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ORIGINAL PAPER

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ASSESSMENT OF GENETIC VARIABILITY AMONG RASPBERRY ACCESSIONS USING MOLECULAR MARKERS

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ABSTRACT

In this study, random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) loci were used to investigate the genetic relationships in a group of 22 raspberry accessions. Fifteen RAPD primers generated a total of 324 bands, among them 94.1% were polymorphic. From ten used SSR pairs of primers, nine generated only polymorphic bands and the average percentage of polymorphism was 97.8%. Genetic similarity indices calculated on the basis of RAPD and SSR data indicated a wide range of genetic variability of the analyzed raspberry collection. Cluster analysis by UPGMA (Unweighted Pair-Group Method with Arithmetic averaging) and PCA (Principal Component Analysis) clearly delineated the genetic relationships among all the accessions. The highest genetic similarity, determined on the basis of RAPD and SSR markers, was found between two Polish cultivars – 'Polesie' and 'Polesie Żółte', whilst 'Jewel' from USA, belonging to *Rubus occidentalis*, was found to be the cultivar that varied most from all the accessions. The obtained results confirmed the usability of RAPD and SSR markers for discriminating among closely related raspberries and for determining the genetic variability among cultivars. It might be helpful for breeders to plan their breeding strategy.

Key words: genetic similarity, raspberry, *Rubus,* RAPD, SSR

INTRODUCTION

Raspberries belong to the genus *Rubus* that is one of the most diverse genera in the plant kingdom due to the number of species and ploidy level. Raspberries are diploid (2n = 2x = 14) in nature with very small genome (275 Mbp) [Graham et al. 2007]. Members of the genus can be difficult to classify into distinct species for a number of reasons including hybridization between species and apomixes. Raspberry cultivars are propagated clonally from their roots or *in vitro* using meristem cultures [Graham and Jennings 2009]. Raspberries are grown in many countries in the world and the interest in that species is currently increased mainly due to the fruits that are good source of antioxidants, such as ascorbic acid, anthocyanins, catechins, flavonols and flavones. Growing interest in raspberries has resulted in increased production and increased outlays on breeding programs for new varieties. The European red raspberry *Rubus idaeus* subsp. *idaeus* and the North American red raspberry *R. idaeus* subsp. *strigosus* Michx, as well as black raspberry *R. occidentalis* L. are the most important domesticated plants and those that are used in breeding programs [Graham and Jennings 2009]. Domestication has reduced the morphological and genetic variability of that species and for breeding the narrow pool of the ancestry cultivars

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has been used. The consequence is that modern cultivars show high similarity. To improve the efficiency of crossing, breeders need a detailed information on the genetic relationships among germplasms. Due to a reduction in morphological diversity, progress in raspberry breeding requires the use of molecular tools for germplasm assessment. The RAPD is a useful and powerful technique for various polymorphism analyses including genetic diversity, identification of cultivars and phylogenetic analysis of many plant species [Khanuja et al. 2000, Besnard et al. 2001, Lisek and Rozpara 2010, Okoń et al. 2014, Gawroński et al. 2017]. Some studies using RAPD markers in *Rubus* have also been reported [Parent et al. 1993, Graham et al. 1994, Graham and McNicol 1995, Graham et al. 1997, Parent and Page 1998, Antonius-Klemola 1999, Badjakov et al. 2006, Stafne et al. 2003, Patamsyte et al. 2010, Umar et al. 2010]. SSR loci are highly polymorphic co-dominant markers also useful for studying the genetic diversity and genetic fingerprinting of many species. SSR markers used for red raspberry and blackberry genome analysis have been developed from expressed sequence tag (EST) and genomic libraries of both species [Graham et al. 2004, Marulanda et al. 2007, Fernandez-Fernandez et al. 2011, Dosset et al. 2012, Marulanda et al. 2012].

The aim of this study was to investigate genetic variability among 22 raspberry cultivars representing different geographical regions: USA, UK, Switzerland, Italy and Poland. The information generated from this study can be used by breeders to optimize selection of diverse parents and broaden the germplasm base in the raspberry breeding programs.

MATERIAL AND METHODS

Plant materials

Twenty two accessions of raspberry were used in our study (Tab. 1). They were cultivars representing different geographical origin. All the accessions are included in the germplasm collection of NIWA Berry Breeding Ltd., Poland.

