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ASSESSMENT OF GENETIC DIVERSITY AND RELATIONSHIPS AMONG GRAPEVINE CULTIVARS ORIGINATING IN CENTRAL AND EASTERN EUROPE AND NORTH AMERICA USING ISSR MARKERS

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ABSTRACT

The study shows genetic diversity of 38 *Vitis vinifera* L. cultivars and hybrids originating in North America and Europe, including cultivars selected in Poland, which have not been characterized with the use of DNA markers yet. The agrobiological features of the genotypes selected for testing indicate that they may be useful for the breeding of new cultivars and grape production. The use of 12 ISSR primers allowed to obtain 94.4% of polymorphism. The polymorphic information content (PIC) value was high and varied between 0.829 and 0.953 with an average of 0.897. The resolving power (Rp) ranged between 3.678 and 8.892 with an average of 6.347. Primers UBC 809, UBC 810, UBC 812, UBC 855, UBC 891 and UBC 810 were found to be highly effective (informative). Similarity coefficient ranged between 0.167 and 1.0, which indicates high degree of diversity of tested grape cultivars. Tested cultivars were grouped in 3 main clusters; one of them was further divided into 6 subclusters. 'Pannonia Kincse' and 'Danmarpa Polonia' were not differentiated. Phenotypic differences among those two cultivars suggest that 'Danmarpa Polonia' might be a clone of 'Pannonia Kincse' and other molecular techniques must be used to differentiate them. Morphological and agrobiological characters of cultivars support the results obtained by ISSR markers.

Key words: *Vitis* spp., interspecific crossings, germplasm resources, genetic distance

INTRODUCTION

More than 3.2 million tons of fruits are produced every year in Poland, most of all apples, cherries, pears and soft fruits such as currant, strawberries and raspberries [Lemanowicz and Krukowski 2009]. Compared to such large production of those fruits, grapevine in Poland is cultivated on a relatively small scale, mainly due to cool climate [Lisek 2008]. Nevertheless, wine-growing has recently become more popular in cooler regions of Europe, including Poland where cultivation area exceeds 700 ha and new wine-producing regions have appeared [EUROSTAT 2012, Gąstoł 2015]. On 20 December 2005 the Council of the European Union (EU) decided to classify Poland in wine-growing region A (the coldest), similar to Denmark and some regions of Germany. This means that wine produced in Poland can be sold in the EU market (Council Regulation (EC) No. 2165/ 2005) [Tarko et al. 2010]. The gradual climate warming favours wine-growing in Poland [Lisek 2008]. Also, new cultivars are being constantly introduced, mainly interspecific and inter-intraspecific hybrids first grown in countries with a cold climate such as the USA or

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Russia. Introduction of cultivars suitable for growing in cool climate also contributes to the increase in cultivation area in Poland [Lisek 2008, 2010]. 'Solaris', 'Regent', 'Rondo', 'Riesling' and 'Pinot Noir' are the most commonly grown cultivars in Poland.

The Research Institute of Horticulture maintains a collection of grapevine cultivars which presently consists of 321 taxa including several cultivars selected in Poland. The following aspects are assessed in the research: morphological traits of bushes (shape of leaves, berries and bunches; colour of fruit skin), quality of fruits, the course of phenological phases and agrobiological traits including plant growth, resistance to frost, diseases and pests [Lisek 2008, 2010]. Collected genotypes may serve as starting plant material for obtaining new valuable cultivars. The genotypes are classified as common grapevine (*Vitis vinifera* L.) cultivars (original cultivars and intraspecific hybrids), interspecific and inter-intraspecific hybrids. Correct identification of collected cultivars, clones and forms on the basis of phenotypic traits might be difficult as the same taxa are referred to by different names (synonyms) or different taxa are referred to by the same name (homonyms). For this reason, DNA markers are used to differentiate and identify cultivars, which allows for a quick and precise identification of collected taxa, independent of environmental conditions or the stage of plant growth. Furthermore, the obtained DNA profiles of particular cultivars allow to determine their genetic similarity. This expands the knowledge on the collected cultivars which, in turn, makes it possible to characterize them even more precisely. The following techniques were used for DNA profiling and assessment of genetic relationships among grapevine cultivars: RAPD [Benjak et al. 2005, Karataş and Ağaoğlu 2010], SSR [Benjak et al. 2005, Martínez et al. 2006, Upadhyay et al. 2007, Jahnke et al. 2009, Cipriani et al. 2010, Dong et al. 2018, Labagnara et al. 2018], AFLP [Upadhyay et al. 2007, Theocharis et al. 2010, Alba et al. 2011], SRAP [Guo et al. 2012], EST-SSR [Huang et al. 2011], SAMPL, M-AFLP [Meneghetti et al. 2012a, Meneghetti et al. 2012b], as well as retrotranspon-based markers such as IRAP, REMAP and SSAP [D'Onofrio et al. 2010]. Additionally, ISSR technique based on the analysis of repeated genome sequences is used in the assessment of genetic relationships. ISSR markers are common in plant genomes and are polymorphic, which makes them a useful tool in assessing genetic diversity of cultivars [Zietkiewicz et al. 1994]. Repeatability and low cost of analysis are other advantages of ISSR markers [Argade et al. 2009]. They were therefore repeatedly used in the assessment of relationships among grapevine varieties [Dhanorkar et al. 2005, Sabir et al. 2008, 2009, Jing and Wang 2013, Argade et al. 2009, Seyedimoradi et al. 2012, Zeinali et al. 2012, Choudhary et al. 2014, Salayeva et al. 2016].

