

Do geographical barriers play any role in isolation of powdery mildew populations?

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It has been supposed that wheat powdery mildew populations (*Blumeria graminis* f. sp. *tritici*) in Dobrá Voda and Turiec Valley may be isolated from other mildew populations by geographical barriers surrounding them. Mildew isolates were obtained from exposed trap plants in mobile and static nurseries (Dobrá Voda). The single colony isolates were subsequently tested on detached leaves of seedlings of sets of differential wheat cultivars/lines with known specific resistance genes. Virulence frequencies in the mildew populations from Dobrá Voda and Turiec Valley were compared to virulence frequencies from their neighbouring regions. Euclidian distances agreed with geographical distances in populations from Dobrá Voda and its neighbouring populations. Cluster analysis has repeatedly proved that mildew population from Turiec Valley belongs to a separate cluster. Differences in virulence frequencies between these clusters were not significant, however, mildew population from Turiec Valley is considered to be different.

Geographical barriers, mountains surrounding Dobrá Voda and Turiec Valley, do not maintain any significant isolation for the migration of the pathogen spores on prevailing winds, even though the spread of the similar host genotypes within Slovakia and Europe makes the study of geographical isolation more difficult.

Key words: wheat, powdery mildew, *Blumeria graminis* f. sp. *tritici*, resistance, geographical barriers.

Introduction

Geographical isolation in mountain valleys has played an important role in a formation of new crop species in gene-centers (SCHWANITZ, 1969; VAVILOV, 1928). Genotypically and phenotypically different populations could have evolved as a result of geographical isolation, genetical drift and selection.

Powdery mildew, caused by *Blumeria graminis* (DC.) SPEER f. sp. *tritici* MARCHAL (syn.: *Erysiphe graminis* DC.) is a fungal disease that has been recognized as a problem on wheat for centuries. It is widely distributed in all wheat-growing areas throughout the world. Losses up to 45% have been documented (FELSENSTEIN, 1991). *Blumeria graminis* f. sp. *tritici* is an obligate parasite specific to wheat. Multiple races of the fungus exist

and new ones continue to be formed as a result of genetic recombination. Besides the sexual cycle contributing to diversity, the disease is polycyclic and well adapted for aerial dispersal and long distance transport (LIMPERT et al., 1999; LIMPERT et al., 2000). Predominant dispersal over Europe is from west towards east. The distortion of the wind field due to the Alpine obstacles tends towards south-east. However, pathogen spores can be dispersed by wind in any direction.

Geographical isolation has already been monitored within Europe, e.g. MÜLLER et al. (1996) found out that the ranking of the diversity indices of the barley powdery mildew sample from Spain was much in contrast to that of Italy. These two populations were particularly homogenous and differed considerably from populations in the rest of Europe, which could be explained by the geographical barriers of mountain chains (LIMPERT et al. 1990; MÜLLER et al. 1992).

The aim of the study was to monitor wheat powdery mildew populations in Slovakia and to find out if the populations may be isolated by geographical barriers there.

Material and methods

Mildew isolates

The isolates used in the study were collected from the aerial population with a jet spore trap (SCHWARZBACH, 1979). Spores were trapped on segments of primary leaves of a susceptible wheat variety 'Košťutka' placed in Petri-dishes containing 7% agar and 30 ppm benzimidazole. Since the valley of Dobrá Voda is not extensive enough, spores were collected there by means of a stationary nursery: seedlings of the susceptible variety 'Košťutka' grown in pots were exposed to air at two distinct localities (DV I, DV II). Sampling was carried out in May and June 1999 and 2000.

Differential set

Virulence tests were carried out on a differential set consisting of near-isogenic lines with 'Chancellor' background and other varieties with single gene or combination of resistance genes: 'Axminster' (*Pm1*) 8xCC*, 'Torysa' (*Pm2+Pm6*), 'Asosan' (*Pm3a*) 8xCC, 'Chul' (*Pm3b*) 8xCC, 'Sonora' (*Pm3c*) 8xCC, 'Khapli' (*Pm4a*) 8xCC, 'Armada' (*Pm4b*), 'Regina' (*pm5*), 'Timgalen' (*Pm6*), 'Salzmunde' 14/44 (*Pm8*), 'Maris Dove' (*Pm2+Mld*), 'Normandie' (*Pm1+Pm2+Pm9*) and 'Košťutka' as the susceptible control. The varieties were, except for 'Torysa' and 'Regina', kindly provided by Dr. M. WINZELER from Swiss Federal Research Station of Agronomy, Zürich-Reckenholz.

