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PHENOTYPIC AND GENOTYPIC VARIABILITY OF CULTIVARS OF HIGHBUSH BLUEBERRY (*Vaccinium corymbosum* L.) GROWN IN THE LUBLIN REGION

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Abstract. During the years 2012 to 2014 some research connected with highbush blueberry was taken. It described phenotypic and genotypic variability of 19 cultivars of highbush blueberries grown in the Lublin region. The cultivars included in the study were: 'Bluecrop', 'Bluejay', 'Blueray', 'Bonifacy', 'Bonus', 'Brigitta Blue', 'Chandler', 'Chanticleer', 'Croatan', 'Darrow', 'Duke', 'Earliblue', 'Herbert', 'Jersey', 'Northland', 'Nelson', 'Patriot', 'Toro' and 'Spartan'. The flowering of highbush blueberry can be observed from the end of April to the beginning of June. Meanwhile, ripening depended on cultivar, starting from June 29th and finishing by September 7th. The strongest vegetative growth was characteristic of 'Bluecrop' cultivar on the oldest plantation – Niemce, whereas on the Spiczyn 1 – 'Darrow' cultivar, and 'Patriot' proved to be the best yielding cultivar on the youngest plantation. In the study, berries of the 'Chandler' cultivar were the largest, and berries of the 'Northland' cultivar were considered to be the smallest. To evaluate the tested cultivars at the DNA level RAPD markers were used. The set of 9 analysing primers generated a total of 91 fragments of which 81 (89%) were polymorphic. The average genetic similarity determined on the basis of the similarity matrix of RAPD markers was 0.41. Application of the UPGMA method for grouping varieties showed the highest distinction of cultivars: 'Croatan', 'Chanticleer', 'Herbert' and 'Brigitta Blue', in relation to the others. Among the tested cultivars genetic variation was detected since genetic similarity ranged from 0.22 to 0.60. Nevertheless the same cultivars grown in different locations demonstrated genetic identity.

Key words: flowering and ripening season, fruit weight, genetic diversity, RAPD markers, yield

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INTRODUCTION

The name highbush blueberry refers to the growth type of shrub, which is characterized by a straight or slightly bent shoots 1.5, 1.8–2.0, 4.0 m in length [Ballington 2005, Retamales and Hancock 2012]. Originally, varieties of this species came from *V. corymbosum* L. and were grown in the northern states of the USA. Incorporation of genes from species *V. anguslifolium* Aiton, *V. ashei* Reade, *V. constablei* Grey, *V. darrowi* Camp, *V. pallidum* Aiton *V. tenellum* Aiton into its gene pool resulted in a significant extension of variability range of breeding materials and made it possible to produce new cultivars [Hancock 2006 a, Hancock 2006 b, Lyrene 2008, Ballington 2009, Mainland 2012]. However, not all of them are suitable for cultivation in Polish conditions [Smolarz 2006, Pluta and Żurawicz 2010, Koziński 2013]. The practical value of the cultivar results from the interaction between the genotype and environment, supporting the necessity to test cultivars in the expected areas of cultivation [Finn et al. 2003, McCallum et al. 2012]. In our country, the first small experimental plantation was established in 1971, and the assessment of the economic value of the cultivars of American origin began in 1978, although the blueberry was first imported from the USA as early as in 1924 [Pliszka 2002, Smolarz et al. 2006, Retamales and Hancock, 2012]. The results of the first experiment taken in the years 1996–2000 confirmed the usefulness of climatic and soil conditions in the Lublin region to grow highbush blueberry [Wach 2008, Kęsik and Wach 2010]. At present, Polish production on plantations occupy an area approximately 3200 ha and they are among the largest in Europe [Karwowski 2012]. Currently, the list of the Community Plant Variety Office includes 349 registered cultivars of those cultivated species in European Union [CPVO 2015]. They are characterized by diversity of morphological traits, blooming and ripening time, disease and pest resistance, fruit weight and quality, mechanical handling tolerance, climatic adaptation, etc. Accurate differentiation between cultivars can be made on the basis of their morphological, physiological and biochemical characteristics but such assessments are influenced by environmental conditions [Debnath 2007]. Therefore, RAPD markers (Randomly Amplified Polymorphic DNA) may be used in this case, which have been confirmed as useful for species assessment, parentage investigation and genetic relationship of several plant species including blueberries [Arce-Johnson et al. 2002, Burgher et al. 2002, Giongo et al. 2006, Garriga et al. 2013, Naugzemys et al. 2013]. The advantage of this method is no necessity for prior knowledge of the DNA sequence, are relatively easy to apply and inexpensive as compared to others [Quililongo et al. 2013].

The objective of the study was to distinguish the blueberry cultivars at both the molecular and phenotypic level and to assess their genetic diversity in order to characterise better the *Vaccinium* genetic resources presented in the Lublin region. Furthermore, the estimation of genetic identity connected with plants from different localisation was included in the study.

