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EVALUATION OF RED RASPBERRY CULTIVARS USED FOR BREEDING AND COMMERCIAL GROWING IN THE BALTIC REGION

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The structure of raspberry cultivars and genetic resources in the Baltic countries have been influenced by the historical political situation in the 20th century and climatic conditions, especially winterhardiness. The genetic resources consist of some old European and American cultivars, but mostly of cultivars and hybrids bred in Russia. Currently, targeted breeding programmes are active only in Estonia and Latvia, which aim to develop winterhardy, disease-resistant cultivars, well adapted to the local climate. Therefore, parent material for hybridisation has been chosen from local advanced hybrids and introduced cultivars suitable to the regional climatic conditions. The aim of the study was to estimate the level of genetic diversity of Rubus *germplasm and assess inter-specific and intra-specific relationships using phenotypical characterisation and molecular markers. Forty one* Rubus *genotypes were evaluated by 41 phenotypical traits and 15 previously described SSR markers. Both characterisation approaches discovered high correspondence with pedigree and a low level of diversity. A limited amount of the diversity of raspberry genetic material has been used in various breeding programmes, despite their broad geographical origin. The obtained results indicate the need for including local wild* R. idaeus *plant material into breeding programmes.*

Key words: Rubus*, microsatellites, germplasm, genetic diversity, microsatellites.*

INTRODUCTION

Rubus is a large, diverse and widely distributed genus that includes approximately 900 to 1000 species (Thompson, 1997). Red raspberry (*Rubus idaeus* L.) is the most popular raspberry species grown commercially in temperate climates, including in the Baltic region. The assortment of raspberry cultivars grown in this region has been influenced by several factors (Strautina and Lacis, 2000). From the beginning of the 20th century until the 1940s, mostly Western European varieties were cultivated (Parksepp, 1985). After the Second World War, the majority of introduced germplasm consisted of cultivars originated in Russia, Belorussia and Ukraine (Parksepp, 1985). Unfortunately, both groups of cultivars were rather poorly adapted to the local agro-ecological conditions. For example, winterhardiness is the most important limiting factor for growth and survival, due to sharp temperature fluctuations: winters with temperatures of –36 °C to –38 °C are possible, as well as warm winters with thaws. Under such conditions, the Western European cultivars are not sufficiently cold hardy, while the plant material originated in the continental climate of Russia suffers serious cold damage after winter thaws. This raises the need for local breeding programmes to supply the region with suitable cultivars. Currently, raspberry breeding programmes are active at the Institute of Horticulture, Latvia University of Agriculture (former Latvia State Institute of Fruit-Growing), which started in the 1960s, and at the Estonian University of Life Sciences (EMÜ), Polli Horticultural Research Centre (Kikas *et al.*, 2002; Arus *et al.*, 2008; Kask *et al.*, 2010; Strautina *et al.*, 2012). The aim of both breeding programmes is the development of winterhardy, disease resistant cultivars, well adapted to the local climate. Therefore, parent material for hybridisation was chosen from local advanced hybrids and introduced cultivars, which conform to the defined requirements (Strautina, 1991; 1992a; 1992b; Strautiņa *et al.*, 2008).

Progress in any breeding programme is dependent on the amount of available genetic variability and the effectiveness of target trait selection and evaluation. Phenotypical description is a traditional approach in characterisation of germplasm, which allow identification of agronomically important traits, and development of selection criteria for breeding. Unfortunately, phenotypical description has several drawbacks: high impact of environmental conditions

and plant development stage, time and resource consuming, and assessed traits represent a limited part of the genome. A more accurate method of genetic variability assessment and trait selection would be at the genetic level. Markers can be used to assess allelic diversity across a range of germplasm, or markers can be examined for linkage to the trait or QTL underlying more complex traits. Therefore, different molecular markers are very useful to distinguish between genotypes and for study of genetic diversity or relatedness. Markers such as RAPD (Graham and McNicoll, 1995), AFLP (Graham *et al.*, 2004; Sargent *et al.*, 2007; Miyashita *et al.*, 2015), and SSR (Graham *et al.*, 2002; 2004; 2006; Badjakov *et al.*, 2005; Stafne *et al.*, 2005; Lopes *et al.*, 2006; Sargent *et al.*, 2007) have been applied also for *Rubus* species. Combined germplasm characterisation including both phenotypical and DNA-based methods is the most effective approach to compensate the shortcomings of each method.

The aim of the study was to estimate the level of genetic diversity within *Rubus* germplasm and to assess inter-specific and intra-specific relationships using phenotypical characterisation and molecular markers. The study was expected to provide information for genetic diversity in the raspberry germplasm collection, to facilitate more accurate selection of genotypes with valuable agronomic characters for breeding, and to enable development of cultivars suitable for the Baltic region.

