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Application of marker-assisted selection for resistance to gall mite and Blackcurrant reversion virus in *Ribes* genus

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Abstract

Gall mite (*Cecidophyopsis ribis* Westw.) is a biological vector of Blackcurrant reversion virus (BRV), and both are widespread in the countries, where blackcurrants are cultivated commercially. Blackcurrant (*Ribes nigrum* L.) is the primary natural host of BRV, although natural infestations also occur in other *Ribes* spp. Some *Ribes* species and cultivars are resistant to *C. ribis* and/or BRV. The mechanism and gene structure of resistance are not clear, that is why molecular markers for breeding of resistant blackcurrants are still in the process of searching. Several molecular markers related to resistance to the pathogen and pest in *Ribes* spp. genotypes were identified by quantitative trait loci (QTL) mapping. These molecular markers can be used for acceleration of the breeding process of resistant blackcurrant, because phenotyping in the field is unnecessary. In our study, the presence of the 12 polymerase chain reaction (PCR) markers, amplified fragment length polymorphism (AFLP) and single sequence repeat (SSR), linked to gall mite and virus resistance loci from 4 to 20 cM were analysed. Eleven of them demonstrated suitability for selection of resistant genotypes in this study. The presence of these molecular markers in 8 *Ribes* species, 15 hybrids and 13 *R. nigrum* cultivars was shown. Fully specific molecular marker for detection of genotypes with resistance to gall mite and virus among *Ribes* species was not found. The complex application of eleven molecular markers allowed for significant grouping of *Ribes* species, cultivars and hybrids with different genetic origin of resistance to pest and virus. As well, they can be useful for marker-assisted selection. Six molecular markers: E36M59_107, E40M43_236, E40M43_289, E40M40_219, E41M88_280 and E45M40_222, were distinguished as more suitable for identifying resistant *Ribes* spp. genotypes with *Ce* and *P* genes in this study.

Key words: gall mite, molecular marker, resistance.

Introduction

Blackcurrant reversion disease (BRD) is economically the most significant virus disease in *Ribes* species. Blackcurrant reversion virus (BRV) is the causal agent of BRD (De Lillo et al., 2018). BRV is the first identified mite-transmitted member of the genus *Nepovirus*. The gall mite (*Cecidophyopsis ribis*) is a biological vector of BRV (Martin, Tzanetakis, 2015). Wild species are mostly used for the introgression of disease resistance genes. The natural host range of BRV is limited. *R. nigrum* is the primary natural host for both *C. ribis* and BRV, although natural infestations occur also in some related *Ribes* species: *R. rubrum*, *R. brasteosum* and *R. spicatum*. In general, they are hardly used to improve quantitative traits in breeding programs. Resistance or even immunity to the blackcurrant reversion agent has been identified in *R. aureum*, *R. cereum*, *R. fuscescens*, *R. gordonianum*, *R. dikushka*, *R. nigrum* ssp. *sibiricum*, *R. pauciflorum* and *R. uva-crispa* (Brennan, 2008; Stalažs, Moročko-Bičevska, 2016; De Lillo et al., 2018). Gene *Ce*, responsible for resistance to gall mite and BRV was established in gooseberries (Keep et al., 1982). Another gene *P* is derived from *R. nigrum* ssp. s*ibiricum* and responsible for genetic resistance to gall mite, thereby preventing viral infection in plant (Andersson, 1971).

The quantitative trait loci (QTL) as marker-assisted selection (MAS) in plant breeding programs are successfully applied. A wide range of DNA-based markers have been developed as well as procedures are getting simpler and cheaper. Nuclear based single sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) are some of the commonly

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used markers for genome and QTL mapping in order to understand the evolutionary genetics and sequences controlling traits (Brennan et al., 2008). Instead of inferring marker-assisted selection schemes in breeding populations from QTL results in bi-parental populations, the development of QTL mapping methods for multiparental populations is envisaged as a better alternative. It would allow linking QTL analysis and marker-assisted selection in tandem through generations in the breeding programs (Asins et al., 2010; Ahmad et al., 2017). It is therefore of interest to breed blackcurrant cultivars not only with high nutritional value (high concentrations of phenolic compounds and vitamin C), but also with natural resistance against diseases and pests (Hummer, Dale, 2010).