MATERIAL AND METHODS

Plant material and location. The study was conducted on 3 plantations of highbush blueberry connected with 19 cultivars. The origin of cultivars and features are described mainly by Pliszka [2004], Boches et al. [2006], Smolarz [2009], Retamales and Hancock [2012], Garriga et al. [2013]. The oldest plantation in Niemce near Lublin (N 51°20'; E 22°37') was established in 1993 ('Bluecrop', 'Bluejay', 'Blueray', 'Croatan', 'Darrow', 'Herbert', 'Jersey', 'Northland' and 'Spartan'; there was the name of cultivar + N on the dendrogram). The highbush blueberries were planted at 2 × 1 m spacing, on a soil developed from weakly loamy sand. The rows of plants were mulched with a layer of sawdust, and sward was maintained in the inter-rows. The plantation was irrigated.

Other two experiments were conducted on a plantation in Spiczyn (N 51°19'; E 22°37'); Spiczyn – 1 in 2008: ('Bluecrop', 'Bluejay', 'Brigitta Blue', 'Chandler', 'Darrow', 'Duke', 'Earliblue', 'Nelson', 'Toro' and 'Spartan'; there was the name of cultivar + S1 on the dendrogram), and Spiczyn 2 in 2010 ('Bluecrop', 'Bonifacy', 'Bonus', 'Chandler', 'Chanticleer', 'Darrow', 'Duke', 'Earliblue', 'Patriot', 'Toro' and 'Spartan'; there was the name of the cultivar + S2 on the dendrogram). The highbush blueberry was planted at 3.3 × 1 m spacing, on a soil developed from less that is considered to be weak wheaten complexes (soil of bonitation class IIIb and IVa). The plantation was mulched and irrigated.

Field observations. The study on the flowering, ripening and yielding of highbush blueberry was conducted in years 2012–2014. The first open flowers on the plants was considered to be the beginning of the flowering, while the last flowers meant the end of the flowering. The first harvest was strictly connected with the beginning of berry ripening, because it began one week after the process when fruits change their colour in the cluster. The term of the last harvest is at the same time the end of highbush blueberry fruiting. There was Faedi index used in order to define earliness of the cultivars (it specified number of days from 1 January until the time when 50% of the crop has been collected). The yielding of highbush blueberry was expressed as the average yield of berries from the bush. Berry weight was computed as the average of 100 chosen fruits from each term of the harvest. The results were statistically processed by means of variance analysis method and Tukeys test was evaluated at $P \leq 0.05$, separately for each localisation. Moreover, dendrogram that used average linkage method was created.

DNA extraction. Fresh young leaves from 10 plants of each cultivar were collected in two replicates from each plantation and kept in liquid nitrogen until extraction. DNA was extracted following the CTAB method described by Doyle and Doyle [1987].

RAPD analysis. DNA amplifications were performed in a gradient thermal cycler (Professional Basic Gradient Biometra GmbH) at a final volume of 15 µl for each reaction, which contained 10 mM Tris pH 8.8, 50 mM KCl, 0.08% Nonidet P40, 200 µM of each deoxyribonucleotide triphosphate (dNTP), 0.3 µl of oligonucleotide primer (10 µM), 1.5 mM $MgCl_2$, 0.5 U of Taq DNA Polymerase (Fermentas) and 40 ng template DNA. In the PCR reactions, the samples were initially subjected to 94°C for 4 min

and then 44 cycles of amplification. Each cycle involved the following steps: 94°C for 1 min, 1 min at 36°C and 2 min at 72°C of amplification. After the 44 cycles, the samples were kept at 72°C for 7 min, for a final extension step. The primers used in this experiment were tested two times on the same sample in order to check reproducibility. Amplification products were separated by electrophoresis on 1.5% agarose gels containing 0.1% EtBr (Ethidium bromide) under a 1 × TBE buffer that was run at 100 mV for at least 3 h. The fragments were visualized under UV transilluminator and photographed using GeneSnap ver. 7.09 (SynGene). Marker GeneRuler^{TM} DNA Ladder Mix (MBI Fermentas) was used to determine the size of the DNA fragments.

DNA data analysis. RAPD products were scored as present (1) or absent (0) from the photographs. Only bright and reproducible products were scored. DNA fragments detected in not all cultivars profiles were considered as polymorphic. Genetic pairwise similarities (SI-similarity index) between studied genotypes were evaluated according to Jaccard's coefficient [Sneath and Sokal 1973]. A cluster analysis was conducted using the distance method UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) in the program Past [Hammer et al. 2001].

RESULTS AND DISCUSSION

According to Rejman and Pliszka [1991], highbush blueberry flowering in Polish conditions begins mostly in mid-May and it stands 3 or 4 weeks. Some studies have shown that flowering could start earlier, even in April [Kropp and Kałoń 1986, Chlebowska and Smolarz 1997, 2003], and from the half of May in the Olsztyn region [Kawecki and Kopytowski 1991].