MATERIAL AND METHODS

Plant material and field trial design. Forty-one raspberry accessions from the genetic resources collection established in 2000–2003 at the Institute of Horticulture, Dobele (56°37′0″N, 23°16′0″E) were characterised for phenotypic traits and genotyped (Table 1). The cultivars and selections were grown in a randomised complete block design with 1–4 replications of 10 plants each, without irrigation, in 3 × 0.5 m plots. The growing technologies included plant protection by pesticides and fertilisation according to soil analyses. The characterisation of 41 phenotypic traits was carried out in 2009–2011 for three plants in each replication. The expression of traits was evaluated using a 9-point scale (Table 2).

Isolation of genomic DNA and PCR analysis. Young leaves from phenotypically characterised plants were collected during May – June. Total DNA was isolated using a Genomic DNA Purification Kit (Thermo Scientific™, Lithuania). PCR reactions were performed using 15 previously tested primer pairs (Table 3) in 20 μL reactions with 25 ng DNA, 2 mM each primer, 200 μM of each nucleotide, 1.5 mM $MgCl_2$ and 0.5 U REDTaq® DNA Polymerase (Sigma-Aldrich, USA) per reaction, in a Eppendorf epgradient thermal cycler (Eppendorf, Germany) for 40 cycles with denaturation at 95 °C for 30 s, annealing at 59 °C for 30 s and extension at 72 °C for 30 s, with a final extension step of 10 min at 72 °C.

Table 1

STUDIED RASPBERRY CULTIVARS

Cultivar	Parentage	Country of origin
Alvi	(Golden Queen × Spirina Belaya) × Novostj Kuzmina	Estonia
Babye Leto	September × (Kostinbrodskaya × Novostj Kuzmina)	Russia
Balzam	Newburgh × Bulgarski Rubin	Russia
Brigantina	Ottawa × Sajana	Russia
Canby	Viking × Lloyd George	USA
Carnival	Ottawa × Rideau	Canada
Comet	Ottawa × Madawaska	Canada
Daiga	Arbat × Nr. 143	Latvia
Dita	P.Upītis seedling, unknown	Latvia
Glen Ample	Complex cross, including Glen Prosen and Meeker	UK
Gusar	Canby × pollen mix	Russia
Ina	Ivanovskaja × Taganka	Latvia
Ivars	P.Upītis unknown seedling	Latvia
Jewel *(R.occidentalis)*	(Bristol × Dundee) × Dundee	USA
Kiržač	Malling Promise × Carnival	Russia
Liene (Nr.8-7)	Arbat × Nr. 143	Latvia
Lubetovskaya	Newburgh × Bulgarski Rubin	Russia
Marianushka	unknown	Russia
Maroseika	unknown	Russia
Meeker	Willamette × Cuthbert	USA
Meteor	Kostinbrodskaya × Novostj Kuzmina	Russia
Mirazh 2	unknown	Russia
Newburgh	Newman × Herbert	USA
Norna	Preussen × Lloyd George	Norway
Novokitaevskaya	Kitaevskaya × Novostj Kuzmina	Russia
Nr. 13-4-14	Ivanovskaya × Nr. 150	Latvia
Nr. 4-6	Ivanovskaya × Nr. 150	Latvia
Obilnaya	Novostj Kuzmina × Lloyd George	Russia
Ottawa	Viking × (Raner × Logan)	Canada
Polana	Heritage × Zeva Herbsternte	Poland
Polka	Autumn Bliss × Lloyd George	Poland
Samarskaya Plotnaya	Novostj Kuzmina × Preussen	Russia
Schoenemann	Lloyd George × Preussen	Germany
Skromnitsa	Bulgarski Rubin × Ottawa	Russia
Sputnitsa	Bulgarski Rubin × Ottawa	Russia
Tomo	Superlative × Novostj Kuzmina	Estonia
Tulameen	Nootka × Glen Prosen	Canada
Viktorija (Nr. 16-4-4)	Ivanovskaya × Nr. 150	Latvia
Wilamette	Newburgh × Lloyd George	USA
Zeva 2	Rote Wadenswiler × Lloyd George	Switzerland
Zvjozdochka	1-54-1 × Barnaulskaya	Russia

PCR products were first checked on 1% agarose gels in 1x TAE buffer and visualised by staining with ethidium bromide to test for the presence of PCR products. The same PCR products were subsequently analysed on an ABI