Genome studies of *Ribes* species of these small fruit plants are still limited. AFLP, single nucleotide polymorphism (SNP) and SSR molecular markers for genotyping, QTL mapping and identification of regions associated with morphological traits have been investigated (Brennan et al., 2008; Russell et al., 2011; Mazeikiene et al., 2012; Palmieri et al., 2013). SNP, AFLP and SSR markers related with resistance to gall mite and other phenological and morphological traits in *Ribes* spp. hybrids were analysed. The second generation sequencing based on transcriptome is the approach extremely cost-effective for species with unsequenced genomes; however, informatic analysis of such data is still in its infancy (Russell et al., 2011). AFLP markers related to genes *Ce* (Brennan et al., 2009) and *P* (Mazeikiene et al., 2012) are suggested at this time. Consequently, bi-parental populations were used to make QTL, these markers are not significantly reliable for the evaluation of various *Ribes* species (Mazeikiene et al., 2017), but due to the similarity of genetic resistance mechanisms in plants, they could be applied. Resistance to the gall mite can be associated with several other molecular markers according to QTL data (Brennan et al., 2008; 2009). Their suitability can be studied in the breeding of resistant *Ribes* spp. genotypes for marker-assisted selection.

The aim of our study is to evaluate the suitability of molecular markers related to gall mite resistance in QTLs for marker-assisted selection of resistant *Ribes* spp. genotypes.

Materials and methods

The study was conducted at the Institute of Horticulture of Lithuanian Research Centre for Agriculture and Forestry in 2018.

Plant material and DNA isolation. All investigated blackcurrant (*Ribes nigrum* L.) plants are grown at a collection of Institute of Horticulture (55°60′ N, 23°48′ E), Lithuanian Research Centre for Agriculture and Forestry. Thirteen cultivars – resistant 'Aldoniai', 'Dainiai', 'Domino', 'Minaj Šmyriov', 'Ritmo', 'Senjorai', 'Smaliai', 'Viktor' and susceptible 'Tauriai', 'Titania', 'Gojai', 'Katiusha', 'Vernisaz', eight *Ribes* species – *R. americanum* Mill., *R. aureum* 'Brecht' Pursh, *R. dikuscha* Fisch., *R. nigrum* ssp. *sibiricum* Pavl., *R. pauciflorum* Turcz., *R. sanguineum* Pursh, *R. uva-crispa* L., *R. hudsonianum* Richardson, and fifteen intra or inter species hybrids with different resistance to gall mite according to the data in field conditions was analysed (Table 1).

Table 1. Crossing components of *Ribes* spp. hybrids and resistance to gall mite in field conditions

Hybrid No.	Crossing components	Resistance to gall mite in field conditions
Hybrid 1	'Tauriai' × 'Vernisaz-1'	resistant
Hybrid 2	'Gojai' × 'Ruben-1'	resistant
Hybrid 3	('Ben Lomond' × 'Ben Gain') × 'Dainiai'	resistant
Hybrid 4	*R. nigrum* × *R. uva-crispa* 'Josta'	resistant
Hybrid 5	(*R. nigrum* × *R. americanum*) × ('Titania' × *R. uva-crispa* 'Bedford Yellow') F_2	resistant
Hybrid 6	'Ben Tirran' × *R. usuriensis* F_2	resistant
Hybrid 7	'Titania' × *R. rubrum* 'Jonkher van Tets' F_3	resistant
Hybrid 8	'Tauriai' × ('Titania' × *R. uva-crispa* 'Bedford Yellow') F_2	resistant
Hybrid 9	'Triton' × *R. rubrum* 'Rondom' F_2	susceptible
Hybrid 10	'Titania × *R. uva-crispa* 'Kuršu Dzintars' F_2	susceptible
Hybrid 11	'Vakariai' × *R. janczewskii* F_1	susceptible
Hybrid 12	PC73 × *R. usuriensis* F_2	susceptible
Hybrid 13	('Ben Lomond' × BRi 8315-25) × 'Tauriai-40'	susceptible
Hybrid 14	'Tauriai' × 'Vernisaz-2'	susceptible
Hybrid 15	'Gojai' × 'Ruben-8'	susceptible

Total DNA of the tested genotypes was extracted from 0.2 mg fresh leaves using the modified cetyltrimethylammonium bromide (CTAB) – based extraction protocol by Doyle and Doyle (1990). After extraction, DNA was additionally cleaned with 10 M LiCl solution.