Genetic relationships were determined in multiple groups of grapevine cultivars originating in South America [Martinez et al. 2006, Herrera et al. 2002], Asia [Dhanorkar et al. 2005, Argade et al. 2009, Seyedimoradi et al. 2012, Zeinali et al. 2014, Salayeva et al. 2016, Tang et al. 2018], North Africa [Riahi et al. 2010] and Europe [Vidal et al. 1999, Benjak et al. 2005, Halász et al. 2005, Jahnke et al. 2009, Pelsy et al. 2010, Labagnara et al. 2018]. There is, however, a group of cultivars – especially originating in North America and Europe, including cultivars selected in Poland – whose genetic similarity has not been characterized with the use of DNA markers yet. This concerns in particular interspecific and inter-intraspecific crossings which are increasingly more frequently used in plant breeding and northern winework. In this paper we present the results of the assessment of genetic relationships among grapevine cultivars which have not been characterized with the use of DNA markers. Among the large number of genotypes gathered in the collection, particularly valuable from the point of view of breeding and grape production were selected. Such traits as early fruit ripening, fertility, taste, tolerance to frost and fungal diseases were taken into account.

MATERIALS AND METHODS

Plant material. Tests were carried out on 38 grapevine cultivars either belonging to *Vitis vinifera* L. or to interspecific and inter-intraspecific hybrids (*Vitis* spp.) originated in various European countries or USA (Tab. 1). Grape skin of the cultivars had different colours: yellow/green, pink, blue/black and red/black. 'Cabernet Sauvignon', 'Chasselas Dore', 'Traminer Rot', 'Pinot Noir' i 'Concord' were used as the reference cultivars.

Table 1. List of grape cultivars examined in this study and their basic characteristics

Cultivar and colour of berry skin[a]	Pedigree	Country of origin	Grape utilization[b]
Iza Zaliwska (B)	unknown interspecific hybrid *V. vinifera* L., *V. labrusca* L.	Poland	T
Izopan (B)	Iza Zaliwska × Pannonia Kincse interspecific hybrid *V. vinifera* L., *V. labrusca* L.	Poland	W/T
Danmarpa Polonia (B)	probably mutation or seedling of Pannonia Kincse *V. vinifera* L.	Poland	T
Małgorzatka (N)	Madeleine Angevine × Alden or Royal interspecific (complex[c]) hybrid	Poland	W/T
Czekoladowa (R/N)	Cardinal Ustoichivyi Magaracha × O.P. interspecific (complex) hybrid	Poland	T
Jutrzenka (B)	SV 12-375 × Pinot Blanc interspecific (complex) hybrid	Poland	W
Granatowe z Tychów (N)	Unknown *V. vinifera* L.	Poland	T
Białe z Leszna (B)	Unknown *V. vinifera* L.	Poland	W/T
Triumf Urbańskiego (B)	probably seedling of Madeleine Angevine *V. vinifera* L.	Poland	T
Deserowe Jasne (B)	unknown *V. vinifera* L.	Poland	T
Antracyt (N)	Victoria × Nadiezhda AZOS interspecific (complex) hybrid	Russia	T
Severnyi Rannyi (B)	Severnyi × Malingre precoce interspecific hybrid *V. vinifera* L., *V. amurensis* Rupr.	Russia	T
Timur (B)	Frumoasa Albae × Vostorg interspecific (complex) hybrid	Russia	T
Vostorg (B)	(Zarya Severa × Dolores) × Russkii Rannii interspecific hybrid *V. vinifera* L., *V. amurensis* Rupr.	Russia	T
Ananasnyi Rannyi (B)	Perl de Csaba × Ananasnyi (seedling of Isabella) interspecific hybrid *V. vinifera* L., *V. labrusca* L.	Ukraine	T
Supaga (B)	Madeleine Angevine × Dvietes Zila interspecific hybrid *V. vinifera* L., *V. labrusca* L.	Latvia	T
Opal (B)	Chaouch Rozovyi × Julski Biser *Vitis vinifera* L.	Slovakia	T
Aron (B)	Eger 2 (Seyve Villard 12-375) × Perlette interspecific (complex) hybrid	Hungary	T
Pannonia Kincse (B)	Queen of Vineyard × Cegléd Szépe *Vitis vinifera* L.	Hungary	T
Allegro (N)	Chancellor × Rondo interspecific (complex) hybrid, *V. vinifera* L. by VIVC	Germany	W
Bolero (N)	Geisenheim 6427-5 × Chancellor intra-interspecific (complex) hybrid	Germany	W
Cabernet Cantor (N)	Chancellor × Solaris interspecific (complex) hybrid, *V. vinifera* by VIVC	Germany	W
Garant (B)	Solaris × Muscat Bleu interspecific (complex) hybrid	Germany	T
Monarch (N)	Solaris × Dornfelder inter-intraspecific (complex) hybrid, *V. vinifera* L. by VIVC	Germany	W