DNA isolation and molecular analysis

The youngest leaves of each accession were ground in liquid nitrogen using mortar and pestle, and 100 mg of the obtained tissue powder was transferred into 1.5 ml Eppendorf tube. DNA was isolated using Genomic Mini AX Plant Kit (AA Biotechnology), following the manufacture instructions. The DNA quantity and quality were assessed on the basis of the A_{260} measurement (spectrophotometer NanoDrop 2000c) and standard agarose electrophoresis. The analysis of genetic distance was performed with 15 RAPD primers and 10 SSR primer pairs. The following RAPD primers were used: OPA-01 – CAGGCCCTTC, OPA-02 – TGCCGAGCTG, OPA-03 – AGTCAGCCAC, OPA-10 – GTGATCGCAG, OPA-11 – CAATCGCCGT, OPA-13 – CAGCACCCAC, OPA-19 – CAAACGTCGG, OPB-07 – GGTGACGCAG, OPB-11 – GTAGACCCGT, OPR-01 – TGCGGGTCCT, OPR-02 – CACAGCTGCC, OPR-03 – ACACAGAGGG, OPR-04 – CCCGTAGCAC, OPR-05 – GACCTAGTGG, OPR-06 – GTCTACGGCA. The information about the SSR markers is given in Tab. 2. Amplification with both types of primers was performed in the final volume of 20 µl with 5 ng of genomic DNA. For RAPD, the reaction mixture contained 2 mM $MgCl_2$, 0.25 mM dNTP Mix, 1 µM primer, 1 U *Taq* DNA polymerase in 1X buffer (Fermentas). The PCR was performed according to the following parameters: 94°C for 3 min, 40 cycles of 93°C for 30 s, 42°C for 1 min, 68°C for 2.5 min and 72°C for 5 min. PCR products were separated by electrophoresis in 1% agarose gel and visualized by ethidium bromide. Amplification with SSR primers was performed with 2 mM $MgCl_2$, 0.2 mM dNTP Mix, 0.3 µM each primer, 1 U *Taq* DNA polymerase in 1X reaction buffer (Fermentas). This PCR was performed according to the following parameters: 94°C for 4 min, 10 or 7 cycles of 94°C for 30 s, 65°C (-1 °C/cycle) for 30 s, 72°C for 1 min, 35 cycles of 94°C for 15 s, 55 or 58°C for 30 s, 72°C for 1 min and 72°C for 5 min. PCR products were separated by electrophoresis in 2% agarose gel and visualized by ethidium bromide.

Table 1. Plant material used in this study

Cultivar	Taxon	Origin	Parents	Fruit color
Heritage	*Rubus idaeus* L.	USA	(Milton × Cuthbert) × Durham	red
Jewel	*Rubus occidentalis* L.	USA	*N.Y.29773 × Dundee*	*black*
Radiance	*Rubus idaeus* L.	USA	PS-1616 × PS-1703	red
A	*Rubus idaeus* L.	UK	no access	red
Glen Ample	*Rubus idaeus* L.	UK	EU Application 2729	deep-red
Imara	*Rubus idaeus* L.	UK	unknown pedigree	orange-red
Kwanza	*Rubus idaeus* L.	UK	unknown pedigree	orange-red
Kweli	*Rubus idaeus* L.	UK	EU PVR Application 37396	orange-red
Amira	*Rubus idaeus* L.	Italy	U.S. Plant Patent #22,459 (Polka × Tulameen)	red
Erika	*Rubus idaeus* L.	Switzerland	‘Autumn Bliss’ (op)	orange-red
Sugana	*Rubus idaeus* L.	Switzerland	‘Autumn Bliss’ × ‘Tulameen’	bright-red
Benefis	*Rubus idaeus* L.	Poland	‘Beskid’ × ‘Tulameen’	red
Laszka	*Rubus idaeus* L.	Poland	80408 × 80192	red
Poemat	*Rubus idaeus* L.	Poland	Polka × 03226	deep-red
Polana	*Rubus idaeus* L.	Poland	‘Heritage’ × Zeva Herbsternte	red
Polesie	*Rubus idaeus* L.	Poland	86594 × 87342	red
Polesie Żółte	*Rubus idaeus* L.	Poland	‘Polesie’ (op)	yellow
Polka	*Rubus idaeus* L.	Poland	89141 (op)	red
Polonez	*Rubus idaeus* L.	Poland	86031 × 93561	light-red
Poranna Rosa	*Rubus idaeus* L.	Poland	83291 × ORUS 1098-1	yellow
Radziejowa	*Rubus idaeus* L.	Poland	92271 × 96221	red
Sokolica	*Rubus idaeus* L.	Poland	96131 × 96221	red

A – anonymous, op – open pollination

Table 2. SSR markers used for studying the genetic variability among raspberry cultivars

Primer names	Primer sequences 5'→3'	Amplified motif	Annealing temperatures (°C)*	Source
RhCBA23-f / r	ATCGGGGATTTGGTGTGGGTTTAGG / ATTGTGTGCATCACTCTGAGAACCG	$(GA)_{10}(GA)_5$	65/58	Lopes et al. 2006
RhM003-f / r	CCATCTCCAATTCAGTTCTTCC / AGCAGAATCGGTTCTTACAAGC	$(TG)_{10}$	65/58	Castillo et al. 2010
Rub1C6-f / r	TCTGCCTCTGCATTTTACACAG / GTTTAGGTAAGCAATGGGAAAGCTC	$(CT)_{15}$	65/58	Dossett et al. 2010
RhME0013bG01-f / r	CCCTCCATCTCCACCATAAA / GTAAGGCCACCCCATTGAG	$(GA)_{38}$	65/58	Lewers et al. 2008
RhME0013cE02-f / r	AGGGTGGGTCTGAGATTGTG / AACAGTGCACAGGGGCTAAT	$(TA)_8$	65/58	Lewers et al. 2008
Rh_ME0015cH02-f / r	TGGATTTCCACACGCACATA / TGTTGGATTTGCCTCCTTTC	$(TC)_9$	65/55	Lewers et al. 2008
RhME0013cF08-f / r	TTTGTCTCCGTCTTTTTGCC / CCTCCGAAGAAAAACAGCAG	$(TC)_{15}$	65/55	Lewers et al. 2008
Rh_ME0007aB01-f / r	TGGTGGTTCACCGTTCACTA / GAAATGCTTGAAGCCGAGAG	$(CT)_{15}$	65/55	Lewers et al. 2008
mRaCIRRI1G3-f / r	CTCTACAAAAGGATCTGCATGA / CAGCAAAAGTGAAATGGTTCA	$(GA)_{28}$	65/55	Amsellem et al. 2001
mRaCIRRIV2A8-f / r	TAAAAAGGCGCAACAGTCG / AGACACAGAAACAGGCATCG	$(CA)_{12}(CT)_{11}$	65/55	Amsellem et al. 2001