Identification of polymerase chain reaction (PCR) markers. Eleven amplified fragment length

polymorphism (AFLP) and one single sequence repeat (SSR) marker from three linkage maps (Brennan et al., 2008; 2009; Mazeikiene et al., 2012) were chosen according to association with gall mite morphologic traits (Table 2). AFLP and SSR analysis was ere used for detection of molecular markers.

AFLP analysis was performed according to Vos et al. (1995) method. Samples were prepared using AFLP Plant Fingerprinting Kit (Applied Biosystems, USA). The AFLP template was prepared with restriction endonucleases *Eco*RI and *Mse*I (Thermo Scientific Ltd.). Ligation, preamplification and selective amplification of samples was performed according to Brennan et al. (2009) and Mazeikiene et al. (2012). Forward primer of loci g2-JO8 was labelled with fluorochrome 6-carboxyfluorescein (6FAM) for SSR analysis. Amplification was performed using conditions described by Mazeikiene et al. (2017). For capillary electrophoresis, each PCR product was diluted with 10 times deionised formamide solution containing GeneScan-500 LIZ ladder and analysed using a 3130 Genetic Analyser (Applied Biosystems).

Table 2. Methods and primer pairs used for detection of molecular markers according to distance data (cM) in linkage group (LG) from gall mite resistance (GMres) in field conditions

Code of primer pair / used method for molecular marker obtaining	Primer pair	Fragment size bp	Distance in LG from GMres, cM
E36M59 / AFLP	*Eco*RI ACC – *Mse*I CTA	107	14[3]
E40M40 / AFLP	*Eco*RI AGC – *Mse*I AGC	219	10[1, 2]
E40M41 / AFLP	*Eco*RI AGC – *Mse*I AGG	121	4[1]
E40M42 / AFLP	*Eco*RI AGC – *Mse*I AGT	226	9[1, 2]
E40M43 / AFLP	*Eco*RI AGC – *Mse*I ATA	236	4[1], 14[2]
E40M43 / AFLP	*Eco*RI AGC – *Mse*I ATA	289	11[2], 12[1]
E41M40 / AFLP	*Eco*RI AGG – *Mse*I AGC	222	5[1]
E41M41 / AFLP	*Eco*RI AGG – *Mse*I AGG	163	7[1], 11[2]
E41M43 / AFLP	*Eco*RI AGG – *Mse*I ATA	179	7[1], 8[2]
E41M88 / AFLP	*Eco*RI AGG – *Mse*I TGC	280	4[2]
E45M40 / AFLP	*Eco*RI ATG – *Mse*I AGC	222	4[1], 20[2]
g2-JO8 / SSR	5'CGC CGA GCT CTA ATC ACT GT3' 5'ATA GCC CAT GCC CAT ATT CA3'	166	7[2], 9[1]

Linkage map data according to [1]Brennan et al., 2008, [2]Brennan et al., 2009 and [3]Mazeikiene et al., 2012

Statistical analysis. The identification of AFLP and SSR molecular markers by size of base pair (bp) (Table 2) was performed with the programme *GeneMapper*, version 4.0 (Applied Biosystems). Data of PCR markers frequency was evaluated by the analysis of variance (*ANOVA*) from the package *Selekcija* (Raudonius, 2017). Heterogeneity between distribution frequencies: molecular markers and morphologic resistance to gall mite and Blackcurrant reversion virus (BRV), among 36 *Ribes* spp. genotypes was examined using the Chi-square test or among 11 molecular markers using the dissimilarity between units. Phylogenetic trees were constructed based on unweighted neighbour-joining grouping method in the software package *DarWin*, version 6.0.10 (Perrier, Jacquemoud-Collet, http://darwin.cirad.fr/darwin); a bootstrap analysis with 1000 replications was performed.

