

EVALUATION OF FOLIAR RESISTANCE OF GRAPEVINE GENETIC RESOURCES TO DOWNY MILDEW (*PLASMOPARA VITICOLA*)

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Abstract

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In this study altogether the resistance of 44 grapevine cultivars to downy mildew was evaluated in a long-term field experiment (1996–2003). The resistance was evaluated under conditions of a natural infection pressure using the OIV 452 scale as well as on the base of the evaluation of disease incidence of sporulation and disease incidence of necrosis. The highest degree of resistance to downy mildew was found out in resistance donors Seyve Villard 12 375, Seibel 13 666 and Bianca cultivar. High correlations existing among individual methods of evaluation indicated a good applicability of all tested methods. The evaluation of disease incidence of sporulation and disease incidence of necrosis demonstrated the dependence of traits associated with the resistance to downy mildew on the colour of berries. When evaluating the correlation existing between the resistance and pedigree, it was demonstrated that the highest average resistance existed in cultivars that had the cultivar Seyve Villard 12 375 as the donor of resistance in their pedigree. Basing on obtained results it can be concluded that the OIV 452 scale can be considered as the basic method of resistance evaluation to downy mildew, above all in cases of a long-term evaluation under field conditions.

grapevine, genetic resource, resistance, downy mildew, OIV scale

The downy mildew, which is caused by the oomycetes, *Plasmopara viticola* (Berk & Curt.) Berl. & de Toni, is one of the most important fungal diseases of grapevine plants occurring in vineyards all the world round. The oomycetes *Plasmopara viticola* infests all green parts of the grapevine plant but above all leaves, flowers, inflorescences and berries. The infestation of plants with this pest may result in marked economic losses.

The infection of plants spreads very quickly above all under warm and humid weather conditions because they are very favourable for the formation of sporangiophores as well as for the release and germination of sporangia; this results in the occurrence of new infections or even epidemics. The photosynthetic capacity of infected plants is reduced, inflorescences and berries necrotise and this altogether results in decreased yields and deteriorated quality of berries (Liu *et al.*, 2008). Under vineyard conditions, downy mildew infected leaves

develop a series of disease symptoms. These involve leaf chlorosis, sporulation due to the development of white downy sporangiophores and sporangia on the abaxial surface (as infection progresses), and leaf necroses (Liu *et al.*, 2003).

Plasmopara viticola is an original disease of wild *Vitis* species to North America. In Europe this disease was found for the first time in 1878. It is probable that this disease was introduced together with imports of wild American grapevine species that were used in France as rootstocks for the recovery of vineyards destroyed by phylloxera (Gessler *et al.*, 2011). In the end of the 19th century very extensive selection and breeding activities were started in Europe and their main objective was to select such grapevine cultivars that would show resistance not only to etiological agents causing not only downy mildew but also other fungal pathogens.

North American and East Asian species of the genus *Vitis* are the main source of resistant plant

material (Alleweldt, 1996). A survey of the genetic resources used for early resistance breeding made evident, that just a limited number of resistance donors provided the basis of today's elite lines for wine grapes (Eibach, 1994). A systematic approach to take advantage of genetic resources is the introgression of resistance traits from wild *Vitis* species followed by consecutive backcrosses with *V. vinifera* L. subsp. *vinifera* (Töpfer *et al.*, 2011).

The evaluation of resistance to downy mildew (*Plasmopara viticola*) may be performed either under field conditions (Eibach *et al.*, 1989; Brown *et al.*, 1999a; Kozma, 2000; Boso *et al.*, 2011) or laboratories and greenhouses. Under field conditions, grapevine plants may be infected either artificially or let to be exposed to a natural pressure of this pathogen. Thereafter, the disease develops under natural conditions (Boso *et al.*, 2006). In laboratory and/or greenhouse, the evaluation of resistance may be performed on leaf discs (Staudt and Kassemeyer, 1995; Brown *et al.*, 1999b; Boso *et al.*, 2006; Boso and Kassemeyer, 2008) or using *in-vitro* cultures (Barlass *et al.*, 1986), detached leaves (Kiefer *et al.*, 2002; Boso *et al.*, 2006; Boso and Kassemeyer, 2008), leaf single-node techniques (Liu *et al.*, 2003) and/or whole plants grown in a greenhouse (Brown *et al.*, 1999a; Boso *et al.*, 2006; Gindro *et al.*, 2006; Boso and Kassemeyer, 2008).

