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RAPD and ISSR Methods Used for Fingerprinting of Selected Accessions of *Viburnum*

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Abstract

Randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers were used to investigate genetic variability within thirteen *Viburnum* species (*Viburnum* × *hillieri; V. dilatatum*; *Viburnum* × *carlcephalum; V. opulus*; *V. hupehense*; *Viburnum* × *bodnantense; Viburnum* × *burkwoodii; V. sieboldii*; *Viburnum* × *globosum* 'Jermyns Globe'*; V. alnifolium (lantanoides); V. plicatum* 'Sterile'*; V. plicatum* f. *tomentosum* and *V. plicatum* 'Watanabe') of wide geographical distribution, collected in the Dendrological Garden in Przelewice (the north-west part of Poland). Twenty-three RAPD and fourteen ISSR primers generated a total of 690 and 418 reproducible bands, respectively, and 39% (RAPD) and 55.5% (ISSR) of them were polymorphic for the two marker systems, which suggest high genetic variability within *Viburnum* genus. However, high numbers of genotype-specific bands, i.e. 60.9% (RAPD) and 44.5% (ISSR), were seen in *Viburnum*. Genetic similarity assessed within *Viburnum* species with the RAPD and ISSR analyses ranged from 6 to 42% and from 6 to 31%, respectively. Both RAPD and ISSR-based dendrograms clustered in five main groups. The Mantel test between two Nei's similarity matrices gave correlation coefficient r=0.305*, showing low correlation between RAPD- and ISSR- based matrices. Thus, both marker systems were equally important for the genetic diversity analysis in *Viburnum* genus.

Keywords: accessions, genetic similarity, polymorphism, variability

Introduction

Viburnum genus belongs to Caprifoliaceae family and comprises more than 230 species mainly distributed in the temperate and subtropical zones, particularly in Asia and North America. It is divided into eleven sections based on morphological characters and geographical origin (Lobstein *et al.,* 2003). Only two species occur in Poland in the wild: *V. lantana* and *V. opulus* (Bugała, 2000).

Donoghue (1983) found nine of the ten traditionally recognized sections to be monophyletic. It was a result of an analysis which was performed on the basis of a set of morphological traits. The exception was *Odontotinus* section that contained a clade corresponding to Latin American *Oreinotinus* section.

Donoghue *et al*. (2004) distinguished three main supra-sectional groups within *Viburnum* genus. The largest one included sections occurring in Eurasia, Asia and Latin America, the second one had sections occurring in Asia and the third one included sections of the region of North America and Asia.

Not only according to Donoghue *et al*. (2004), but also to Winkworth and Donoghue (2004, 2005), DNA sequence analysis can be useful in meticulous research on mutual phylogenetic relationships and the direction of evolution within *Viburnum.* The researchers stated that most of the already recognized sections are monophyletic. *Odontotinus* section is an exception, as it was divided into the purple-fruited New World clade, where Latin American *Oreinotinus* section is nested, and Old World clade, closely related *Viburnum cylindricum* (*Megalotinus* section) and the New World clade – *Viburnum acerifolium* (Donoghue *et al*., 2004).

The collection of various, often unique *Viburnum* objects of the Dendrological Garden in Przelewice is a place where many systematically distant objects, including hybrids

and accessions, are gathered. As an extraordinary valuable source of *Viburnum* genes, it enables morphological, phenological and molecular analyses. It can be used for genotyping and description of mutual genetic relationships with the use of different methods, including those based on PCR amplification with randomly amplified polymorphic DNA (RAPD), as well as inter simple sequence repeats (ISSR) (Hyvönen, 2010; Poczai and Hyvönen, 2010; Smolik *et al*., 2010; Sharma *et al*., 2009; Shneyer, 2009, Smolik and Krzysztoszek (2010)). The first one amplifies DNA using 10-mer primers, while the second one involves amplification of DNA sequence between two adjacent and inversely oriented microsatellites usually by using 18-24-mer primers (Williams *et al*., 1990; Ziętkiewicz *et al*., 1994). RAPD and ISSR are popular DNA fingerprinting methods because they are quick, inexpensive, highly polymorphic and also they do not require any sequence information about the under study genome (Gajera *et al*., 2011; Yüzbaşioğlu *et al*., 2011).

Thus, in the present study, we used both RAPD and ISSR methods to perform fingerprinting and examine the level of genetic similarity within *Viburnum* accessions, and to assess to what extent the results of the molecular analysis will be helpful in the assessment of genetic relationships between the selected accessions of *Viburnum* genus collected in the Dendrological Garden in Przelewice.

Materials and methods

The research material comprised 13 objects of *Viburnum* sp. of different origin, a part of the genus collection gathered in the Dendrological Garden in Przelewice (the north-west part of Poland). The research included: *Viburnum* × *hillieri* – hybrid of *V. erubescens* × *V. henryi, V. dilatatum*, *Viburnum* × *carlcephalum* – hybrid of *V. carlesii* × *V. macrocephalum, V. opulus*, *V. hupehense*, *Viburnum* × *bodnantense* – hybrid of *V. farreri* × *V. grandiflorum, Viburnum* × *burkwoodii* – hybrid of *V. carlesii* × *V. tiule, V. sieboldii*, *Viburnum* × *globosum* 'Jermyns Globe' – hybrid of *V. davidii* × *V. calvum, V. plicatum* f. *tomentosum*, *V. alnifolium (lantanoides), V. plicatum* 'Sterile' and *V. plicatum* 'Watanabe'.