Results and discussion

Among all investigated *Ribes* spp. genotypes, 102 fragments associated with molecular markers linked to gall mite resistance were amplified. Frequency of molecular markers ranged from 11.1% to 33.3% in resistant *Ribes* spp. genotypes to gall mite and/or BRV and to 19.4% in susceptible genotypes according to results obtained by the programme *GeneMapper*. The E40M41_121 marker in QTL map linking group (LG) 2 was at 4 cM distance from the phenotypic resistance based on gene *Ce* (Brennan et al., 2008). The study revealed that presence of E40M41_121 molecular marker in resistant genotypes was 25.0%, in susceptible genotypes – 19.4%. We removed E40M41_121 marker from further data analysis, because it is inappropriate for gal mite and BRV resistance. Low frequency of AFLP markers E40M43_236 and E41M40_222 among susceptible genotypes was detected once and that of markers E40M42_219 and E45M40_222 – twice (data not shown).

Molecular markers E36M59_107 (frequency 75%) and E40M43_289 (frequency 50.0%) were detected in resistant to gall mite and BRV genotypes (Fig. 1). These molecular markers were not found in BRV resistant genotypes. It is known that PCR fragment E36M59_107 is a molecular marker of gene *P* (Mazeikiene et al., 2012). DNA fragment E40M43_289 can be used as a marker of resistance to gall mite also. PCR markers E4043_236, E41M40_222, E41M41_163, E41M43_179, E41M88_280, E40M40_219, E40M42_226, E45M40_222 and g2-J08 were identified in resistant to gall mite and BRV *Ribes* spp. genotypes as well in genotypes only with virus resistance. These amplified fragments might identify genotypes that are resistant to the virus as well as molecular marker E41M88_280 suggested by Brennan's and colleagues (2009). Markers shown in Figure 1 jointly could be useful for marker-assisted selection without phenotypic evaluation in *Ribes* species.

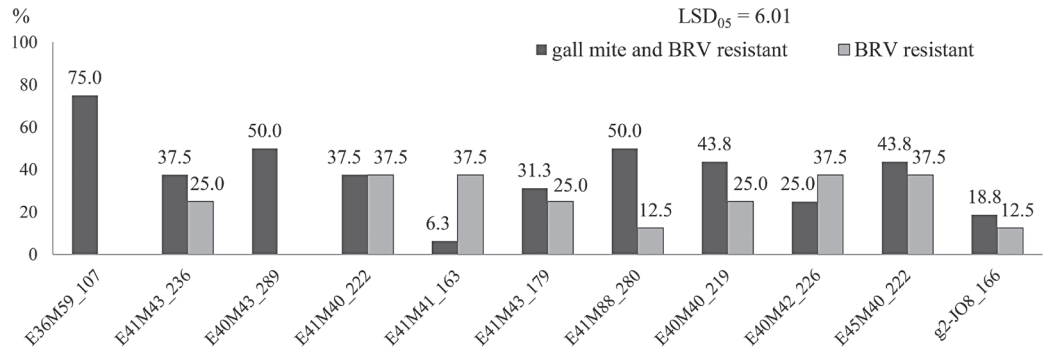
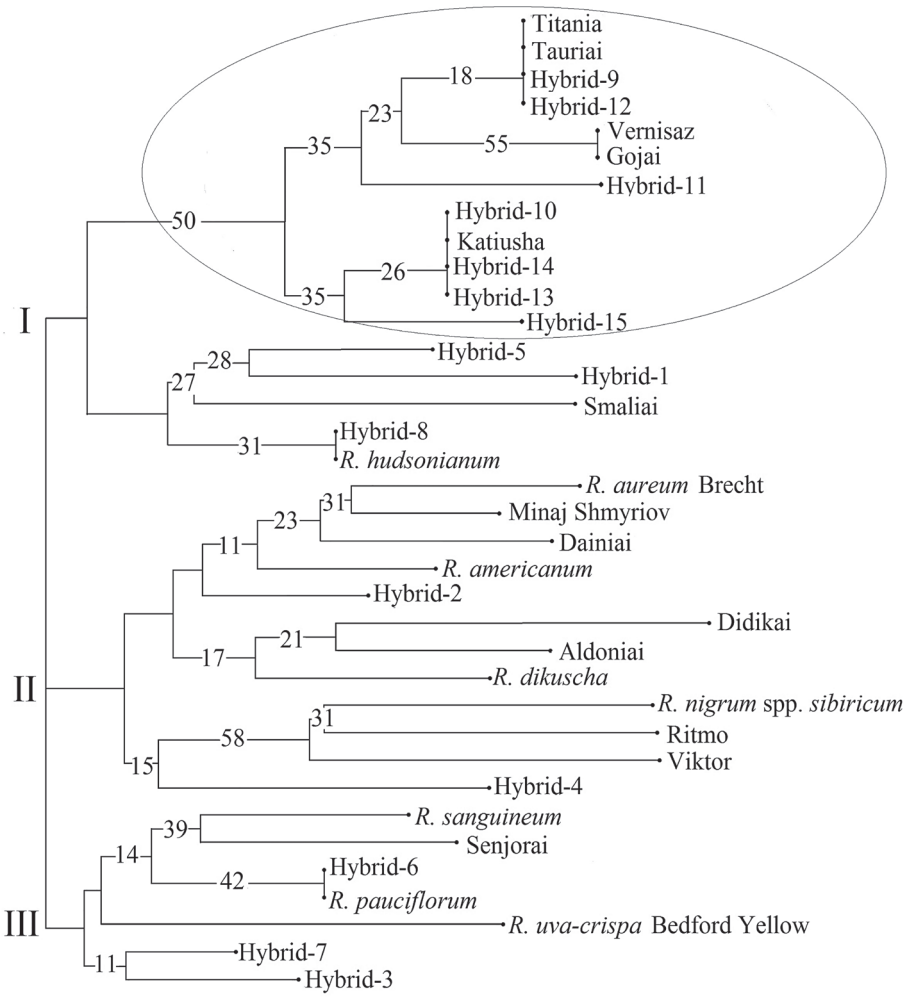