Table 1 cont.

Seneca (B)	Lignan Blanc × Ontario interspecific hybrid *V. vinifera* L., *V. labrusca* L.	USA	T
Century (R/N)	(S.V. 20-347 × Dunstan 3) × (Chasselas Violet × Golden Muscat) interspecific (complex) hybrid	USA	T
Jupiter (R/N)	Arkansas 1258 × Arkansas 1672 interspecific (complex) hybrid including *V. labrusca* L.	USA	T
Price (R/N)	(Hector × Cascade) × (Fredonia × Athens) interspecific (complex) hybrid including *V. labrusca* L.	USA	T
Kay Gray (B)	Elmer Swenson 217 × Onaka interspecific (complex) hybrid	USA	W
Norway Red (N)	Elmer Swenson 283 × Elmer Swenson 193 interspecific (complex) hybrid	USA	W
St. Croix (N)	Elmer Swenson 193 × Elmer Swenson 283 interspecific (complex) hybrid	USA	W
Reliance (R)	Ontario × Suffolk Red interspecific hybrid *V. vinifera* L., *V. labrusca* L.	USA	T
Frontenac (N)	Riparia 89 × Landot 4511 interspecific (complex) hybrid *V. riparia* Michx., *V. vinifera* L. and other species	USA	W
Cabernet Sauvignon[c] (N)	*Vitis vinifera* L.	France	W
Chasselas Dore[d] (B)	*Vitis vinifera* L.	unknown (probably Near East or Caspian Sea region)	W/T
Traminer Rot[d] (R)	*Vitis vinifera* L.	France or Italy	W
Pinot Noir[d] (N)	*Vitis vinifera* L.	France	W
Concord[d] (N)	Catawba × *V. labrusca* L. interspecific hybrid *V. vinifera* L., *V. labrusca* L.	USA	W/T

[a] Colour of berry skin: B (blanc) – yellow/green, R – rose/red, N (noir) – blue/black,
[b] Grape utization: W – wine, T – table, W/T – wine and table
[c] Complex – hybrid with 3 or more species in parentage
[d] Control cultivars

DNA extraction. DNA was isolated from 100 mg of leaf tissue with the use of Plant & Fungi DNA Purification Kit (EURx, Gdańsk, Poland). Obtained DNA was purified with Anti-Inhibitor Kit (A&A Biotechnology, Gdynia, Poland). DNA concentration was measured spectrophotometrically at 260 nm. DNA dilutions of 10 ng/µl were prepared for the analyses.

PCR conditions

PCR reactions were conducted twice with the use of 12 ISSR primers (Tab. 2) in a total reaction volume of 20 µl containing 1x reaction buffer, 0,2mM dNTPs, 0,4µM of each primer, 0,5U of Taq DNA polymerase (Dream TaqTM Green; ThermoScientific, Waltham, USA) and 20 ng of DNA matrix. Reactions were carried out in 35 cycles: 94°C for 45 s, 55°C for 45 s, and 72°C for 90 s, in S1000™ thermal cycler (BioRad, Hercules, USA).