One of missing links for further improvement of breeding is an evaluation of the grapevine genetic resources. The existing grapevine collection could be very helpful and provide the possibility of evaluation at various environmental conditions. A harmonization of evaluation protocols can be envisaged and particularly achieved on the base of OIV descriptors (Töpfer *et al.*, 2011).

The aim of this study was to carry out long-term experiments enabling to evaluate the resistance of grapevine genetic resources to the downy mildew under field conditions. The followed parameters involved effects of cultivar, resistance donor, and colour of berries. Basing on obtained results it allowed us to recommend genetic resources suitable for the breeding and selection of cultivars resistant to *Plasmopara viticola*.

MATERIALS AND METHODS

This research was performed using the material from a collection of grapevine genetic resources of the Faculty of Horticulture, Mendel University in Brno. The plantation is situated in the locality "Na Valtické" in the grape-growing municipality Lednice na Moravě near the Czech-Austrian border.

The evaluation involved altogether 44 cultivars; of these 2 were of European origin (*V. vinifera* L., viz. Riesling and Blaufränkisch), 2 were donors of resistance (Seyve Villard 12 375 and Seibel 13 666) and the remaining 40 were interspecific hybrids. Interspecific hybrids involved 18 cultivars with white berries (Cvetoschnyi, Kunleany, Zolotistyi Ustoichivyi, Rani Riesling, Lela, Liza, Mila, Zlata, Petra, Erilon, Luminica, Bianca, Riton, Phoenix, Augustovskyi, Biona, Malverina and Savilon), 3 with red berries (Rakisch, XIV-1-76, Festivalnyi) and 19 with blue berries (Golubok, Peking-1, Flakera, Mendeum, Merlan, Morela, Laurot, Marlen, Kofranka, Cerason, Nativa, Mi-5-50, Mi-5-55, Mi-5-70, Mi-5-86, Mi-5-114, Mi-5-122, Demetra, Rubin Tairovskiy). A detailed specification of their pedigrees can be found on the internet address www.evigez.cz.

The resistance of plants to downy mildew was analysed under field conditions and without any artificial infection within the period of 1996–2003. In the course of the experiment no fungicides were applied. Pruning, load of vines and vineyard management were identical during the whole experimental period. Depending on the intensity of downy mildew infection, the degree of infestation of plants was estimated within the period July–September. Always 25 leaves from the central part of the leaf wall (i.e. from the region between the 6th–8th nodes) were evaluated. Leaves were sampled on seven randomly selected vines.

The resistance to downy mildew was evaluated using a scale published in the "International List for Grapevine Varieties and Species Evaluation" (OIV, 1983) and this scale is presented in Tab. I.

The disease incidence of sporulation (DIS) and disease incidence of necrosis (DIN) were evaluated under field conditions. DIS and DIN were evaluated on 100 randomly selected leaves collected from 7

I: Evaluation of resistance to downy mildew on leaves using the OIV 452 scale (OIV, 1983)

Evaluation point	Degree of resistance	Symptom description
1	Very low (very high sensitivity)	Not limited, vast attacked patches or totally attacked leaf blades, strong fungus fructifications, pronounced and dense mycelium, very early leaf drop
3	Low (susceptible cultivar)	Vast, no limited attacked patches, very strong fungus fructification, numerous mycelia, leaf drop not as early as with note 9
5	Medium (medium sensitivity)	Limited attacked patches 1–2 cm in diameter – more or less severe fungus fructification, irregular formation of mycelia
7	High (low sensitivity)	Less necrosis attacked patches, less fructification, few mycelium
9	Very high (no sensitivity)	Punctuated necroses or no symptoms, neither fructification nor mycelium

grapevine plants under study. The DIS value was calculated using the equation:

$$\frac{\text{number of leaves with symptoms of sporulation}}{\text{total number of evaluated leaves}} \times 100.$$

The DIN value was calculated according to the equation:

$$\frac{\text{number of leaves with symptoms of necrotisation}}{\text{total number of evaluated leaves}} \times 100.$$

The obtained results were statistically analysed using the programme UNISTAT and the following basic characteristics were computed: mean, standard values of each trait. Effects of cultivar, donor of resistance and colour of berry were estimated using the ANOVA and Tukey test ($p < 0.05$).