Figure 1. Frequency of molecular markers related to resistance to gall mite and/or Blackcurrant reversion virus (BRV) in resistant *Ribes* spp. genotypes

The phylogenetic tree of *Ribes* spp. cultivars, species and hybrids with different genetic resistance was constructed using the data of eleven PCR markers related to resistance to gall mite and reversion virus. *Ribes* spp. genotypes constitute three groups depending on the inherited molecular markers. Distinct cluster with significant difference (at 50% bootstrap) of susceptible *Ribes* spp. cultivars to gall mite and BRV was grouped in the separate branch, which was circumscribed in Figure 2.

It was established that 'Titania', 'Tauriai', 'Vernisaz', 'Gojai', 'Katiusha' and seven inter and intra hybrids were highly susceptible to gall mite and BRV in field conditions according to our and other authors' data (Šikšnianas, 2005; Rubauskis et al., 2006; Siksnianas et al., 2006). Resistant genotypes constitute three groups depending on the inherited molecular markers. Low susceptibility to gall mite is known for *R. hudsonianum* and cultivar 'Smaliai', but they are completely BRV resistant (Šikšnianas, 2005; Brennan, 2008). These genotypes are



Note. Susceptible genotypes are framed in the circle.

Figure 2. Phylogenetic tree based on neighbour-joining unweighted analysis generated using the Chi-square test representing the relationships of *Ribes* spp. genotypes based on 11 molecular markers linked to resistance to *Cecidophyopsis ribis* and Blackcurrant reversion virus (BRV)

