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Genetic control of resistance to fungal diseases, winterhardiness and productivity in orchard plants and the use of DNA and isoenzyme markers for donor identification

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Systematic crosses of apple and black currant varieties were made and the resistance of progenies to fungal diseases, productivity and apple seedling winterhadines was evaluated. Significant differences of combining ability for the investigated traits were ascertained. Inheritance of the traits was determined predominantly by genes with additive effects. DNA polymorphism of black currant was estimated and the molecular markers common for the genus *Ribes* and specific molecular markers for different species were identified. The polymorphism of the peroxidase enzyme system in apples enables to ascertain the representative bands or band combinations specific for scab-resistant and scab-susceptible varieties. The variability of polyphenoloxidase isozymes in black currant was determined during the vegetation period, and more winterhardy species were found to have a larger number of bands.

Key words: apple, black currant, combining ability, scab, powdery mildew, apple blotch, septoria leaf spot, inheritance

INTRODUCTION

In big commercial currant plantations, conditions are favorable for the spreading of fungal diseases. The earlier developed varieties lost resistance to newly formed mildew strains [1]. An efficient way to reduce harm incurred by diseases is development of resistant varieties. When for creation of new varieties the crossing components are selected according to the phenotype, usually the desirable results are not obtained [2]. In this case the breeding value of the varieties utilized in crossings could be successfully established by assessing their combining ability and genetic control of traits [3]. If the genetic control of individual traits and their combination and the heritability regularities are known, it is possible to select the crossing components deliberately, to provide the parameters of future varieties and at the same time to favour the breeding work and speed up variety development.

The objective of the work was to assess the combining ability of apple and black currant varieties, to establish genetic control resistance to fungal diseases, winterhardiness and productivity as well to identify DNA and isoenzyme markers and donors.

MATERIALS AND METHODS

The investigation was carried out at the Horticultural Plant Breeding Department in 1998–2000. Black currant varieties were crossed according to a top-cross mating design [4], when 3 cultivars (‘Minaj Shmiriov’, ‘Titania’ and ‘Ben Lomond’) were used as a female (testers) and 8 as a male (‘Belorusskaya sladkaya’, ‘Zagadka’, ‘Naslednica’, ‘Storklas’, ‘Polar’, Intercontinental’, D16/1/-25, and ‘Ben Gairn’). Hybrids were obtained in 24 cross combinations and planted in the orchard in 1995. Eight apple varieties (‘Orlik’, ‘Noris’, ‘Auksis’, ‘Katja’, ‘Kaunis’, ‘Tellissaare’, ‘Prima’, ‘Idared’) were crossed according to the half diallel mating design [5]. Seedlings of 28 crosses were planted in the orchard in 1991. The combining ability of black currant was calculated according to Chotiliova [4] and of apple varieties according to [5] Griffing’s method 4. Resistance of apple and black currant seedlings to fungal diseases was estimated on a 0–5 scale: 0 – no infection on leaves, 5 – more than 75% of leaf area infected. Winterhardiness was estimated according to the 0–5 scale: 0 – no injuries, 5 – pith of one-year shoot is brown.

DNA was isolated from young leaf tissue of 11 *Ribes* accessions including 6 species (*R. nigrum* ssp.

sibiricum, R. fontaneum, R. pauciflorum, R. sanguineum, R. usuriensis, R. hudsonianum), one cultivar ('Minaj Shmiriov') and four interspecific hybrids No. 2, No. 3, No. 9, No. 11 (received from cross No. 79-197-7 × *R. sanguineum*) following the hexadecyltrimethylammonium bromide (CTAB) method described by [6]. Isoenzymes were extracted from skin, buds and leaves of two-year-old black currant shoots and apple leaves by using Tris-Glyc (pH 8.3) buffer and studied by the electrophoresis method according to the modified Jaaska methodology [7]. Polyphenoloxidase (PPO) isoforms were established by staining gel by L-3,4-dihydroxyphenylalanine with p-phenylenediamine.