RESULTS AND DISCUSSION

The OIV 452 scale is used as the basic method of the evaluation of grapevine resistance to downy mildew. Nowadays, this scale is very often used also for the phenotypical evaluation of grapevine plants to downy mildew in studies focused on the research of genes and/or resistance mechanisms (Peressotti *et al.*, 2010; Malacame *et al.*, 2011).

Results of evaluation of resistance to downy mildew on the base of the OIV 452 scale are presented in Tab. II. The lowest level of resistance was found out in *V. vinifera* L. cultivars Riesling and Blaufränkisch. Both can be classified as cultivars with a low resistance. There is no significant genetic variability in the response to pathogens infestation but, nevertheless, the majority of them are sensitive to downy mildew (Patil *et al.*, 1989; Brown *et al.*, 1999c; Alonso-Villaverde *et al.*, 2011). In contradistinction to the preceding study, there was no significant difference between two aforementioned cultivars of *V. vinifera* L.

However, in other evaluated interspecific cultivars a highly significant variability of their resistance was observed (Tab. II). These differences were evaluated by means of Tukey's test. The resistance donor Seyve Villard 12 375 showed the highest degree of resistance 8.75; next ones were cultivars Seibel 13 666 (7.50) and Bianca (7.13). In all these cultivars the resistance was manifested as a hypersensitive response, either in the form of spot necroses (Seyve Villard 12 375) or as small necrotic patches (Seibel 13 666, Bianca). The resistance to downy mildew can be manifested also as a hypersensitive reaction, i.e. as the necrotisation of the leaf tissue. In grapevine plants, this hypersensitive reaction is associated with a low or zero sporulation and can be classified as small necrotic spots and/or small but better defined, localised necrotic lesions or spots (Liu *et al.*, 2008); this observation was corroborated also in this study.

A higher level of resistance (according to the OIV 452 scale evaluated with 6 to 7 points) was observed in a great number of cultivars under study.

Also the cultivar Laurot showed a high degree of resistance (6.86). A high resistance (evaluated in average with 6.75 points) was found out in cultivars Merlan, Morela, Riton, and Augustovskiy. Although among cultivars that were evaluated as cultivars with a higher degree of resistance it is possible to observe a marked cultivar variability (Tab. II). Only a medium level of resistance to downy mildew as well as a medium level of sporulation was observed in cultivars originating from crossings with the East Asian species *V. amurensis* Rupr. although species *V. amurensis*, *V. riparia* and *V. rupestris* show a high level of DIN and a low level of DIS (Boso and Kassemeyer, 2008).

Methods of DIS and DIN evaluation were used to obtain a more detailed survey of the level and manners of resistance of individual cultivars against downy mildew. When evaluating the resistance of grapevine plants to downy mildew under field conditions, various types of symptoms may be observed namely from a hypersensitive reaction with the occurrence of necroses to diffuse necroses with a limited sporulation and to sporulation without the occurrence of necrosis (Dai *et al.*, 1995).

DIS values indicate the capability of pathogen agents to thrive on the leaf area of grapevine plants. Again, a high level of sporulation was observed in both cultivars of the species *V. vinifera* L. (i.e. Riesling and Blaufränkisch) that showed a higher level of DIS than in the study published by Boso *et al.* (2011); these authors mentioned that in *V. vinifera* L. cultivars Loureira, Caino Blanco, Moscatel de Grano, Mediano, Moscatel de Grando Menudo Rojo, Prieto Picudo, Albarino, Tempranillo a Morrastrel Bouschet the disease incidence of sporulation was higher than 50%. On the other side, however, in donors of resistance the incidence of sporulation was very low (Seyve Villard 12 375 (4.38) and Seibel 13 666 (8.75). The variability of observed sporulation was much more obvious (Tab. II). A very low level of sporulation was observed in cultivars Merlan, Morela, Laurot, and Cerason. Similar results were obtained also when evaluating DIN. Values of DIN are associated with a high level of resistance to downy mildew. A high level of DIN was found out in cultivars that were evaluated as more resistant also when using other methods of evaluation.