Electrophoresis of PCR products

Amplification products were separated in 2% agarose gel. Agarose gels were stained with ethidium bro-

Table 2. Characterization of ISSR primers used in this study and their polymorphism and diversity analysis

Primer	Repeat motif	Scored bands	Polymorphic bands	Polymorphism (%)	PIC	Resolving power
UBC 809	$(AG)_8G$	10	10	100	0.937	8.892
UBC 810	$(GA)_8T$	8	8	100	0.894	7.574
UBC 812	$(GA)_8A$	8	8	100	0.942	5.418
UBC 823	$(TC)_8C$	9	8	80	0.875	3.678
UBC 826	$(AC)_8C$	8	7	87,5	0.851	5.786
UBC 827	$(AC)_8G$	8	8	100	0.915	4.894
UBC 829	$(TG)_8C$	8	8	100	0.901	6.048
UBC 830	$(TG)_8G$	5	5	100	0.829	5.102
UBC 847	$(CA)_8RC$	6	6	100	0.853	6.414
UBC 855	$(AC)_8YT$	7	7	100	0.933	8.418
UBC 890	VHV(GT)7	7	6	85.7	0.88	6.05
UBC 891	HVH(TG)7	10	8	80,0	0.953	7.89
Total		94	89	–	–	76.164
Minimum		5	5	80.0	0.829	3.678
Maximum		10	10	100.0	0.953	8.892
Mean		7.8	7.4	94.4	0.897	6.347

R = A/G, Y = C/T

mide and visualized in UV light (GelDoc-It®Imaging System, UVP, Upland, USA). Size of PCR products was measured with GeneRuler 1 kb DNA Ladder (Thermo Fisher Scientific).

Data analysis. Only clear and repeatable DNA fragments were selected for the analysis. They were specified as present (1) or absent (0). The number of monomorphic and polymorphic DNA fragments and the level of polymorhism were determined on the basis of acquired data. A similarity matrix was defined based on the Jaccard coefficient. A dendrogram was constructed using the unweighted pair-group mean analysis (UPGMA) method, using XLSTAT software (Addinsoft 2006). Polymorphism information content (PIC) values were specified for each primer, in accordance with the formula $PIC = 1 - \Sigma(P_{ij})^2$, where P_{ij} is the frequency of the $_i$th pattern revealed with the $_j$th primer [Botstein et al. 1980]. Resolving power was obtained by Prevost and Wilkinson's [1999] equation: $RP = \Sigma Ib$, $(Ib = 1 - (2 \times |0.5 - p|)$, where p represents the rate of I band in total genotypes.

RESULTS AND DISCUSSION

Screening of ISSR primers and their information potential. 94 reaction products sized from 300 bp to 2300 bp, 94.4% of which were polymorphic, were obtained in the analysis conducted for 38 grapevine genotypes with the use of 12 ISSR primers (Tab. 2). Depending on the primer used, 5 (UBC 830) to 10 (UBC 809) polymorphic products were obtained, 7.4 on average. The polymorphic information content (PIC) value varied between 0.829 (UBC 830) and 0.953 (UBC 891) with an average of 0.897. The resolving power (Rp) of 12 ISSR primers ranged between 3.678 (UBC 823) and 8.892 (UBC 809) with an average of 6.347.

The results are similar to data obtained by other authors assessing the genetic diversity of grapevine. In previous research on the DNA polymorphism in grapevine done with the ISSR technique, 2 to 13 polymorphic products were obtained per primer, 6.7 to 7.9 on average [Argade et al. 2009, Sabir et al. 2009,

Dhanorkar et al. 2005]. When analyzing four grapevine cultivars, Choudhary et al. [2014] obtained a mean PIC value of 0.85 and medium resolving power of 6.14 and primer 810 had the highest Rp ratio (10). Salayeva et al. [2016] assessed genetic diversity of 65 grape accessions from Azerbaijan with the use of five ISSR primers and obtained average polymorphism of 87.69, mean PIC value of 0.94 and mean Rp of 5.65. Those authors point out high effectiveness of primer 810 which might be useful in further research. The assessment of genetic diversity of 21 local grapevine cultivars with the use of 10 ISSR primers showed the polymorphism level of 64% and PIC value of 0.43 [Seyedimoradi et al. 2012]. The analysis of molecular diversity of 44 grapevine cultivars from Turkey with the use of 20 ISSR primers showed polymorphism at the level of 88.6%, mean PIC value of 0.762 and Rp ratio varying between 0.349 and 1.272, 0.75 on average [Sabir et al. 2009]. Assessment of genetic relationships among 43 grape cultivars in India with the use of 13 ISSR primers allowed to obtain polymorphism level of 69% [Dhanorkar et al. 2005]. The analysis of genetic diversity of 32 seeded grape varieties with the use of 11 ISSR primers showed the polymorphism was at the level of 83.3% [Dhane et al. 2006].

In our study, the following primers were found to have the highest polymorphic information content (PIC) which is one of the indicators of primer effectiveness: UBC 891 (0.953), UBC 812 (0.942), UBC 809 (0.937) and UBC 855 (0.933). Highest resolving power was obtained in reactions with primers UBC 809 (9.892), UBC 855 (8.418), UBC 891 (7.89) and UBC 810 (7.574).