Plant DNA extraction

Fresh leaves were collected from 13 *Viburnum* genotypes (selected as one plant per genotype). All leaf samples were ground in liquid nitrogen using a mortar and a pestle to fine powder. Total genomic DNA was extracted using a *Genomic Mini AX Plant* kit from 100 mg of fresh leaf material. Contaminating RNA was removed by digestion with RNase A (20 mg·cm^{-3}).

RAPD amplification

PCR amplification was performed with a set of 23 decamer primers (professional kit) according to the protocol described by Williams *et al*. (1990). Amplifications were performed in 25 μl reaction volume containing 10×PCR buffer with $(NH_4)_2SO_4$ (750mM Tris-HCl pH 8.3, 200mM $(NH_4)_2SO_4$, 0.1% Tween 20), 0.2mM of dNTPs, 2.0mM $MgCl_2$, 0.25 μM of primer, 1.0 unit of *Taq* DNA polymerase enzyme (Thermo Scientific) and 30-40 ng of template DNA. Amplifications were performed in an Eppendorf Master cycler 5333. The program of thermal cycling was as follows: initial activation step at 95°C for 15 min, followed by 34 cycles for 1 min at 94°C, 1 min at 36°C and 2 min at 72°C, with the final extension step at 72° for 10 min. The amplification was conducted by final extension at 72°C for 5 min.

ISSR amplification

ISSR amplifications were performed with a set of 14 ISSR primers (collection of the University of British Columbia, Canada). Reaction mixtures (25 μl) contained: 10×PCR buffer with $(NH_4)_2SO_4$ (750 mM Tris-HCl pH 8.3, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 2.0 mM $MgCl_2$, 1 out of 14 different 0.2 μM primers, 0.2 mM of each dNTP (Thermo Scientific), 1.0 unit of *Taq* DNA polymerase (Thermo Scientific) and 100 ng genomic DNA. Amplification was performed in an Eppendorf Master cycler 5333 using the following program: initial denaturation at 94°C for 7 min, 35 cycles of 30 s at 94°C, 50 s at annealing temperature, 2 min at 72°C and 7 min at 72°C for final extension. The annealing temperature was adjusted according to the T_m of the primers used in the reaction.

Electrophoresis

PCR products were mixed with 6 × Orange Loading Dye Solution and analyzed by electrophoresis (SubCell GT). O'RangeRuler 200 bp DNA Ladder (3000-200 bp) was used as a size marker. Amplified products were loaded on 1.5% agarose gel and separated in 1× TBE buffer. A constant voltage of 100 was provided for 2 h. DNA fragments were visualized under UV light after staining with ethidium bromide (5.0 mg·cm^{-3}). The RAPD and ISSR bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity.

Data analysis

Data analyses were conducted using the PhylTool software (Buntjer, 2001). Similarity matrix for both RAPD and ISSR primers was constructed using the Nei's similarity coefficient values to find genotypic relationships (Nei and Li, 1979). The 0/1 matrix data obtained from RAPD and ISSR primers were arranged to get separate similarity matrices which were subjected to UPGMA (unweighted pair-group method with arithmetic averages) analysis to generate a dendrogram and compared using the Mantel matrix correspondence test (XLSTAT software). The strength of the internal branches from the resulting tree was tested with the Treecom bootstrap analysis application using 2,000 resamplings (Van de Peer and De Wachter, 1994). The matrices were also compared using the Mantel's test (Mantel, 1967) for matrix correspondence.

Results and discussion

RAPD analysis

For thirteen examined *Viburnum* objects, altogether 690 loci were amplified (1160 amplicons), including 271 (39%)

polymorphic ones, 418 (60.9%) accession-specific, and only 1(0.1%) monomorphic (Tab. 1). Altogether, on average ca. 30 loci were amplified in a reaction with one RAPD primer (including 12 polymorphic and 18 accession-specific loci). The length of the amplified loci ranged from 2530 (OPW_02) to 190 bp (OPM_05). The greatest number of loci (45) was obtained in a reaction with primer OPW_12, and the lowest (20) with primer OPW_03.

Monomorphic loci

Among the 23 RAPD primers used in the experiment, a monomorphic product of the length of 190 bp was amplified only in a reaction with primer OPM_05 (Tab. 1).

Polymorphic loci

The examined *Viburnum* genotypes exhibited a high level of genetic diversity (Fig. 1). Among all the amplified RAPD loci, 271 (39%) were described as polymorphic (Tab. 1). The greatest number (19) was amplified in a reaction with primer OPW_02, and the least (6) – with primers OPW_09 and OPW_10.

Genotype-specific loci

60.9% of amplified loci were described as accession-specific (Tab. 2). For each examined subject, accession-specific RAPD products were amplified. The greatest number (28) was generated in a reaction with RAPD primer OPW_12, and the lowest number (11) – with primer OPA_1 (Tab. 2). The highest number (47) of products of this type was observed for *V.* × *globosum* 'Jermyns Globe' accession, and the lowest number (14 genotype-specific) for *V. dilatatum* species (Tab. 2). They were amplified in reactions with 20 and 10 RAPD primers, respectively.