The terms of the highbush blueberry flowering in the years 2012–2014 are given in Table 1. The 'Northland' cultivar in Niemce as well as 'Duke', 'Earliblue' and 'Patriot' in Spiczyn first started flowering. Flowering could be even until 3 June in Niemce ('Darrow', 'Jersey'). The results of the observation connected with the terms of the flowering confirm earlier studies taken in the Lublin region that flowering could depend on the cultivar and year of research and usually falls between the end of April and the beginning of June [Wach 2008, Kęsik and Wach 2010, Wach 2010, 2012].

The terms of the highbush blueberry ripening in the years 2012–2014 are given in Table 1. 'Chanticleer' berries started to ripen as first (29 June), the next were 'Earliblue' and 'Duke' berries (2 July) cultivated in Spiczyn. 'Darrow' berries ripened as last (25 July in Niemce) whereas the first yield of 'Chandler' fruits was taken as a last (6 August). The harvest of fruits finished at the earliest 15 July ('Chanticleer' – Spiczyn 2) until 7 September ('Darrow' – Spiczyn 1). In recent literature, some information can be found that berries ripening in the Skierniewice region depended on the year of study (28 June concerning 'Duke'), and it could vary even 2 weeks [Chlebowska and Smolarz 2003].

Table 1. Time of flowering and fruit ripening of 19 cultivars of highbush blueberry (average in the years of research)

	Cultivar	Time of flowering		Time of fruit ripenning	
		beginning	end	beginning	end
Niemce	Bluecrop	1 V–10 V	21 V–27 V	17 VII–20 VII	20 VIII–6 IX
	Bluejay	30 IV–10 V	21 V–27 V	10 VII–18 VII	8 VIII–13 VIII
	Blueray	2 V–12 V	21 V–28 V	18 VII–21 VII	14 VIII–18 VIII
	Croatan	5 V–12 V	23 V–30 V	17 VII–20 VII	4 VIII–14 VIII
	Darrow	5 V–3 V	26 V–3 VI	25 VII–1 VIII	25 VIII–6 IX
	Herbert	11 V–14 V	24 V–2 VI	21 VII–25 VII	20 VIII–25 VIII
	Jersey	11 V–14 V	26 V–3 VI	25 VII–28 VII	30 VIII–4 IX
	Northland	28 IV–9 V	20 V– 25 V	9 VII–16 VII	10 VIII–13 VIII
	Spartan	30 IV–9 V	21 V–25 V	9 VII–17 VII	10 VIII–20 VIII
Spiczyn – 1	Bluecrop	5 V–11 V	23 V–30 V	15 VII–18 VII	10 VIII–19 VIII
	Bluejay	1 V–10 V	21 V–30 V	9 VII–10 VII	31 VII–6 VIII
	Brigitta Blue	6 V–12 V	25 V–29 V	17 VII–31 VII	6 VIII–19 VIII
	Chandler	6 V–12 V	23 V–1 VI	29 VII–6 VIII	29 VIII–30 VIII
	Darrow	6 V–13 V	25 V–1 VI	30 VII–2 VIII	22 VIII – 7 IX
	Duke	27 IV–10 V	20 V–30 V	2 VII–7 VII	24 VII–12 VIII
	Earliblue	27 IV–8 V	20 V–29 V	2 VII–5 VII	25 VII–1 VIII
	Nelson	6 V–13 V	23 V–1 VI	20 VII–31 VII	19 VIII–23 VIII
	Toro	5 V–12 V	26 V–30 V	11 VII–20 VII	6 VIII–11 VIII
	Spartan	28 IV–9 V	20 V–28 V	6 VII–10 VII	25 VII–12 VIII
Spiczyn – 2	Bluecrop	5 V–11 V	23 V–30 V	15 VII–18 VII	3 VIII–10 VIII
	Bonifacy	8 V–12 V	21 V–28 V	17 VII–20 VII	30 VII–1 VIII
	Bonus	6 V–12 V	22 V–30 V	25 VII–26 VII	5 VIII–8 VIII
	Chandler	6 V–12 V	23 V–1 VI	29 VII–5 VIII	14 VIII–18 VIII
	Chanticleer	28 IV–5 V	19 V–24 V	29 VI–1 VII	15 VII–22 VII
	Darrow	6 V–13 V	25 V–1 VI	28 VII–2 VIII	10 VIII–14 VIII
	Duke	27 IV–10 V	20 V–30 V	2 VII–7 VII	24 VII–25 VII
	Earliblue	27 IV–8 V	20 V–29 V	2 VII–5 VII	16 VII–20 VII
	Patriot	27 IV–9 IV	18 V–25 V	9 VII–12 VII	27 VII–28 VII
	Toro	5 V–12 V	26 V–30 V	11 VII–20 VII	28 VII–3 VIII
	Spartan	28 IV–9 V	20 V–28 V	6 VII–10 VII	20 VII–24 VII