Summing up, the level of polymorphism as well as the PIC and Rp values obtained in this study indicate high effectiveness of five primers: UBC 809, UBC 891, UBC 855, UBC 810 and UBC 812. Results show the usefulness of those primers in analyses of genetic diversity of grapevine cultivars on account of the possibility to obtain highly informative loci.

Genetic relationships among grape accessions. Genetic similarity among 38 grapevine cultivars belonging to *Vitis vinifera* L. and interspecific hybrids calculated with Jaccard's coefficient was found to range from 0.167 to 1.0. This indicates high degree of diversity among tested grape cultivars. This high degree of genetic diversity results from the origin of tested cultivars: most of them are hybrids and some of the genotypes have as many as 7 *Vitis* species in their origin. So far, highest genetic diversity ranging from 0.08 to 0.93 was obtained for genotypes belonging to seventeen Chinese wild *Vitis* species, American and European cultivars belonging to *V. vinifera*, *V. riparia* and seven interspecific hybrids [Jing and Wang 2013]. Genetic similarity among two cultivars belonging to two species, *V. vinifera* and *V. labrusca* and their hybrids, was determined at 0.14−0.68 [Sabir et al. 2009], 0.48−0.89 [Dhane et al. 2006] and 0.65−0.96 [Dhanorkar et al. 2005]. For *V. vinifera* cultivars the similarity coefficient ranged from 0.33 to 0.87 [Zeinali et al. 2012], from 0.23 to 0.81 [Seyedimoradi et al. 2012] and from 0.24 to 0.63 [Choudhary et al. 2014]. Highest degree of similarity was observed in seedless *V. vinifera* varieties, where the similarity coefficient ranged from 0.79 to 1.0 [Argade et al. 2009].

A dendrogram was drawn up on the basis of the conducted analyses; it presents the relationships among the studied grape cultivars (Fig. 1). Tested cultivars were divided into 3 main clusters. One of them was further divided into 6 subclusters. Detailed breakdown and comparison of parental forms based on literature as well as field assessment of some phenotypic traits (own, unpublished data) confirm the usefulness of the method employed to assess the genetic diversity of studied cultivars, with the exception of one pair ('Pannonia Kincse', 'Danmarpa Polonia'), and the validity of the division into clusters.

First cluster consists of four cultivars belonging to interspecific hybrids, including three cultivars with blue/black berries, 'Cabernet Cantor', 'Allegro', 'Frontenac' and one cultivar, 'Garant', with yellow/green berries. Cultivars from this cluster are complex hybrids with a number of species in their origin. Three of them are different generations of *V. amurensis* Rupr.: 'Garant' – F4 in mother line, 'Allegro' – F3 in father line and 'Cabernet Cantor' – F4 in father line [VIVC 2018]. 'Frontenac' is F1 generation of *V. riparia* Michx. in mother line; in father line it contains *V. vinifera* and American species *V. labrusca* L., *V, rupestris* S., *V. berlandieri* Planch., *V. lincecumii* Buckley (*V. aestivalis* var. *lincecumii* (Buckley) Munson), and *V. riparia* Michx. [HORT 3040 2016, VIVC 2018]. American species are also present in the parentage of other cultivars from this cluster. The validity