ISSR analysis

Assessment of genetic diversity between thirteen *Viburnum* genotypes was carried out in a reaction with fourteen ISSR primers (Tab. 1). Altogether, 418 ISSR loci (873 amplicons) were amplified in the experiment, of which 232 (55.5%) were described as polymorphic and 186 (44.5%) as genotype-specific (Tab. 1). Monomorphic products were not amplified. In a reaction with one primer, on average 30 ISSR loci were amplified, of which 17 were polymorphic, and 13 – genotype-specific (Tab. 1). Their length ranged from 3130 bp (primer 863) to 230 bp (primer 816 and 863).

Polymorphic loci

A relatively wide range of variability was observed between the examined *Viburnum* objects (Fig. 1), resulting from the presence of PCR products, determined as polymorphic (55.5%), in electropherograms. Reactions with primers 818, 848 and 851 generated the highest number of

Tab. 1. Characteristic of generated RAPD and ISSR products for 13 genotypes of *Viburnum*

Technique, primers etc.				Loci			Genotypes of *Viburnum*													
Technique	Primer number	Fragment size range (bp)	Total/Mean loci amplified	Monomorphic	Polymorphic	Genotype-specific	*V. ×hillieri*	*V. dilatatum*	*V. ×carlcephalum*	*V. opulus*	*V. hupehense*	*V. ×bodnantense*	*V. ×burkwoodii*	*V. sieboldii*	*V. ×globosum 'Jermyns* 'Globe'	*V. abnifolium (lantanoides)*	*V. plicatum* 'Sterile'	*V. plicatum f. tomentosum*	*V. plicatum* 'Watanabe'	Total generated amplicons (mean for 1 genotype)
				Mean for 1 primer used																
RAPD	OPA_1, OPA_2, OPA_3, OPA_4, OPG_14, OPJ_01, OPJ_13, OPM_01, OPM_02, OPM_03, OPM_05, OPM_12, OPW_01, OPW_02, OPW_03, OPW_05, OPW_07, OPW_08, OPW_08, OPW_09, OPW_10, OPW_17, OPW_13, OPW_1	2530 - 190	690/30	1 (0,1%)	271 (39%)/12	418 (60,9%)/18	87	72	84	94	87	85	107	113	113	90	103	68	97	1160/50
ISSR	802, 809, 815, 816, 817, 818, 821, 827, 834, 837, 845, 848, 851, 863	3130 - 230	418/30	0	232 (55,5%)/17	186 (44,5%)/13	57	73	77	51	73	75	63	70	69	64	77	69	55	873/62

Tab. 2. Genotype-specific RAPD products generated for 13 genotypes of *Viburnum* investigated (profiled)

