

Powdery Mildew Resistance in the Peach Cultivar Pamirskij 5 Is Genetically Linked with the *Gr* Gene for Leaf Color

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Abstract. Peach powdery mildew (*Podosphaera pannosa* var. *persicae*) is a major disease of peach trees [*Prunus persica* (L.) Batsch]. Various studies on powdery mildew resistance have been conducted in peach. The present study was initiated to determine the inheritance of powdery mildew resistance found in the green leaf peach rootstock ‘Pamirskij 5’. Crosses were carried out between ‘Pamirskij 5’ and the susceptible red leaf peach rootstock ‘Rubira’[®]. Segregation analysis performed in F₁ (1:0), F₂ (3:1), and test cross (1:1) progenies indicated single dominant gene control of peach powdery mildew resistance in ‘Pamirskij 5’. Cosegregation analysis provided clear evidence that the loci for powdery mildew resistance and the leaf color trait are very closely linked in ‘Pamirskij 5’. The estimated distance between these loci was 0 ± 3.26 cM for the F₂ and 0 ± 0.00 cM for the test cross populations.

Peach powdery mildew caused by *Podosphaera pannosa* (Wallr.) var. *persicae* is a fungal disease of the cultivated peach (*Prunus persica* L. Batsch) spread throughout all European production areas. Circular whitish spots on leaves, young twigs, and fruits are typical symptoms of powdery mildew infection. The disease may induce necrosis and deformations resulting in unmarketable fruit, premature leaf drop, and shoot stunting harmful to the crop. Most peach cultivars are susceptible to powdery mildew and numerous applications of fungicide are required from prebloom until after harvest. To satisfy consumers’ request for a reduction of chemical inputs (Byrne, 2002) and for an environmentally safe solution, resistant cultivars are a desirable alternative to fungicides for effective control of powdery mildew.

Various studies have been conducted to evaluate peach cultivars and *Prunus* species for their response to powdery mildew. Several sources of resistance have been identified in related wild species close to *P. persica* (Smykov et al., 1982). The polygenic resistance of *P. davidiana* (Carr.) Franch. is already being exploited in a breeding program (Foulongne et al., 2003a, 2003b). The *Ee* locus controlling the presence/absence and shape of foliar glands in peach has been reported to be associated with very high susceptibility to this fungus (Watkin and Brown, 1956). This has led peach breeders to select only glandular cultivars. Among glandular cultivars, a single

dominant gene conferring resistance to powdery mildew was found in ‘Ustoichivy Pozdni’ (Tsukanova et al., 1982). Powdery mildew resistance in *P. ferganensis* (Kost. and Rjab) Kov. and Kost. (Dabov, 1983) was found to involve two loci: the partially dominant allele *Vr* controls high resistance and is epistatic to the dominant allele *Sr* controlling low resistance.

In France, seeds of a new resistance source called ‘Pamirskij 5’ (clone S 6146) have been introduced from the Nikita Botanical Garden of Yalta (Crimea, Ukraine) through the courtesy of Professor Eremin States (personal communication) that the powdery mildew resistance of ‘Pamirskij 5’ was higher than resistant cultivars such as Malo Konare developed by Dabov (1985). In our environmental conditions, ‘Pamirskij 5’ has shown a high level of resistance to powdery mildew in different experiments over several years. The main objective of this study was to determine the genetic control of resistance to powdery mildew in the peach rootstock cultivar Pamirskij 5. The second was to assess linkage relationships between resistance to powdery mildew and two major genes brought by the peach rootstock cultivar Rubira[®] (clone S2605), used here as the powdery mildew-susceptible parent and homozygous, respectively, for the resistance to the green peach aphid *Myzus persicae* (Pascal et al., 2002) and the red leaf character (*Grgr*).

Materials and Methods

Two hundred fifty F₁ hybrids were obtained from a first cross between ‘Rubira’[®] and ‘Pamirskij 5’. F₂ families were produced

by self-pollination of five F₁ hybrid plants and a pseudo-test cross (TC₁) population was generated by crossing one F₁ hybrid with ‘Big Top’[®] (clone 5811), a nectarine cultivar susceptible to powdery mildew with green foliage. Selfed progenies (S₁) of the parental cultivars were created at each step of the genetic study. Seeds from all crosses were harvested at full maturity, extracted, and stratified. After radicle emergence, seeds were planted individually in 1-L plastic pots containing a commercial horticultural mix and randomized in a greenhouse. Temperatures were maintained at 23 ± 5 °C and seedlings were watered daily ad libitum with a commercial solution containing micronutrients.