A good suitability of all methods of resistance evaluation was indicated also by correlations. The highest values of Pearson's coefficient of correlation were recorded between DIS and DIN ($r = -0.7875$). High correlations were observed also between results obtained by means of OIV 452 scale and DIS ($r = -0.7371$) and between OIV 452 values and DIN ($r = 0.7031$). Based on these results it can be concluded that the evaluation on the base of OIV 452 scale can be used as a basic method of evaluation of resistance of grapevine plants to downy mildew, especially in long-term experiments performed under field conditions.

Results of long-term evaluation of resistance to fungal pathogens obtained under field conditions

II: Evaluation of resistance to downy mildew using the OIV 452 scale, disease incidence of sporulation and disease incidence of necrosis. Results were evaluated using ANOVA and the Tukey's test.

Grapevine cultivar	Origin/ Pedigree	Evaluation with OIV scale	Disease incidence of sporulation	Disease incidence of necrosis
Blafränkisch	<i>V. vinifera</i>	2.57 ± 0.63a	76.25 ± 11.88v	0.00 ± 0.00a
Riesling	<i>V. vinifera</i>	2.50 ± 0.53a	71.25 ± 14.58v	0.00 ± 0.00a
Cvetoschnyi	<i>V. amurensis</i>	5.38 ± 0.74def	44.38 ± 4.17tu	25.00 ± 5.35e
Kunleany	<i>V. amurensis</i>	5.88 ± 0.64fghi	37.50 ± 7.07pqrs	13.75 ± 5.18b
Zolotistyi Ustoichivyi	<i>V. amurensis</i>	4.75 ± 0.46bc	39.38 ± 7.76qrst	15.63 ± 4.96bc
Golubok	<i>V. amurensis</i>	5.13 ± 0.83bcd	46.25 ± 10.26u	15.00 ± 5.35bc
Peking-1	<i>V. amurensis</i>	6.13 ± 0.35ghijk	33.75 ± 9.16opq	17.50 ± 4.63bcd
Rani Rizling	<i>V. amurensis</i>	6.13 ± 0.64ghijk	30.00 ± 7.56mno	16.25 ± 3.54bc
Lela	<i>V. amurensis</i>	5.88 ± 0.83fghi	36.25 ± 5.18pqr	16.88 ± 5.30bc
Liza	<i>V. amurensis</i>	5.75 ± 0.71efgh	36.25 ± 5.18pqr	17.50 ± 2.67bcd
Mila	<i>V. amurensis</i>	5.75 ± 0.71efgh	40.00 ± 5.35rst	18.75 ± 4.43bcd
Zlata	<i>V. amurensis</i>	5.63 ± 0.74defg	38.75 ± 6.41qrst	16.25 ± 4.43bc
Petra	<i>V. amurensis</i>	5.63 ± 0.74defg	37.50 ± 8.86pqrs	19.38 ± 3.20cd
Erilon	Seibel 13666	6.63 ± 0.52klmn	18.13 ± 2.59cdefghi	47.50 ± 8.86klmn