Genotype	Primers and genotypes-specific RAPD products
V. × *hillier*	OPA_1[1330, 1170]; OPA_2[660]; OPA_3[420]; OPA_4[1170]; OPG_14[1070]; OPM_01[920, 400]; OPM_02[1130, 530]OPM_03[860]; OPM_12[1300, 1140, 620]; OPW_01[780, 430]; OPW_03[860]; OPW_05[560]; OPW_07[800]; OPW_08[730]; OPW_09[1350]; OPW_10[1220]; OPW_12[900]; OPW_13[1220]; OPW_1[1110, 460]
V. dilatatum	OPA_3[1130]; OPG_14[1310, 740]; OPJ_13[760]; OPM_01[530]; OPM_12[760]; OPW_05[570]; OPW_07[300]; OPW_09[860]; OPW_10[1210, 460]; OPW_12[1060, 720, 330]
V. × *carlcephalum*	OPA_3[330]; OPA_4[510, 430]; OPG_14[1350, 500]; OPJ_01[460]; OPM_05[310]; OPM_12[600]; OPW_01[1930, 1160, 960]; OPW_05[640]; OPW_07[920, 240]; OPW_08[820, 570]; OPW_09[1790, 1200]; OPW_10[480]; OPW_12[990, 310]; OPW_13[1050]; OPW_1[1320, 300]
V. opulus	OPA_3[700, 430, 350]; OPG_14[1430, 480]; OPJ_01[380]; OPJ_13[390]; OPM_01[720, 540, 480]; OPM_02[940, 690, 470, 340]; OPM_03[960, 600, 410]; OPM_05[450]; OPM_12[1370, 1210, 400]; OPW_01[1810, 890]; OPW_09[1380]; OPW_12[1190, 1040, 910, 790, 530, 260]; OPW_1[1080, 780, 550]
V. hupehense	OPA_2[470]; OPG_14[910]; OPJ_01[410]; OPM_02[1120, 590]; OPM_03[920]; OPM_05[650, 560, 340, 280]; OPM_12[810]; OPW_02[620, 370]; OPW_07[1330, 1040, 610]; OPW_08[1080, 720, 590]; OPW_12[690]; OPW_13[780]; OPW_1[430];
V. × *bodnantense*	OPA_1[1370]; OPA_2[940, 670]; OPA_3[280]; OPA_4[1190]; OPG_14[970, 760]; OPJ_01[610]; OPJ_13[840, 550]; OPM_01[990, 440]; OPM_02[980]; OPM_05[480]; OPM_12[800, 700, 260]; OPW_05[600]; OPW_07[970]; OPW_09[1690, 900]; OPW_10[1050, 660, 430]; OPW_13[1000]; OPW_1[800, 420]
V. × *burkwoodii*	OPA_1[1110, 850, 480]; OPA_2[550]; OPA_3[1620]; OPA_4[550]; OPG_14[1370, 800, 380]; OPJ_01[490]; OPJ_13[980, 650]; OPM_01[770]; OPM_02[820, 210]; OPM_03[1020]; OPM_05[380]; OPM_12[1490, 630]; OPW_01[1010, 870, 370]; OPW_02[1400, 330]; OPW_03[1620, 1460]; OPW_05[1040]; OPW_07[1200, 1010, 640]; OPW_08[1240, 750, 550]; OPW_10[870, 550]; OPW_13[610]; OPW_1[950, 810, 350]
V. sieboldii	OPA_2[730]; OPG_14[920, 630]; OPJ_01[780]; OPM_01[960, 750, 510]; OPM_03[1540, 1110, 880]; OPM_05[900, 810, 540, 390, 330]; OPM_12[1420, 1160, 550]; OPW_03[1440, 1080]; OPW_02[2530, 1210, 660, 300]; OPW_03[1420, 760]; OPW_05[1190]; OPW_07[770]; OPW_08[1390, 800]; OPW_09[970, 700]; OPW_10[820, 530]; OPW_12[320]; OPW_1[1490, 1040, 940]
V. × *globosum* 'Jermyns Globe'	OPA_1[500]; OPA_2[760];OPA_3[1330];OPA_4[940, 680, 480]; OPJ_01[1650, 1210, 960, 790, 690, 450]; OPM_01[1560, 1270, 1070, 840]; OPM_02[1510, 950, 770, 630]; OPM_03[1040]; OPM_05[960, 470]; OPM_12[970]; OPW_01[580]; OPW_02[420]; OPW_03[1720, 720, 620]; OPW_05[970, 680, 610, 520, 460]; OPW_07[1260, 860, 500]; OPW_09[1960]; OPW_10[810, 710]; OPW_12[1120, 980, 760, 270]; OPW_13[520]; OPW_1[1050, 320];
V. alnifolium (lantanoides)	OPA_2[800, 600]; OPA_3[510, 240]; OPA_4[790, 650, 520]; OPG_14[750]; OPJ_01[870]; OPJ_13[1460, 500]; OPM_01[550]; OPM_02[1030, 870, 600]; OPM_03[590]; OPM_05[500]; OPM_12[460]; OPW_01[930]; OPW_07[1000, 830, 420]; OPW_08[320]; OPW_09[270]; OPW_10[1310, 990, 850]; OPW_12[730]; OPW_13[1210, 1090, 940]; OPW_1[1580, 960, 580];
V. plicatum 'Sterile'	OPA_1[1140]; OPA_2[410, 310]; OPA_4[810]; OPG_14[1110, 980, 580, 360]; OPJ_01[900, 680, 550]; OPJ_13[640]; OPM_01[1690, 850]; OPM_02[510]; OPM_03[1090, 830, 660]; OPM_05[760, 490]; OPM_12[1280, 980]; OPW_01[700, 420]; OPW_02[970, 850, 800]; OPW_03[1490, 1250, 1110, 970]; OPW_05[810]; OPW_07[1180, 840]; OPW_08[780]; OPW_09[1030, 610]; OPW_10[670, 340, 240]; OPW_12[670, 570, 280]; OPW_13[640, 470]; OPW_1[1240];
V. plicatum f. *tomentosum*	OPA_2[1900, 1430, 980]; OPA_3[1400, 740]; OPA_4[1180, 620, 390]; OPJ_13[610]; OPM_01[1020, 310]; OPM_02[1990, 1050, 890]; OPM_03[540, 470]; OPM_12[1820, 720]; OPW_02[920]; OPW_05[740, 500]; OPW_07[1320]; OPW_08[1220]; OPW_09[2000, 470]; OPW_12[1650, 1150, 880]; OPW_1[1150];
V. plicatum 'Watanabe'	OPA_1[1420, 940, 350]; OPA_3[930]; OPA_4[1240, 850]; OPG_14[1220, 930, 810]; OPJ_01[970, 630]; OPJ_13[1140, 790, 700, 410]; OPM_02[540]; OPM_03[1390, 550]; OPM_12[430]; OPW_01[1120]; OPW_02[1220]; OPW_03[680]; OPW_05[330]; OPW_07[1300, 670]; OPW_08[740]; OPW_09[1010]; OPW_10[840]; OPW_12[1010, 850, 410]; OPW_13[1300, 230]; OPW_1[1420, 1060, 760, 570, 270];

polymorphic ISSR products (26 and 24) and their length ranged from 2650 to 440 bp.

