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Genetic Analysis of Thirteen Accessions of *Hordeum vulgare* ssp. *spontaneum* Resistant to Powdery Mildew

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Thirteen accessions of wild barley (*Hordeum vulgare* ssp. *spontaneum*) resistant to powdery mildew caused by the fungus *Blumeria graminis* f. sp. *hordei* were studied with the aim of determining the number of resistance genes and their allelic relationships to the *Mla* locus on the short arm of chromosome 1H. In five accessions (PI391130, PI466193, PI466200, PI466495 and PI466510), the resistance was caused by one gene, in seven accessions (PI354949, PI391081, PI466158, PI466197, PI466211, PI466297 and PI466461) by two independent genes and in PI301004 by three independent genes. The type of inheritance of all analysed genes except two was dominant or semi-dominant; only one of two genes in PI391081 and PI466297 was recessive. Allelism tests confirmed that in 10 accessions one gene was allelic with the *Mla* locus, and in three accessions (PI391081, PI466193 and PI466297) the resistance genes were different from the *Mla* locus.

Keywords: allelism, barley, *Blumeria graminis* f. sp. *hordei, Mla* locus, resistance, wild barley

Introduction

Barley (*Hordeum vulgare* L.) is one of the most wide-spread crops in the world and its production is adversely affected by many diseases. In the Czech Republic, powdery mildew caused by *Blumeria graminis* f. sp. *hordei* DC. f. sp. *hordei* Ém. Marchal *(Bgh)* is the most common and economically important disease of barley (Dreiseitl 2003a). Powdery mildew epidemics lead to the reduction of grain yield,

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feeding and malting quality, and profitability for growers. The high frequency of virulences to resistance genes carried by current winter barley varieties (Dreiseitl 2004) contributes to a frequent and heavy infection of both spring and winter barley. Winter barley serves as an important source of inoculum where new and widely virulent pathotypes of the powdery mildew pathogen can arise and reproduce, subsequently attacking both barley types. This results in a faster adaptation of the pathogen to the resistances present in cultivated barley varieties (Dreiseitl 2003b).

Jørgensen (1994) summarised known genes for powdery mildew resistance in barley, including their location on four out of seven barley chromosomes. Later, Schönfeld et al. (1996) mapped three genes on other two chromosomes, 5H and 7H, and Pickering et al. (1995) mapped one gene on chromosome 2H. The complex *Mla* locus on the short arm of chromosome 1H is the most important among the known powdery mildew resistance genes (Panstruga and Schulze-Lefert 2002).

Resistance to powdery mildew plays a significant role in the breeding of barley. To counter the pathogen, varieties should be bred with resistance genes for which no corresponding virulence factor in the pathogen population has been found within a given epidemiological unit. Wild barley *H. vulgare* ssp. *spontaneum* and *H. bulbosum* represent promising sources of resistance to important barley diseases (Williams 2003). Screening of wild barley accessions from the USDA National Small Grains Collection revealed that a high proportion of these accessions exhibited useful resistance to powdery mildew (Dreiseitl and Bockelman 2003).

We studied a set of wild barley accessions resistant to powdery mildew aiming: (1) to find the number of genes/loci conferring the resistance, (2) to identify the modes of inheritance of these genes, and (3) to define their relationships to the *Mla* locus.

Materials and Methods

Plant material and population development

Preparation of biological material and all experiments were carried out at the Agricultural Research Institute Kroměříž Ltd. Thirteen wild barley accessions (PI354949, PI391004, PI391081, PI3191130, PI466158, PI466193, PI466197, PI466200, PI466211, PI466297, PI466461, PI466495 and PI466510) from the USDA National Small Grains Collection, with resistances to powdery mildew

(Dreiseitl and Bockelman 2003, Dreiseitl and Dinoor 2004), and the two-row winter barley variety 'Tiffany' carrying powdery mildew resistance genes *Mla7, MlaMu2* were used. 'Tiffany', as a female parent, was crossed with the thirteen resistant accessions. The dormancy of harvested seed was routinely interrupted at 38 °C for 48 h, and F_1 generations were consecutively sown in vegetation pots. During vernalisation, young plants were grown in a cool room at 5±2 °C for 42 days and then moved into a greenhouse until harvest. The seeds of F_2 generations were obtained after selfing of F_1 plants.

Pathogen isolates

Two selected pathotypes of *Bgh* held in the gene bank of the pathogen at the Agricultural Research Institute Kroměříž Ltd. were used for the inoculation of the tested plants. A virulent *(Va7, VaMu2)* pathotype 0323 was used for determination gene number conferring resistance in each accession and their inheritance. An avirulent *(Aa7)* pathotype 1002 was employed for the tests of allelism for the *Mla* locus. Each pathotype had previously been purified, verified for the correct virulence/avirulence phenotype on the differential hosts and increased on the varieties 'Tiffany' (0323) or 'Algerian' (1002).

