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EVALUATION OF RESISTANCE TO DOWNY MILDEW IN GRAPEVINE GENETIC RESOURCES

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Abstract

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The evaluation of grapevine genetic resources is very important especially from the viewpoint of the use of some varieties within the process of grape breeding. At present, the breeding of grapevine is more and more focused upon selection of varieties showing an increased resistance to fungal diseases and, especially to downy mildew. At the Faculty of Horticulture, we have studied the resistance of 32 grapevine varieties to downy mildew resistance on leaves. This evaluation took place under field conditions in a vineyard that was not treated with pesticides within the period of 1996–2000. The resistance to downy mildew was evaluated using a scale published in the "International List for Grapevine Varieties and Species Evaluation". The highest degree of resistance was observed in the following varieties: *‚Augustovskii'*, *‚Yalovenskii Ustoichyvii'*, *‚Pőlőskei Muskotály', Pleven ustoichyvii, ‚Aivaz', ‚Dachnyi', ‚Kutuzovskii', ‚Nero', and ‚Smuglyanka Moldavskaya'*. These varieties can be used as starting material for a further breeding. It can be therefore concluded that studies on genetic resources are very important.

grapevine, genetic resource, leaf, resistance, breeding

The grapevine (*Vitis vinifera* L.) is one of the economically most important cultural plants and it is grown practically in all parts of the world. Its berries are used above all for making wine and partly also juices; they are consumed as table grapes and also as raisins.

One of major concerns of modern agriculture is the conservation and utilisation of valuable genetic resources of agricultural plants.

For the time being, the species *Vitis vinifera* is the most frequently cultivated species, above all due to a high quality of its grapes. On the other hand, however, fungal diseases are the most important problem for grapegrowers worldwide (Ferreira et al., 2004).

Downy mildew, a fungal disease caused by *Plasmopara viticola* **(**Berk. et Curt.) Berl. et De Toni) is an important, if not the most serious disease that threatens the yields and quality of grapes in many of the world's viticultural areas (Dai et al., 1995).

The obligate biotrophic parasitic fungus *P. viticola* is the causal agent of the downy mildew disease infesting members of the genus *Vitis*. Its symptoms appear as yellowish, oily spots on the top surface of leaves and the characteristic signs are white sporangiophores and sporangia that penetrate through the stomata (Kast et al., 2000).

Since the genome of *V. vinifera* does not carry any resistance to the downy mildew fungi, multiple fungicide applications per growing season became indispensable for all traditional varieties. Grapevine breeding to combine the resistance of e.g. American *Vitis* species with a good quality of wine made of *V. vinifera* grapes became an important strategy to combat the fungal diseases. Already around 1880, Millardet concluded that a combination of resistance and quality should be possible (Fischer et al., 2004).

The use of resistant varieties represents an alternative way how to prevent infections caused by *P. viticola*. In grapevine, the resistance mechanisms are complex and involve some constitutive substances that act as fungicide-like molecules (Gindro et al., 2003).

The crossing of some varieties of *V. vinifera* with those carrying the genes of resistance to fungal diseases might activate or to a different degree stimulate some of these protective mechanisms and this could explain the different heritability of these characteristics in individual varieties of *V. vinifera* (Eibach, 2000).

The disease resistance of vines can be tested both in field and in laboratory. In the field, vines can either be artificially inoculated or, if the natural conditions are favourable, they may be left untouched so that the disease can develop in a natural way (Boso et al., 2006).

Many studies on the resistance against downy mildew were performed under laboratory conditions using the method of inoculation on leaf discs (Stein et al., 1985; Staudt and Kassemeyer, 1995), leaves (Kiefer et al., 2002), and/or whole plants (Rumbolz et al., 2002).

The evaluation of genetic resources and new candidate varieties of the resistance to downy mildew under field conditions is also a very popular method of testing (Volynkin et al., 1995; Basler and Pfenninger, 2003; Csikász and Kozma, 2003).

The aim of this study was to evaluate the resistance to downy mildew in some selected genetic resources of grapevine under field conditions. The obtained results could be used as a starting point of and a basis for selection and breeding new grape varieties resistant to downy mildew.