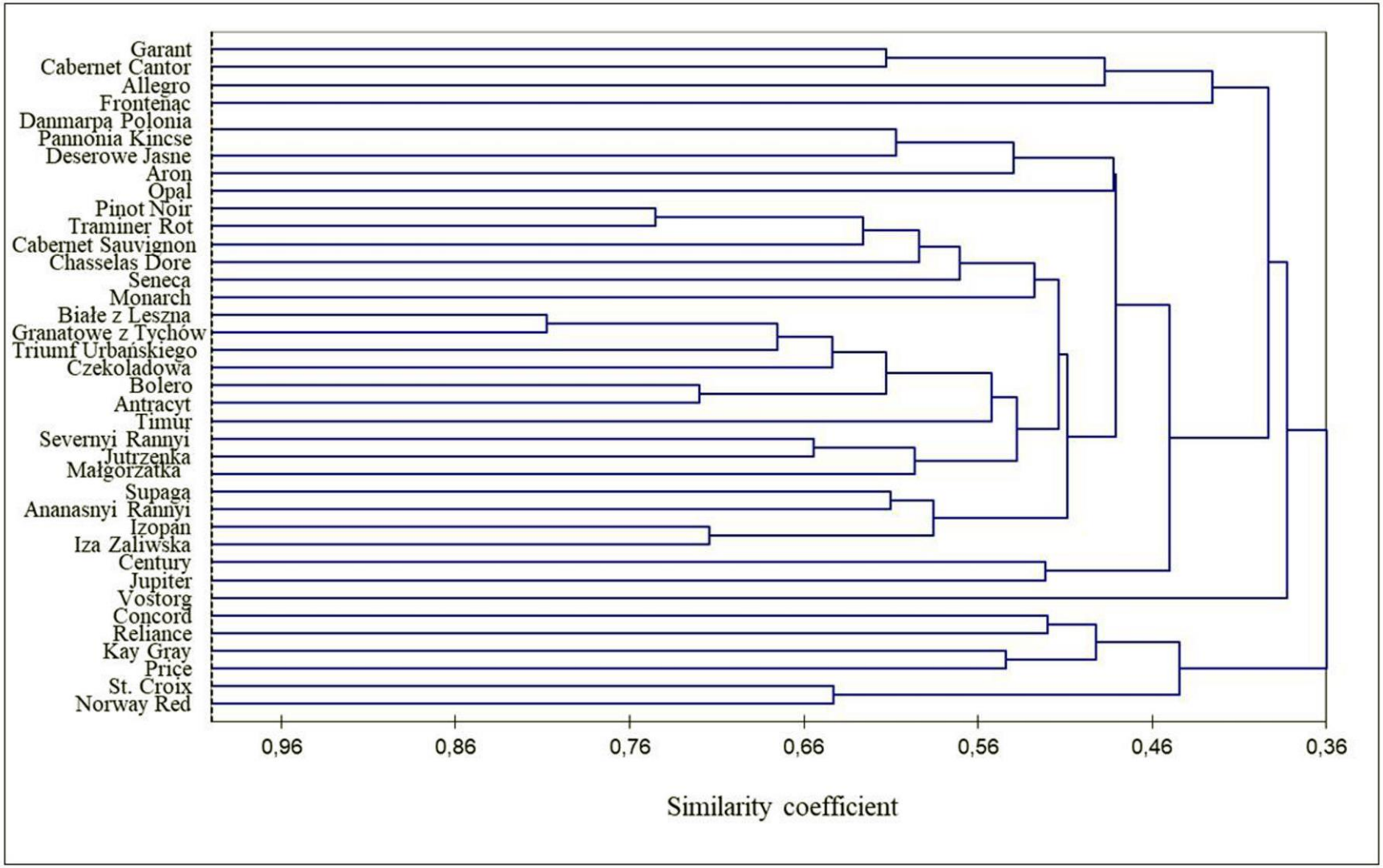

Fig. 1. Dendrogram based on ISSR data showing relationships among grape cultivars using the unweighted pair-group mean analysis (UPGMA)

of such a composition of this cluster corresponds with statements of Di Gaspero et al. [2000] who created a similarity tree of 9 *Vitis* species, based on point mutations observed along the microsatellite flanking regions. According to results achieved by those authors, two American species, *V. riparia* and *V. rupestris*, were grouped together with *V. amurensis*, an Asian species, because they showed identical nucleotides at all mutation sites.

Second cluster consists of interspecific hybrids originating in USA with coloured berries (blue/black, red/black or red), such as 'Price', 'St. Croix', 'Norway Red', 'Concord' and 'Reliance', and 'Kay Gray' with yellow/green berries. Cultivars in this cluster are hybrids of *V. labrusca* in different generations: 'Concord' - F1 in father line and F2 in mother line; 'Reliance' – F3 (twice) in mother line and F3 in father line; 'Price' – F2 and F3 in father line and F4 in mother line, 'Kay Gray' – F3 and F4 in father line and F4 and F5 (twice) in mother line; 'St. Croix' – F4 and F5 (twice) in mother line and F5 in father line. Some of the cultivars have other American species in their origin. 'Kay Gray' is generation F4 in father line and F5 in mother line of *V. riparia*. 'St. Croix' is generation F4 of *V. riparia* in mother line, F4 and F5 of *V. rupestris* in father line and F5 of *V. lincecumii* in father line. 'Price' also has *V. riparia* and *V. lincecumii* in its lineage. 'Norway Red' has the same parents as 'St. Croix' interspecific (complex) hybrid (Tab. 1) but with reversed roles, hence the great similarity among those cultivars, visible in the results of the analyses (similarity coefficient 0.658).

'Vostorg', a cultivar originating in Russia with yellow/green berries was separated from other cultivars. For this cultivar the similarity coefficient was low and ranged from 0.189 to 0.487. It is an interspecific hybrid derived from *V. vinifera* and *V. amurensis* [VIVC 2018]. The reason for 'Vostorg' being so different probably lies in high genetic similarity to *V. amurensis*, much higher than to other genotypes. 'Vostorg' is an offspring of *V. amurensis*: F2 in mother line and F3 in father line. It was not grouped in the same cluster either with 'Severnyi Rannyi', a generation F2 of *V. amurensis* in mother line (similarity coefficient 0.354), or with complex hybrids, including *V. amurensis* and American species ('Garant', 'Cabernet Cantor', 'Allegro').