Highbush blueberry is a fertile plant [Smolarz 2000]. The yield of young plants is rather small but when it is the time of fruiting, we can obtain more than 7 kg berries from bush [Chlebowska and Smolarz 2003, Smolarz 2003, Koziński 2006, Smolarz et al. 2006, Glonek and Komosa 2013]. In the years 2012–2014 a study on the yielding of highbush blueberry was connected with the age of plantation and varied in cultivars (tab. 2). The yields of highbush blueberry on the plantation in Niemce varied from 3.30 kg bush^{-1} (‘Blueray’) to 4.39 kg bush^{-1} (‘Bluecrop’). In earlier studies, ‘Bluecrop’ was considered to be also a prolific plant [Wach 2008]. The yields of highbush blueberry on the plantation in Spiczyn 1 varied from 1.40 kg bush^{-1} (‘Brigitta Blue’) to 2.57 kg bush^{-1} (‘Darrow’). The large yielding was characteristic of ‘Darrow’, which is in the

agreement with other literature data [Wach 2012]. The youngest planting of highbush blueberry (Spiczyn – 2) consisted of bushes that started fruiting. Under the conditions of the experiment the yields varied from 0.43 ('Chandler') to 1.32 kg bush^{-1} ('Patriot').

Table 2. Yield (kg·bush^{-1}), berry weight (g) and Faedi Index of 19 cultivars of highbush blueberry (average 2012–2014)

	Cultivar	Yield (kg·bush^{-1})	Berry weight (g)	Faedi Index
Niemce	Bluecrop	4.39a*	1.65c	217b
	Bluejay	3.73b–d	1.54c	202d
	Blueray	3.30e	1.60c	217b
	Croatan	3.41de	1.65c	214c
	Darrow	3.96b	2.27a	228a
	Herbert	3.77bc	2.16b	229a
	Jersey	3.73b–d	1.42d	229a
	Northland	3.91bc	1.37d	199e
	Spartan	3.59c–e	1.61c	199e
Spiczyn – 1	Bluecrop	2.42ab	1.59d	211bc
	Bluejay	2.27ab	1.58d	203cd
	Brigitta Blue	1.40b	1.64d	216ab
	Chandler	1.95ab	2.45a	222ab
	Darrow	2.57a	2.20b	223a
	Duke	2.38ab	1.57d	198d
	Earliblue	2.33ab	1.54d	199d
	Nelson	2.22ab	1.78c	219ab
	Spartan	2.06ab	1.43e	199d
	Toro	2.17ab	1.81c	205cd
Spiczyn – 2	Bluecrop	0.93a–c	1.79c–e	203b
	Bonifacy	0.57bc	1.84c	203b
	Bonus	0.61a–c	2.08b	206b
	Chandler	0.43c	3.10a	213a
	Chanticleer	1.15ab	1.70de	190cd
	Darrow	0.46c	3.06a	214a
	Duke	1.23ab	1.68de	193cd
	Earliblue	0.94a–c	1.61e	195cd
	Patriot	1.32a	1.78cd	196c
	Spartan	0.64a–c	1.60e	189d
	Toro	0.82a–c	1.79cd	203b

* – means in the column for each plantations folllowed by the same letter do not significantly differ at $p < 0.05$

The weight of highbush blueberry fruit depends on the cultivar, technology as well as yielding [Siefker and Hancock 1986, Glonek and Komosa 2006]. Different cultivars of highbush blueberry varied in weight of a single fruit (tab. 2). The study showed that the largest berries were characteristic of 'Chandler' (2.45–3.1 g). The next decreasing order of hierarchy of cultivar in terms of that feature included 'Darrow' with fruits var-

ied from 2.2 g to 3.06 g. According to Siefker and Hancock [1986], the fruit weight is correlated negatively with yield, and for this reason differences in the fruit weight resulted from the size of the yield. The smallest fruits in the experiment were produced by 'Northland' (1.37 g), 'Jersey' (1.42 g) and 'Spartan' (1.43 g). The results of this experiment are in agreement with all literature data [Nelson 1985, Smolarz 1997, 2003, Tamada 1997, Chlebowska and Smolarz 2003, Koziński 2006, Smolarz et al. 2006, Wach 2008, 2010, 2012, Kęsik and Wach 2010]. Nevertheless, Paprstein and Ludvikowa [2006] obtained fruits 2.4 g produced by 'Spartan' cultivar, 'Duke' –2.34 g, 'Croatan' – 2.58 g, 'Brigitta Blue' – 1.98 g, whereas 'Darrow' berries only 1.85 g.

It can be observed that Faedi index was associated with earliness of the flowering and the term of the ripening (tab. 2). On the plantation in Niemce Faedi index averaged from 199 to 229 ('Northland', 'Spartan' – 'Jersey', 'Herbert'); in Spiczyn 1 from 198 ('Duke') to 223 ('Darrow'), and in Spiczyn 2 from 189 ('Spartan') to 214 ('Darrow'). Earliness of a ripening presentation by using Faedi index in most cases corresponded with a bonitation mark of this feature. With regard to concentrated ripening of berries on young plants and with low yields, lower value of Faedi index can be expected (the same date connected with the beginning of ripening, e.g. 'Bluecrop', 'Chandler' in Spiczyn and different values of Faedi index).