MATERIAL AND METHODS

This research was performed using the material from a collection of grapevine genetic resources in the Faculty of Horticulture, Mendel University of Agriculture and Forestry Brno. The plantation is situated in the locality "V Mendeleu" in the grape-growing municipality Lednice na Moravě near the Czech-Austrian border. A survey of varieties under this study is presented in Tab. IV.

The resistance of plants to downy mildew was analysed under field conditions and without any artificial infection within the period of 1996–2000. In the course of the experiment no fungicides were applied. Pruning, load of vines and vineyard management were identical during the whole experimental period.

Each year the infection observation took place during period from July to September. The obtained dates reflect maximum infection in the course of vegetation period. Evaluated were always 25 leaves from the central part of the leaf wall (i.e. from the region between the 6^{th}–8^{th} nodes). Leaves were sampled on seven randomly selected vines.

The variety '*Müller Thurgau'* was utilized for monitoring of intensity of attack and the date of the occurrence of first symptoms of downy mildew infestation *(Plasmopara viticola)* in Lednice na Moravě. This sensitive variety '*Müller Thurgau'* was planted together with table varieties under study and grown without any chemical treatment.

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.

I: *Evaluation of resistance to downy mildew on leaves – OIV 452 (OIV, 1983)*

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

The obtained results were statistically analysed using the programme UNISTAT and the following basic characteristics were computed: mean, standard deviation and the maximum and the minimum values of each trait. Effects of year and variety were estimated using the Kruskall-Wallis analysis and Tukey test ($p < 0,05$).

RESULTS AND DISCUSSION

The evaluation of the resistance to fungal diseases and above all to downy mildew is very important especially with regard to the selection and breeding of new varieties, which should show a high degree of resistance to this disease.

When improving genetic traits of grapevine, the main attention should be paid to its viticultural traits since the quality and yields of grapes of existing varieties are good and quite sufficient. Characteristics of resistance are therefore the most desired traits to be improved, above all due to the fact that especially grapes and their quality require a very intensive and also strict protection and phytosanitary measures.

Under natural conditions and without artificial infections, the occurrence of downy mildew infection and its intensity are significantly influenced by the year. The first symptoms, i.e. oily spots on leaves, intensity of infestation and extent of damage were evaluated on vines of the variety '*Müller Thurgau'*, which is known to be very sensitive to this fungal disease.

The first symptoms of infestation (oily spots on leaves) and its intensity in the variety '*Müller Thurgau'* are presented in Tab. II.

II: *Intensity of attack and the date of the occurrence of first symptoms of downy mildew infestation (Plasmopara viticola) in Lednice*

	1996	1997	1998	1999	2000
Downy mildew on leaves	Strong attack	Strong attack	Medium attack	Medium attack	Medium attack
First symptoms on leaves	20 June	8 July	17 June	24 June	20 July

Barlass et al. (1986) showed, that the expression of resistance to downy mildew in *Vitis* spp. are controlled by local climatic parameters that, and especially the relative air humidity, determine the intensity of infection.

It is believed that the capacity of oospores to infect diminishes rapidly from May to June; thereafter, abundant secondary infections produced by only a few genotypes are assumed to lead to a costly expansion of epidemics until the leaf fall in the autumn (Gobbin et al., 2005).

Although the downy mildew can infest the developing green tissues during the whole growing season, its epidemics usually occur from June to September. Traditionally, the secondary sporangia were viewed as the major cause of the spatial and temporal dissemination of this disease (Blaise et al., 1999).

The effect of individual years on the score of resistance to downy mildew under field conditions, its average values in individual years, and results of a detailed statistical analysis by means of Tukey test are presented in Tab. III.

Vintage influence resistance to downy mildew at leaves was observed using Kruscal-Wallis single factorial analysis. Q = 57.53 was the value that was calculated for the effect of vintage to the resistance against downy mildew on leaves. Basing on a comparison of this value with table values $\chi^2_{(0.05)} = 9.49$ and $\chi^2_{(0.01)} = 13.28$ it was demonstrated that the effect of vintage on the resistance to downy mildew on leaves was statistically highly significant.