(* – eight-times backcrossed to 'Chancellor')

Experimental conditions and virulence analysis

The infected leaves were kept under continuous light (2000 lux) at 17–18 °C. Untreated seeds of the differential set varieties were sown in plastic pots. The seedlings were protected against infection by cellophane bags. Primary leaves of 10–12 days old seedlings were used for the experiments. Fifteen millimeters long leaf segments of each variety were laid out in a particular arrangement in Petri-dishes on a medium containing 5% agar and 25 ppm benzimidazole. Each differential set was inoculated with the progeny of the single colony isolate by drawing spores into a pipette and blowing them onto a settling tower. Inoculum density was approximately 250 conidia/cm². After 10 days of incubation at 18±1 °C under continuous light (Tungsram, 36W) severity of disease was scored relative to the susceptible control ('Košťutka') in the set. Sporulation of more than 50% was considered to be a susceptible reaction and, vice versa, sporulation of less than 50% was considered to be resistant.

Statistical evaluation

Cluster analysis was used to characterise powdery mildew populations by K-means and genetic distance (genetic distance = 1 – Pearson correlation coefficient). Calculations were carried out by a computer program SYSTAT. The program also makes it possible to evaluate data by analysis of variance (ANOVA), which recognizes the significant differences in virulence frequencies between clusters. Computer program VIRAN was used to calculate the virulence frequencies.

Geographical localities and their barriers

Two geographical localities were chosen: 1. Dobrá Voda, a small village situated 248 m a.s.l. in a valley surrounded by hills of Malé Karpaty covered by forests. The height of the surrounding hills reaches 400–500 m a.s.l.. 2. Turiec Valley (SK8, 410–470 m a.s.l.), a large geographical area protected by high mountains of Malá

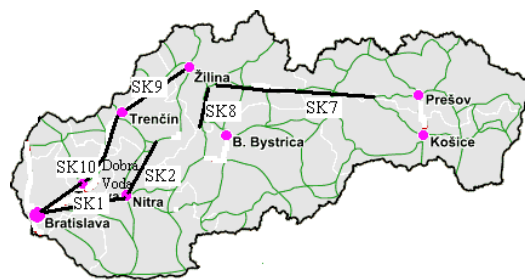


Fig. 1. Routes of the mobile spore sampling in Slovakia. SK1 – Bratislava-Nitra (Danubian Valley); SK2 – Nitra-Prievidza (the river Nitra region); SK7 – Poprad-Ružomberok-Martin (Liptov); SK8 – Martin-Budiš-Martin (Turiec Valley); SK9 – Žilina-Trenčín (the river Váh region); SK10 – Trenčín-Piešťany-Bratislava (Carpathian region).

Fatra (1441 m a.s.l.) and Velká Fatra (1709 m a.s.l.), Kremnica Mountains and Žiar. Climate of the valley is mild, while the surrounding mountains belong to a cold climatic region. Wheat is grown excessively in both Dobrá Voda and Turiec Valley.

Powdery mildew populations from the two regions were compared to other populations obtained by mobile sampling from Slovakia (Fig. 1), Czech Republic (Znojmo-Hodonín route; CZ) and Austria (Kittsee-Wiener Neustadt-Wien route; A1; Wien-Znojmo route; A2)

Results and discussion

Specific resistance genes and their corresponding virulence frequencies in mildew populations from Dobrá Voda, Danubian Valley and Carpathian region in 1999 are given in Table 1. In 2000, the mobile spore sampling was also carried out in Czech Republic and Austria (Tab. 2). Powdery mildew populations from these regions have subsequently been divided into three clusters according

Table 1. Specific resistance genes and their corresponding virulence frequencies in mildew populations from Dobrá Voda (DV I, DV II), Danubian Valley (SK1) and Carpathian region (SK10) in 1999.

