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# Genetic diversity and chemical characterization of selected Polish and Russian cultivars and clones of blue honeysuckle (*Lonicera caerulea*)

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**Abstract:** In traditional medicine, *Lonicera caerulea* has a notable place, and it has been used for thousands of years in East Asian countries for treating cancer, inflammation, hepatic complications, influenza, and wounds. However, the molecular and chemical characterization of this plant is not well defined. In this study, inter-simple sequence repeat (ISSR) markers were used to determine the genetic variability of 7 blue honeysuckle cultivars and 3 clones. To characterize these 10 accessions, total flavonoids, phenolic acids contents, and antioxidant activities were estimated. Twelve primers generated 101 polymorphic ISSR-polymerase chain reaction bands. Based on the ISSR amplification profiles, a cluster dendrogram was obtained, which showed that the similarity coefficients among the 10 genotypes of *L. caerulea* ranged from 0.53 to 0.69. The study also identified promising genotypes in terms of flavonoid and phenolic acid contents and high antioxidant activities, as follows: cultivar Atut and clone P. A systematic cluster analysis conducted on the basis of chemical data showed that the 10 tested genotypes can be classified into 2 groups. The ISSR analysis together with data for antioxidant activities as well as flavonoid and phenolic acid contents in honeysuckle could be used to evaluate the genetic diversity and to make more efficient choices of parents in current blue honeysuckle breeding programs.

**Key words:** DPPH (diphenyl picrylhydrazyl), flavonoids, genetic diversity, ISSR markers, *Lonicera caerulea*, phenolic acids

## 1. Introduction

*Lonicera caerulea* var. *kamtschatica* (honeysuckle) belongs to the family Caprifoliaceae, which comprises more than 200 species (Hummer, 2006; Svarcova et al., 2007; Miyashita et al., 2009; Sun et al., 2011).

Positive properties of haskap berries, belonging to *L. caerulea*, have been known for centuries. From ancient times in Japan, honeysuckle has been used to treat urinary disorders, fever, and headache, and in Korea it was used widely for upper respiratory tract infections, diabetes mellitus, and rheumatoid arthritis (Skupień et al., 2007). The fruits are rich in ascorbic acid, macro- and microelements, polyphenolic compounds (especially phenolic acids), anthocyanins, proanthocyanidins, flavonoids, and other bioactive substances. The major anthocyanins are glucosides and rutinosides (Frejnagel, 2007; Rop et al., 2011; Jurikova et al., 2012). The studies presented by Frejnagel (2007), Małodobry et al. (2010), and Ochmian et al. (2012) indicated that haskap berries exhibit antifungal and anticancer properties, antiadherence, and chemoprotective effects. Compounds contained in the fruits provide protection from cancer, tumor growth, diabetes mellitus, and cardiovascular diseases, reducing blood pressure, decreasing the risk of heart attack, preventing osteoporosis and anemia, and slowing the aging process. According to Hoppula and Karhu (2006), the biochemical composition of the fruit is strongly affected by the genotype, harvest date, and environmental conditions. Total antioxidant activity, as well as phenolic acid and flavonoid content, can vary among cultivars. There is a pressing need to develop reliable methods for identifying blue honeysuckle cultivars/clones and for assessing genetic diversity in *Lonicera* genotypes for practical breeding purposes. To start a breeding program it is essential to characterize the available germplasm, either morphologically or molecularly. Germplasm characterization by DNA-based molecular markers allows a better understanding of genetic variability. This is due to the determination of the level of genetic divergence and the molecular pattern of each cultivar. With these data, the breeder can plan crosses using molecular and field data, which can result in the development of superior cultivars more quickly and economically (Langridge and Chalmers, 2004; Morales et al., 2011). Molecular

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markers are increasingly used in breeding programs of many horticultural crops. Random polymorphic DNA analysis was used to assess the genetic variation of 51 accessions of blue honeysuckle, including 19 elite cultivars and 32 genetic lines derived from seeds collected in wild populations (Naugžemys et al., 2011).

Inter-simple sequence repeat (ISSR) markers represent a successful tool for the assessment of genetic diversity in blue honeysuckle because they are known to be highly informative and can be used to detect polymorphism in genotypes with both wide and narrow genetic backgrounds (Smolik et al., 2006; Wang et al., 2008; Sestili et al., 2011; Chen et al., 2012). Smolik at al. (2010) estimated genetic similarity among 14 Polish and Russian blue honeysuckle accessions using ISSR markers.