III: *Mean, standard deviation and multiple comparison of vintage influence for leaf resistance to downy mildew using Tukey test ($p < 0.05$)*

Year	Mean (x)	Standard deviation (sx)
1996	5.93 ^{ab}	1.79
1997	5.89 ^{a}	1.76
1998	6.12 ^{b}	1.73
1999	6.45 ^{c}	1.73
2000	7.10 ^{d}	1.14

In the average of all varieties, the lowest resistance score was calculated in 1996 and 1997 (5.93 and 5.89, resp.). As shown in Tab. II, the pressure of downy mildew was strong in both years while the highest resistance was observed in 2000 (7.10).

Because the genome of Vitis vinifera does not contain any factors of resistance it is necessary to introduce the genes of resistance to powdery and downy mildew from wild species (e.g. Vitis riparia, Vitis berlandieri, Vitis labrusca etc.). In order to achieve a long-lasting and broad level of resistance, a combination of various resistance factors originating from diffe-

rent genetic sources and used within the framework of pyramidization strategies is anticipated. An evaluation as well as characterisation of genetic resources is hence strongly required (Zyprian et al., 2003).

The genotypic variation in downy mildew resistance must be described visually and estimated on the base of disease severity using a specific scoring system (Stein et al., 1985; Staudt and Kassemeyer, 1995; Brown et al., 1999a, b, c).

Because of this, altogether 32 genetic resources of grapevine resistance to downy mildew were evaluated within the period of 1996–2000 with a special regard to the degree of their resistance and potential to be used in a programme of breeding new varietals of table grapes.

The effect of varieties on the resistance to downy mildew on leaves was evaluated also using Kruscal-Wallis single factorial analysis. Q = 1499.85 was the value calculated for the effect of the variety to the resistance against downy mildew on leaves. y influence evaluation. Basing on a comparison of this calculated value with table values $\chi^2_{(0.05)} = 43.80$ and $\chi^2_{(0.01)} = 50.90$ it was demonstrated that the effect of the variety on the resistance to downy mildew on leaves was also statistically highly significant.

A detailed analysis of observed mean values of resistance of individual varieties is presented in Tab. 4. Besides, this table also contains the maximum and the minimum observed values of resistance, standard deviations, and statistically significant differences evaluated by means of Tukey test ($p < 0.05$).

In the majority of varieties, the strongest attack and the lowest resistance were observed in 1996. As a rule, downy mildew developed already within 5–7 days after the end of flowering and in this time the infection was also the most intensive.

The lowest resistance of Vitis vinifera L. varieties to the occurrence of downy mildew infection on leaves was found out in the varietals 'Chasselas rouge'(2.04).

In 1996, the most resistant varieties to the occurrence of downy mildew on leaves were 'Pölöskei muskotály' (7.32), 'Yalovenskii Ustoichyvii' (7.24), Pleven ustoichyvii (7.16), and 'Dachnyi' (7.16) while the lowest resistance was observed in interspecific hybrids V-46-40 (5.80) and 'Ananasnyi '(5.80).

In 2000, the mean score of resistance for all varieties under study was 7.10 and the first symptoms occurred on 20 July.

The variety 'Chasselas rouge' showed the lowest resistance against downy mildew on leaves (2.60).

In 2000, the highest resistance among interspecific varieties showed 'Aron' (7.56), 'Aivaz' (7.56) and 'Pölöskei muskotály' (7.56) while the lowest one was observed in the interspecific varietal VII-25-41 (5.96).

Within the period of 1996 – 2000, the highest degree of resistance among all interspecific varieties showed 'Pölöskei muskotály '(7.28), 'Dachnyi' (7.26), 'Aivaz' (7.18), and 'Yalovenskii Ustoichyvii' (7.16). Of Vitis vinifera varieties, the highest resistance to the occurrence of downy mildew on leaves was observed in 'Olsava' (2.82).

In 1996–2000, the lowest degree of resistance among all interspecific varieties showed 'Ananasnyi' (6.12), 'Startovyi' (6.08), L-2-14 (6.04) and VII-25-41 (5.84).

Statistically significant differences among individual genetic resources, as defined by means of Tukey test, are presented in Tab. IV. Basing on results of this analysis, the variety 'Pölöskei muskotály' showed the highest degree of resistance (7.56) and markedly differed from all others.

The resistance to downy mildew under field conditions was investigated by many authors. In some variety, similar results were obtained.