Table 2

DESCRIPTIVE STATISTICAL ANALYSIS OF PHENOTYPIC TRAITS CHARACTERISED IN 41 RASPBERRY CULTIVARS

Trait	Rating scale	Min[a]	Max[b]	Mean	SD[c]	CV%[d]
Habit	1-upright, 2-semi upright, 3-arching	1.00	3.00	1.88	0.60	32.1
Number of current season‘s canes	3-few, 5-medium, 7 –many, 9 –very many	1.00	7.00	5.08	1.17	23.0
Very young shoot:						
– anthocyanin coloration of apex during rapid growth	1-absent, 9-present	1.00	9.00	8.61	1.75	20.3
– intensity of anthocyanin coloration of apex during rapid growth	3-weak, 5-medium, 7-strong	1.00	7.67	4.50	1.73	38.5
Current season's cane:						
– bloom	1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong	1.00	7.00	3.83	1.90	49.7
– hairiness	1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong	1.00	7.00	2.13	1.92	90.1
– anthocyanin coloration	1-absent or very weak, 3-weak, 5-medium, 7-strong, 9-very strong	1.00	9.00	4.94	1.62	32.9
– length of internode	3-short, 5-medium, 7-long	2.00	5.67	3.89	0.93	23.9
Spines: presence	1-absent, 9-present	1.00	9.00	8.61	1.75	20.3
Cultivars with spines present only (spines):						
– density	3-sparse, 5-medium, 7-dense	3.67	8.00	5.77	0.98	17.0
– size of base	1-very small, 3-small, 5 medium, 7-large, 9-very large	1.00	8.00	4.26	1.65	38.6
– length	3-short, 5-medium, 7-long	3.00	7.00	5.09	1.02	20.1
– colour	1-green, 2-brownish green, 3-greenish brown, 4-purplish brown, 5-brownish purple, 7-purple	1.00	7.00	6.29	1.35	21.4
Leaf:						
– green colour of upper side	3-light, 5-medium, 6-dark	3.00	7.00	5.31	0.77	14.5
– predominant number of leaflets	1-three, 2-equally three and five, 3- five	1.00	3.00	1.74	0.76	43.4
– profile of leaflets in cross section	1-concave, 2-stright, 3-convex	1.00	3.00	1.84	0.55	30.1
– rugosity	1-very weak, 3-weak, 5-medium, 7-strong, 9-very strong	1.00	6.00	3.88	1.36	35.1
– relative position of lateral leaflets	1-free, 2-touching, 3-overlapping	1.00	3.00	2.29	0.71	30.9
Terminal leaflet:						
– length	3-short, 5-medium, 7-long	5.00	7.00	6.24	0.77	12.4
– width	3-narrow, 5-medium, 7-broad	4.00	7.00	5.13	0.77	15.1
Pedicel: number of spines	1-absent or very few, 3-few, 5-medium, 7-many, 9-very many	1.00	9.00	3.23	2.46	76.1
Peduncle:						
– presence of anthocyanin coloration	1-absent, 5-present	1.00	9.00	8.81	1.25	14.2
– intensity of anthocyanin coloration	1-very weak, 3-weak, 5-medium	1.00	7.00	4.91	1.64	33.4
Fruit:						
– length	3-short, 5-medium, 7-long	3.00	7.00	5.54	1.27	22.9
– width	3-narrow, 5-medium, 7-broad	3.00	7.00	5.04	0.66	13.1
– ratio length/width	-small, 5-medium, 7-large	3.00	7.00	5.28	1.11	20.9
– general shape in lateral view	1-circular, 2-broad conical, 3-conical, 4-trapezoidal	1.00	4.00	2.49	0.85	34.2
– size of single drupe	3-small, 5-medium, 7-large	3.00	7.00	5.24	0.81	15.4
– colour	1-yellow, 2-orange, 3-light red, 4-medium red, 5-dark red, 6-purple, 7-dark purple	1.00	6.00	4.31	0.80	18.7
– glossiness	3-weak, 5-medium, 7-strong, 9-very strong	1.00	7.00	4.96	1.16	23.3
– firmness	1-very soft, 3-soft, 5-medium, 7-firm, 9-very firm	3.00	7.00	5.11	0.81	15.9
– adherence to plug	1-very weak, 3-weak, 5-medium, 7-strong, 9-very strong	3.00	6.33	4.85	0.53	10.9
– main bearing type	1-only on previous years cane in summer, 2- both on previous year’s cane in summer and on current year’s cane in autumn, 3- only on current year’s cane in autumn	1.00	3.00	1.32	0.57	43.4
Cultivars, which fruit on previous season’s cane in summer:						
– Dormant cane length	3-short, 5-medium, 7-long	5.00	7.00	5.97	0.83	13.9
– Dormant cane colour	1-brownish grey, 2-greyish brown, 3-brown, 4-purplish brown, 5-brownish purple	1.00	4.00	2.05	0.80	38.9
– Fruiting lateral attitude	1-erect, 2-semi erect, 3-horizontal to drooping	1.00	2.67	2.02	0.23	11.5
– Fruiting lateral length	1-very short, 3-short, 5-medium, 7-long, 9-very long	4.00	7.00	5.52	0.81	14.7
– Time of beginning of fruit ripening	3-early, 5-medium, 7-late	1.00	7.00	5.08	1.11	21.9
– Length of fruiting period on previous year’s cane	3-short, 5-medium, 7-long	3.00	7.00	5.37	0.93	17.3
Cultivars, which fruit on current season’s cane in autumn:						
– Current season’s cane length	3-short, 5-medium, 7-long	3.33	8.00	6.09	0.96	15.7
– Time of beginning of fruit ripening	3-early, 5-medium, 7-late	3.00	5.00	4.00	1.00	25.0

[a] Minimum value, [b] Maximum value, [c] Standard deviation, [d] Variation coefficient expressed in percentage