Locality	<i>n</i>	Resistance genes												
		<i>Pm1</i>	<i>Pm2</i>	<i>Pm3a</i>	<i>Pm3b</i>	<i>Pm3c</i>	<i>Pm4a</i>	<i>Pm4b</i>	<i>pm5</i>	<i>Pm6</i>	<i>Pm8</i>	<i>Mld</i>	<i>Pm9</i>	<i>Pm3d</i>
DV I	54	26	41	30	15	76	78	61	96	80	96	9	7	31
DV II	63	33	42	27	22	81	79	52	98	78	92	22	14	25
SK10	35	40	37	29	6	66	83	66	100	71	97	6	9	6
SK1	36	25	56	44	5	72	69	53	97	75	97	17	3	19

n – number of isolates

Table 2. Specific resistance genes and their corresponding virulence frequencies in mildew populations from Dobrá Voda (DV), Danubian Valley (SK1), Carpathian region (SK10), Czech Republic (route Znojmo-Hodonín; CZ) and Austria (route Kittsee-Wiener Neustadt-Wien; A1; route Wien-Znojmo; A2) in 2000.

Locality	<i>n</i>	Resistance genes												
		<i>Pm1</i>	<i>Pm2</i>	<i>Pm3a</i>	<i>Pm3b</i>	<i>Pm3c</i>	<i>Pm4a</i>	<i>Pm4b</i>	<i>pm5</i>	<i>Pm6</i>	<i>Pm8</i>	<i>Mld</i>	<i>Pm9</i>	<i>Pm3d</i>
DV	40	45	47	52	27	80	90	62	100	88	100	13	15	27
SK10	57	53	53	81	28	95	89	77	100	100	100	23	12	54
SK1	28	29	54	39	43	86	93	64	100	100	100	7	0	21
CZ	50	32	58	50	26	90	94	74	98	98	100	34	14	54
A1	69	51	67	82	27	91	99	94	99	97	99	61	22	47
A2	46	41	65	58	9	80	96	78	100	100	98	4	6	30

n – number of isolates

Table 3. Powdery mildew populations from Dobrá Voda (DV I, DV II), Danubian Valley (SK1) and Carpathian region (SK10) collected in 1999 and divided into three clusters according to virulence frequencies to resistance genes *Pm1*, *Pm2*, *Pm4b*, *Pm9* and *Pm3d*.

Variability source (Resistance genes)	Cluster I	Cluster II	Cluster III
	DV I, DV II	SK10	SK1
<i>Pm1</i>	29.6	40.0	25.0
<i>Pm2</i>	41.2	37.1	55.6
<i>Pm4b</i>	56.8	65.7	52.7
<i>Pm9</i>	10.6	8.6	2.8
<i>Pm3d</i>	28.4	5.7	19.4

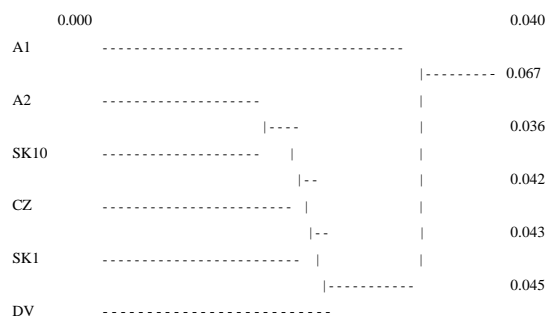
Table 4. Powdery mildew populations from Dobrá Voda (DV), Danubian Valley (SK1), Carpathian region (SK10), Czech Republic (route Znojmo-Hodonín; CZ) and Austria (route Kittsee-Wiener Neustadt-Wien; A1; route Wien-Znojmo; A2) collected in 2000 and divided into three clusters according to virulence frequencies to resistance genes *Pm1*, *Pm2*, *Pm4b*, *Pm9* and *Pm3d*.