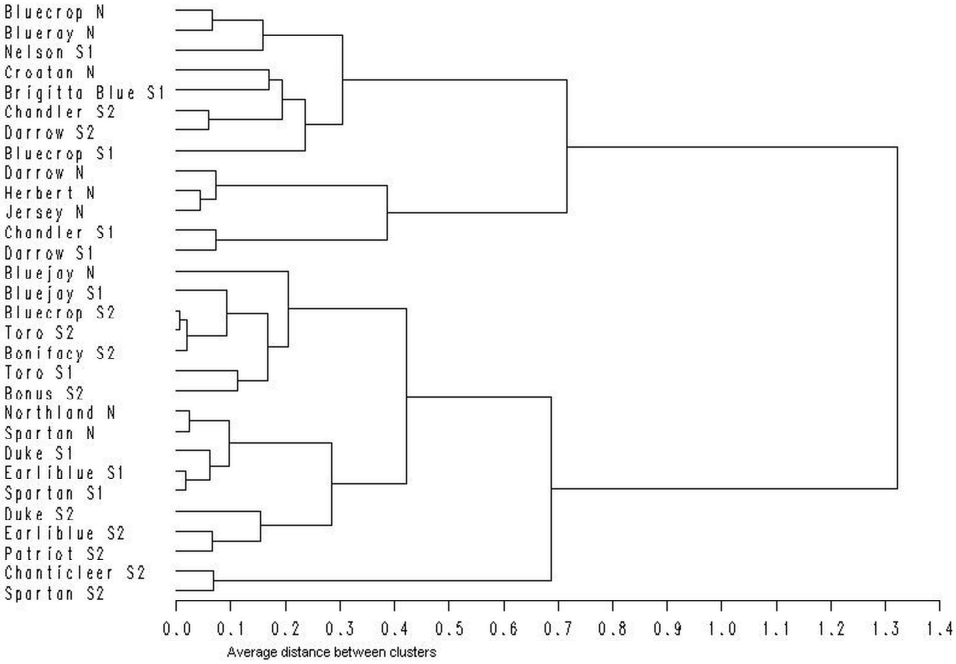

Fig. 1. Dendrogram estimating distance between 19 blueberry cultivars based on yield, berry weight and Faedi index (N – plantation in Niemce, S 1 – plantation in Spiczyn 2008, S 2 – plantation in Spiczyn 2010)

Table 3. Characteristics of banding patterns obtained with nine selected oligodeoxynucleotide primers used for RAPD analysis of *Vaccinium corymbosum* L.

RAPD primer (Sigma)	Sequence 5'-3'	Total bands	Polymorphic bands	%P[a]	Size range of DNA fragments (bp)
BJ3	CGATTGGACG	9	8	88.9	2800–500
BJ7	ATGCCGCGAT	11	10	90.9	3100–300
BJ10	TAGCGCCAAT	11	11	100.0	2500–400
BJ11	CACCCGATGA	12	10	83.3	4600–400
BJ18	ATGTGCCGTA	12	9	75.0	4000–500
BJ24	TGGCGCAATA	10	9	90.0	3200–100
BJ27	ACAACGCCTC	9	8	88.9	4100–300
BJ32	GACCGCTTTG	7	7	100.0	2700–100
BJ35	CCTCCTCATC	10	9	90.0	3000–300
Total		91	81	–	4600–100
Mean		10.1	9.0	89.0	–

[a] – percentage of polymorphism

Table 4. Matrix of genetic similarity among 19 *Vaccinium corymbosum* L. cultivars based on RAPD markers calculated by Jaccard's coefficient