Although the scientific literature does include some reports of ISSR analysis for blue honeysuckle (Smolik et al., 2006; Wang et al., 2008; Smolik et al., 2010; Chen et al., 2012), antioxidant activity and phenolic acid and flavonoid contents in relation to molecular analysis have not been documented in a given set of blue honeysuckle. The purpose of this study was to examine the genetic variation at DNA level among 7 blue honeysuckle cultivars and 3 clones and their chemical markers for antioxidant activity and phenolic acid and flavonoid content. The genotypes were chosen due to their good fruit quality and suitability to Polish climatic conditions. Genetic diversity was assessed with the use of ISSR-polymerase chain reaction (PCR) fingerprints and compared with the chemical markers.

## 2. Materials and methods

### 2.1. Plant material

The plantation of 7 blue honeysuckle (*Lonicera caerulea* var. *kamtschatica*) cultivars derived from Russia (Czelabinka, Dlinnopłodna, and Wołoszebnica) and from Poland (Duet, Atut, Warszawa, and Wojtek) and 3 clones (Nr 9, P, and T2) was established in the spring of 2007 at the Felin Experimental Station, eastern Poland (51°13′N, 22°39′E). These genotypes were derived from the experimental station of the Research Institute of Horticulture in Brzezna. Breeding clones Nr 9, P, and T2 originated from the breeding program of this species, also carried out at this station. Field trials were established on gray-brown podzolic soil deposited on chalky clay soil. Bushes were planted at the spacing of 1.5 × 2 m in 3 replicates on 5 shrubs in a random subblock system. During the experiment, no chemical protection of plants was used. Irrigation and artificial fertilization were applied when necessary.

### 2.2. DNA extraction

DNA was isolated from 10 plants (fresh young leaves collected separately from each cultivar) in 2 replicates and kept in liquid nitrogen until extraction. DNA was extracted following the CTAB method described by Doyle and Doyle (1987).

#### 2.2.1. ISSR analysis

The DNA amplifications were performed in a gradient thermal cycler (TProfessional Basic Gradient Biometra GmbH) at a final volume of 15 µL for each reaction, which contained 20 mM Tris-HCl (pH 8.3), 100 mM KCl, 0.1% Triton X-100, 130 µM of each dNTP, 470 pM oligonucleotide primer, 1.5 mM $MgCl_2$, 1 U of Taq DNA polymerase (Fermentas), and 60 ng of template DNA. In the PCR reactions, the samples were initially subjected to 94 °C for 7 min and then 35 cycles of amplification. Each cycle involved the following steps: 94 °C for 30 s, 30 s at the primer annealing temperature, and 2 min at 72 °C of amplification. After the 35 cycles, the samples were kept at 72 °C for 7 min for a final extension step. The annealing temperature was adjusted according to the $T_m$ of the primers used in the reaction. In order to check reproducibility, the primers used in this experiment were tested 2 times on the same sample. Amplification products were separated by electrophoresis on 1.5% agarose gels containing 0.1% ethidium bromide under 1X TBE buffer and run at 100 mV for at least 3 h. Fragments were visualized under a UV transilluminator and photographed using GeneSnap ver. 7.09 (SynGene). The GeneRuler DNA Ladder Mix (MBI Fermentas) was used to determine the size of the DNA fragments.

#### 2.2.2. DNA data analysis

The ability of the 12 most informative primers to distinguish accessions at DNA level was assessed by calculating their resolving power (Rp) (Prevost and Wilkinson, 1998). This function is given by the following formula: $Rp = \sum I_b$, where band informativeness is $I_b = 1 - (2 \times |0.5 - p|)$ and p is the proportion of accessions containing band I.

ISSR products were scored as present (1) or absent (0) from the photographs. Only bright and reproducible products were scored. DNA fragments not detected in all cultivar and clone profiles were considered as polymorphic. Genetic pairwise similarities (similarity index) between studied genotypes were evaluated according to Dice's formula after Nei and Li (1979). A cluster analysis was conducted using the unweighted pair-group method with arithmetic mean (UPGMA) in the program NTSYS (Rohlf, 2001).