Table 3

GENOTYPING RESULTS FOR RASPBERRY ACCESSIONS USING FIFTEEN SSR LOCI

Locus	Data set characteristics *					
	Na	Ne	I	Ho	He	Allele range, bp
Ru108[a]	5.0	2.5	1.133	0.561	0.608	148-161
Ru118[a]	11.0	6.4	2.040	0.756	0.844	102-163
Ru126[a]	13.0	8.1	2.259	0.732	0.876	137-201
Ru157[a]	11.0	5.2	1.904	0.829	0.807	205-281
Ru223[a]	6.0	2.9	1.242	0.463	0.653	137-167
Ru26[a]	8.0	4.7	1.685	0.350	0.788	118-152
Ru277[a]	5.0	1.8	0.903	0.500	0.459	231-248
Rub103a[b]	3.0	1.3	0.398	0.128	0.207	149-153
Rub222e[c]	4.0	2.7	1.123	0.189	0.630	213-228
Rub236b[c]	9.0	3.6	1.561	0.512	0.718	127-181
RubNebA09b[d]	4.0	2.8	1.171	0.732	0.648	231-240
Rubus118b[c]	8.0	4.5	1.743	0.526	0.780	96-135
Rubus24a[c]	4.0	2.4	0.970	0.683	0.575	154-170
Rubus25a[c]	3.0	1.8	0.749	0.366	0.449	134-155
Rubus45c[c]	3.0	2.0	0.747	0.132	0.506	214-227

* Na, number of alleles, Ne, number of effective alleles, I, Shannon's information index, Ho, observed heterozygosity, He, expected heterozygosity

[a] Graham *et al*., 2002; [b] Sargent *et al*., 2007; [c] Graham *et al*., 2004; [d] Graham *et al*., 2006

PRISM® 3100 Genetic Analyser (Applied Biosystems, USA) and genotyped using GeneMapper® Software v4.0 (Applied Biosystems, USA).

Data analysis. Statistical analysis of phenotypic data was performed on average values of three-year measurements. Descriptive statistics, evaluation of significant differences between evaluated cultivars by ANOVA was done using IBM SPSS Statistics software version 21 (Anonymous, 2012) to characterise variation of traits in the tested raspberry germplasm.

Marker characteristics (number of effective alleles, Shannon's information index, observed heterozygosity, expected heterozygosity) were computed by GENALEX 6.5 software (Peakall and Smouse, 2012) using data of identified amplification fragments.

SSR marker data were transformed into a binary matrix registering 1 for the presence of a particular amplification fragment and 0 for its absence. Principal Component Analysis (PCA) was applied to identify patterns of multi-trait variation, phenotypic and genetic structure, as well as to evaluate relationships among genotypes. Pair-wise similarity was calculated using Gower's General Similarity Coefficient (Gower, 1971), which is defined as a measure of proximity for mixed data types. PCA, calculation of similarity matrices and their evaluation was performed by software PAST version 3 (Hammer *et al.*, 2001).

RESULTS

Phenotypic characterisation. Phenotypical traits showed high variability between tested raspberry genotypes, with average coefficient of variability 27.1% (Table 2), significant differences among cultivars were found for all of them. The highest variability was identified for current season's cane hairiness, following by number of spines on pedicels and current season's cane bloom intensity (Table 2). In general, higher variability was observed for traits characterising vegetative growth, but lower variability for fruit and yield component traits. Analysed traits had a high level of mutual correlation — average correlation coefficient was 0.44, significant correlations over 0.5 were identified in 42.5% of cases, while correlations over 0.75 — in 20.9% of cases. Complete correspondence ($r = 1.0$) was identified for seven traits: presence of spines and density, base size, length and colour of spines; presence of anthocyanin colouration on pedicel and intensity of anthocyanin coloration on pedicel.

Due to high mutual correlation of traits, thirteen components with eigenvalues larger than 1.0 were extracted, which described 80.5% of the original observed variability, while the first two PC's described 25.0% of original variability. Traits with the highest loadings on PC1 were main bearing type of fruits, dormant cane colour of previous season cane, current season's cane anthocyanins colouration, and on PC2 — fruit traits (beginning of fruit ripening for current year cane, fruit length/width ratio, size of single drupe in fruits). These traits had the highest impact on raspberry cultivar grouping (Fig. 1). According to results of PCA, the cultivars 'Dita', 'Ivars', 'Jewel', and 'Meteor' were grouped separately from other analysed raspberry genotypes. The cultivar 'Dita' is distinguishable due to yellow colour of berries, green colour of shoots and spines, the lack of new shoot anthocyanin colouration, which is typical for majority of local cultivars, whereas 'Ivars' and 'Meteor' differ from other genotypes due to very early ripening of berries and a secondary crop in autumn on the tops of young shoots. The cultivar 'Jewel' represents species *R. occidentalis*, the only one with black, dense and tiny berries. A fairly compact group was formed by cultivars with a large fruit mass: 'Daiga', 'Ina', and 'Maroseika', which are the L_1 gene donors, other large-fruited cultivars 'Glen Ample', 'Polka', 'Tulameen' were also located in the same scatter plot sector (Fig. 1). Common features of these cultivars were large number of fruit laterals on shoots and number of berries on fruit lateral as well as high fruit length / width ratio. Cultivars with small and soft berries like 'Carnival', 'Comet', 'Kirzhach', and 'Ottawa' were located in the opposite sector of the scatter plot and showed mutual relationships — they all have the cultivar 'Ottawa' in different generations of their pedigrees. The grouping of cultivars was not affected by production type, which has only one harvest either on primocane or floricane shoots, therefore the two primocane cultivars 'Polana' and 'Polka' were located distantly from each other. The PCA grouping of cultivars using phenotypical traits had no significant correspondence with