Table. **Combining ability of apples and black currants**

Trait	Mean squares			GCA/SCA	
	GCA		SCA		
Apples					
Scab	0.86**		0.07**	12.3	
Powdery mildew	0.52**		0.04	13.0	
Apple blocth	0.61**		0.05**	12.2	
Winterhardiness	0.76**		0.08**	9.5	
Yield	121.66**		4.23	28.8	
Black currants					
	GCA, maternal varieties (testers)	GCA, paternal varieties		Maternal varieties	Paternal varieties
American mildew	4.93**	4.10**	0.53	9.30	7.74
Leaf spot	4.05**	2.59**	0.56	7.23	4.63
Yield, 1998–2000	1.22*	3.23**	0.51*	2.39	6.33

$^{**}P < 0.01$, $^{*}P < 0.05$

RESULTS AND DISCUSSION

Variance analysis of combining ability demonstrated significant GCA differences (P < 0.01) within the investigated apple and black currant varieties and forms for fungal diseases, yield and winterhardiness in apple (Table). SCA differences (P < 0.05) were established only for yield in black currant, for scab apple blotch resisance and winterhardiness in apple-trees. Black currant resistance to American mildew and septoria leaf spot and apple resistance to scab, powdery mildew, apple blotch was determined by genes with additive effects and as well as yield and winterhardiness of apple varieties, because the mean squares of GCA were several times less than the mean squares of SCA, but the impact of the dominant and epistatic genes for the latter traits was significant.

The effects of GCA (g_i) of the apple varieties indicated that 'Katja' can be used in breeding as a donor of polygenic resistance to scab, 'Orlik', 'Auksis' and 'Katja' as powdery mildew resistance donors, and 'Prima' as an apple blotch resistance donor. The GCA effect for winterhardiness of 'Tellissaare' significantly differed form the effects other apple varieties. 'Prima' was selected as a donor for high yield among the varieties studied.

The effects of GCA of black currant show that in breeding for improvement of American mildew resistance the most suitable form is D16/1/25. In breeding for leaf spot resistance, the variety 'Belorusskaya sladkaya' is most valuable, because the GCA effects of this variety for this trait are the highest.

The highest values of GCA effects throughout the whole study period were found in varieties 'Intercotinental' and 'Ben Gairn'. These varieties first should be employed in crosses for development of more productive forms. Other varieties in this respect were close and their progeny will be medium productive.

A band map (Fig. 1) demonstrated that the study genotypes had distinct profiles. The lowest number of positive scores for one variety was present for hybrid No. 2 (10) and the highest for *R. pauciflorum* (20). Four RAPD markers (number 11, 1350 kb; 18, 960 kb; 32, 420 kb; 36, 330 kb) were presented in one variety. The marker number 19 (929 kb) was present in all genotypes and obviously is common for the genus *Ribes*. The high level of polymorphism observed in this study is in contrast with previous results demonstrated in *Rubus* [8]. Two RAPD markers (number 5, 1857 kb; number 27, 580 kb) were common for species of the *Eucariosma* section. *R. sanguineum* lacked such markers. Thus, their two markers were common for species of *Eucariosma* section. There is a contradictory evidence to that linking *Eucariosma* and *Calobotria* sections [9].

Peroxidase (PRX) showed a considerable variation among the apple varieties and selections (Fig. 2). The PRX band at $R_f = 0.21$ occurred in all apple accessions. In the zone with R_f values 0.46–0.78, four to ten bands were detected and 17 phenotypes were observed among all varieties and selections. 'Vista Bella', 'Auksis' and 'Prima' had the same PRX banding pattern. 'Antonovka' and 'Exeter', *M. prunifolia* 4630 and *M. prunifolia* 4633 showed the uniform banding pattern, respectively. The

Species	Number of genotypes with particular RAPD marker																	
	11	10	9	9	8	8	8	7	7	7	6	6	6	6	5	5	5	4
R. nigrum ssp. *sibiricum*	■	■		■		■	■	■	■	■		■	■			■		■
R. fontaneum	■	■	■	■	■	■	■		■	■	■	■		■			■	
R. pauciflorum	■	■	■	■	■	■	■	■	■		■		■	■			■	
R. usuriensis	■	■	■	■	■	■	■	■		■		■	■			■		
R. hudsonianum	■	■	■	■	■	■	■	■	■	■	■						■	■
R. nigrum var. Minaj Shmiriov	■	■	■		■	■				■		■		■	■		■	
R. sanguineum	■			■	■		■	■				■	■	■		■		
No. 2	■	■	■		■					■			■	■	■			
No. 3	■	■	■	■	■	■		■	■		■	■	■		■	■		■
No. 9	■	■	■	■			■	■	■	■	■			■	■			
No. 11	■	■	■	■		■	■		■		■				■	■	■	■
No. of marker	19	27	28	13	29	5	14	21	10	7	34	23	30	22	35	8	2	3