### 2.3. Chemical data analysis

The fruits were collected (2 kg of bulk sample) on 28 June 2013. The contents of flavonoids (mg 100 $g^{-1}$) and phenolic acids (mg 100 $g^{-1}$) in fresh samples were determined in the Laboratory for Vegetable and Herbal Material Quality. The ethanol extracts of the tested materials were subject to determination of the binding strength of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals.

**2.3.1. Total flavonoids estimation**

Total flavonoids were estimated according to the spectrophotometric method of Christ and Müller (Polish Pharmaceutical Society, 2011) after their extraction. For this purpose, 2.0 g of crushed berries was added to a round-bottomed flask; 20 mL of acetone, 2 mL of HCl (281 g $L^{-1}$), and 1 mL of methenamine (5 g $L^{-1}$) were then added and the mixture was maintained for 30 min under reflux on a water bath. The hydrolysate was filtered through cotton wool into a volumetric flask of 100 mL, then placed in a flask together with the cotton pellet and 20 mL of acetone and refluxed for 10 min. Next, 20 mL of solution was dispensed into a separatory funnel with 20 mL of water and extracted with ethyl acetate in 15-mL portions 3 times with 10 mL. The combined organic layers were washed twice with 40 mL of water, filtered into a volumetric flask of 50 mL, and supplemented with ethyl acetate. To determine flavonoid content, 2 samples were prepared: to 10 mL of the stock solution was added 2 mL of a solution of aluminum chloride (20 g $L^{-1}$), supplemented with a mixture (1:19) of acetic acid (1.02 kg L–$^{1}$) and methanol (25 mL). To prepare the comparative solution, stock was supplemented with 10 mL of a mixture (1:19) of acetic acid (1.02 kg $L^{-1}$) and methanol

(25 mL). After 45 min, the absorbance of the solutions was read at $\lambda$ = 425 nm using the reference solution for comparison. Samples were analyzed in 3 replicates. The total content of flavonoids (mg 100 $g^{-1}$) was expressed as quercetin equivalent according to the following formula:

$$X = \frac{A \times k}{m}$$

where X is total flavonoids (mg 100 $g^{-1}$), A is the absorbance of the solution being studied, k is the convection factor for quercetin and equal to 8.750, and m is the sample with the raw material (g).

**2.3.2. Total phenolic acids estimation**

The content of total phenolic acids in the fresh berries was determined using the spectrophotometric method with Arnov reagent according to the procedure described by the Polish Pharmacopoeia VII (Polish Pharmaceutical Society, 2005). To 5.0 g of homogenized raw material placed in a round-bottomed flask was added 20 mL of methanol and the mixture was heated for 30 min at 70 °C in a water bath at reflux. The hydrolysate was filtered through a hard filter paper into an Erlenmeyer flask of 100 mL. The filtered medium was returned to the round-bottomed flask with 20 mL of methanol and heated at reflux for 30 min. This process was repeated 3 times. The combined filtrates were taken to the tube with 1 mL of blueberry extract, 1 mL of 0.5 N hydrochloric acid, 1 mL of Arnov reagent, and 1 mL of 1 N sodium hydroxide, made up to 10 mL with distilled water. The absorbance was measured at $\lambda$ = 490 nm. The total phenolic acid content, expressed as acid equivalent weight of fresh caffeic acid in the fruit, was calculated from the equation obtained from the calibration curve of caffeic acid ($y = 1.7319x + 0.0225$; $R^2 = 0.9994$). Samples were analyzed in triplicate.

**2.3.3. Antioxidation activity**

Antioxidation activity (%) was evaluated on the basis of the ability to neutralize DPPH radicals by means of spectrophotometry according to Chen and Ho (1997). To do this, water extracts were prepared from berries of the 7 cultivars and 3 clones, and then extracts were evaporated until dry and lyophilized. Analyses were performed for a concentration of 20 μg $mL^{-1}$. Samples were analyzed in 3 replicates. The absorbance measurements were made at wavelength $\lambda$ = 517 nm after 30 min using a Hitachi U-2900 spectrophotometer (Canberra Packard).

The chemicals used in this study were purchased from Sigma-Aldrich Chemical Co. (France) and/or Merck Company (Germany). A Hitachi U-2900 spectrophotometer was used for absorbance measurements.

**2.4. Statistical analysis**

Results achieved from the laboratory experiments were statistically processed by means of a variance analysis method and Tukey's test was evaluated at $P \leq 0.05$. A dendrogram was created using the average linkage method available in SAS Enterprise Guide 3.0.

