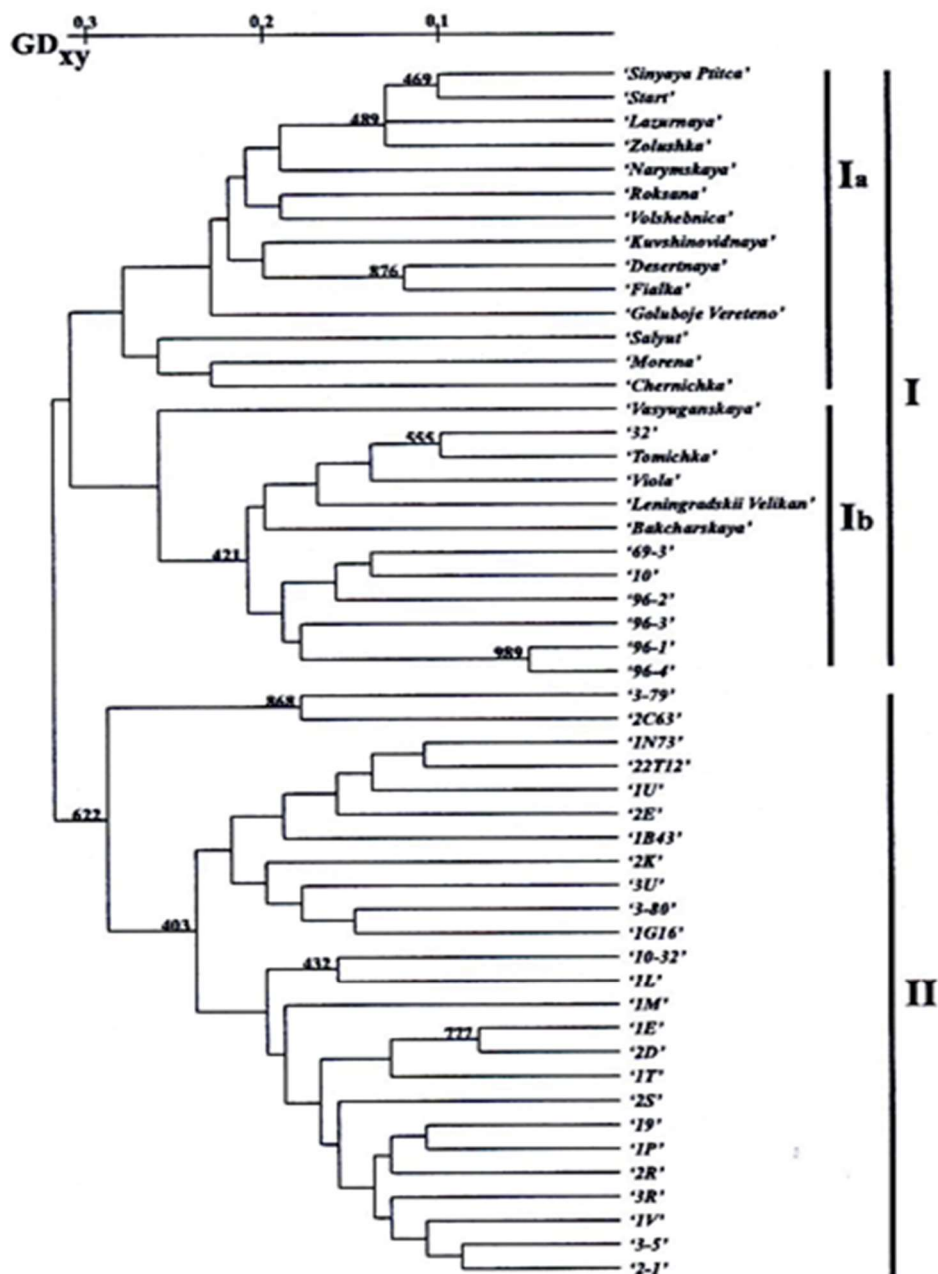


Figure 1. Dendrogram of *Lonicera caerulea* L. cultivars and genetic lines based on RAPD data.

The first cluster includes all cultivars and seven genetic lines and the second cluster includes all genetic lines. The genetic lines from the two main clusters are significantly different from the elite cultivars and can be

position of *L. boczkarnikowae* Plekh. and *L. venulosa* Maxim within this complex. *L. chrysantha* Turcz. ex Ledeb., *L. orientalis* Lam., and *L. xylosteum* L. were used as the outgroup species (Naužemys et al., 2014).

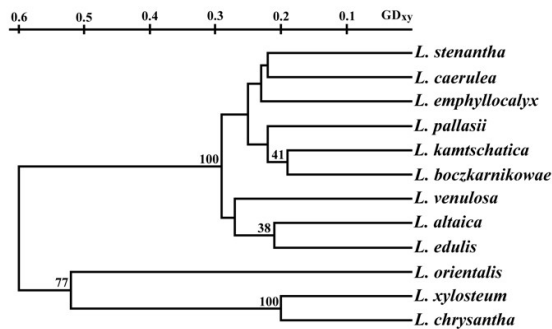


Figure 2. Genetic relationship among studied *Lonicera* L. taxa based on RAPD markers. The dendrogram was generated by UPGMA clustering method using the Nei and Li (1979) genetic distance matrix. The bootstraps (%) were calculated by 1000 iterations.