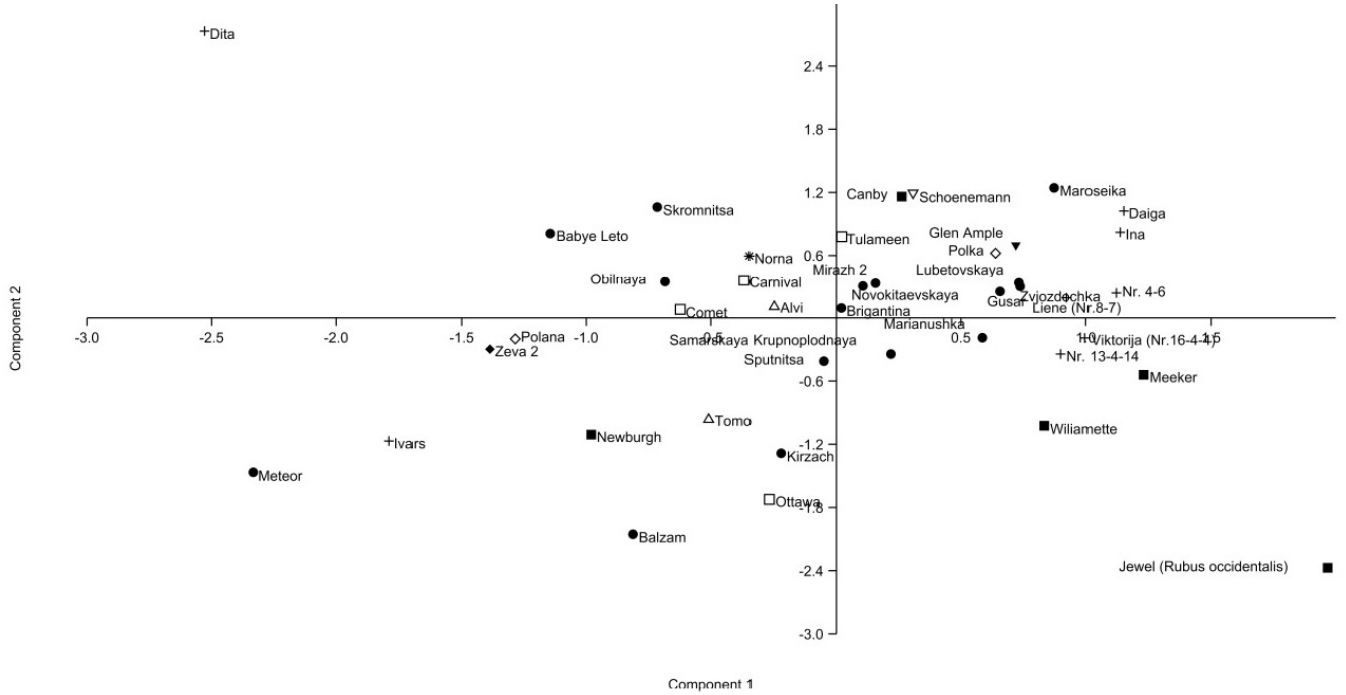

Fig. 1. Relationships among raspberry cultivars shown in two-dimensional scatter plot of the first principal components, based on phenotypic characterisation of 41 traits. Origin: □ - Canada, △ - Estonia, ▽ - Germany, + - Latvia, ★ - Norway, ✧ - Poland, ● - Russia, ♦ - Switzerland, ▼ - United Kingdom, ■ - USA.

genotype geographical origin, but showed high relationship with parentage.

Characterisation by SSR molecular markers. The 15 tested primer pairs generated SSR fragments for all 41 raspberry genotypes. Three to 13 alleles were identified for each locus with 6.5 alleles per marker on average (Table 3). The highest number of alleles (13) was found at locus *Ru 126*, the lowest (3) at loci *Rub 25a* and *Rub 45c*. The number of effective alleles ranged from 1.26 (*Rub 103a*) to 8.08 (*Rub 126*) with 3.52 effective alleles per marker on average (Table 3). Observed heterozygosity was very variable among loci. There was a group of loci with high (values ranging from 0.500 (*Ru277*) to 0.829 (*Ru157*)) and low (values ranging from 0.128 (*Rub103a*) to 0.463 (*Ru223*)) observed heterozygosity. The length of alleles (in base pairs) was widely distributed for some loci, in particular *Ru 157* (205 – 281 bp), *Ru 126* (137 – 201 bp), *Ru 26* (118 – 152 bp) and *Rub 236b* (127 – 181 bp) (Table 3).