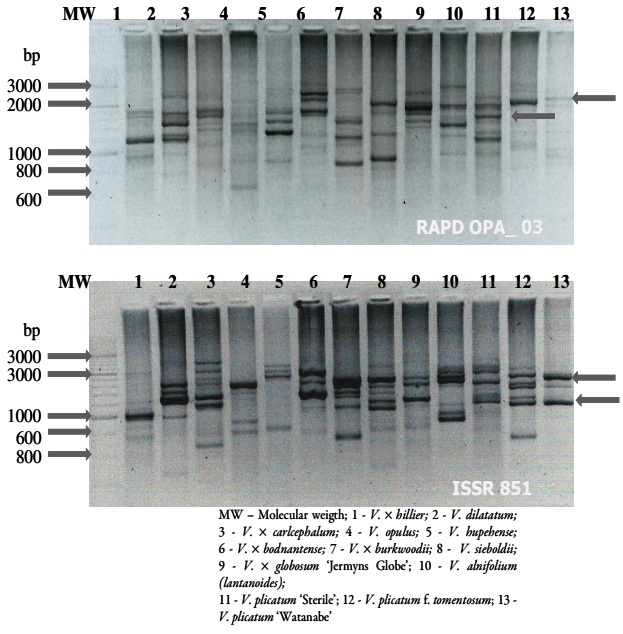
Genotype-specific loci

Analysis of electropherograms of the examined *Viburnum* genotypes described 186 (44.5%) loci as accession-specific (Tab. 1). The most loci (20 and 18) were amplified in reactions with primers: 837, 818 and 863 (Tab. 3). The least loci (8) were noted for *V. opulus* species. Analysis of the sequence of primers used for DNA amplification of the examined *Viburnum* forms revealed that the highest number of genotype-specific ISSR loci (20) was amplified with primer 837 [$(AC)_8YG$], while the lowest number (6) was obtained with the use of primer 834 [$(AG_8GT$].

Polymorphic loci

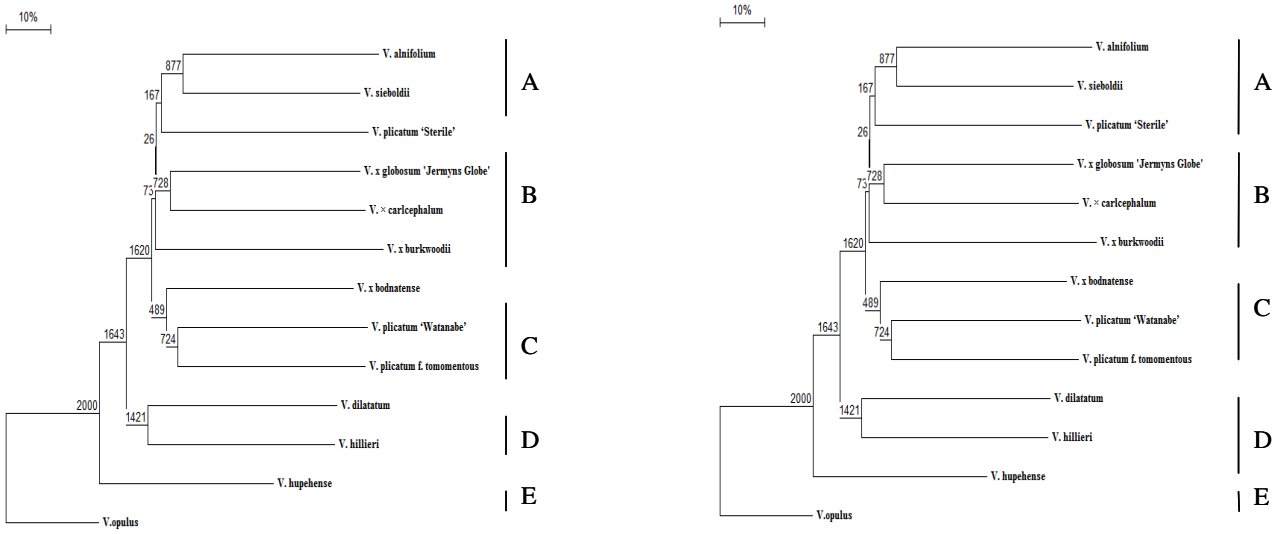
A relatively wide range of variability was observed between the examined *Viburnum* objects (Fig. 1), resulting from the presence of PCR products, determined as polymorphic (55.5%), in electropherograms. Reactions with primers 818, 848 and 851 generated the highest number of polymorphic ISSR products (26 and 24) and their length ranged from 2650 to 440 bp.