* maximal/minimal (touchdown PCR)

Data analysis

All tested RAPD primers produced informative electrophoretic profiles across the tested accessions and therefore all these primers were used for further analysis. Each amplification was performed in duplicate to control band regularity. The presence and absence of bands were scored as 1 or 0, respectively. Bands with the same migration distance were considered monomorphic. The level of polymorphism (polymorphic bands/total bands) and polymorphic information content (PIC = 2fi(1-fi), where fi is the frequency of ith bands present) [Anderson et al. 1993] were calculated. For the analysis of genetic relationships among the studied accessions, the Jaccard similarity coefficient was used. Dendrograms were generated using the Unweighted Pair-Group Method with Arithmetic averaging (UPGMA) and Principal Component Analysis (PCA), with 1000 permutations of bootstrapping using *PAST* software.

RESULTS AND DISCUSSION

Efficiency of polymorphism assessment

Identification of raspberry cultivars has been traditionally carried out by morphological, agronomical and chemical traits. These phenotypic characters only partially reflect the heritable genetic variability, due to the modifying impact of the environment on growth and development. Recognizing the limitation of studies on morphological variability, molecular techniques have been developed. All the accessions tested in our studies in general could be distinguished using RAPD and SSR markers. The percentages of polymorphism found on the basis of the RAPD and SSR marker analysis were 94.1 and 97.8%, respectively. It is in agreement with results of Fernández et al. [2008], who also found higher level of polymorphism for SSR markers compared with RAPD markers used for genetic characterization of raspberry cultivars. We used 15 RAPD primers that generated a total of 324 bands of which 307 were polymorphic. The number of total bands produced by each primer ranged from 14 to 31; consequently, polymorphism varied among the primers used. The highest ratio of polymorphism (100%) was generated by seven primers (OPA-01, OPA-03, OPA-10, OPA-19, OPB-11, OPR-03, OPR-05), while the primer OPA-02 yielded the lowest polymorphism, amounting to 76% (Tab. 3). The 10 SSR primer pairs used in the study generated a total of 60 bands of which 58 were polymorphic. The range of bands produced by each primer combination varied from 2 (Rh_ME0015cH02-f/r) to 11 (mRaCIRRIV2A8-f/r). Only one primer pair generated monomorphic bands (Rh_ME0013cE02-f/r) (Tab. 3). This indicates that both types of molecular markers are powerful techniques that can be used for differentiation of raspberry cultivars. There are some reports concerning the use of RAPD markers in the analysis of *Rubus* populations. Badjakov et al. [2006] identified 87.5% of polymorphic bands using only four arbitrary primers. Also Patamsyte et al. [2010] generated more than 80% of polymorphic bands in the analysis of genetic structure of red raspberry populations from Lithuania. High level of polymorphism in wild and cultivated *Rubus* species was also detected by Marulanda et al. [2007]. Using SSR markers, these authors identified approximately 20% of variation among populations and 80% within populations. SSR markers were also used by Lamoureux et al. [2011] to investigate the genetic diversity among Russian raspberry collections. In turn, Dossett et al. [2012] used SSR primers for discrimination of wild and cultivated black raspberries.

Genetic diversity analysis

In our studies, dendrograms derived from UPGMA cluster analysis based on the Jaccard genetic similarity coefficient matrix were obtained separately for the RAPD and SSR data. Both analytical approaches displayed a high correspondence to each other. The correlations between the RAPD and SSR variation patterns were already reported [Fernandez et al. 2008, Gawroński et al. 2017]. In our studies, in both dendrograms, grouping according to affinity and the region of origin were observed. However, Dossett et al. [2012] on the basis of neighbor-joining (NJ) dendrogram constructed on SSR data, showed lack of grouping based on geographical location.

The cluster analysis, prepared on the basis of the RAPD data, clearly separated all the tested accessions and the bootstrap values (n = 1000) supported significantly their locations. The USA cultivar 'Jewel' was placed outside the main cluster. However, within the main cluster, also one cultivar was excluded from the remaining accessions – it was 'Radziejowa'. Among the main cluster, two groups of accessions were identified. In Group A, two subgroups were found. Subgroup A1 was divided into two clads and outside them 'Polonez" was classified. In Clad A1-1, only two UK cultivars – A and 'Glen Ample' – were grouped, whilst most of the tested cultivars were grouped into Clad A1-2. In that clad, two Subclads can be distinguished. In Subclad A1-2-1, the most similar 'Imara', 'Kweli' as well as 'Poemat', 'Kwanza', and 'Radiance' were classified. On the other hand, Subclad A1-2-2 contained the most similar 'Amira' and 'Sugana' as well as 'Eryka', 'Benefis' – in which 'Tulameen or 'Autumn Bliss' were used as one of the parental forms and 'Laszka'. Subgroup A2 also can be divided into two clads. In a Clad A2-1, three Polish cultivars were grouped – the most similar ' Polesie' and 'Polesie Żółte' as well

Table 3. Number of bands, number of polymorphic bands, polymorphism percentage and PIC values of 22 raspberry accessions generated by 15 RAPD primers and 10 SSR primer pairs