## 3. Results

**3.1. ISSR amplification**

Of the 40 primers tested for their capacity to differentiate among 10 blue honeysuckle cultivars and clones, the best 12 primers showed a polymorphism between accessions and gave reproducible banding patterns. Among these 12 primers tested, 5 were designed to anneal to dinucleotide repeats, 4- to 3-nucleotide repeats, and 3- to 4-nucleotide repeats. Table 1 shows characteristics of banding patterns obtained with selected primers. These 12 primers amplified 113 loci and 101 of them were polymorphic (89.7% polymorphism). The total number of ISSR bands scored per primer in the presented work varied from 6 (primers ISSR 1 and ISSR 11) to 13 (primer ISSR 2). An average of 9.4 bands was obtained per primer and their sizes ranged from 3720 to 380 bp (data not shown).

The ability of the 12 most informative primers to diagnose blue honeysuckle accessions was assessed on the basis of Rp. The Rp values varied from 2.0 for primer ISSR 1 to 6.6 for primer ISSR 3. The collective Rp value for all 12 primers was 54.2 (Table 1). Three of the ISSR primers (3, 6, and 12) possessed the highest Rp values (from 6.6 to 5.6) and were able to distinguish all 10 genotypes. The remaining primers were unable to fully separate all cultivars and clones (data not shown).

**Table 1.** Characteristics of banding patterns obtained with 12 selected oligodeoxynucleotide primers used for ISSR analysis of *Lonicera caerulea*.

| Primer code | ISSR primer sequence[a] | Polymorphic bands | | | Resolving power, Rp |
|---|---|---|---|---|---|
| | | Number | Size range (bp) | %P[b] | |
| ISSR 1 | VBVACACACACACACAC | 4 | 570–1640 | 66.7 | 2.0 |
| ISSR 2 | BDBCACACACACACACA | 11 | 400–3500 | 84.6 | 5.2 |
| ISSR 3 | HBHCTCTCTCTCTCTCT | 11 | 490–3660 | 91.7 | 6.6 |
| ISSR 4 | GCVTCTCTCTCTCTCTC | 9 | 480–2880 | 100 | 4.8 |
| ISSR 5 | VCGTCTCTCTCTCTCTC | 10 | 410–3720 | 100 | 3.8 |
| ISSR 6 | HVHTGTTGTTGTTGTTGT | 10 | 380–2560 | 90.9 | 5.6 |
| ISSR 7 | BDBCACCACCACCACCAC | 9 | 500–3620 | 81.8 | 4.8 |
| ISSR 8 | BDVCAGCAGCAGCAGCAG | 7 | 660–2340 | 87.5 | 3.6 |
| ISSR 9 | ATGATGATGATGATGATG | 8 | 480–3520 | 72.7 | 5.4 |
| ISSR 10 | GATAGATAGATAGATAGATA | 7 | 510–3070 | 100 | 4.0 |
| ISSR 11 | GACAGACAGACAGACAGACA | 6 | 790–3080 | 100 | 2.8 |
| ISSR 12 | AGTGAGTGAGTGAGTG | 9 | 570–2750 | 100 | 5.6 |
| Mean | | 8,4 | - | 89.7 | 4.5 |
| Total | | 101 | 380–3720 | - | 54.2 |

[a]: Key to symbols: V = G + A + C, B = G + T + C, H = A + T + C, D = G + A + T; [b]: Percentage of polymorphism.

Examples of typical ISSR banding patterns produced by primers ISSR 6, ISSR 7, and ISSR 8 are shown in Figure 1.

The genetic similarity matrix was produced on the basis of ISSR markers using Dice's coefficient (Table 2). ISSR-based mean genetic similarity was calculated as 0.56. The highest degree of similarity occurred between the cultivars Czelabinka and Dlinnopłodna (0.69) from Russia, while the lowest (0.53) was found from 24 out of 45 possible comparisons.

In the present study, the genetic similarity matrix was applied for cluster analysis through the UPGMA method (Figure 2). The generated dendrogram of 10 accessions showed exactly 2 clusters. The larger group includes accessions Dlinnopłodna, Czelabinka, Wojtek, and Atut and clones Nr 9 and P. The phylogenetic similarity within the group ranged from 0.56 to 0.69 (between Russian cultivars Dlinnopłodna and Czelabinka). Cultivars Warszawa, Wołoszebnica, and Duet and clone T2 were classified in the second group with about 0.58 similarity.