Plants were evaluated for resistance to the green peach aphid (Pascal et al., 2002), scored for red or green leaf color, and pruned before artificial inoculation by *S. pannosa* once they developed succulent shoots. Initially, the inoculum source derived from the populations endemic to Avignon (France) was maintained on potted susceptible peach seedlings. Inoculations were carried out by blowing or shaking fungal spores from these heavily infected plants onto young test progenies. In addition, heavily infected susceptible plants were grown adjacent to the progenies being tested as a continuing source of inoculum. Plants were evaluated three times over a 4-week period using a well-tried linear rating scale consisting of the following: 0 = no visible symptoms to 4 = white spots completely covering leaves and young stems (Dirlewanger et al., 1996; Foulongne et al., 2003a, 2003b; Pascal et al., 1998). Plants scored 0 and 1 were considered resistant. Segregation ratios were analyzed using standard χ^2 procedures. Maximum likelihood tables (Allard, 1956) were used to calculate recombination rates for close linkages. The recombination rates were converted to map distances in centiMorgans (cM) using the formula of Kosambi (1944).

Results and Discussion

All S₁ progenies of ‘Rubira’[®] were uniformly and highly susceptible to infection by powdery mildew in both experiments. Conversely, all S₁ progenies of ‘Pamirskij 5’ as well as all F₁ plants were uniformly and highly resistant (Table 1). These results show that ‘Pamirskij 5’ and ‘Rubira’[®] are homozygous for resistance and susceptibility to powdery mildew, respectively. The high level of resistance expressed by all F₁ plants indicates that resistance is dominant to susceptibility. Segregation data from the F₂ and TC₁ progenies were tested for goodness-of-fit to expected ratios using χ^2 analysis. The results of the χ^2 analysis indicate that the F₂ and TC₁ progenies fit 3:1 and 1:1 ratios, respectively. The data are consistent with powdery mildew resistance in ‘Pamirskij 5’ being controlled by a single homozygous dominant gene that we propose to call *Vr2*.

The use of single dominant genes for the development of resistant cultivars is highly controversial because of resistance durability aspects. However, in a perennial plant such

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as peach, little effort has been devoted to pest improvement and few sources of resistance are available. The use at short term by peach breeders of *Vr2* could constitute an essential first step to the development of more stable and long-lasting resistance. Furthermore, this use would be fully justified by the high level of resistance to powdery mildew of 'Pamirskij 5', which is clearly more resistant than most current conventional peach cultivars and than resistant cultivars developed by Dabov (Arregui et al., 1999). Nevertheless, to better characterize the resistance genes originating from different genetic backgrounds, it would be necessary to test for allelism the resistance originating from 'Pamirskij 5' and 'Malo Konare'.

Two linkage tests were conducted involving powdery mildew resistance from 'Pamirskij 5' and green peach aphid resistance and the red leaf traits from 'Rubira'. χ^2 analysis of segregation data were consistent with independent assortment of powdery mildew resistance and green peach aphid resistance (data not shown). Conversely, distorted segregation ratios were observed in the joint distributions of powdery mildew resistance and the leaf color character (Table 2). In F_2 populations, there was an excess of parental types (susceptible red and resistant green)

and a large shortage of recombinant types (resistant red and susceptible green). Recombinants with susceptibility to powdery mildew and green foliage phenotype were not observed in $\approx 940 F_2$ plants. Similarly, no recombinant types were observed in the TC_1 population. From these results we could postulate that the resistant red leaf progeny were heterozygous at both loci (*Vr2vr2Grgr*) analogous to the F_1 hybrids. These results clearly indicate a very strong linkage in the repulsion phase ($\chi^2 = 105.3, P = 0$) between the *Vr2* resistant allele and the red leaf color allele in 'Rubira', which constitutes a new case of linkage in peach.

On the basis of pooled F_2 and TC_1 data (Table 2), the recombination rate between these two genes was calculated as equal to 3.26% and 0%, respectively. The genetic distance converted from the F_2 recombination rate was 0 ± 3.26 cM. Based on TC_1 data, the recombination rate was equal to 0 ± 0.00 cM.