Primer/s	Number of total bands	Number of polymorphic bands	Polymorphism (%)	PIC
		RAPD		
OPA-01	25	25	100	0.49
OPA-02	17	13	76	0.50
OPA-03	14	14	100	0.50
OPA-10	29	29	100	0.33
OPA-11	20	19	95	0.47
OPA-13	24	23	96	0.46
OPA-19	31	31	100	0.40
OPB-07	17	15	88	0.49
OPB-11	17	17	100	0.39
OPR-01	31	28	90	0.45
OPR-02	27	26	96	0.48
OPR-03	18	18	100	0.47
OPR-04	15	13	87	0.49
OPR-05	20	20	100	0.42
OPR-06	19	16	84	0.47
Total	324	307	–	6.81
Average/primer	21.6	20.47	94.1	0.45
Average/genotype	14.73	13.95	–	0.31
		SSR		
RhCBA23-f / r	6	6	100	0.44
RhM003-f / r	3	3	100	0.46
Rub1C6-f / r	4	4	100	0.41
RhME0013bG01-f / r	5	5	100	0.35
R hME0013cE02-f / r	9	7	78	0.49
RhME0015cH02-f / r	2	2	100	0.50
RhME0013cF08-f / r	7	7	100	0.41
RhME0007aB01-f / r	8	8	100	0.32
mRaCIRRI1G3-f / r	5	5	100	0.43
mRaCIRRIV2A8-f / r	11	11	100	0.31
Total	60	58	–	4.12
Average/primer	6	5.8	97.8	0.41
Average/genotype	2.73	2.64	–	0.19

as 'Poranna Rosa'. In the second Clad A2-2, only 'Polana' and 'Sokolica' were found. As regards to Group B, it consisted of only 'Heritage' and 'Polka' (Fig. 1). The PCA analysis in most cases confirmed the distribution of accessions produced by the UPGMA analysis. The cultivars 'Jewel' and 'Radziejowa' were clearly distinguished from other studied accessions (Fig. 2). Also Stafne *et al.* [2003] and Umar *et al.* [2010] discriminated raspberry cultivars using RAPDs. RAPD markers were also suitable for the genetic structure analysis of *R. idaeus* accessions from different agroclimatic regions of Lithuania [Patamsyte *et al.* 2010]. This method was also used for studying the genetic diversity between *Rubus* species [Graham and McNicol, 1995] and between wild populations and cultivated clones of *R. idaeus* [Graham et al. 1997].