According to the PCA of 15 SSR marker data, the first two PCs characterise 22.6% of the total variability, discriminating different *Rubus* species: a single, compact group including all cultivars of *R. idaeus*, and the only *R. occidentalis* sample, which had a large genetic distance (data not shown). Therefore detailed analysis of raspberry cultivar mutual relatedness was performed by PCA, excluding the *R. occidentalis* accession (Fig. 2). Genotypes of one species did not show a pronounced grouping; however, the tested raspberry cultivars could be divided into three groups: Group I — the largest with 30 cultivars, Group II with three cultivars (cvs. 'Carnival', 'Comet', and 'Mirazh 2') and Group III with seven cultivars ('Balzam', 'Skromnitsa', 'Polka', 'Polana', 'Sputnitsa', 'Marianushka', and 'Ottawa') (Fig. 2). The cultivar grouping did not show any association with country of origin and/or breeding programme, e.g. Group II included cultivars from Russia, Poland, and Canada. However, molecular marker data had close relationship with known cultivar pedigree (Table 1), for example, a close sub-group was formed by cultivars with 'Novostj Kuzmina' as an ancestor ('Babye Leto', 'Meteor', 'Tomo', 'Alvi', 'Samarskaya Plotnaya', 'Novokitaevskaya', and 'Obilnaya'). Cultivars bred in Latvia also were grouped according to pedigree: Group 1 — genotypes obtained from cross 'Ivanovskaya' × No. 150 ('Lina', 'Viktorija', No. 4-6, No. 13-4-14); Group 2 — obtained from cross 'Arbat' × No. 143 ('Daiga', 'Liene') and Group 3 — unknown origin seedlings obtained by P. Upītis ('Dita', 'Ivars'), which shows close genetic relationship with cultivars developed from cv. 'Novostj Kuzmina' (Fig. 2). A similar relationship between pedigree and genetic similarity was found also for cultivar 'Canby' and 'Gusar' ('Canby' × pollen mix). Two pairs of cultivars ('Brigantina' and 'Kirzach', 'Carnival' and 'Comet') showed complete genetic identity.

DISCUSSION

According to D. Jennings (1988), some phenotypic traits directly characterise the presence of a particular gene, e.g. cane bloom — gene *B*, cane hairiness — gene *H*, yellow colour of berries and green spines — gene *t*, canes without spines — gene *s*. Not all such traits affected PCA grouping; a significant impact was identified for yellow colour of berries determined by the *t*-gene (cultivar 'Dita'). An important criterion for cultivar selection is yield, which is a multicomponent trait influenced by the following elements: number of producing shoots, number of fruit laterals on shoots,