Szöke and Kozma (1997) mentioned 'Teréz', 'Eszter', and 'Pőlőskei muskotály' as varietals with a very high degree of resistance.

Bankovskaya and Molchanova (1999) obtained similar results and classified varieties 'Kutuzovskii', 'Krystal' and 'Augustovskii' among those with a high resistance and 'Aivaz' only as tolerant.

Volynkin et al. (1995) observed a low resistance in 'Chasselas Blanc'. A high degree of resistance was found out in varieties 'Augustovskii', 'Yalovenskii Ustoichyvii', 'Kutuzovskii', 'Smuglyanka Moldavskaya' and 'Kodryanka'.

The classification of individual varieties according to their resistance to the occurrence of symptoms of downy mildew on leaves is presented in Tab. V.

The resistance to downy mildew infections, which enables to reduce the application of plant protective agents, is very important above all with regard to the protection of public health, environmental protection and economics of production.

Liu et al. (2003) mentioned that, with regard to the need of an improved reliability of resistance screening techniques, the obtained results indicate that when determining the genotypic resistance and/or susceptibility to the disease it is essential to asses the degree of leaf chlorosis and sporulation.

Plant genetic resources available for food production and agriculture represent a base of global food security and comprise of genetic material occurring in traditional varieties, modern varieties, crop wild relatives and other wild species (Kameswara Rao, 2004).

This group includes the following varieties: '*Augustovskii*', '*Yalovenskii Ustoichyvii*', '*Pőlőskei Muskotály*', *Pleven ustoichyvii*, '*Aivaz*', '*Dachnyi*', '*Kutuzovskii*', '*Nero*', and '*Smuglyanka Moldavskaya*'; however, it should be emphasised in this context that it is quite impossible to consider them automatically as varieties showing a very good resistance.

IV: *Results of resistance evaluation of individual genetic sources of grapevine. Mean, standard deviation and multiple comparison of variety influence for leaf resistance to downy mildew using Tukey test ($p < 0.05$)*

Variety	Colour of berries	Mean (x)	Standard deviation (Sx)	Min.	Max.
Ananasnyi	**B**	6.12^{cde}	0.99	5.80	6.28
Aron	**B**	6.88^{ijklm}	1.13	6.44	7.56
Augustovskii	**B**	7.12^{klm}	1.06	7.00	7.32
Cvetochnyi	**B**	6.70^{fghijkl}	1.31	6.20	7.48
Chasselas blanc	**B**	2.70^{ab}	1.22	2.20	3.40
Yalovenskii Ustoichyvii	**B**	7.46^{klm}	1.27	7.00	7.32
Jolanka	**B**	6.34 ^{cdefghi}	0.94	6.04	6.60
Kristalli	**B**	6.64 ^{efghijk}	1.15	6.04	7.24
L-2-14	**B**	6.04 ^{cd}	1.00	5.96	6.12
Panonia kincse	**B**	2.44 ^{ab}	1.07	2.20	2.84
Pölöskei muskotály	**B**	7.28 ^{m}	1.06	6.76	7.56
Palatina	**B**	6.36 ^{cdefghi}	1.02	6.04	6.68
Rusmol	**B**	6.20 ^{cdefg}	1.06	5.96	6.36
Startovyi	**B**	6.08 ^{cde}	1.08	5.80	6.36
Suzi	**B**	6.18 ^{cdefg}	0.99	5.96	6.36
Ustoichivii Dokuchaevoi	**B**	6.42 ^{defghi}	1.07	6.04	6.76
Vostorg	**B**	6.40 ^{cdefghi}	1.08	6.12	6.92
Pleven ustoichyvi	**B**	7.14 ^{klm}	0.99	7.00	7.24
V-46-40	**B**	6.14 ^{cdef}	1.03	5.80	6.44
BV-18-29	**RG**	6.84 ^{defghij}	1.26	6.12	7.00
Chasselas rouge	**RG**	2.24 ^{a}	1.02	1.96	2.60
VII-25-41	**RG**	5.84 ^{c}	1.03	5.56	5.96
XV-7-72	**RG**	6.78 ^{hijklm}	1.17	6.12	7.40
Agat Donskoi	**N**	6.72 ^{ghijklm}	1.01	6.36	7.24
Aivaz	**N**	7.18 ^{klm}	1.01	6.84	7.56
Alden	**N**	6.24 ^{cdefgh}	1.09	5.96	6.84
Dachnyi	**N**	7.26 ^{lm}	1.05	7.08	7.48
Kodryanka	**N**	6.20 ^{cdefg}	0.98	5.96	6.68
Kutuzovskii	**N**	7.00 ^{jklm}	1.06	6.84	7.24
Nero	**N**	7.30 ^{jklm}	1.14	6.84	7.48
Olsava	**N**	2.82 ^{b}	1.14	2.36	3.56
Smuglyanka Moldavskaya	**N**	7.00 ^{jklm}	1.14	6.68	7.48