Cultivar	1.*	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.
1. Chanticleer	1.00																		
2. Earliblue	0.41	1.00																	
3. Duke	0.41	0.45	1.00																
4. Spartan	0.43	0.44	0.56	1.00															
5. Patriot	0.46	0.44	0.50	0.52	1.00														
6. Bonus	0.36	0.36	0.44	0.46	0.49	1.00													
7. Bonifacy	0.43	0.47	0.41	0.49	0.55	0.46	1.00												
8. Bluejay	0.28	0.43	0.46	0.37	0.43	0.48	0.42	1.00											
9. Chandler	0.40	0.35	0.40	0.48	0.46	0.43	0.48	0.41	1.00										
10. Toro	0.37	0.53	0.47	0.52	0.55	0.49	0.52	0.39	0.45	1.00									
11. Bluecrop	0.41	0.39	0.42	0.52	0.60	0.47	0.49	0.45	0.46	0.49	1.00								
12. Nelson	0.39	0.46	0.52	0.43	0.43	0.45	0.39	0.41	0.44	0.54	0.57	1.00							
13. Darrow	0.35	0.28	0.33	0.37	0.40	0.31	0.38	0.29	0.50	0.41	0.37	0.29	1.00						
14. Brigitta Blue	0.26	0.29	0.29	0.25	0.33	0.35	0.31	0.30	0.31	0.34	0.36	0.33	0.29	1.00					
15. Northland	0.38	0.33	0.41	0.46	0.49	0.41	0.52	0.34	0.45	0.59	0.58	0.45	0.45	0.32	1.00				
16. Croatan	0.35	0.27	0.41	0.29	0.42	0.44	0.43	0.39	0.37	0.40	0.36	0.40	0.33	0.28	0.47	1.00			
17. Jersey	0.40	0.33	0.38	0.48	0.57	0.43	0.42	0.38	0.50	0.51	0.57	0.47	0.47	0.29	0.51	0.43	1.00		
18. Blueray	0.39	0.30	0.35	0.37	0.45	0.40	0.42	0.33	0.44	0.42	0.45	0.38	0.46	0.28	0.56	0.39	0.46	1.00	
19. Herbert	0.22	0.25	0.27	0.24	0.36	0.38	0.27	0.36	0.37	0.35	0.36	0.34	0.33	0.28	0.38	0.35	0.40	0.45	1.00

* – sequence cultivars in the columns is the same as in rows

Grouping of cultivars on dendrogram (fig. 1) included features mentioned above indicate primarily significant phenotypic similarity of cultivars within specific plantations (because direct concentration was characteristic for 7 out of 9, 5 out of 10, and 10 out of 11 cultivars on the plantation in Niemce, Spiczyn 1 and Spiczyn 2 respectively). Grouping of the same cultivars can go by order of plantation creating. It could means that there is an aggregation of cultivar created on the plantation in Niemce with the same cultivar on the plantation in Spiczyn 1 (‘Bluecrop’ N-S1, ‘Bluejay’ N-S1, ‘Darrow’ N-S1, ‘Spartan’ N-S1), cultivar from the youngest plantation Spiczyn 2 (‘Bluecrop’ N-S1-S2, ‘Darrow’ N-S1-S2) is joined at last. It can be claimed, that regardless existence of differential cultivars on a molecular level, phenotypic features charatieristic for specific cultivar are revealed as plants reach maturity, which in the case of this species may take a few years. According to Retamales and Hancock [2012], blueberries grown in warm southern climates can reach mature size in as little as 3 to 4 years, while those grown in colder northern climates take as many as 6 to 8 years to reach full maturity.

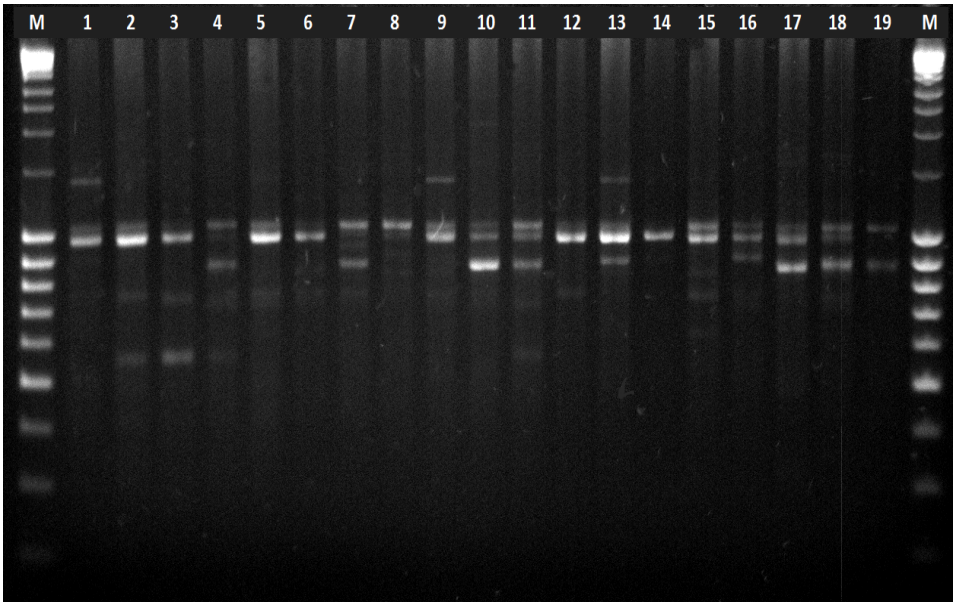

Fig. 2. RAPD fingerprints of nineteen blueberry genotypes using primer BJ18. M – standard of DNA fragment size GeneRuler^{TM} DNA Ladder Mix (100-10000 bp). The arabic numerals on the top of the picture indicate the code number of the cultivars: 1 – ‘Chanticleer’, 2 – ‘Earliblue’, 3 – ‘Duke’, 4 – ‘Spartan’, 5 – ‘Patriot’, 6 – ‘Bonus’, 7– ‘Bonifacy’, 8 – ‘Bluejay’, 9– ‘Chandler’, 10– ‘Toro’, 11 – ‘Bluecrop’, 12 – ‘Nelson’, 13 – ‘Darrow’, 14 – ‘Brigitta Blue’, 15 – ‘Northland’, 16 – ‘Croatan’, 17 – ‘Jersey’, 18 – ‘Blueray’, 19 – ‘Herbert’