located in the first branch of the phylogenetic tree and represent blackcurrants with low susceptibility to gall mite and resistance to virus. The second branch consisted of genotypes with full genetic resistance, which in most cases is determined by gene *P*. Cultivars located in this branch 'Minaj Shmyriov', 'Dainiai', 'Didikai', 'Aldoniai', 'Ritmo' and 'Viktor' have resistance to gall mite and its transmitted BRV, determined by gene *P* as in *R. nigrum* ssp. *sibiricum* and *R. americanum.* Gall mite resistant hybrids show distorted segregation at expected Mendelian ratio 1:1 in hybrids of bi-parental families when one parent is with dominant gene of resistance (Mazeikiene et al., 2017). Presence of molecular markers in intra species hybrids 'Tauriai' × Vernisaz-1' and 'Gojai' × 'Ruben-1' and absence in 'Tauriai' × 'Vernisaz-2' and 'Gojai' × 'Ruben-8' demonstrate different resistance of these genotypes derived from 'Tauriai' (Šikšnianas, 2005) and 'Ruben' (Piotrowski et al., 2016). Genotypes with resistance determined by gene *Ce* were branched in the third group of phylogenetic tree. The presence of *R. uva-crispa* and *R. sanguineum* as donors of gene *Ce* (Brennan et al., 2009; Mazeikiene et al., 2017) proves the statement mentioned before. A complex study of eleven molecular markers correctly identifies resistant *Ribes* spp. cultivars, species and hybrids to gall mite and BRV in short time.

Ribes spp. genotypes with resistance to gall mite or BRV in field conditions and presence or absence of molecular markers are shown in Table 3. Presence of molecular markers varied between 4 and 8 in *R. nigrum* cultivars. Eight molecular markers were found in 'Didikai' genome. It is a new cultivar with a rich pedigree, combining features from three *Ribes* spp. genomes (Mažeikienė et al., 2017). Other Lithuanian cultivars are also characterized by a large number of resstance molecular markers.

Table 3. Presence of molecular markers in *Ribes* spp. genotypes with resistance to gall mite (GM) and Blackcurrant reversion virus (BRV)

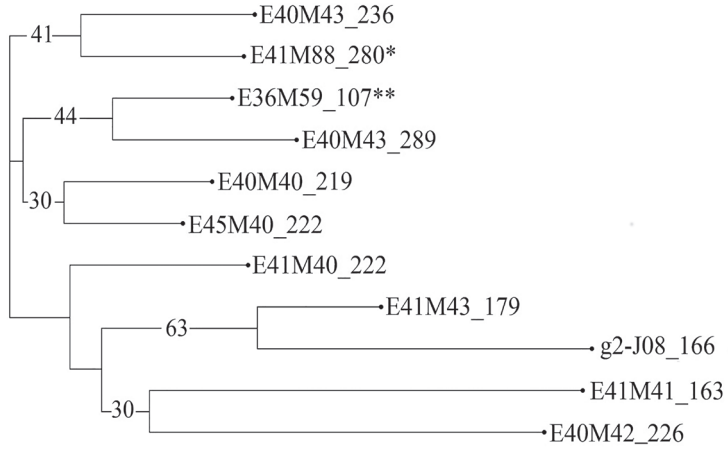
Genotype resistant to gall mite and/or virus	Molecular markers											Total
	E36M59_107	E40M43_236	E40M43_289	E41M40_222	E41M41_163	E41M43_179	E41M88_280	E40M40_219	E40M42_226	E45M40_222	g2-JO8_166	
'Aldoniai' (GM, BRV)	×	×					×	×		×		5
'Dainiai' (GM, BRV)	×		×	×			×	×		×		6
'Didikai' (GM, BRV)	×	×	×	×	×		×	×		×		8
'Minaj Shmyriov' (GM)	×		×	×				×				4
'Ritmo' (GM, BRV)	×	×	×			×		×		×		6
'Senjorai' (BRV)				×		×			×	×	×	6
'Smaliai' (BRV)				×	×	×		×		×		6
'Viktor' (GM, BRV)	×	×	×	×		×	×			×		7
Hybrid 1 (BRV)		×			×				×			3
Hybrid 2 (GM)	×										×	2
Hybrid 3 (GM, BRV)	×						×					2
Hybrid 4 (GM, BRV)	×					×	×				×	4
Hybrid 5 (BRV)					×							1
Hybrid 6 (BRV)									×			1
R. americanum (GM)	×		×									2
R. aureum 'Brecht' (GM)	×		×	×				×	×	×		6
R. dikuscha (BRV)		×					×	×		×		4
R. nigrum ssp. *sibiricum* 'Rus' (GM, BRV)	×	×		×		×	×	×			×	7
R. pauciflorum (BRV)				×					×			2
R. sanguineum (GM)						×	×		×	×		4
R. uva-crispa (GM, BRV)		×	×				×		×			4
Total	12	8	8	9	4	7	10	9	7	10	4	87