Genotype-specific loci



MW – Molecular weigth; 1 - *V.* × *hillier;* 2 - *V. dilatatum;* 3 - *V.* × *carlcephalum;* 4 - *V. opulus;* 5 - *V. hupehense;* 6 - *V.* × *bodnantense;* 7 - *V.* × *burkwoodii;* 8 - *V. sieboldii;* 9 - *V.* × *globosum* 'Jermyns Globe'; 10 - *V. alnifolium (lantanoides);* 11 - *V. plicatum* 'Sterile'; 12 - *V. plicatum* f. *tomentosum*; 13 - *V. plicatum* 'Watanabe'

Fig. 1. PCR amplification profiles of 13 *Viburnum* genotypes. (a) RAPD primer no. OPA_03, and (b) ISSR primer no. 851



$r_{(RAPD\ ISSR)}$ = 0.305* p-value (two tailed) = 0.015

Fig. 2. Dendrograms generated using unweighted pair group method with arithmetic average analysis, showing relationship between *Viburnum* genotypes, using RAPD a) and ISSR b) data. The number at the forks indicate the confidence limits for the grouping of those species in a branch occurred, based on 2.000 pseudoreplications in bootstrap analysis

Analysis of electropherograms of the examined *Viburnum* genotypes described 186 (44.5%) loci as accession-specific (Tab. 1). The most loci (20 and 18) were amplified in reactions with primers: 837, 818 and 863 (Tab. 3). The least loci (8) were noted for *V. opulus* species. Analysis of the sequence of primers used for DNA amplification of the examined *Viburnum* forms revealed that the highest number of genotype-specific ISSR loci (20) was amplified with primer 837 [$(AC)_8YG$], while the lowest number (6) was obtained with the use of primer 834 [$(AG_8GT$].

Phylogenetic analysis

On the basis of the genetic profiles obtained with the RAPD and ISSR techniques, respectively two dendrograms of phylogenetic similarity were generated for the thirteen examined *Viburnum* genotypes (Fig. 2a, b). Genetic similarity assessed between the accessions of *Viburnum* with the RAPD and ISSR analyses ranged from 6 to 42% and from 6 to 31%, respectively. The thirteen examined genotypes of *Viburnum* were grouped in five clusters according to both RAPD and ISSR data. Clustering of genotypes within groups was similar when RAPD- and ISSR- derived dendrograms were compared. *V. dilatatum*, *V. hillieri* and also *V. plicatum* 'Watanabe' and *V. plicatum* f. *tomentosum* were clustered in similar groups when RAPD- and ISSR- derived dendrograms were compared. The correlation between the matrices of cophenetic correlation values for the dendrogram based on RAPD and ISSR data was low (r=0.305*), yet significant (p-value = 0.015).

Discussion

Molecular markers have been widely used to evaluate genetic variability within and between species for several

Tab. 3. Genotype-specific ISSR products generated for 13 genotypes of *Viburnum*

Genotype	Primers and genotype-specific ISSR products
V. × *hillieri*	816 [600]; 818 [1400]; 821 [2690]; 827 [880, 720]; 837 [1000, 890, 460]; 845 [720]; 851 [1210]; 863 [860]
V. dilatatum	802 [1560, 640, 550]; 815 [1210, 570]; 816 [1360, 960, 800]; 818 [2650, 1130]; 821 [320]; 827 [1750]; 834 [2550, 1180]; 837 [1110, 700, 610, 320]; 851 [440]; 863 [1330, 270]
V. × *carlcephalum*	802 [690]; 815 [800]; 816 [770]; 817 [1210]; 818 [1430, 1250]; 821 [930]; 827 [690, 590, 410]; 845 [1900, 1320, 700]; 848 [1110, 840]; 851 [680]; 863 [740];
V. opulus	802 [450]; 809 [630]; 815 [930]; 818 [610]; 834 [790]; 845 [550]; 848 [640]; 851 [810];
V. hupehense	802 [1000, 620]; 809 [1100, 850, 760]; 815 [1590, 600]; 818 [1020]; 827 [330]; 837 [560]; 851 [2290, 830]; 863 [1100];
V. × *bodnantense*	817 [1460, 1120]; 821 [2460]; 827 [370]; 837 [1640, 1130, 660, 290]; 845 [1600]; 848 [2970]; 851 [2580, 1950];
V. × *burkwoodii*	802 [1240]; 809 [530, 260]; 816 [370]; 817 [700, 590]; 818 [700]; 821 [550, 410, 340]; 827 [1670, 1350, 280]; 848 [1600, 850]; 851 [750]; 863 [420, 230];
V. sieboldii	802 [1410]; 809 [450]; 815 [340]; 816 [720]; 818 [1870, 990, 760]; 827 [740]; 834 [430]; 845 [1260]; 848 [1720]; 851 [460]; 863 [730];
V. × *globosum* 'Jermyns Globe'	802 [460]; 809 [540, 490, 400]; 815 [620]; 816 [280]; 818 [980, 870, 420]; 837 [1540, 450]; 851 [2850];
V. alnifolium (lantanoides)	802 [740]; 815 [670]; 818 [520, 450]; 821 [720]; 827 [2450, 1640, 1440]; 837 [1820, 820]; 848 [2600, 1590]; 863 [660];
V. plicatum 'Sterile'	809 [2940, 2490, 700]; 815 [1470]; 816 [2890, 1980, 1670, 1040, 940, 680]; 818 [570]; 821 [1710, 510, 430]; 827 [2320]; 837 [540]; 848 [1140]; 863 [3130, 2120, 1720, 1090, 990, 540];
V. plicatum f. *tomentosum*	815 [310]; 816 [460]; 817 [2830, 1870, 1250, 750]; 818 [910]; 821 [740]; 827 [560]; 834 [1350, 450]; 837 [1290, 1160, 620]; 851 [710]; 863 [520];
V. plicatum 'Watanabe'	817 [1710, 1000, 920]; 821 [1250]; 827 [790]; 848 [2090, 1000]; 863 [1210, 650];