Species	Number of genotypes with particular RAPD marker																			A
	4	4	4	3	3	3	2	2	2	2	2	2	2	2	2	1	1	1	1	
R. nigrum ssp. *sibiricum*				■		■	■			■			■							17
R. fontaneum						■			■								■			16
R. pauciflorum	■	■			■					■				■	■	■				20
R. usuriensis	■			■	■			■	■			■								18
R. hudsonianum				■			■	■												16
R. nigrum var. Minaj Shmiriov		■	■		■	■												■	■	16
R. sanguineum	■		■																	11
No 2		■													■					10
No 3	■	■	■									■	■							19
No 9			■								■									13
No 11											■			■						14
No of marker	20	16	9	17	24	1	15	31	26	25	12	6	4	37	33	18	11	32	36	

[1] A – number of markers present in each genotype. A gray box represents the presence of a marker.

Fig. 1. RAPD markers detected in *Ribes* species and interspecific hybrids

band at R_f = 0.48 had four of ten scab immune varieties ('Štaris', 'Aldas', 'Chistotel', SR 0523 and 'Katja' which is a donor of polygenic resistance to scab) [10]. The previously mentioned immune varieties had two more bands (R_f = 0.50, R_f = 0.54) joint with the band at R_f = 0.48, but it was not common for 'Katja'. The band at R_f = 0.50 was common for most (eight of ten) scab immune apple varieties. Varieties not immune to scab ('Noris', 'Ranger', 'Antonovka' and 'Exeter') had PRX band at R_f = 0.50 as well and it joined with other two bands at R_f = 0.73 and R_f = 0.76.

The morphological traits of plants and the PPO isozimes were changing in the course of currant development. Threfore, isozymes of currant species were established in different stages of organgenesis, *i.e.* stages V^a and XI (Fig. 3). PPO isoforms of currant species of Eucoreosma section, whose Rf 0.51–0.54, were found in all species. Comparing bark isoforms of currant species in stage V^a and XI we observed that part of isoforms were the same and part were new ones. In stage V^a in the bark of all species there were found 1–3 isoforms, except *R. pauciflorum,* which had 6 isoforms. In stage XI *R. janzcewskii* had 1 isoform, *R. nigrum* ssp. *sibiricum* 2, *R. nigrum* ssp. *europaeum*, *R. hudsonianum* and *R. americanum* 3, *R. dikuscha* and *R. pauciflorum* 5. In buds (stage V^a) many isoforms were found in *R. hudsonianum, R. dikuscha* and *R. nigrum* ssp. *sibiricum* (8, 7 and 6 isoforms, respectively), and little in *R. nigrum* ssp. *europaeum* (1 isoform), *R. americanum* had 3

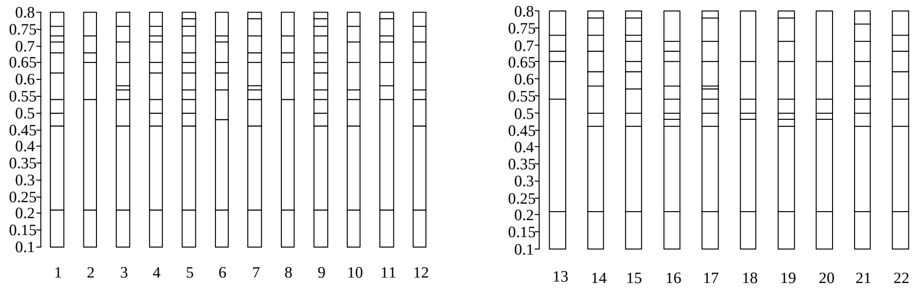

Fig. 2. Schematic representation of the variation in peroxidase isozymes in apple leaf extracts in July. 1 – *Noris,* 2 – *Vista Bella,* 3 – *Papirovka*, 4 – *Ranger*, 5 – *Antonovka,* 6 – *Katja,* 7 – *Tellissaare,* 8 – *Auksis,* 9 – *Exeter,* 10 – *M. prunifolia* 4630, 11 – *M. prunifolia* 4632, 12 – *M. prunifolia* 4633, 13 – *Prima,* 14 – *Priam,* 15 – *Florina,* 16 – *Štaris,* 17 – *Liberty,* 18 – *Aldas, 19 – Chistotel*, 20 – SR 0523, 21 – *Arbat*, 22 – No 22001