Due to the fact that the phenotypic features are strongly influenced by environmental conditions, randomly amplified polymorphic DNA (RAPD) markers were used to study genetic variation in cultivated blueberry. Of the 40 primers tested for their capacity to differentiate among 19 highbush blueberry cultivars, the best 9 primers showed polymorphism between accessions and gave reproducible banding patterns. Table 3 shows characteristics of banding patterns obtained with selected primers. These 9 primers amplified 91 loci and 81 of them were polymorphic (89.0% polymorphism). A similar level of polymorphism (88.3, 80.7%) was obtained by Xu et al. [2008] and Carvalho et al. [2014] respectively. The total number of RAPD bands scored per primer in the presented work varied from 7 (primer BJ32) to 12 (primer BJ11 and BJ18). This is consistent with the results acquired by Burgher et al., [2002] who selected twenty six genotypes of lowbush blueberry (*Vaccinium angustifolium* Aiton) (they represent four geographical zones) to estimate genetic similarity by randomly amplified polymorphic DNA analysis. An average of 10.1 bands was obtained per primer and their size ranged from 4600 to 100 bp. Examples of typical RAPD banding patterns produced by primer BJ18 are shown in Figure 2.

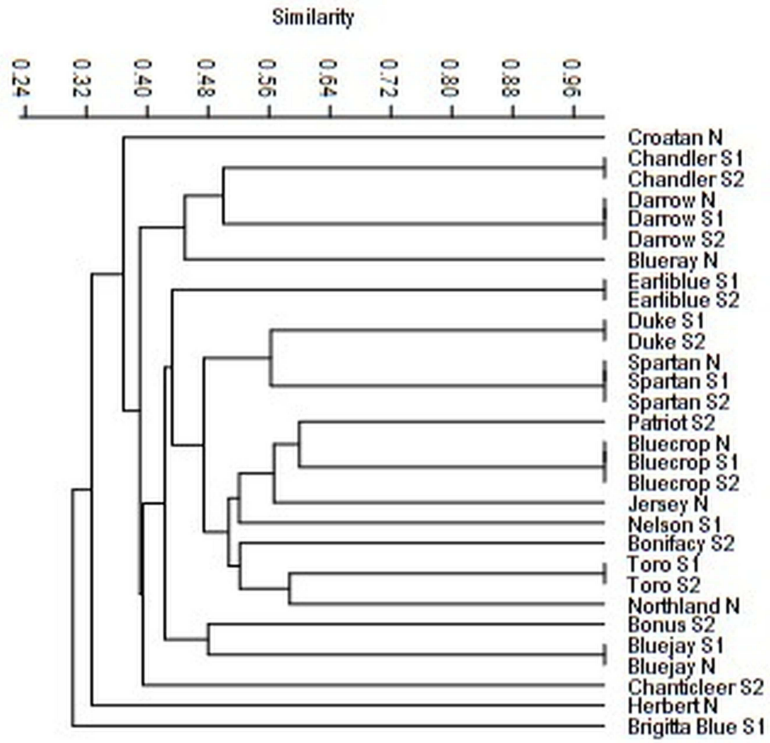

Fig. 3. Dendrogram of genetic similarity among nineteen blueberry cultivars obtained from RAPD markers using UPGMA method (N – Niemce, S 1, S 2 – Spiczyn)

The genetic similarity matrix was produced on the basis of RAPD markers using the Jaccard's coefficient (tab. 4). Obtained banding patterns for the same cultivars grown on two or three plantations were identical as well as values of their genetic similarity in relation to other cultivars was the same. This demonstrates the genetic identity of plants of the same cultivar grown in different locations (Similarity Index equals 1.00), and for this reason the cultivars in the table 4 were presented one time. Thus, it confirms that after many years of cultivation and production the cultivars are still genetic stable. RAPD based mean of genetic similarity was calculated at 0.41 and was similar to the value reported by Burgher et al. [2002] and slightly lower than reported by Carvalho et al. [2014]. The highest degree of similarity occurred between the cultivars 'Patriot' and 'Bluecrop' (0.60), while the lowest value (0.22) estimated for the cultivars 'Herbert' and 'Chanticleer'. There was also a wide range of similarity (40–97.4%) among accessions obtained by Giongo et al. [2006].