Flakera	Seibel 13666	5.88 ± 0.35fghi	43.13 ± 8.43stu	25.00 ± 5.35e
Mendeleum	Seibel 13666	5.25 ± 0.46cde	38.75 ± 5.82qrst	17.50 ± 3.78bcd
Merlan	Seibel 13666	6.75 ± 0.46lmn	13.13 ± 3.72bcd	53.75 ± 7.44op
Morela	Seibel 13666	6.75 ± 0.46lmn	13.75 ± 3.54bcde	60.00 ± 7.56q
Luminica	Seibel 13666	4.63 ± 0.52b	41.25 ± 13.56rstu	18.13 ± 2.59bcd
Laurot	Seibel 13666	6.86 ± 0.35mn	13.13 ± 3.72bcd	53.75 ± 7.44op
Marlen	Seibel 13666	6.14 ± 0.64ghijk	23.75 ± 3.54ijkl	43.75 ± 5.18jkl
Kofranka	Seibel 13666	6.43 ± 0.49ijklm	16.88 ± 2.59cdefg	53.75 ± 5.18op
Cerason	Seibel 13666	6.71 ± 0.45klmn	15.63 ± 4.17cdef	52.50 ± 7.07nop
Nativa	Seibel 13666	5.13 ± 0.35bcd	27.50 ± 3.78klmn	22.50 ± 7.07de
Mi-5-50	Seibel 13666	6.00 ± 0.76ghij	23.13 ± 2.59hijk	36.25 ± 4.43gh
Mi-5-55	Seibel 13666	5.13 ± 0.35bcd	28.13 ± 2.59klmno	33.75 ± 5.18gh
Mi-5-70	Seibel 13666	6.50 ± 0.93jklm	18.75 ± 4.43defghi	50.00 ± 5.35mno
Mi-5-86	Seibel 13666	6.00 ± 0.93ghij	20.63 ± 4.96fghij	26.25 ± 5.18ef
Mi-5-114	Seibel 13666	6.25 ± 0.89hijkl	17.50 ± 2.67cdefgh	48.75 ± 8.35lmno
Mi-5-122	Seibel 13666	5.63 ± 0.52defg	20.00 ± 3.78fghij	25.63 ± 4.96e
Seibel 13666	Seibel 13666	7.50 ± 0.93o	8.75 ± 3.54ab	71.25 ± 8.35r
Bianca	SV 12375	7.13 ± 0.35no	13.75 ± 3.54bcde	60.00 ± 7.56q
Rakisch	SV 12375	6.13 ± 0.35ghijk	31.88 ± 2.59nop	31.25 ± 6.41fg
Riton	SV 12375	6.75 ± 0.46lmn	12.50 ± 5.98bc	56.25 ± 9.16pq
Phoenix	SV 12375	5.38 ± 0.74def	37.50 ± 8.86pqrs	18.13 ± 2.59bcd
XIV-1-76	SV 12375	6.25 ± 0.46hijkl	22.50 ± 2.67ghijk	46.25 ± 5.18klm
Seyve Villard 12 375	SV 12375	8.75 ± 0.71p	4.38 ± 3.20a	85.00 ± 5.35s
Augustovskyi	SV 18315	6.75 ± 0.46lmn	19.38 ± 4.96efghi	43.75 ± 5.18jkl
Biona	SV 18315	6.13 ± 0.35ghijk	29.38 ± 4.17lmno	38.75 ± 6.41hij
Demetra	SV 23657	6.25 ± 0.46hijkl	25.63 ± 4.17jklm	38.75 ± 6.41hij
Festivalnyi	SV 23657	6.50 ± 0.53jklm	17.50 ± 2.67cdefgh	51.25 ± 6.41mnop
Rubin Tairovskyi	SV 23657	6.38 ± 0.52ijklm	18.75 ± 5.18defghi	42.50 ± 7.07ijk
Malverina	Seibel 13 666 SV 12 375	5.12 ± 0.63bcd	37.50 ± 7.07pqrs	25.00 ± 4.63e
Savilon	Seibel 13 666 SV 12 375	6.13 ± 0.64ghijk	18.75 ± 3.54defghi	37.50 ± 4.63hi
Significance (p)		0.000	0.000	0.000