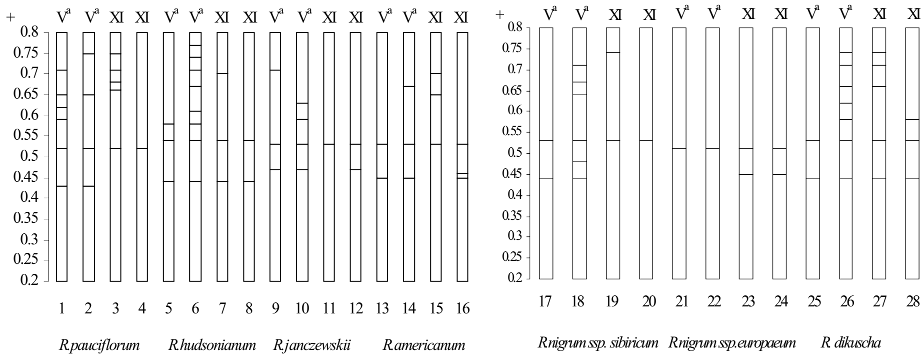

Fig. 3. Electrophoregram of polyphenoloxidase isoforms of *Eucoreosma* section in currant species. 1, 2, 3, 4 – *R. pauciflorum,* 5, 6, 7, 8 – *R. hudsonianum,* 9, 10, 11, 12 – *R. janzcewskii,* 13, 14, 15, 16 – *R. americanum*, 17, 18, 19, 20 – *R. nigrum ssp. sibiricum,* 21, 22, 23, 24 – *R. nigrum ssp. europaeum,* 25, 26, 27, 28 – *R. dikuscha.* 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 – bark, 2, 6, 10, 14, 18, 22, 26 – buds, 4, 8, 12, 16, 20, 24, 28 – leaves

and *R. pauciflorum*, *R. janzcewskii* 4. In leaves, all species had a few isoforms (1–3). It should be noted that *R. americanum* and particularly *R. nigrum* ssp. *europaeum* in Lithuania are insufficiently cold resistant and winterhardy. It is possible to suppose that the species with a small number of isoforms are not winterhardy. The currant species in the dormancy period (stage V^a), under low temperatures, have more PPO isoforms in comparison with the summer period. The best way to identify the wild species of *Eucoreosma* section is to study PPO isoforms from bark and buds in organogenesis stage V^a and in stage XI from bark and leaves.

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SODO AUGALŲ ATSPARUMO GRYBINĖMS LIGOMS, IŠTVERMINGUMO ŽIEMĄ IR PRODUKTYVUMO GENETINĖ KONTROLĖ, TAIP PAT DNR IR IZOFERMENTINIŲ ŽYMEKLIŲ PANAUDOJIMAS ATRENKANT DONORUS

Santrauka

Atlikus sisteminius obelų ir juodųjų serbentų veislių kryžminimus, įvertintas palikuonių atsparumas grybinėms ligoms, jų produktyvumas, taip pat obelų sėjinukų ištvermingumas žiemą. Nustatyti patikimi tirtų požymių bendrosios kombinacinės galios skirtumai. Tirtų požymių paveldėjimą labiausiai determinuoja genai su adityviais efektais. Ištyrus juodųjų serbentų rūšių ir formų DNR polimorfizmą, nustatyti molekuliniai žymekliai, būdingi *Ribes* genčiai, bei specifiniai žymekliai atskiroms jos rūšims. Tirta obelų peroksidazės fermentinė sistema yra polimorfiška, o tai leido išskirti specifines fermento izoformų elektroforetines linijas arba jų derinius, būdingus jautrioms arba rauplėms atsparioms obelų veislėms ir formoms. Polifenoloksidazės polimorfizmas juoduosiuose serbentuose leidžia teigti, jog izoformų linijų skaičius per vegetaciją kinta ir šalčiams ištvermingesnės rūšys turi didesnį izoformų skaičių.