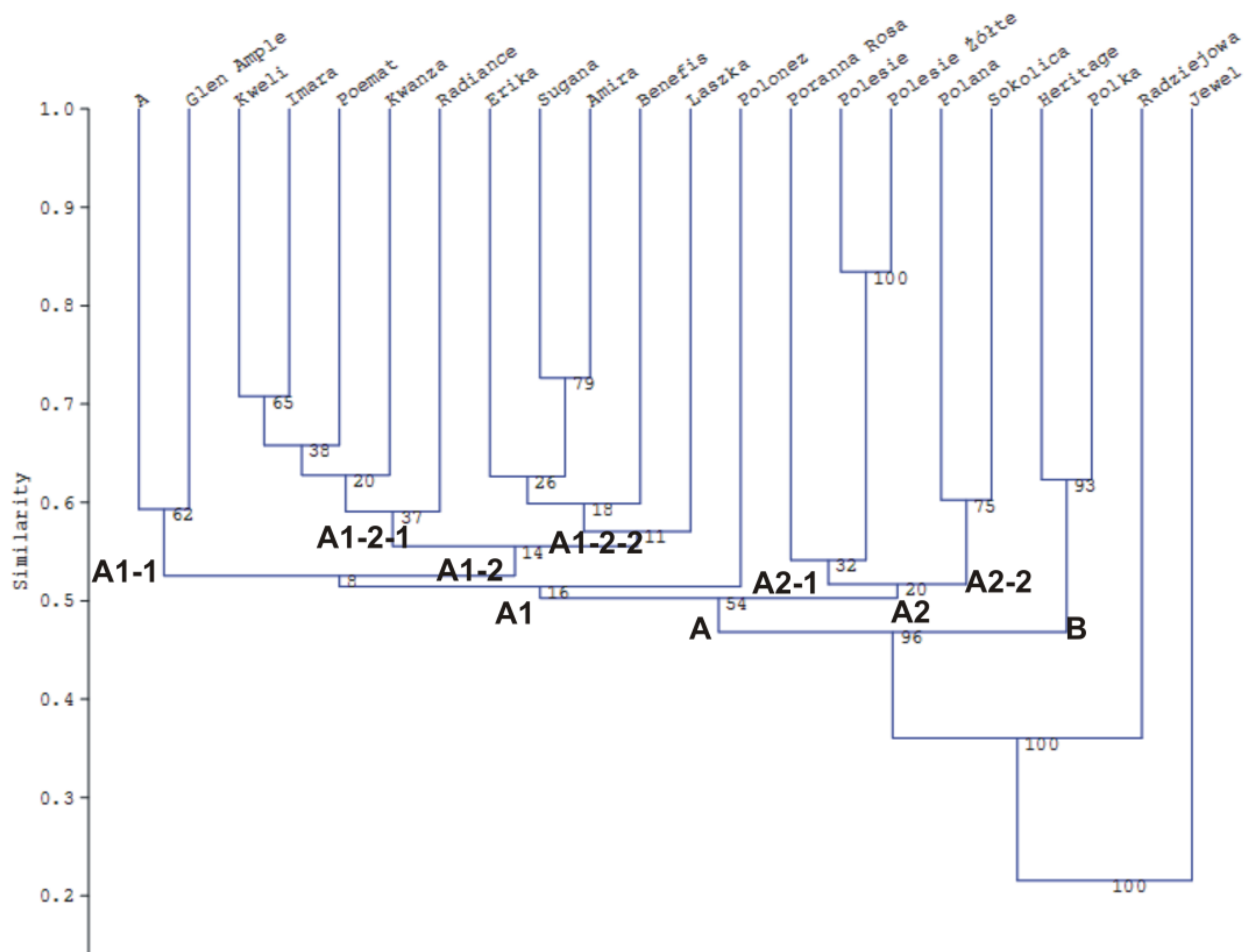

Fig. 1. Dendrogram of studied raspberry cultivars constructed with the Jaccard similarity coefficient using UPGMA cluster analysis based on the RAPD data (cophenetic correlation r = 0.93)

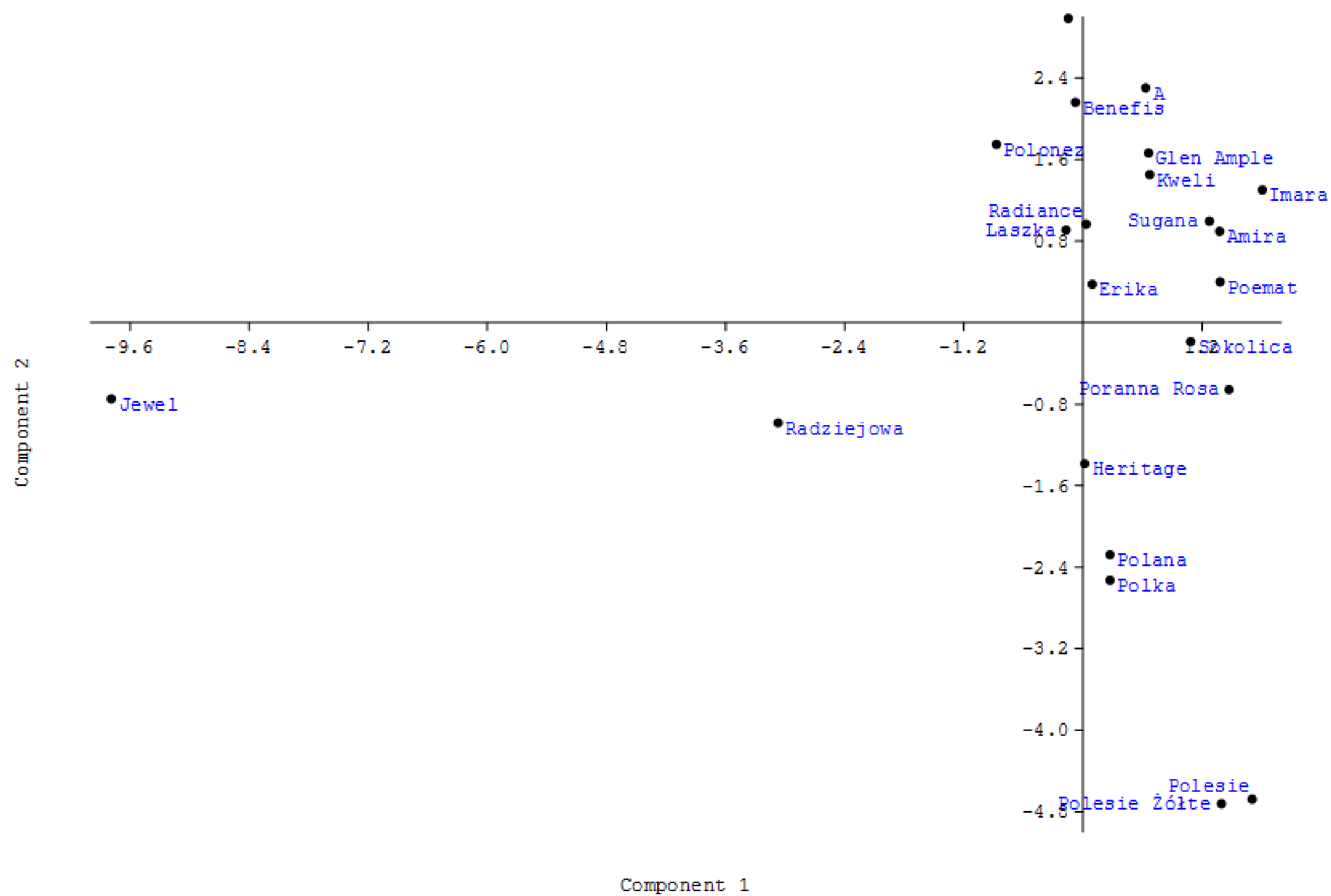

Fig. 2. Principal component analysis (PCA) of studied raspberry cultivars based on the RAPD data (the first and the second axes explained 13.8 and 10.2% of variance, respectively)