Variability source (Resistance genes)	Cluster I	Cluster II	Cluster III
	SK10, CZ	A2	DV
<i>Pm1</i>	42.1	41.1	44.8
<i>Pm2</i>	55.5	65.4	47.5
<i>Pm4b</i>	75.8	78.2	62.3
<i>Pm9</i>	13.1	6.4	14.9
<i>Pm3d</i>	54.0*	30.4*	27.5*

* significant at error probability of $P \leq 0.05$



Tree diagram 1. Genetical distances between powdery mildew populations from Dobrá Voda (DV I, DV II), Danubian Valley (SK1), Carpathian region (SK10) in 1999. (metric distance = 1 – Pearson correlation coefficient)



Tree diagram 2. Genetical distances between powdery mildew populations from Dobrá Voda (DV), Danubian Valley (SK1), Carpathian region (SK10), Znojmo-Hodonín route (CZ) and Austria (Kittsee-Wiener Neustadt-Wien route A1, Wien-Znojmo route A2) in 2000 (metric distance = 1 – Pearson correlation coefficient).

to virulence frequencies (Tabs 3–4) Mildew samples trapped in both localities from Dobrá Voda form together one cluster No. I (Tab. 3). This clearly indicates that the mildew populations are

similar and there are no other subpopulations in Dobrá Voda. Although the two other mildew populations were arranged into two different clusters, the differences between the three clusters were not significant. The results in Tree diagram 1 prove that the two populations from Dobrá Voda are close together not only geographically but also genetically. Similarities between genetical and geographical distances are clearly shown in Tree diagram 2, where mildew population from Austria (A1) is the most genetically distant population. But t-test has not proved any significant differences between genetical distances. Therefore, we do not find powdery mildew population from Dobrá Voda to be isolated from other mildew populations in south west Slovakia. The locality is situated in a vast agricultural area with an intensive wheat growing activities, and thus strongly influenced by these populations from which pathogen pathotypes migrate on the prevailing winds. Migration of pathotypes has already been observed by many authors (DREISEITL & SCHWARZBACH, 1994; LIMPET et al., 2000; DREISEITL, 2000). The height of the hills surrounding Dobrá Voda does not create a sufficient obstacle for a spore dispersal by wind since pathogen migration overcoming greater distances and higher mountain ranges have been reported (e.g. from Spain in the west to Ukraine in the east; LIMPET et al., 2000). Wheat cultivars having a similar resistance pattern are grown in whole Europe. Therefore, it would be better to monitor geographical isolation in a region where cultivars carrying other than generally used resistance genes are grown.

Specific resistance genes and their corresponding virulence frequencies in powdery mildew populations from Turiec Valley and the surrounding regions in 1999 and 2000 are given in Tables 5 and 6. Note that cluster analysis (Tabs 7–8) has

Table 5. Specific resistance genes and their corresponding virulence frequencies in mildew populations from the river Nitra region (SK2), Liptov (SK7), Turiec Valley (SK8) and the river Váh region (SK9) in 1999.

Locality	n	Resistance genes												
		<i>Pm1</i>	<i>Pm2</i>	<i>Pm3a</i>	<i>Pm3b</i>	<i>Pm3c</i>	<i>Pm4a</i>	<i>Pm4b</i>	<i>pm5</i>	<i>Pm6</i>	<i>Pm8</i>	<i>Mld</i>	<i>Pm9</i>	<i>Pm3d</i>
SK2	74	31	49	35	15	78	80	57	100	80	96	16	14	35
SK7	55	26	65	47	9	74	78	49	96	84	93	5	14	13
SK8	26	38	46	31	8	69	65	42	100	85	96	16	27	23
SK9	57	29	55	37	14	77	70	48	100	88	96	11	9	18

n – number of isolates

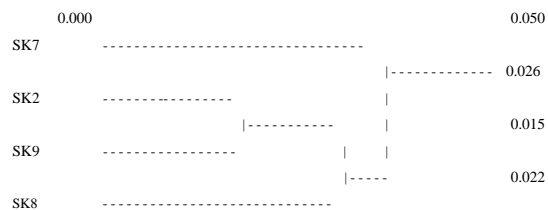
Table 6. Specific resistance genes and their corresponding virulence frequencies in mildew populations from The river Nitra region (SK2), Liptov (SK7), Turiec Valley (SK8) and The river Váh region (SK9) in 2000.