The linkage between *Gr* and *Vr2* could be used for marker-assisted selection in the introduction of the 'Pamirskij 5' resistance gene to peach powdery mildew in fruit cultivars. The *Gr* gene controlling red leaf color has been mapped to linkage group 6 both in a genetic map derived from an interspecific cross with GN22 (Dirlewanger et al., 2004)

and another from an intraspecific cross between Japanese peach cultivars Akame and Juseitou (Yamamoto et al., 2005). This would suggest starting the search for linked molecular markers on linkage group 6 and would be consistent with the fact that in *P. davidiana*, the quantitative trait locus (QTL) with the strongest effect for powdery mildew resistance, was on this linkage group (Foulongne et al., 2003a, 2003b). *Vr2* would thus be located on a linkage group distinct from the major quantitative trait locus (QTL) of resistance to peach powdery mildew from *P. ferganensis*, which mapped on linkage group 7 (Dettori et al., 2001; Verde et al., 2002) and is associated with the *Ee* foliar gland locus. The diversity of genomic loci implicated in powdery mildew resistance in peach and related species should make possible the pyramiding of different resistant genes to build more durable resistance.

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Table 1. Segregation ratios for reaction to peach powdery mildew and leaf color of parental selfs, F_1 , F_2 , and test cross progenies derived from the resistant peach cultivar Pamirskij 5 and the susceptible peach cultivar with red foliage, 'Rubira'.

Cross	Progeny observed		Expected ratios ^a	χ^2 (1 df)	P
	Resistant	Susceptible			
(Rubira) ² S1	0	45	0: 1	—	—
(Pamirskij 5) ² S1	131	0	1: 0	—	—
Rubira \times Pamirskij 5 F1	250	0	1: 0	—	—
[(Rubira \times Pamirskij 5)1] ² F2	210	67	3: 1	0.097	0.754
[(Rubira \times Pamirskij 5)2] ² F2	53	19	3: 1	0.074	0.785
[(Rubira \times Pamirskij 5)3] ² F2	197	63	3: 1	0.082	0.774
[(Rubira \times Pamirskij 5)4] ² F2	217	63	3: 1	0.933	0.333
[(Rubira \times Pamirskij 5)5] ² F2	37	14	3: 1	0.163	0.686
[(Rubira \times Pamirskij 5)1] \times Big Top TC1	257	264	1: 1	0.094	0.759
	Red	Green			
(Rubira) ² S1	45	0	1: 0	—	—
(Pamirskij 5) ² S1	0	131	0: 1	—	—
Rubira \times Pamirskij 5 F1	250	0	1: 0	—	—
F2 pooled data for leaf color	696	244	3: 1	0.459	0.497

^aExpected segregation based on a one-gene model with powdery mildew resistance and red leaf trait dominant.

Table 2. Evidence for repulsion phase linkage between the genes for resistance to peach powdery mildew and leaf color in peach.

Cross	Progeny observed				Expected ratios ^a	χ^2	df	P
	Resistant		Susceptible					
	Red	Green	Red	Green				
[(Rubira \times Pamirskij 5)1] ² F2	135	75	67	0	9:3:3:1	34.7	3	3.10 ⁻⁹
[(Rubira \times Pamirskij 5)2] ² F2	28	25	19	0	9:3:3:1	20.3	3	6.10 ⁻⁶
[(Rubira \times Pamirskij 5)3] ² F2	129	68	63	0	9:3:3:1	30.1	3	4.10 ⁻⁸
[(Rubira \times Pamirskij 5)4] ² F2	154	63	63	0	9:3:3:1	21.7	3	3.10 ⁻⁶
[(Rubira \times Pamirskij 5)5] ² F2	24	13	14	0	9:3:3:1	7.2	3	0.007
F2 pooled data	470	244	226	0	9:3:3:1	105.3	3	0
F2 homogeneity						12.7	8	0.11
[(Rubira \times Pamirskij 5)1] \times Big Top TC1	0	257	264	0	1:1:1:1	521.1	3	0

^aExpected segregation based on a two independent gene model with powdery mildew resistance and red leaf trait dominant.

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