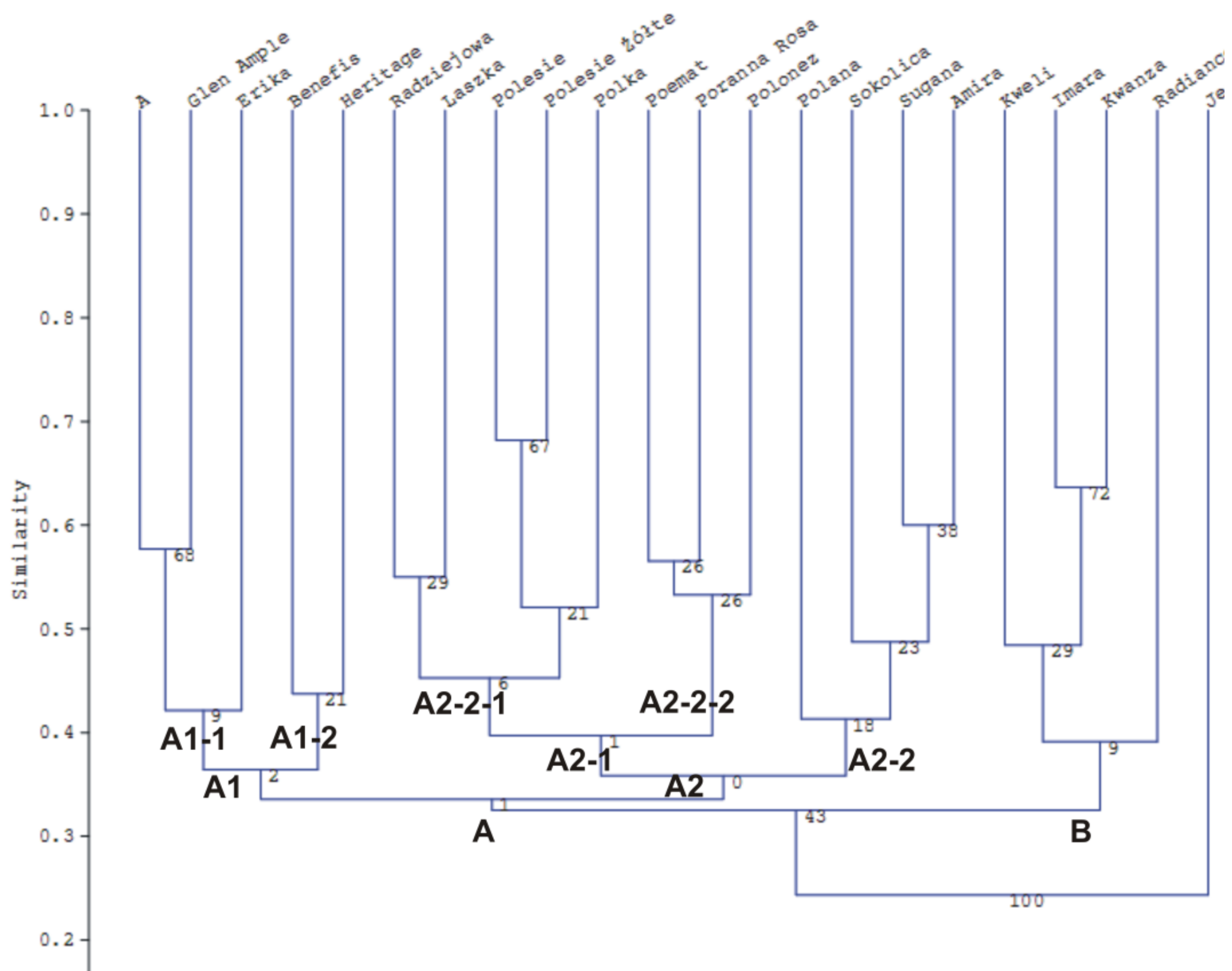

Fig. 3. Dendrogram of studied raspberry cultivars constructed with the Jaccard similarity coefficient using UPGMA cluster analysis based on the SSR data (cophenetic correlation r = 0.71)