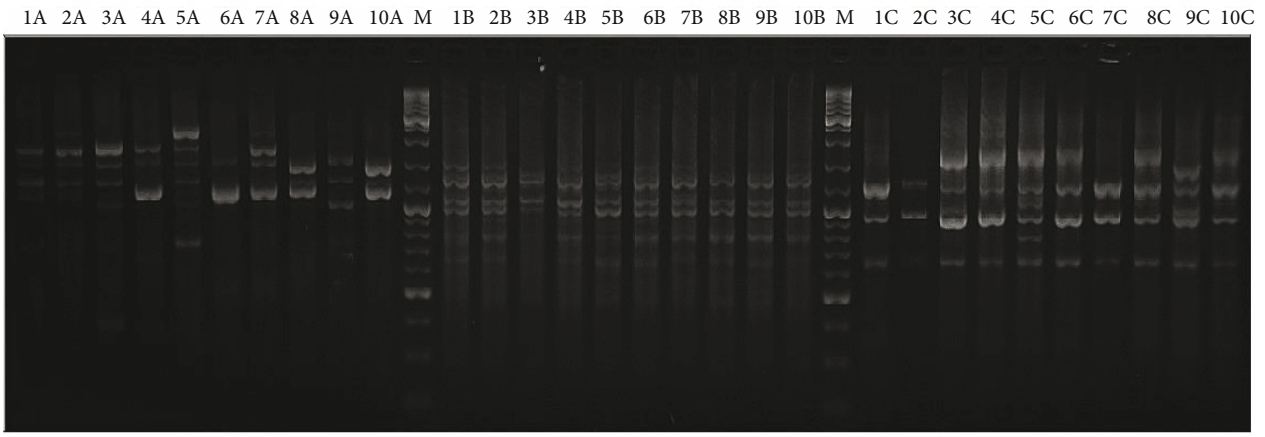


**Figure 1**. ISSR fingerprints of 10 honeysuckle genotypes using primers ISSR 6 (Arabic number + A), ISSR 7 (Arabic number + B), and ISSR 8 (Arabic number + C). M: Standard of DNA fragment size GeneRuler DNA Ladder Mix (100–10,000 bp). The numerals at the top of the picture indicate the code number of the individuals: 1-Warszawa, 2- clone T2, 3- Dlinnopłodna, 4- Wołoszebnica, 5- Czelabinka, 6- Wojtek, 7- Duet, 8- clone Nr 9, 9- Atut, 10- clone P.

**Table 2.** Matrix of genetic similarity among 10 *Lonicera caerulea* cultivars and clones based on ISSR markers calculated by Dice's coefficient.

| Cultivar/clone | 1▲ | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.00 | | | | | | | | | |
| 2 | 0.64 | 1.00 | | | | | | | | |
| 3 | 0.53 | 0.53 | 1.00 | | | | | | | |
| 4 | 0.58 | 0.58 | 0.53 | 1.00 | | | | | | |
| 5 | 0.53 | 0.53 | 0.69 | 0.53 | 1.00 | | | | | |
| 6 | 0.53 | 0.53 | 0.64 | 0.53 | 0.64 | 1.00 | | | | |
| 7 | 0.58 | 0.58 | 0.53 | 0.60 | 0.53 | 0.53 | 1.00 | | | |
| 8 | 0.53 | 0.53 | 0.56 | 0.53 | 0.56 | 0.56 | 0.53 | 1.00 | | |
| 9 | 0.53 | 0.53 | 0.64 | 0.53 | 0.64 | 0.67 | 0.53 | 0.56 | 1.00 | |
| 10 | 0.53 | 0.53 | 0.56 | 0.53 | 0.56 | 0.56 | 0.53 | 0.65 | 0.56 | 1.00 |

▲**:** The numerals indicate the code number of the individuals. 1- Warszawa, 2- clone T2, 3- Dlinnopłodna, 4- Wołoszebnica, 5- Czelabinka, 6- Wojtek, 7- Duet, 8- clone Nr 9, 9- Atut, 10- clone P.