III: Evaluation of resistance to downy mildew according to the berry colour. Results were evaluated using ANOVA and the Tukey's test.

Origin	Evaluation with OIV scale	Disease incidence of sporulation	Disease incidence of necrosis
Blanc	5.87 ± 1.33	31.91 ± 16.18a	29.70 ± 21.00a
Noir	5.97 ± 1.14	25.86 ± 15.88b	37.53 ± 18.39b
Rouge	6.00 ± 0.72	27.34 ± 8.89ab	38.44 ± 12.14b
Significance (p)	0.7131	0.0023	0.0060

IV: Evaluation of resistance to downy mildew according to the resistance donor. Results were evaluated using ANOVA and the Tukey's test.

Origin	Evaluation with OIV scale	Disease incidence of sporulation	Disease incidence of necrosis
V. vinifera	2.53 ± 0.57a	73.75 ± 13.10d	0.00 ± 0.00a
V. amurensis	5.64 ± 0.76b	38.18 ± 8.07c	17.44 ± 5.14b
Seibel 13666	6.12 ± 0.93c	22.33 ± 10.85a	41.11 ± 16.58d
SV 12375	6.73 ± 1.18d	20.42 ± 12.54a	49.48 ± 22.44e
SV 18315	6.44 ± 0.51cd	24.38 ± 6.80ab	41.25 ± 6.19d
SV 23657	6.38 ± 0.49cd	20.63 ± 5.38a	44.17 ± 8.30de
Seibel 13 666 SV 12 375	5.62 ± 0.80b	28.13 ± 11.09b	31.25 ± 7.85c
Significance (p)	0.0000	0.0000	0.0000

are important above all when selecting genetic resources for further selection and/or when introducing new, prospective cultivars into the viticultural practice. Results of this study also fully corroborated that – when evaluating the resistance of plants to *Plasmopara viticola* under field conditions – the obtained results cultivar-dependent (Boso *et al.*, 2006; Boso and Kassemeyer, 2008; Boso *et al.*, 2011).

Regarding the longevity of plants grown under field conditions and because of high costs associated with the establishment of new vineyards it can be concluded that a durable resistance to diseases represents a required property of grapevine cultivars (Cadle-Davidson *et al.*, 2008).

The aim of this study was also to find out correlations existing between the resistance of grapevine plants to downy mildew and the colour of berries. Obtained results are presented in Tab. III.

The evaluation of resistance on the base of OIV 452 scale did not enable to demonstrate the effect of berry colour on the resistance of grapevine plants to downy mildew. Similar results were obtained also in experiments evaluating the resistance of *V. vinifera* L. cultivars to downy mildew because there was no correlation between the sensitivity to *Plasmopara viticola* and the colour of grapevine berries (Boso *et al.*, 2011). In our experiments, the evaluation of DIS and DIN enabled to demonstrate that both these parameters were dependent on the colour of berries. A significantly different level of resistance

was observed in cultivars with white berries because DIS values were the highest while those of DIN the lowest ones. Although the observed differences were only slight, they significantly demonstrated the correlation existing between the colour of berries and the behaviour of the fungal pathogen on grapevine plants.

From the viewpoint of grapevine breeding and selection it is very important to know that it is possible to compare the resistance of genetic resources to downy mildew also on the base of the pedigree of plants. A wide spectrum of cultivars evaluated in this study enabled such a comparison. The obtained results are presented in Tab. IV.

CONCLUSIONS

The highest degree of resistance was observed in cultivars with Seyve Villard 12 375 donor of resistance in their pedigree; they showed lowest average values of DIS and the highest average values of DIN. On the other hand, the lowest degree of resistance to downy mildew was found out in cultivars with *V. amurensis* Rupr. in their pedigree. It was concluded that the method based on the OIV 452 scale represented the basic method of evaluation of resistance of grapevine plants to downy mildew under field conditions. Similar results were obtained also when using the DIN and the DIS methods.

SUMMARY

This study evaluated the degree of resistance of 2 cultivars of *V. vinifera* L. and 42 hybrids and cultivars originating from interspecific crossings to downy mildew (*Plasmopara viticola*). Experiments were performed under field conditions within the period of 1996–2003. The resistance to downy mildew was evaluated by means of the OIV 452 scale and methods of disease incidence of sporulation (DIS), and disease incidence of necrosis (DIN). When using the OIV 452 scale for the evaluation, the highest

degree of resistance was recorded in resistance donors Seyve Villard 12 375 and Seibel 13 666, and in the cultivar Bianca. A high degree of resistance was observed also in cultivars Laurot, Merlan, Morela, Riton, and Augustovskyi. It was concluded that the method based on the OIV 452 scale represented the basic method of evaluation of resistance of grapevine plants to downy mildew under field conditions. Similar results were obtained also when using the DIN and the DIS methods. A good applicability of all methods of resistance evaluation was corroborated also by high values of correlation coefficients. The aim of this study was to reveal mutual relationships and dependences existing between resistance of plants to downy mildew and the colour of berries as well as between the resistance and the pedigree of individual cultivars. The evaluation on the base of DIS and DIN values enabled to demonstrate the dependence of these parameters on the colour of berries. When evaluating the correlation existing between resistance and pedigree, it was found out that the highest average resistance existed in cultivars that had the resistance donor Seyve Villard 12 375 in their pedigree.

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