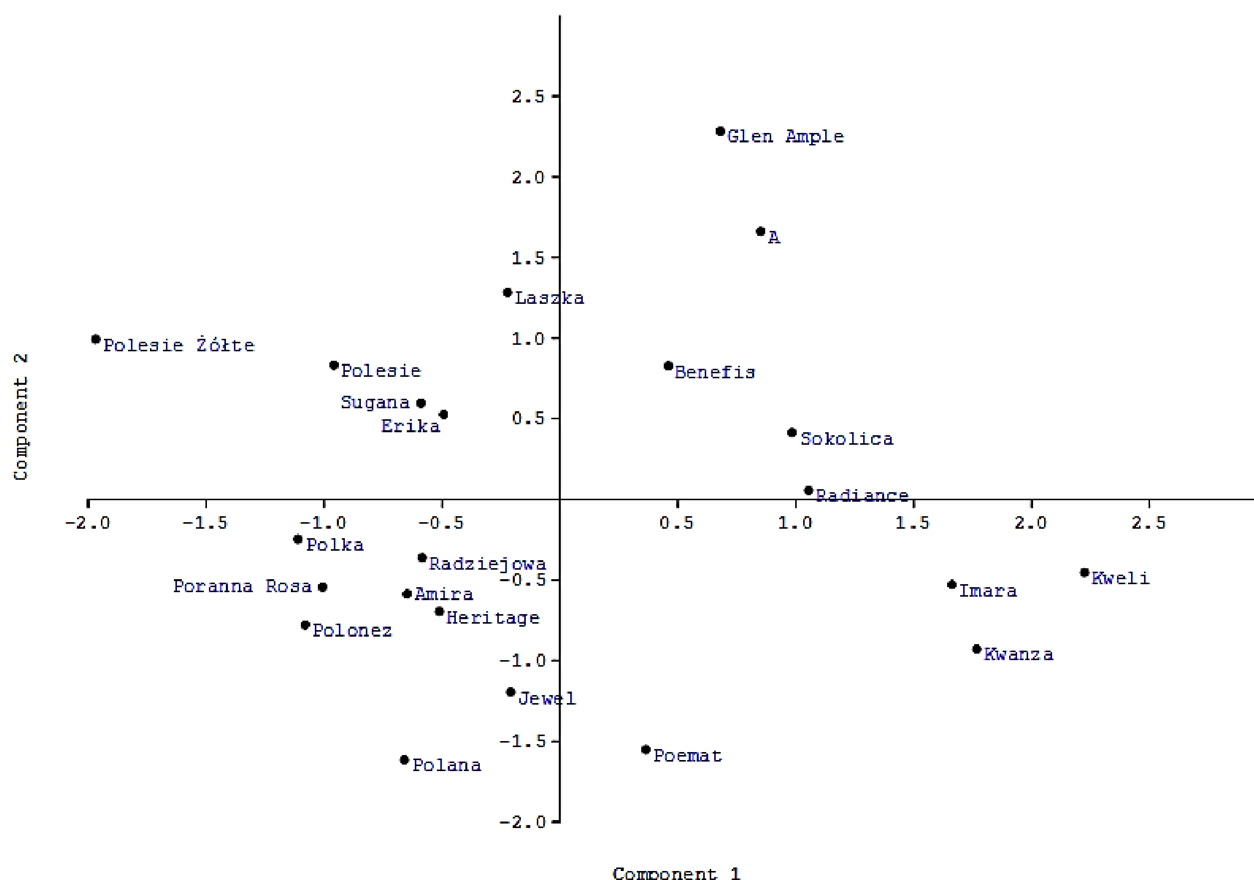

Fig. 4. Principal component analysis (PCA) of studied raspberry cultivars based on the SSR data (the first and the second axes explained 14.4 and 13.1% of variance, respectively)

The dendrogram based on Jaccard's coefficient and the UPGMA clustering method obtained for SSR data showed one main cluster and one outside accession, which was 'Jewel' – the USA cultivar. The main cluster was divided into two groups – A and B. In Group A, two subgroups were identified. In the Subgroup A1, two clads were separated. In Clad A1-1, cultivar A with 'Glen Ample', as well as 'Eryka' were identified. Among the accessions, which clustered into Clad A1-2, 'Benefis' and 'Heritage' were grouped. In the second Subgroup A2, also two clads were distinguished. Clad A2-1 contained two subclads. Subclad A2-2-1 including five Polish cultivars, among them 'Polesie' and 'Polesie Żółte' was the most similar. Other Polish cultivars formed Subclade A2-2-2. In Clad A2-2, 'Amira' and 'Sugana', as well as 'Sokolica' and 'Polana' were grouped. As regards to Group B, it consisted of one of the most similar accessions – 'Imara' and 'Kwanza', as well as 'Kweli' and 'Radiance' (Fig. 3). Principal component analysis supports some of the clusters already observed in the dendrogram. 'Heritage' that clustered with the Polish cultivars (Fig. 3) was also grouped with them on the negative side of this axis. Generally, the distribution of the accessions based on PCA analysis was in most cases similar to that prepared by the UPGMA method (Fig. 4). The one exception is cultivar Jewel, which was not separated using PCA based on SSRs. Already, Dosset et al. [2012] reported the problem with identification of 'Jewel' using SSR

markers. Likely, a different set of SSRs is needed for differentiation of 'Jewel' from the remaining accessions.'

CONCLUSIONS

1. Based on genetic distances, the grouping of cultivars in most cases reflects the pedigree relations between cultivars and sites of origin.

2. Analyzed cultivars characterized high genetic variability, where 'Jewel' was the most distinct from others cultivars, while among 'Polesie' and 'Polesie Żółte' the highest similarity was estimated – and these two cultivars were closely located on the obtained dendrograms.

3. The presented study has demonstrated that the RAPD and SSR markers may provide useful information on the level of polymorphism and diversity in raspberry cultivars, indicating their suitability for the characterization of even closely related genotypes.

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REFERENCES

Anderson, J.A., Churchill, G.A., Autrique, J.E., Sorells, M.E., Tansley, S.D. (1993). Optimizing parental selection for genetic-linkage maps. Genome, 36, 181–186.

Amsellem, L., Dutech, C., Billotte N. (2001). Isolation and characterization of polymorphic microsatellite loci in *Rubus alceifolius* Poir. (*Rosaceae*), an invasive weed in La Réunion island. Mol. Ecol. Notes, 1, 33–35.

Antonius-Klemola, K. (1999). Molecular markers in Rubus (*Rosaceae*) research and breeding. J. Hortic. Sci. Biotechnol., 74, 149–160.

Badjakov, I., Todorovska, E., Kondakova, V., Boicheva, R., Atanassov, A. (2006). Assessment the genetic diversity of Bulgarian raspberry germlpasm collection by microsatellite and RAPD markers. J. Fruit Ornam. Plant Res., 14 (Suppl. 1), 61–76.

Besnard, G., Breton, C., Baradat, P., Khadari, B., Bervillé, A. (2001). Cultivar identification in olive based on RAPD markers. J. Am. Soc. Hort. Sci., 126, 668–675.

Castillo, N., Reed, B., Graham, J., Fernandez-Fernandez, F., Bassil, N.V. (2010). Microsatellite markers for raspberry and blackberry. J. Am. Soc. Hort. Sci., 135, 271–278.