In the present study, the genetic similarity matrix was applied to cluster analysis through the UPGMA method (fig. 3). Dendrogram obtained on the basis of the genetic similarity matrix presents mainly genetic identity of the same cultivars grown on different plantations. For this reason, use of the RAPD technique can be a useful tool for certification of plant material also in the nursery industry [Belaj et al. 2001]. The generated dendrogram of nineteen accessions showed two main clusters. The first consists of 'Darrow', 'Chandler' (whose female form is cv. 'Darrow') and the 'Blueray'. The largest group includes other twelve cultivars among which 'Patriot' and 'Blucrop' formed the closest group because of the highest value of genetic similarity amounting 0.60. The common ancestor of these accessions is cv. 'Stanley' (occurring in the pedigree of cv. 'Earliblue'). Within this cluster, the high phylogenetic similarity – 0.56 between cultivars 'Duke' and 'Spartan' was estimated in accordance with a common parent 'Earliblue'. Moreover, on the basis of the calculated average value of genetic similarity (0.41), the four cvs: 'Croatan', 'Chanticleer', 'Herbert' and 'Brigitta Blue' (which is a selected seedling from the free pollination of cv. 'Lateblue') have not been linked to any grouping. The moderate level of genetic diversity between *V. corymbosum* cultivars could be explained by the fact that in their pedigree there are often the same genotypes used as a maternal or paternal component. The high genetic diversity was observed between wild and domesticated accessions, and between northern and southern types at various levels [Boches et al. 2006]. However, in some cases closely related accession does not group together [Burger et al. 2002], while Xu et al. [2008] have reported that in their studies there was a certain correlation between the clustering and the pedigrees of cultivars. Moreover, clustering based on RAPD data was different from that one based on phenotypic data. As Debnath and Ricard claim [2009], the reason of this situation can be related with the fact that markers were distributed throughout the genome, and in the majority of casus most regions of the genome were not expressed at the phenotypic level. This result is similar regarding strawberry [Garcia et al. 2002] or blue honeysuckle [Kaczmarska et al. 2015].

CONCLUSIONS

1. There was a variability of blueberry cultivars at both the molecular and phenotypic level.

2. In the study, the flowering of highbush blueberry can be observed from the end of April to the beginning of June, whereas the ripening of highbush blueberry started on 29 June and finished 7 September.

3. On the oldest plantation the highest yield of berries was characteristic of 'Bluecrop' cultivar, on the next 'Darrow' cultivar, and on the youngest plantation 'Patriot' cultivar.

4. 'Chandler' cultivar produced the largest fruit, whereas berries of the 'Northland' cultivar were the smallest.

5. It is possible to establish banding patterns, obtained by PCR–RAPD markers, to determine the cultivars of blueberry in order to confirm their genetic identity.

6. Analysed *Vaccinium corymbosum* genotypes characterized quite a high genetic diversity with mean polymorphism at level of 0.41.

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ZRÓŻNICOWANIE FENOTYPOWE I GENOTYPOWE ODMIAN BORÓWKI WYSOKIEJ (*Vaccinium corymbosum* L.) UPRAWIANYCH NA LUBELSZCZYŹNIE

Streszczenie. W latach 2012–2014 przeprowadzono badania określające zróżnicowanie na poziomie fenotypowym i genotypowym 19 odmian borówki wysokiej uprawianych na Lubelszczyźnie. Doświadczenie uwzględniało odmiany: Bluecrop, Bluejay, Blueray, Bonifacy, Bonus, Brigitta Blue, Chandler, Chanticleer, Croatan, Darrow, Duke, Earliblue, Herbert, Jersey, Northland, Nelson, Patriot, Toro i Spartan. Kwitnienie kwiatów borówki wysokiej obserwowano od końca kwietnia do początku czerwca. W zależności od odmiany dojrzewanie owoców borówki wysokiej rozpoczynało się 29 czerwca, a kończyło 7 września. Na najstarszej plantacji (Niemce) największy plon jagód wydała odmiana Bluecrop, w Spiczynie 1 – Darrow, a na najmłodszej plantacji – odmiana Patriot. Największą masą charakteryzowały się jagody odmiany Chandler, a najmniejszą Northland. Wartość indeksu Faediego zależała od klasy wczesności odmiany, zaś różnice dla tej samej odmiany wynikały z wieku krzewów i wielkości plonu, a nie terminu dojrzewania. Celem badań była również ocena zróżnicowania badanych odmian na poziomie DNA przy wykorzystaniu markerów RAPD. Analizowany zestaw starterów generował łącznie 91 fragmentów, z czego 81 (89%) było polimorficznych. Średnia wartość podobieństwa określona na podstawie matrycy markerów RAPD wynosiła 0.41. Zastosowanie grupowania odmian metodą UPGMA wykazało największą odrębność odmian: Croatan, Chanticleer, Herbert i Brigitta Blue w stosunku do pozostałych. Uzyskane wyniki potwierdzają przydatność markerów RAPD w ocenie zróżnicowania genetycznego odmian borówki wysokiej.

Słowa kluczowe: termin kwitnienia i owocowania, plon, masa owocu, zróżnicowanie genetyczne, markery RAPD

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