In *Ribes* species the quantity of molecular markers varied between 2 and 7. Species *R. americanum*, *R. aureum*, *R. dikuscha*, *R. nigrum* ssp. *sibiricum*, *R. pauciflorum*, *R. sanguineum* and *R. uva-crispa* are known as donors of resistance to gall mite and/or BRV (Anderson, 1971; Keep et al., 1982; Brennan, 2008; Stalažs, Moročko-Bičevska, 2016; Mazeikiene et al., 2017). The origin of resistance in *R. dikuscha* and *R. pauciflorum* was unknown as well as molecular markers. Specific markers for *R. dikuscha* (E40M43_236, E41M88_280, E40M40_219 and E45M40_222) and *R. pauciflorum* (E41M40_222 and E40M42_226) were found in this study.

AFLP markers are characterized by a moderate level of polymorphism in intra species studies (Ahmad et al., 2017), therefore markers obtained in bi-parental

population studies (Brennan et al., 2008; Mazeikiene et al., 2012) were suitable for the analysis of most *Ribes* spp. genotypes in this research. Specific combinations of resistance molecular markers for species *R. americanum*, *R. aureum*, *R. nigrum* ssp. *sibiricum*, *R. sanguineum* and *R. uva-crispa* were found (Table 3) and could be used for marker-assisted selection in the future. No molecular markers were detected in *R. hudsonianum* and in hybrids 'Titania' × *R. rubrum* 'Jonkher van Tets' F_3 and 'Tauriai' × ('Titania' × *R. uva-crispa* 'Bedford Yellow') F_2, although they had resistance to gall mite and BRV in field conditions. *R. hudsonianum* is known as a genetic donor of resistance (Brennan, 2008), but this study did not validate any molecular marker for this species. Genetic origin of *R. hudsonianum* resistance is still unclear and more researches in the future are required. Frequency of molecular markers in cultivars and hybrids was determined by targeted selection of resistant seedlings in hybridization process. We suggest that genetically determinate resistance to gall mite and BRV loci in promising donors must be recombined with several molecular markers.

Phylogenetic tree based on neighbour-joining unweighted analysis shows that the tested molecular markers grouped according to the dependence on resistance genes to *C. ribis* or BRV (Fig. 3). It was assumed that there are several resistance mechanisms available in *Ribes* species according to the data of clustering analysis. With reference to already known marker E41M88_208 (Brennan et al., 2009), marker E40M43_236 may be grouped in marker-assisted selection of resistance determined by gene *Ce*. The marker E36M59_107 of gene *P* (Mazeikiene et al., 2012) shows similarity of distribution in gall mite resistant genotypes with marker E40M43_289. Molecular markers E40M40_219 and E45M40_222 also can be used for identification of *Ribes* spp. genotypes with gene *P*. The reliability of selection is much greater when flanking markers are used (Pathania et al., 2017). According to the data of QTL by Brennan et al. (2008), marker E45M40_222 in combination with other investigated markers can flank the target locus of resistance. Markers E40M42_226, E41M41_163, g2-J08_166, E4143_179 and E41M40_222 were specific for resistant genotypes, but were located in the third branch of the phylogenetic tree, separately from markers of genes *Ce* and *P*. We assume the presence of other resistance mechanisms in *Ribes* species and hybrids, which can be identified by markers from the third branch.



Note. Phylogenetic tree based on neighbour-joining unweighted analysis generated using the dissimilarity between units; * – the molecular marker of gene *Ce* (Brennan et al., 2009); ** – the molecular marker of gene *P* (Mazeikiene et al., 2012).