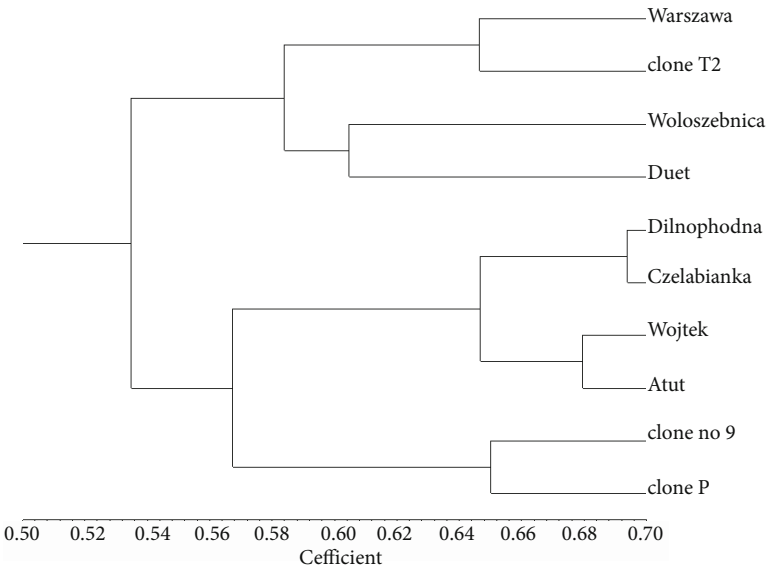


**Figure 2**. Dendrogram of genetic similarity among 10 blue honeysuckle cultivars and clones obtained from ISSR markers using the UPGMA method.

### 3.2. Flavonoid and phenolic acids content and antioxidant activity

The tested set of genotypes was characterized by significantly different levels of flavonoids, phenolic acids, and DPPH (Table 3). The content of flavonoids ranged from 30.5 to 34.4 mg 100 $g^{-1}$. The highest content was observed in cultivars Warszawa, Wojtek, and Czelabinka and the lowest in clone T2, cultivar Duet, and clone P. Mean concentration of phenolic acids ranged from 12.2 mg 100 $g^{-1}$ in cultivar Warszawa to 28.1 mg 100 $g^{-1}$ in Atut. High levels of antioxidant activity were seen in cultivars of Russian (Wołoszebnica, Czelabinka, and Dlinnopłodna) and Polish (Atut and Duet) origin. In general, this trait ranged from 20.9% to 42.4%. The relationships among the 10 genotypes revealed by average linkage method based on flavonoid and phenolic acid content and antioxidant activity data are presented in Figure 3. Two major groups of clusters were distinguished. The first includes 2 breeding clones and cultivar Wojtek, while the second includes 6 other cultivars and clone P.

**Table 3.** Content of flavonoids (as quercetin, mg 100 $g^{-1}$) and phenolic acids (as caffeic acid, mg 100 $g^{-1}$) and antioxidation activity (DPPH, %) in berries of different cultivars and clones of blue honeysuckle. All values are presented as mean ± standard deviation (n = 3).

| Cultivar/clone | Flavonoids | Phenolic acids | DPPH |
|---|---|---|---|
| Warszawa | 34.4 ± 0.35 a[1] | 12.2 ± 0.56 g | 37.7 ± 0.57 c |
| Clone T2 | 30.5 ± 0.34 f | 12.3 ± 0.35 g | 20.9 ± 0.85 g |
| Dlinnopłodna | 33.4 ± 0.17 abc | 20.1 ± 0.36 d | 38.8 ± 0.71 bc |
| Wołoszebnica | 31.9 ± 0.38 de | 15.6 ± 0.27 f | 42.4 ± 0.93 a |
| Czelabinka | 34.0 ± 0.25 ab | 18.8 ± 0.70 de | 39.0 ± 0.65 bc |
| Wojtek | 34.1 ± 0.39 ab | 24.5 ± 0.36 b | 29.4 ± 1.14 e |
| Duet | 31.0 ± 0.36 ef | 18.0 ± 0.36 e | 38.5 ± 1.13 bc |
| Clone Nr 9 | 33.1 ± 0.41 bc | 22.8 ± 0.70 c | 24.6 ± 0.92 f |
| Atut | 32.8 ± 0.39 cd | 28.1 ± 0.63 a | 40.6 ± 0.86 ab |
| Clone P | 31.4 ± 0.41 ef | 22.5 ± 0.53 c | 34.6 ± 1.53 d |
| Mean | 32.6 | 19.5 | 34.7 |

[1]: Means in columns marked with the same letters do not differ significantly at P ≤ 0.05.