Other cultivars formed the third cluster, composed of *V. vinifera* cultivars and interspecific hybrids, which was further divided into six subclusters.

First subcluster consists of five cultivars with yellow/green berries, belonging to *V. vinifera* – 'Pannonia Kincse', 'Danmarpa Polonia', 'Opal', 'Deserowe Jasne' and 'Aron', an interspecific hybrid. 'Pannonia Kincse' and 'Danmarpa Polonia' were not differentiated. They do, however, have different phenotypic traits such as shape, size and skin thickness of berries (own unpublished data). Obtained results show that 'Danmarpa Polonia' is most probably a clone of 'Pannonia Kincse'. Clones of grapevine appear during vegetative reproduction as a result of spontaneous somatic mutations in the regenerative cells [Pelsy et al. 2010]. Differentiating among clones of grapevine with the use of techniques based on microsatellite markers such as ISSR and SSR is often impossible [Moreno et al. 1998, Baneh et al. 2009, Pelsy et al. 2010]. So far, partial differentiation among clones was possible only with the FLP technique [Baneh et al. 2009]. Therefore, differentiation among 'Pannonia Kincse' and 'Danmarpa Polonia' requires further study and the use of other molecular techniques.

Second subcluster consists of cultivars with yellow/green or coloured berries, including four control *V. vinifera* cultivars such as 'Pinot Noir', 'Traminer Rot', 'Cabernet Sauvignon' and 'Chasselas Dore'. Interspecific hybrids 'Seneca' and 'Monarch' are also in this subcluster. In literature, 'Seneca' is defined as interspecific hybrid and 'Monarch' as *V. vinifera* cultivar [VIVC 2018], but, more specifically, 'Seneca' is an intra-interspecific hybrid with a *V. vinifera* cultivar in mother line and 'Monarch' is an inter-intraspecific hybrid with a *V. vinifera* cultivar in father line.

V. vinifera cultivars such as 'Białe z Leszna', 'Granatowe z Tychów', 'Triumf Urbańskiego' and interspecific hybrids 'Antracyt' and 'Timur' [Vinograd-info 2018], 'Czekoladowa' and 'Bolero' constitute the third subcluster. Those cultivars have both yellow/green and blue/black berries. There are two pairs of cultivars showing high degree of genetic similarity: 'Białe z Leszna' with 'Granatowe z Tychów' and 'Bolero' with 'Antracyt'. The similarity coefficient for those pairs was 0.804 and 0.756 respectively.

Fourth subcluster consists of three interspecific hybrids with yellow/green or coloured berries including

Polish cultivars 'Małgorzatka' and 'Jutrzenka' (complex hybrids) and Russian 'Severnyi Rannyi' (*V. vinifera* × *V. amurensis* crossing). 'Severnyi Rannyi' and 'Jutrzenka' had a high similarity coefficient: 0.681. This may be due to the fact that in father line 'Severnyi Rannyi' is generation F2 of 'Pinot' [Nordvin.by 2018, VIVC 2018] and 'Jutrzenka' is generation F1 of 'Pinot Blanc' [Gąstoł 2015]. 'Severnyi Rannyi' had leaves with clearly indicated 5 lobes, yelow/green skin adherent to semi-crisp flesh of broad ellipsoid berries and had morphological traits similar to *V. vinifera*, especially to 'Opal'.

Four interspecific hybrids with yellow/green berries, including Polish cultivars 'Iza Zaliwska' and 'Izopan' with similarity coefficient of 0.72, Ukrainian 'Ananasnyi Rannyi' [Abuzov 2009] and Latvian 'Supaga' formed the fifth subcluster. Obtained results seem to confirm that, in line with a statement of a Polish breeder (Bolesław Jabłoński), 'Iza Zaliwska' is a mother form of 'Izopan' (unpublished data). 'Ananasnyi Rannyi' and 'Supaga' originated from the crossing of *V. vinifera* cultivars characterized by early ripening season with seedlings of *V. labrusca* hybrids.

The sixth subcluster consists of 'Century' and 'Jupiter', hybrids originating in USA with red/black berries.