Tab. 4. Similarity matrix of 13 *Viburnum* genotypes based on RAPD and ISSR markers

ISSR \ RAPD	1	2	3	4	5	6	7	8	9	10	11	12	13
V. × *hillieri*	**1.00**	0.42	0.29	0.22	0.22	0.20	0.11	0.09	0.15	0.10	0.06	0.09	0.06
V. dilatatum	0.28	**1.00**	0.28	0.23	0.27	0.23	0.15	0.06	0.12	0.07	0.06	0.08	0.11
V. × *carlcephalum*	0.18	0.17	**1.00**	0.31	0.34	0.20	0.07	0.11	0.13	0.10	0.15	0.07	0.12
V. opulus	0.17	0.19	0.20	**1.00**	0.34	0.29	0.17	0.15	0.17	0.07	0.12	0.10	0.07
V. hupehense	0.17	0.26	0.16	0.31	**1.00**	0.41	0.24	0.12	0.14	0.17	0.11	0.11	0.10
V. × *bodnantense*	0.15	0.18	0.22	0.13	0.20	**1.00**	0.25	0.11	0.13	0.10	0.09	0.13	0.08
V. × *burkwoodii*	0.12	0.12	0.17	0.12	0.06	0.20	**1.00**	0.21	0.12	0.11	0.13	0.14	0.11
V. sieboldii	0.11	0.08	0.22	0.12	0.18	0.21	0.17	**1.00**	0.15	0.21	0.20	0.06	0.21
V. ×*globosum* 'Jermyns Globe'	0.10	0.11	0.26	0.13	0.15	0.18	0.15	0.17	**1.00**	0.21	0.18	0.10	0.07
V. alnifolium	0.15	0.09	0.10	0.10	0.12	0.16	0.13	0.28	0.24	**1.00**	0.11	0.17	0.19
V. plicatum 'Sterile'	0.09	0.16	0.17	0.09	0.16	0.17	0.16	0.18	0.23	0.23	**1.00**	0.16	0.16
V. plicatum f. *tomentosum*	0.16	0.11	0.15	0.10	0.13	0.24	0.08	0.27	0.23	0.21	0.18	**1.00**	0.20
V. plicatum 'Watanabe'	0.18	0.11	0.12	0.13	0.16	0.28	0.14	0.26	0.18	0.13	0.17	0.27	**1.00**

reasons, such as delimitation of species, conservation of endangered species, analysis of the population' structure of a species and construction of phylogenetic relationships among species in fungi, plants and animals (Yüzbaşioğlu *et al.,* 2011). The invention of PCR led to the development of fast and inexpensive molecular markers, such as RAPD and ISSR (Arseniuk 2000; Czembor and Sharma *et al*., 2009; Gajera *et al*., 2011; Godwin *et al*., 1997; Williams *et al*., 1990; Yüzbaşioğlu *et al*., 2011). Because of the longer primers used, ISSR markers reported to be more reproducible than RAPD markers (Godwin *et al*., 1997). Since both of them are PCR-based methods, they require only small amounts of template DNA. Furthermore, they do not require any sequence information for primer construction. The main limitation associated with the RAPD and ISSR method is their dominant inheritance. The second main drawback of RAPDs is low reproducibility due to their sensitiveness to reaction conditions. However, they produce repeatable band profiles when reaction conditions are optimized and kept constant (Gillings and Holley 1997, Godwin *et al*., 1997; Kumar *et al*., 2009; Williams *et al*., 1990).

There are few reports on morphological and genetic characterization of *Viburnum* genus, difficult in systematization, in the literature on the subject matter (Donoghue *et al*., 2004). The first study on the subject included the analysis of phenotypic and phenological traits (Donoghue *et al*., 2004). Another studies used methods based on molecular analysis of DNA variability (Ackerly and Donoghue 1998; Grimm *et al*., 2006; Pfosser *et al*., 2002; Suh *et al*., 2000). Techniques describing the variability of restriction-digested cDNA, as well as analysis of DNA sequences (Donoghue and Sytsma 1993; Donoghue and Baldwin 1993; Donoghue *et al*., 2004; Winkworth and Donoghue 2004, 2005) were also studied. Then an attempt was made to describe the direction and scale of migration, development and origin of species (Donoghue and Baldwin 1993; Donoghue *et al*., 2004, Winkworth and Donoghue 2004). In broad terms, the results of the above-presented studies have agreed on the monophyly and non-monophyly of traditionally recognized taxonomic sections, on relationships between several sections, and on species relationships within several section-level clades. However, more accurate understanding of phylogenetic relationships within *Viburnum* species requires further research on a considerably greater amount of material, as well as supplementation of the research with the use of other available molecular techniques.