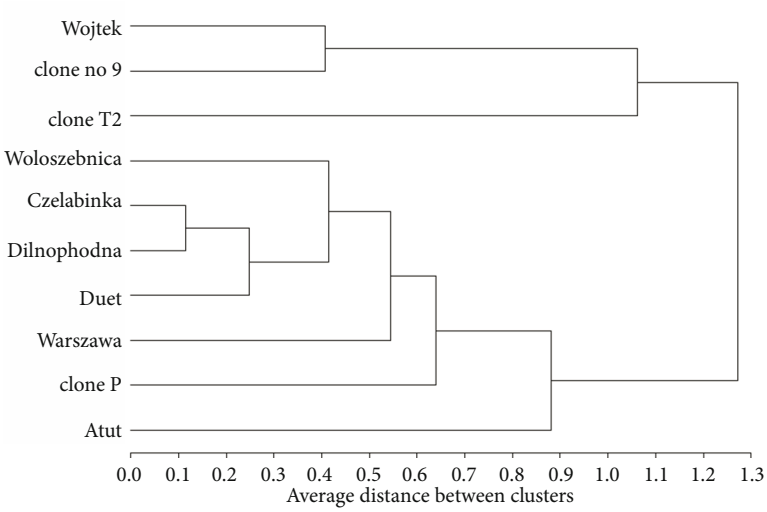


**Figure 3**. Dendrogram estimating genetic distance among 10 blue honeysuckle genotypes based on flavonoid and phenolic acid contents and DPPH.

## 4. Discussion

The practical advantage of a molecular approach for germplasm control is partly determined by the ability to differentiate between large numbers of genotypes. The ISSR primers used in this study varied in their ability to diagnose *Lonicera caerulea* accessions, and primers with higher Rp values were generally able to distinguish more genotypes (data not shown) and showed higher polymorphic bands. This is in agreement with the findings in potato cultivars (Prevost and Wilkinson, 1999), lupin (Gilbert et al., 1999), lingonberry (Debnath, 2007), and strawberry (Debnath and Ricard, 2009). The results clearly indicate that ISSR markers can be used in genetic diversity studies as well as in genotypic identification of blue honeysuckle, as noted by Smolik et al. (2006, 2010), Wang et al. (2008), Smolik et al., and Chen et al. (2012), and in helping breeders to