The RAPD and cpDNA analyses indicated that all the studied taxa form a single cluster consisting of two subclusters. The second cluster includes the outgroup species (Figs. 2, 3). According to the cpDNA analysis, *L. bozkarnikowae* and *L. venulosa* belong to the subcluster containing the taxa of the polymorphic complex *L. caerulea*. A separate subcluster within the cluster contains *L. altaica* and *L. edulis*. *L. venulosa*, *L. bozkarnikowae* should not be considered separate species but as intraspecific taxa.

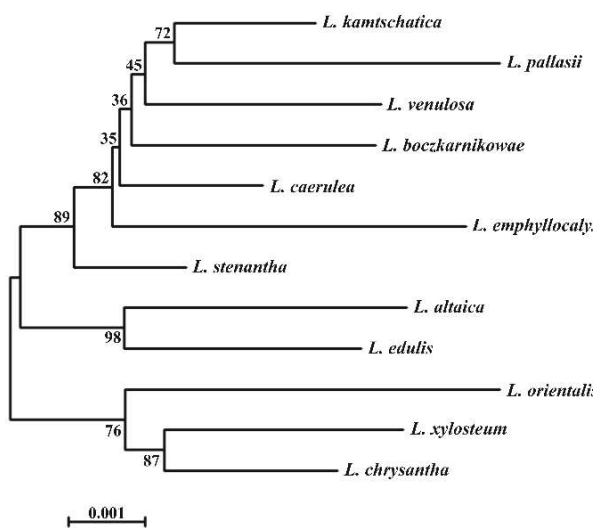


Figure 3. Genetic relationship among *Lonicera* L. taxa evaluated using sequencing data of seven non-coding chloroplast DNA regions. The dendrogram was generated by applying the ML method and the algorithm of the Tamura-Nei model (2011). The bootstrap values (1000 iterations) are above the dendrogram branches.

Investigation of genetic diversity of some populations

The blue-berried honeysuckle (*Lonicera caerulea* subsp. *pallasii* (Ledeb.) Browicz) populations closest to Lithuania are found in small areas of Latvia and Estonia. We collected samples in Latvia (Kemeri, Kandava, Mikeltonis, Ventpils) and Estonia (Kalli). Other samples we obtained from Japan and Russia. Dr.

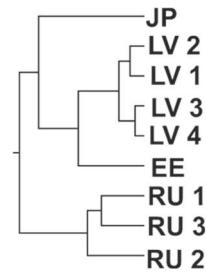


Figure 4. Genetic relationship of *Lonicera caerulea* L. populations from Latvia (LV), Estonia (EE), Russia (RU) and Japan (JP). The UPGMA dendrogram is based on the Nei genetic distances estimated using ISSR markers data using software Popgene 1.32.

Yoichiro Hoshino (Hokkaido University) sent 24 DNA samples of blue-berried honeysuckle (*Lonicera caerulea* L.) from Hokkaido Island. Dr. Nina Sedova (Kamchatka State Technical University) provided Kamtchatka populations (Russia)samples.

DNA polymorphism of populations was studied using ISSR assay. Analysis of molecular variance (AMOVA) revealed high differentiation between the studied populations. Genetic relationships among honeysuckle populations were evaluated using Popgene 1.32 computer program (Fig. 4).

A more detailed study of samples from Japan revealed high differentiation inside Hokkaido samples (Fig. 5). These samples were grouped into five clusters, and this clustering in general correlates with sample collection sites.

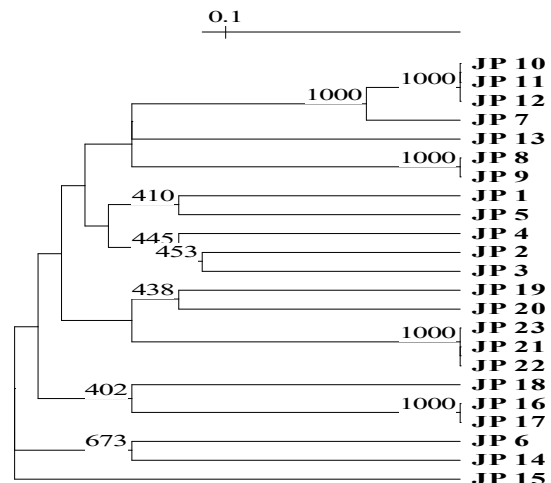


Figure 5. UPGMA dendrogram of *Lonicera caerulea* L. samples from Japan, Hokkaido generated from ISSR data based on the Nei and Li (1979) genetic distance matrix.

Conclusion

Our results confirm the high genetic variability of blue-berried honeysuckle at different levels (species, subspecies, populations, cultivars, and genetic lines), meaning that they can be used to improve the process of new cultivar breeding might conserve genetic resources.

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