Colours of berries: B – red, N – black, RG – rosé

Some of them can be held for valuable donors of genes for breeding programmes and selection of plants resistant to various diseases, but first of all to downy mildew.

The evaluation of genetic grapevine resources under field conditions is therefore very important and the obtained results represent a very useful information and valuable material for those plant breeders that occupy themselves with breeding of varieties resistant to downy mildew.

SUMMARY

Study of plants genetic resources is very important for breeding of new plant varieties. Genetic resources of grapevine are important for breeding to resistance of biotic and abiotic factors and grape quality.

V: *Classification of varieties according to their resistance of downy mildew on leaves.*

Resistance score	Variety
9–8	
8–7	*Augustovskii, Yalovenskii Ustoichyvii, Pőlőskei muskotály, Pleven ustoichyvii, Aivaz, Dachnyi, Kutuzovskii, Nero, Smuglyanka Moldavskaya*
7–6	*Ananasnyi, Aron, Tschvetochnyi, Jolanka, Kristalli, L-2-14, Palatina, Rusmol, Startovyi, Suzi, Ustojchyvii Dokuchaevoi, Vostorg, V-46-40, BV-18-29, XV-7-72, Agat Donskoi, Alden, Kodryanka*
6–5	*VII-25-41*
5–4	
4–3	
3–2	*Chasselas blanc, Panonia Kincse, Chasselas rouge, Olsava*
2–1	

The aim of this research was evaluation of resistance to downy mildew in the field condition. I evaluated 32 grapevine varieties. The resistance to downy mildew was evaluated using a scale OIV. This research was performed in the Faculty of Horticulture, Mendel University of Agriculture and Forestry Brno in the years 1996–2000.. The highest degree of resistance was observed in the following varieties: *‚Augustovskii‘*, *‚Yalovenskii Ustoichyvii‘*, *‚Pőlőskei Muskotály‘, Pleven ustoichyvii, ‚Aivaz‘, ‚Dachnyi‘, ‚Kutuzovskii‘, ‚Nero‘, and ‚Smuglyanka Moldavskaya‘*. These varieties can be used as starting material for a further breeding for resistance to downy mildew.

SOUHRN

Hodnocení odolnosti k plísni révové u genových zdrojů révy vinné

Studium genových zdrojů rostlin je velmi významné z pohledu šlechtění nových odrůd. Genové zdroje révy vinné jsou velmi významné z pohledu šlechtění na odolnost k nepříznivým biotickým a biotickým faktorům a na kvalitu hroznů a vína. Cílem práce bylo vyhodnocení odolnosti 32 odrůd révy vinné k plísni révové v polních podmínkách. Hodnocení bylo prováděno metodikou OIV pro sledování odrůd révy vinné. Hodnocení probíhalo v letech 1996–2000 na Zahradnické fakultě v Lednici. Nejvyšší stupeň odolnosti byl pozorovaný u následujících odrůd: *‚Augustovskii‘*, *‚Yalovenskii Ustoichyvii‘*, *‚Pőlőskei Muskotály‘, Pleven ustoichyvii, ‚Aivaz‘, ‚Dachnyi‘, ‚Kutuzovskii‘, ‚Nero‘, and ‚Smuglyanka Moldavskaya‘*. Tyto odrůdy mohou být použity jako výchozí materiál pro další šlechtění révy vinné na odolnost k plísni révy.

réva vinná, genové zdroje, list, rezistence, šlechtění

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