The following hybrids, offspring of *V. labrusca* –'Iza Zaliwska' of undetermined origin defined as *V. labrusca* × *V. vinifera* crossing [Sękowski and Myśliwiec 1996], 'Izopan'; 'Seneca', 'Ananasnyi Rannyi', and 'Supaga' (F3 in father line) and 'Jupiter' (F5 in mother line, twice) were included in the third cluster together with *V. vinifera* cultivars and some intespecific crossings and not in the second cluster composed of interspecific hybrids containing *V. labrusca* and bred in the USA. Cultivars from the second cluster showed phenotypic traits typical of *V. labrusca*: leaves with three broad, vaguely indicated lobes, skin non-adhering to flesh, where typical slip-skin fruits were characteristic of 'Concord', 'Reliance', and 'Price'. Morphological traits of leaves and fruits of *V. labrusca* hybrids from the third cluster were varied, but some of them were different from the traits typical of *V. labrusca*. Leaves of 'Iza Zaliwska', 'Seneca', 'Supaga', and most of all 'Izopan' had distinctively indicated lobes (most often 5). Berries of those cultivars had skin adherent to flesh which was semi-crisp in 'Seneca', 'Jupiter' and 'Anasnyi Rannyi'. Berries of 'Seneca' and 'Jupiter' had the shape of broad ellipsoid whereas the fruits of *V. labrusca* are globose. Bushes of hybrids of *V. labrusca* from the third cluster, similarly to bushes of *V. vinifera* cultivars, were more infested by powdery and downy mildew than cultivars included in the second cluster (own unpublished data).

Genetic similarity among some interspecific hybrids and *V. vinifera* cultivars and the consequent placing them in the same cluster may result from multiple crossings of *V. vinifera* genotypes. The clustering of cultivars belonging to the same species and its derivatives was observed in *V. labrusca* [Dharnokar et al. 2009]. Those authors also observed clustering together cultivars belonging to different species. 'James', a *V. rotundifolia* cultivar, was clustered with *V. labrusca* cultivars and their derivatives. When comparing 34 cultivars originating from *V. vinifera*, *V. labrusca*, and *V. amurensis*, two major clades in the dendrogram, *V. vinifera* with a hybrid of *V. vinifera* and *V. labrusca* or hybrid of *V. amurensis* and *V. vinifera*, were clearly differentiated [Dong et al. 2018], but the authors did not present a common dendrogram for the three species and their hybrids. However, the comparison among 10 species of Vitaceae, including 3 original *V. vinifera* cultivars and *Parthenocissus quinquefolia*, based on the point mutation in the microsatellite flanking regions, allowed to place *V. labrusca* and *V. vinifera* cultivars, 'Syrah' and 'Cabernet Sauvignon', in the same cluster, while within Vitis, *V. rotundifolia*, *V. berlandieri*, *V. riparia*, *V. amurensis* and *V. rupestris* differed most from *V. vinifera* [Di Gaspero et al. 2000]. Relations described by Di Gaspero et al. [2000] and Dong et al. [2018] justify clustering together some of the *V. vinifera* and *V. labrusca* hybrids with *V. vinifera* cultivars but separating *V. labrusca* hybrids and American species in another cluster, also including forms containing *V. labrusca* to a large degree. Study on genetic similarity among 18 grapevine cultivars, conducted by Theocharis et al. [2010], allowed to create a cluster consisting of interspecific hybrids and another cluster composed of inter-intraspecific hybrids and original *V. vinifera* cultivars, but the genetic similarity, expressed as Dice coefficient, among two *V. vinifera* cultivars – 'Scheurebe' and 'Perlan' was 0.596 and was lower than among 'Scheurebe' (*V. vinifera*) and 'Rondo' (inter-intraspecific crossing), which was 0.711.

In this study some clusters contained cultivars with the same colour of fruits while other clusters contained cultivars with different colour of fruits. High similarity coefficient (0.804) was observed in the pair 'Białe z Leszna' and 'Granatowe z Tychów' which have fruits of different colour. High similarity was also found among 'Bolero' and 'Antracyt', cultivars with blue/black fruits (0.756) and among 'Iza Zaliwska' and 'Izopan', with yellow/green fruits (0.72). In previous studies on genetic diversity most grapevine cultivars were clustered on the basis of the colour of their fruits, but some cultivars with yellow/green berries were grouped with those having red/black/purple berries [Dharnokar et al. 2005, Dhane et al. 2006, Argade et al. 2009].

CONCLUSIONS

Presented results for the first time show the assessment of genetic relationships among grapevine cultivars selected in Poland and cultivars originating in other European and North American countries which have not been characterized with the use of molecular markers. Results of the research show that ISSR technique proved a useful tool for identification of grapevine cultivars. Developed ISSR markers may be applied in the identification of grapevine cultivars in vineyards, as well as in germplasm collections of *V. vinifera* genotypes and interspecific hybrids.

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