Locality	n	Resistance genes												
		<i>Pm1</i>	<i>Pm2</i>	<i>Pm3a</i>	<i>Pm3b</i>	<i>Pm3c</i>	<i>Pm4a</i>	<i>Pm4b</i>	<i>pm5</i>	<i>Pm6</i>	<i>Pm8</i>	<i>Mld</i>	<i>Pm9</i>	<i>Pm3d</i>
SK2	25	12	52	56	8	80	100	76	100	100	100	24	4	8
SK7	29	44	69	41	24	72	83	48	100	100	100	30	21	41
SK8	36	61	69	69	30	94	86	78	94	97	100	58	25	33
SK9	20	35	65	55	10	75	90	85	100	100	100	20	10	25

n – number of isolates

Table 7. Powdery mildew populations from the river Nitra region (SK2), Liptov (SK7), Turiec Valley (SK8) and the river Váh region (SK9) collected in 1999 and divided into three clusters according to virulence frequencies to resistance genes *Pm1*, *Pm2*, *Pm4b*, *Pm9* and *Pm3d*.

Variability source (Resistance genes)	Cluster I	Cluster II	Cluster III
	SK2, SK9	SK7	SK8
<i>Pm1</i>	30.3	25.7	38.4
<i>Pm2</i>	51.7	65.4	46.2
<i>Pm4b</i>	52.3	49.5	42.4
<i>Pm9</i>	11.0	14.5	27.1
<i>Pm3d</i>	25.8	12.8	23.1

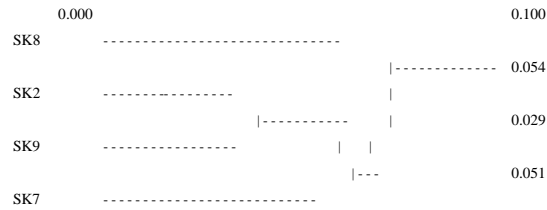


Tree diagram 3. Genetical distances between powdery mildew populations from the river Nitra region (SK2), Liptov (SK7), Turiec Valley (SK8) and the river Váh region (SK9) in 1999 (metric distance = 1 – Pearson correlation coefficient).

repeatedly placed mildew population from Turiec Valley into the last and the most distant cluster No. III in both 1999 and 2000. The results might indicate the different distribution of virulence frequencies in Turiec Valley, and thus even a certain degree of isolation. In 1999, the population from Turiec Valley belonged to two genetically most distant mildew populations (Tree diagram 3), and Tree diagram 4 proves that the population from Turiec Valley was the most genetically distant one in 2000. Despite of non-significant differences in virulence frequencies in the three clusters and in the genetical distances, which might have been

Table 8. Powdery mildew populations from the river Nitra region (SK2), Liptov (SK7), Turiec Valley (SK8) and the river Váh region (SK9) collected in 2000 and divided into three clusters according to virulence frequencies to resistance genes *Pm1*, *Pm2*, *Pm4b*, *Pm9* and *Pm3d*.

Variability source (Resistance genes)	Cluster I	Cluster II	Cluster III
	SK7, SK9	SK2	SK8
<i>Pm1</i>	39.9	12.0	61.1
<i>Pm2</i>	67.0	52.2	69.4
<i>Pm4b</i>	68.0	76.0	77.9
<i>Pm9</i>	14.1	3.9	24.8
<i>Pm3d</i>	32.8	8.0	33.3



Tree diagram 4. Genetical distances between powdery mildew populations from the river Nitra region (SK2), Liptov (SK7), Turiec Valley (SK8) and the river Váh region (SK9) in 2000 (metric distance = 1 - Pearson correlation coefficient).

caused by a small number of isolates tested, the mildew population from Turiec Valley can be referred to as different from the other three populations. Certain conditions favour isolation in Turiec Valley. The Turiec Valley is i) more spacious, thus the wheat is grown in larger fields than in Dobrá Voda, and therefore the wheat cultivars may influence new pathotype selection into a greater extent, ii) surrounded by higher mountains, and iii) wheat growing conditions are not very favourable there as it is situated in higher heights above a sea level. The sample size might have played an important role as well. If the sample size was higher, the significant differences might have been observed.

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