Dossett, M., Bassil, N.V., Finn, C.E. (2010). Transferability of *Rubus* microsatellite markers to black raspberry. Acta Hortic., 859, 103–106.

Dossett, M., Bassil, N.V., Lewers, K.S., Finn, C.E. (2012). Genetic diversity in wild and cultivated black raspberry (*Rubus occidentalis* L.) evaluated by simple sequence repeat markers. Genet. Resour. Crop. Evol., 59, 1849–1865.

Fernández, MP., Hernáiz, S., Ibáñez, J. 2008. Genetic characterization of raspberry cultivars using molecular markers. Acta Hortic., 777, 125–132.

Fernandez-Fernandez, F., Antanaviciute, L., Govan, C.L., Sargent, D.J. (2011). Development of a multiplexed microsatellite set for fingerprinting red raspberry (*Rubus ideaus*) germplasm and its transferability to other *Rubus* species. J. Berry Res., 1, 177–187.

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

Graham, J., Hein, I., Powell, W. (2007). Raspberry. In: Genome mapping and molecular breeding in plants, Kole, C. (ed.). Springer, Berlin–Heidelberg, 4, 207–216..

Graham, J., Jennings, N. (2009). Raspberry breeding. In: Breeding plantation tree crops: temperate species. Springer, New York.

Graham, J., McNicol, R.J. (1995). An examination of the ability of RAPD markers to determine the relationships within and between *Rubus* species. Theor. Appl. Genet., 90, 1128–1132.

Graham, J., McNicol, R.J., Greig, K. Van de Ven, W.T.G. (1994). Identification of red raspberry cultivars and an assessment of their relatedness using fingerprints produced by random primers. J. Hortic. Sci., 69, 123–130.

Graham, J., Smith, K., Mackenzie, K., Jorgensen, L., Hackett, C.A., Powell, W. (2004). The construction of a genetic linkage map of red raspberry (*Rubus idaeus* subsp. *idaeus*) based on AFLPs, genomic-SSR and EST-SSR markers. Theor. Appl. Genet., 109, 740–749.

Graham, J., Squire, G.R., Marshall, B., Harrison, R.E. (1997). Spatially dependent genetic diversity within and between colonies of wild raspberry *Rubus idaeus* detected using RAPD markers. Mol. Ecol., 6, 1001–1008.

Khanuja, S., Shasany, A., Srivastava, A. Kumar, S. (2000). Assessment of genetic relationships in Mentha species. Euphytica, 111, 121–125.

Lamoureux, D., Sorokin, A., Lefèvre, I., Alexanian, S., Eyzaguirre, P., Hausman, J.-F. (2011). Investigation of genetic diversity in Russian collections of raspberry and blue honeysuckle. Plant Genet. Res., 9, 202–205.

Lewers, K.S., Saski, C.A., Cuthbertson, B.J., Henry, D.C., Staton, M.E., Main, D.S., Dhanaraj, A.L., Rowland, L.J., Tomkins, J.P. (2008). A blackberry (*Rubus* L.) expressed sequence tag library for the development of simple sequence repeat markers. BMC Plant Biol., 8, 9–76.

Lisek, A., Rozpara, E. (2010). Identification of pear cultivars with RAPD and ISSR markers. J. Fruit Ornam. Plant Res., 18, 17–22.

Lopes, M.S., Belo Maciel, B., Menconca, D., Sabino, G.F., Da Camara Machado, A. (2006). Isolation and characterization of simple sequence repeat loci in *Rubus hochstetterorum* and their use in other species from the Rosaceae family. Mol. Ecol. Notes, 6, 750–752.

Marulanda, M.L., López, A.M., Aguilar, S.B. (2007). Genetic diversity of wild and cultivated *Rubu*s species in Colombia using AFLP and SSR markers. Crop Breed. Appl. Biot., 7, 242–252.

Marulanda, M., López, A.M., Uribe, M. (2012). Molecular characterization of the Andean blackberry, *Rubus glaucus*, using SSR markers. Genet. Mol. Res., 11, 322–331.

Okoń, S., Paczos-Grzęda, E., Łoboda, M., Sugier, D. (2014). Identification of genetic diversity among *Arnica montana* L. genotypes using RAPD markers. Acta Sci. Pol. Hortorum Cultus 13, 63–71.

Parent, J.-G., Fortin, M.G., Page, Â.D. (1993). Identification of raspberry cultivars by random amplified polymorphic DNA (RAPD) analysis. Can. J. Plant Sci., 73, 1115–1122.

Parent, J.-G., Page, D. (1998). Identification of raspberry cultivars by sequence characterized amplified region DNA analysis. HortScience, 33, 140–142.

Patamsytė, J., Kleizaitė, V., Čėsnienė, T., Rančelis, V., Žvingila, D. (2010). The genetic structure of red raspberry (*Rubus idaeus* L.) populations in Lithuania. Cent. Eur. J. Biol., 5, 496–506.

Stafne, E.T., Clark, J.R., Pelto, M.C., Lindstrom, J.T. (2003). Discrimination of *Rubus* cultivars using RAPD markers and pedigree analysis. Acta Hortic. 626, 119–124.

Umar, G., Vasanthaiah, H.K., Kambiranda, D., Basha, S.M., Phills, B.R., Hunter, W. (2010). Assessment of genetic diversity among selected raspberry cultivars. Proc. Fla. State Hort. Sci., 123, 26–28.