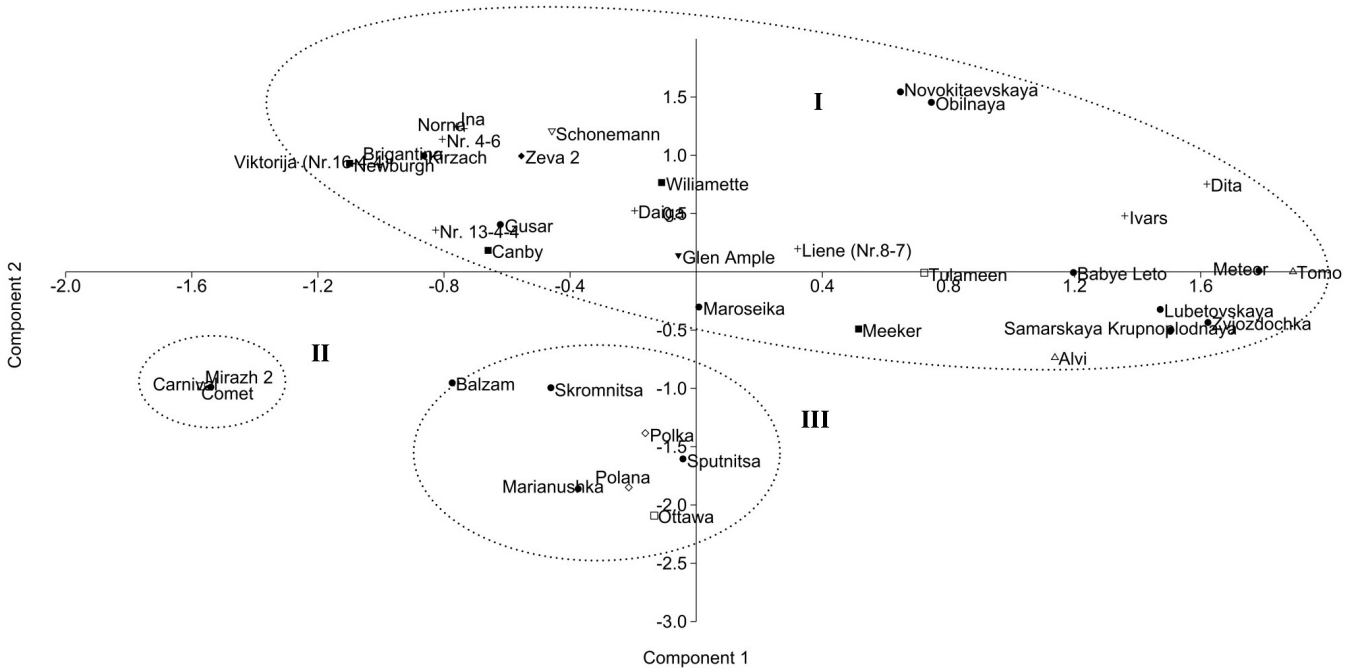

Fig. 2. Relationships among raspberry cultivars of species *Rubus idaeus* shown in two-dimensional scatter plot of the first principal components, based on data of 15 SSR molecular markers. Origin: □ - Canada, △ - Estonia, ▽ - Germany, + - Latvia, ★ - Norway, ✧ - Poland, ● - Russia, ♦ - Switzerland, ▼ - United Kingdom, ■ - USA.

number of flowers and berries per shoot and weight of individual berries. Gene L_1, which determines large berries, is relatively unstable, but it is the unifying element of cultivar grouping according to fruit size. Donors with this gene have been used quite frequently in breeding, especially in Russia (Kazakov, 2001; Kazakov and Jevdokimenko, 2007). Inheritance of berry weight is polygenic and depends on the weight of drupe and number of drupes in berry. Small drupes usually have cultivars with small seeds, e.g. 'Ivars' and 'Meteor'. Obtaining large berries with small seeds is very challenging due to the existing correlation where small berries and large seeds are dominating. In addition, there is a positive correlation between weight of fruit and individual drupe. Cultivars 'Brigantina', 'Glen Ample', 'Polka', and 'Tulameen' can be selected as donors for high berry weight taking into account this correlation and results of PCA. Productivity is also characterised by short internodes and large number of fruit laterals. Unfortunately, these traits had insufficient impact on the PCA results. I. Kazakov (2001) described the cultivars 'Carnival', 'Newburgh', and 'Ottawa' as donors for a large number of nodes on shoots and number of fruit laterals, while a large number of berries from fruit laterals can be obtained in cross combinations with the cultivar 'Carnival'. Shoot pubescence, determined by the *H* gene did not significantly affect the distribution of cultivars, therefore PCA does not allow selection of cultivars according this trait (e.g., cv. 'Canby', which would be important for breeding), to obtain raspberry cultivars resistant to raspberry spur blight *Didymella applanata* and grey mould *Botrytis cinerea*. The local Latvian genotypes from the current breeding programme ('Daiga', 'Ina', 'Liene', Nr. 13-4-14, Nr. 4-6, and 'Viktorija') showed high phenotypical similarity (Fig. 1) that can be explained by a common pedigree: these genotypes were obtained from crosses between the full-sib cultivars 'Ivanovskaya' and 'Arbat' ('Carnival' × 'Bulgarski Rubin'), which are characterised by high winter-hardiness, and selections with high fruit weight and productivity, but medium to low cold hardiness (Strautina *et al*., 2012). The PCA based on phenotypical traits did not allow discrimination of groups of cultivars with valuable traits in further breeding, but showed high similarity and accordance with known pedigree (Table 1). Phenotypical similarity of tested plant material is defined mostly by targeted breeding work towards valuable traits and a common gene pool. However, PCA showed good applicability in characterisation of germplasm based on a complete set of traits, which is not possible by analysis of separate traits as well as by evaluation of chosen traits.

The SSR markers used in this study were selected from several previously applied sets of markers (Graham *et al.*, 2002; 2004; 2006; Badjakov *et al.*, 2005). Therefore, comparison of obtained results was possible for loci *Ru 26*, *Ru 118*, *Ru 223*, *Ru 108*, *Ru 277*, *Ru 126*, *Ru 157*, which were used in characterisation of collections with a comparable number of accessions. Other loci (*Rubus 118b*, *Ru 103a*, *Rubus 24a*, *Rubus 25a*, *NebA09b*, *Rub 222e*, *Rubus 45c*, *Rub 236b*) have been used only in mapping projects, which included a limited number of accessions (Graham *et al.*, 2004; 2006). Our results identified a lower number of alleles than identified in other investigations on raspberries with a similar number of accessions (Graham *et al.*, 2002). Most loci identified a slightly lower number of alleles, which can be explained by the lower number of accessions in our investigations (41 compared to 50), or to the genetic diversity of the analysed plant material. Significantly lower

polymorphism was found for loci *Ru 26*, *Ru 223* and *Ru 277* (Table 3). Our data were very similar to data published by Badjakov *et al.* (2005), acquired from analysis of 16 Bulgarian raspberry varieties. Nevertheless, the set of 15 SSR markers allowed discrimination of raspberry cultivars by unique genotypes, except for two pairs of cultivars: the Russian cultivars 'Brigantina' and 'Kirzach', and the Canadian cultivars 'Carnival' and 'Comet'. Although these cultivars were phenotypically distinct (Fig. 1), the utilised set of markers could not discriminate them. The close pedigree relationship of these cultivars might be one of the reasons for this result. The cultivars 'Carnival' and 'Comet' have a common mother — cv. 'Ottawa', while both pollinators (cvs. 'Madawaska' and 'Rideau', respectively) are selected from the same cross 'Lloyd George' × 'Newman'. A similar situation was also found for the cvs. 'Brigantina' and 'Kirzach', which also have cv. 'Ottawa' in their pedigrees. This demonstrates the need for combined evaluation of genetic resources, since genotyping of only closely related plant material can not reveal the phenotype differences. Another option is increasing the number of analysed markers to ensure better genome coverage or utilisation of gene specific markers.