select initial materials for breeding programs. The study of genetic divergence by similarity coefficients or dendrograms helps to identify parents suitable for obtaining hybrids with higher heterosis effects and with wider ranges of recombination resulting from generative reproduction so that superior individuals can be selected. Twelve primers used in our study generated 101 polymorphic ISSR-PCR bands. Smolik et al. (2006) characterized genetic variation and relationships among forms of the honeysuckle accessions representing 4 populations of this species occurring in northern Poland and 2 varieties using the ISSR method. The analysis involved 30 ISSR primers on various di-, tri-, tetra-, and penta-SSR motifs containing additional selective 3' anchor nucleotides. Out of the 30 primers, 21 were chosen for final study. These amplified a total of 984 bands, out of which 900 (91.4%) were polymorphic. Blue honeysuckle accessions were also genotyped by Lamoureux et al. (2011) with 5 ISSR markers, generating more than 1100 polymorphic fragments across the 194 accessions. Statistical analysis of these data showed that the subspecies level was key in explaining blue honeysuckle diversity. In the present study, the average number of total bands generated per primer (9.4) depended on the primer's sequence. In the study conducted by Chen et al. (2012), 20 ISSR primers amplified 186 bands with 103 (54.63%) polymorphic bands and 58 sequence-related amplified polymorphism (SRAP) primer combinations amplified 591 bands with 347 (55.46%) polymorphic bands. Both ISSR and SRAP analyses revealed a middle level of genetic diversity in *Lonicera macranthoides* cultivars. The dendrograms based on SRAP and ISSR markers were not all the same. The results of the present study showed a significant level of genetic relatedness among blue honeysuckle cultivars and clones. The investigated blue honeysuckle genotypes showed 53% to 69% genetic similarity. Smolik et al. (2006) found a level of similarity for 6 populations of *Lonicera periclymenum* ranging from 82.3% to 86.6%, indicating their closely related nature. ISSR amplification was used by Smolik et al. (2010) to analyze polymorphisms of microsatellite sequences in the honeysuckle genome and to evaluate genetic diversity among 14 Polish and Russian blue honeysuckle accessions. The applied 11 primers gave interpretable banding patterns in all tested genotypes. Identified level of similarity ranged from 0.44 to 0.95. Random amplified polymorphic DNA (RAPD) analysis was used by Naugžemys et al. (2011) to assess the genetic relationships among 51 accessions of blue honeysuckle. The pairwise genetic distance ($GD_{xy}$) values among studied accessions ranged from 0.054 to 0.479; the mean $GD_{xy}$ was 0.283. The UPGMA dendrogram based on $GD_{xy}$ estimated genetic relationships derived from RAPD analysis among the studied accessions. The UPGMA analysis in this study grouped all accessions into 2 main clusters. Of the 2 presented dendrograms of genetic distance based on ISSR markers and chemical composition, each demonstrated division of the tested blue honeysuckle genotypes into 2 groups (Figures 1 and 2). In both, Russian cultivars Dlinnopłodna and Czelabinka were the most closely grouped. However, clustering based on ISSR data was different from that based on chemical data. Debnath and Ricard (2009), investigating 10 strawberry cultivars and 9 breeding lines, reported that cluster analysis based on anthocyanin content and antioxidant activity data was different to that obtained on the basis of molecular markers. Garcia et al. (2002) also did not find direct correlation between morphological and RAPD characterization in strawberry. Debnath and Ricard (2009) reported that ISSR markers were distributed throughout the genome and in the majority of cases most regions of the genome were not expressed at the phenotypic level. In recent years, cultivated berries have become very attractive for consumers because of potentially beneficial phytochemicals contained in these fruits. Higher contents of flavonoids, phenolic acids, and anthocyanins in fruits contribute to their higher antioxidant activity. Due to these compounds, the fruits have a positive impact on the body's functions, but their content in berry fruits varies among species and cultivars. It can also be affected by growth conditions including environmental factors and cultivation techniques. Knowledge of the content of secondary metabolites in individual genotypes allows us to choose the best in *Lonicera* breeding programs in order to increase the nutritional value and health benefits. In the literature, studies give very different values for the content of the same chemical compounds in the fruit of blue honeysuckle. Zadernowski et al. (2005) determined the content of phenolic acid in the fruit of blue honeysuckle at 542 mg per 100 g dry matter. Ochmian et al. (2012) determined it in the range of 126–184 mg per 100 g while studying 2 cultivars. Strelcina et al. (2006) evaluated the total content of bioflavonoids in the fruits of 51 blue honeysuckle genotypes in the range of 782–1890 mg per 100 g. The content of phenolic acids (expressed as caffeic acid equivalents) in genotypes in this study ranged from 12 to 28 mg per 100 g and was slightly lower in relation to the quantities obtained by Zadernowski et al. (2005); however, the authors did not indicate the specific varieties for which the value was estimated. The contents of flavonoids, expressed as quercetin equivalent in the fruits of genotypes analyzed in this experiment, ranged from 30.5 to 34.4 mg per 100 g and was higher in comparison to those observed in genotype Czarna and seedling N (Skupień et al., 2009) and varieties of Czarna and Wojtek (Ochmian et al., 2012). Significant differences in ability to neutralize the free DPPH radical by extracts made of fruits from examined cultivars and clones of blue honeysuckle were recorded. In the presented study, the mean value of DPPH for tested genotypes was 34.7%.

According to Skupień et al. (2007), neutralizing capacity was 51.7 for cultivar Zielona. Free radical levels were slightly lower in our analysis but comparable with antioxidant activity of such species as blueberries, blackberries, and black currants (Chaovanalikit et al., 2004) and about 2 times higher than in *Fragaria vesca* (Najda et al., 2014). The results obtained in our research are useful for future breeding programs for *Lonicera* spp. aimed at the selection of genotypes with higher nutraceutical fruit values. The most promising among all analyzed cultivars and clones to achieve this purpose seem to be Atut and Wojtek, which were characterized by the high content of flavonoids and phenolic acids as well as a high level of antioxidant activity.

In conclusion, the presented study indicated a high level of genetic relatedness between blue honeysuckle cultivars and clones. However, similarity at the DNA level was different from that obtained on the basis of the chemical data. This may be due to the fact that the noncoding regions of genome are not accessible for phenotypic expression and might have resulted in disagreement between the chemical and molecular diversity. The low correspondence between genetic similarity from chemical and ISSR data most likely suggest that these markers differ in their degree of genomic coverage.

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