In the author's research, genotypic variability of *Viburnum* × *hillieri, V. dilatatum*, *Viburnum* × *carlcephalum, V. opulus, V. hupehense, Viburnum* × *bodnantense, Viburnum* × *burkwoodii, V. sieboldii, Viburnum* × *globosum* 'Jermyns Globe', *V. alnifolium*, *V. plicatum* 'Sterile', *V. plicatum* f. *tomentosum* and *V. plicatum*

'Watanabe' was assessed with 14 ISSR and 23 RAPD primers. As a result of the amplifications with the use of the examined *Viburnum* genotypes, 1160 (RAPD) and 873 (ISSR) amplicons were scored as the effect of amplification of 690 RAPD and 418 ISSR loci, respectively. The length of the amplified products corresponded to the number of products amplified, the number of primers used, and the length of products mostly generated with these methods and presented in papers by Sharma *et al*. (2009), Williams *et al*. (1990), Yüzbaşioğlu *et al*. (2011) and Zietkiewicz *et al*. (1994).

We demonstrated high genetic variability among the examined *Viburnum* objects. It was manifested by a great number of products – both polymorphic (39% for RAPD and 55.5% for ISSR) and genotype-specific (60.9% for RAPD and 44.5% for ISSR) in electropherograms. In only one case a monomorphic product was obtained, present in electrophoretic images of all the examined genotypes, generated in amplification of RAPD primer OPM_05. The results prove that the Dendrological Garden in Przelewice is a rich collection of both cultivated and botanical accessions, as well as hybrids of *Viburnum* species of different origin. Similar results were observed for several other species, such as black gram (Souframanien and Gopalakrishna 2004), where the percentage of polymorphism ranged from 16.6 to 66.6%; sesame (Sharma *et al*., 2009), where 56.18 and 66.78% of polymorphic bands were amplified by both RAPD and ISSR primers, and *Lobathallia radiosa* (Yüzbaşioğlu *et al*., 2011), where all the obtained bands were polymorphic.

A high level of genetic polymorphism of *Viburnum* species was confirmed in a study by Donoghue *et al*. (2004) and Winkworth and Donoghue (2005), where analysis of ITS sequences and chloroplast trnK intron with the PCR technique, as well as the analysis of sequences of GBSSI genes, were used for examination of evolutionary relations between different species and accessions of *Viburnum*, migration, development and origin of species. On the basis of the obtained results, Donoghue *et al*. (2004) identified three major supra-sectional groups. The largest of these lineages consisted of four subclades, i.e. two directly corresponding to traditional sections *Opulus* (circum-boreal) and *Tinus* (Eurasia); one containing *Oreinotinus* section (Latin America) plus the purple-fruited New World members of *Odontotinus* section, and one consisting of predominantly red-fruited Old World *Odontotinus* species, purple-fruited New World *Viburnum acerifolium* species, and *Viburnum cylindricum* of *Megalotinus* section (Asia). The second supra-sectional lineage included *Viburnum plicatum* of *Tomentosa* section (Asia) and the clade containing most representatives of *Solenotinus* section (Asia). In the third supra-sectional lineage, the clade corresponding to *Pseudotinus* section (North America and Asia) was sister to the one containing *Lentago* section (North America) and the core group of species from *Viburnum* section (Eurasia).

In our study, the RAPD- and ISSR- based dendrograms clustered *Viburnum* accessions in five main groups. Comparison of the two dendrograms showed that only *V. dilatatum* and *V. hillieri* and also *V. plicatum* 'Watanabe' and *V. plicatum* f. *tomentosum* belong to the same cluster. However, similarity observed among *Viburnum* genotypes on the basis of the RAPD (42%) and ISSR (31%) analysis was low. This evaluated diversity was sufficient to distinguish *Viburnum* genotypes individually with specific bands and banding patterns. The correlation coefficient between the RAPD and ISSR analysis was low (r=0.305*), yet statistically significant, which confirms that it was possible to describe a similar range of variability with the use of both the RAPD and ISSR technique. What is more, the low value of the correlation coefficient with a high level of significance might be due to the fact that PCR-amplified profiles in the two marker assay originated from different repetitive and non-repetitive regions of the genomes, and/or background noise due to non-homologous co-migrating band amplification, and/or error in scoring complex banding patterns, which could have possibly influenced the results.

Our results showed great genetic variability among the genotypes of *Viburnum* obtained from the Dendrological Garden in Przelewice. RAPD and ISSR of the two-marker system applied in the present study were used as effective tools for evaluating genetic similarity and selecting the core collection to enhance efficiency of germplasm management for use in *Viburnum* breeding and conservation.

Conclusions

Twenty-three RAPD primers produced a total of 690 loci, of which 271 (39%) were polymorphic and 418 (60.9%) – genotype-specific. The ISSR analysis produced 418 loci, of which 232 (55.5%) were polymorphic and 186 (44.5%) – genotype-specific. The UPGMA clustering of genotypes was similar when RAPD- and ISSR- derived dendrograms were compared. Genetic similarity between the examined objects ranged from 6 to 42% for RAPD matrices, and from 6 to 31% for ISSR matrices. The Mantel test between two similarity matrices of the markers revealed low, but significant, correlation (r = 0.305*). At the same time, significance of the coefficient suggests that it is possible to describe the same range of variability with the use of both the RAPD and ISSR technique. Randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers are useful tools to detect DNA polymorphism among thirteen *Viburnum* species of wide geographical distribution obtained from the Dendrological Garden in Przelewice.

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