Genotyping data were analysed by PCA to characterise genetic structure, diversity and relationships among cultivars. The set of applied markers differentiated raspberry cultivars according to *Rubus* species — cultivar 'Jewel' was located distantly from other cultivars and represents the species *R. occidentalis*, which was previously described as genetically distinct (Badjakov *et al.*, 2005; Bassil *et al.*, 2012). Cultivars of *R. idaeus* did not show strict grouping (Fig. 2). Similar to analysis of phenotypical data (Fig. 1), there was no clear clustering of raspberry cultivars according to country of origin and breeding programme; however correspondence with known pedigree was observed. The average values of Gower's distances for both types of characterisation were similar — 0.257 and 0.252 for phenotypical and molecular characterisation, respectively. According to the Mantel test, a relatively high correlation between molecular and phenotypic data was found ($r = 0.3075$, $p = 0.0003$), although SSR markers are localised in non-coding DNA regions.

According to phenotypic and genetic characterisation, the raspberry germplasm grown and used in breeding in Baltic region is relatively similar, resulting from long-term selection for suitability to local conditions. Mutual relatedness has also been confirmed by pedigree data (Table 1), since parentage information was available for 36 of the 41 tested genotypes. A few cultivars have been regularly used as parents in several breeding programmes (e.g. 'Bulgarski Rubin', 'Lloyd George', 'Newburgh', 'Novostj Kuzmina', 'Ottawa'). Of the analysed accessions, the cultivar 'Ottawa' is the parent of five, whereas 'Lloyd George' is the parent of seven cultivars. Regardless of country of origin, the parent plants used in breeding programmes overlap and cultivars developed in geographically remote regions have a similar genetic background. This can pose problems in the future since the material is quite homogeneous, which can lead to both production and breeding problems, due to the lack of diversity of novel valuable breeding characteristics. Therefore, new, genetically differentiated germplasm should be introduced and/or the use of wild relatives should be promoted (Patamsytė *et al.*, 2010) to continue successful breeding efforts. This finding supports previous reports about the very limited genetic diversity in raspberry breeding programmes and the necessity to introduce new germplasm (Dale *et al.*, 1993).

The use of selected SSR markers set in conjunction with pedigree information and phenotypical evaluation will allow the monitoring of the genetic diversity in the raspberry breeding programmes, planning of future crosses in order to broaden the genetic base, as well as maintenance of desired agronomic traits. This study discovered limited genetic diversity of raspberries used in various breeding programmes, despite their differing geographical origin. It also points to the need for research on local wild *R. idaeus* plant material to select genotypes suitable for breeding of cultivars with high ecological plasticity for cultivation in the Baltic Sea region.

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BALTIJAS REĢIONĀ SELEKCIJĀ UN KOMERCIĀLAJĀ AUDZĒŠANĀ IZMANTOTO AVEŅU ŠĶIRŅU NOVĒRTĒJUMS

Aveņu šķirņu sortimentu Baltijas valstīs ir ietekmējusi divdesmitajā gadsimtā dominējošā vēsturiskā situācija un klimatiskie apstākļi, it īpaši ziemcietība — ģenētiskos resursus veido dažas senas Eiropas un Amerikas šķirnes, bet lielākā daļa audzēto šķirņu un hibrīdu izveidoti Krievijā. Šobrīd mērķtiecīgas selekcijas programmas ir tikai Igaunijā un Latvijā, kuru mērķis ir radīt ziemcietīgas, pret slimībām izturīgas šķirnes, kas labi pielāgotas vietējiem klimata apstākļiem. Tāpēc hibridizācijā izmantojamie vecākaugi izvēlēti no labākajiem vietējiem genotipiem un reģionālajiem klimata apstākļiem piemērotām introducētajām šķirnēm. Pētījuma mērķis bija novērtēt *Rubus* augu materiāla ģenētisko daudzveidību un genotipu iekšsugas un starpsugu radniecību, izmantojot fenotipisko raksturojumu un molekulāros marķierus. Četrdesmit viens *Rubus* genotips vērtēts, izmantojot 41 fenotipisko pazīmi un 15 iepriekš aprakstītus SSR marķierus. Abu pētījuma metožu rezultāti uzrādīja ciešu saistību ar genotipu izcelsmi, kā arī ierobežotu selekcijas programmās izmantoto aveņu ģenētisko daudzveidību, neatkarīgi no to ģeogrāfiskās izcelsmes, kā arī norāda uz vietējā savvaļas *R. idaeus* augu materiāla izmantošanas nepieciešamību selekcijā.