Figure 3. Relationships of eleven molecular markers closely related to the pathogen and pest resistance

All investigated markers were selected from QTLs of bi-parental *Ribes* spp. populations (Brennan et al., 2008; 2009; Mazeikiene et al., 2012) and the data of these QTLs was transferred to *Ribes* spp. genotypes from different populations in our research. Results of this study showed that eleven investigated PCR markers could be used for the identification of resistant hybrids in different breeding populations according to genetic inheritance at this time. Data of similar scientific studies would be more reliable using meta-QTL of multiple populations and analyses need to be improved in the future (Ahmad et al., 2017). The investigated molecular markers of resistance to gall mite and BRV might be tested using a DNA sample without phenotyping in the field at this time. Assays of PCR markers are non-destructive for plants; therefore further comprehensive research of plant is possible.

Conclusions

1. A complex study of eleven molecular markers: E36M59_107, E40M43_236, E40M43_289, E41M40_222, E41M41_163, E41M43_179, E41M88_280, E40M40_219, E40M42_226, E45M40_222 and g2-J08_166, reliably identifies *Ribes* spp. cultivars, species and hybrids with resistance to *Cecidophyopsis ribis* and Blackcurrant reversion virus (BRV).

2. Different origin of genetic resistance (genes *P* and *Ce* or other) occurs in *Ribes* spp. genotypes according to the distribution of molecular markers, which can be determined without phenotyping.

3. The locus of resistance to gall mite, determined by gene *P*, had close recombination with E36M59_107, E40M43_289, E40M40_219 and E45M40_222.

Molecular markers E40M43_236 and E41M88_280 are suitable for identification of gene *Ce*. Other markers E40M42_226, E41M41_163, g2-J08_166, E4143_179 and E41M40_222 can be grouped together with already mentioned markers in breeding programs by marker-assisted selection of resistant *Ribes* spp. genotypes.

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Atsparumo serbentinei erkutei ir juodojo serbento reversijos virusui molekulinių žymeklių taikymas *Ribes* genties augalų selekcijoje

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Santrauka

Serbentinė erkutė (*Cecidophyopsis ribis* Westw.) yra juodojo serbento (*Ribes nigrum* L.) reversijos viruso (BRV) biologinis vektorius. Šie kenkėjai ir patogenai plačiai paplitę šalyse, kuriose yra juodųjų serbentų plantacijų. *Ribes nigrum* yra pirminis natūralus BRV šaltinis, tačiau viruso infekcija aptinkama ir kitose *Ribes* rūšyse. Kai kurios *Ribes* rūšys ir veislės yra atsparios *C. ribis* ir/ar BRV. Atsparumo mechanizmas ir genų struktūra nėra aiški, todėl vykdant juodojo serbento veislių atranką ieškoma jiems atsparių molekulinių žymeklių. Remiantis kiekybinių požymių lokusų (QTL) žemėlapiais, *Ribes* spp. genotipuose nustatyta keletas molekulinių žymeklių, susijusių su atsparumu erkutei ir virusui. Šie žymekliai padeda paspartinti atsparių juodojo serbento veislių selekciją ir tada nebereikia augalų fenotipinio vertinimo.

Tirta 12 PGR (AFLP ir SSR) žymeklių, per 4–20 cM nutolusių nuo atsparumo serbentinei erkutei ir virusui lokusų. Tyrimo metu 11 iš jų pasirodė tinkami atsparių genotipų atrankai. Molekulinių žymeklių ieškota 8 *Ribes* rūšyse, 15 hibridų ir 13 *R. nigrum* veislių. Nustatyti specifiniai molekuliniai žymekliai atsparių erkutei ir virusui *Ribes* spp. genotipų atrankai. *Ribes* spp. genomuose nustatyta skirtinga genetinio atsparumo *C. ribis* ir BRV kilmė. Vienuolikos molekulinių žymeklių kompleksinis panaudojimas leido patikimai identifikuoti *Ribes* rūšis, veisles ir hibridus su nevienodu genetiniu atsparumu. Jie gali būti naudojami vykdyti žymekliais paremtai selekcijai. Buvo nustatyti šeši molekuliniai žymekliai: E36M59_107, E40M43_236, E40M43_289, E40M40_219, E41M88_280 ir E45M40_222, tinkami identifikuoti *Ribes* spp. genotipams su *Ce* ir *P* genais.

Reikšminiai žodžiai: atsparumas, molekulinis žymeklis, serbentinė erkutė.