Resistance tests

Four seeds per genotype (parental, F_1 and F_2 generations) were sown in pots (80 mm upper diameter) in the greenhouse, and the plants were grown at a continuous temperature of 17±2 °C and under natural daylight. Four segments of about 25 mm in length were cut from the central part of each fully expanded primary leaf of eighteen-day-old plants and placed in four dishes with 0.6% agar and 35 ppm of benzimidazole; inoculation was carried out with each pathotype separately with two replications (Dreiseitl and Dinoor 2004) with inoculum density of ca. 8 conidia mm^{-2}. Eight days after inoculation, reaction types (RTs) of leaf segments were scored on the 0–4 scale (Torp et al. 1978). Reaction types 2–3 and lower were considered resistant. Twenty to forty plants of each parent, 22 to 74 F_1 plants and 190 to 542 F_2 plants of individual crosses were evaluated.

Inheritance of resistance genes

The numbers of plants in the two phenotypic categories (resistant and susceptible) found in F_2 populations were compared with theoretical Mendelian segregation ratios by a chi-square test, and the number of resistance genes in each accession

was estimated. The comparison of RTs between parental and F_1 generations enabled the determination of the modes of inheritance of resistance genes (dominant, semi-dominant or recessive).

Allelism tests

The results of resistance tests of F_2 populations with *Va7* and *Aa7* pathotypes were compared and conclusions on allelism for the *Mla* locus were drawn. If both resistant and susceptible plants in the F_2 population were found after inoculation with the *Va7* pathotype and all F_2 plants showed only the resistant phenotype after inoculation with the *Aa7* pathotype, the resistance was considered to be determined by an allele of the *Mla* locus. If resistant and also susceptible F_2 plants were identified after inoculation with the *Aa7* pathotype, the resistance genes were considered to be different from the *Mla* locus.

Results

Table 1 summarises the numbers of resistant and susceptible plants in the F_2 populations of 13 powdery mildew resistant accessions and significance of considered segregation ratios. The numbers of F_2 plants sorted according to evaluated RTs are given in Figure 1. The evaluation of plants of F_2 populations after inoculation with the *Va7* pathotype revealed the whole range of RTs, including the susceptible ones. Only resistant plants were found after inoculation with the *Aa7* pathotype with the exception of PI391081, PI466193 and PI466297. This indicated the presence of an allele of the Mla locus in all accessions excluding the three exceptions. The following conclusions were drawn for individual, tested, resistant accessions after analysis of the parental, F_1 and F_2 plants.

In PI354949, dominant alleles of two independent genes were present ($P = 0.37$), and the allelism test indicated that one gene was located at the *Mla* locus (Table 1). All parental plants showed RT0; F_1 plants showed RTs 0 and 1 as a consequence of one gene resembling a dominant and the other a semi-dominant type of inheritance (Figure 1A). In PI391004, three independent resistance genes ($P = 0.70$) were detected, and the allelism test confirmed at least one resistance gene at the *Mla* locus. The parental plants proved to be of RTs 0 and 1 and the same RTs were found in plants of the F_1 generation. This clearly indicated dominant inheritance (Figure 1B). In PI391081, two independent resistance genes ($P = 0.16$) were confirmed, one dominant and the other recessive. The allelism test also revealed susceptible plants, which indicated that none of the genes was at the *Mla* locus. The parental plants with the RT1-2 and the F_1 generation with RTs ranging be-

Table 1. The number of resistant and susceptible plants in the F_2 populations of 13 powdery mildew resistant accessions of *Hordeum vulgare* ssp. *spontaneum* after inoculation with virulent *(Va7)* and avirulent *(Aa7)* pathotypes of *Blumeria graminis* f. sp. *hordei* and the significance of considered segregation ratios

Resistant accession	*Va7* pathotype No. plants		Segregation Ratio	χ^2	*Aa7* pathotype No. plants	
	Resistant[a]	Susceptible[b]			Resistant	Susceptible
PI354949	422	33	15:1	0.78*	455	0
PI391004	206	4	63:1	0.16*	210	0
PI391081	162	28	13:3	2.01*	183	7
PI391130	370	114	3:1	0.54*	484	0
PI466158	352	22	15:1	0.09*	374	0
PI466193	241	73	3:1	0.51*	300	14
PI466197	431	31	15:1	0.17*	462	0
PI466200	175	71	3:1	1.96*	246	0
PI466211	372	16	15:1	2.99*	389	0
PI466297[c]	425	117	13:3	2.86*	532	10
PI466461	468	30	15:1	0.04*	498	0
PI466495	318	94	3:1	1.05*	412	0
PI466510	145	53	3:1	0.33*	198	0

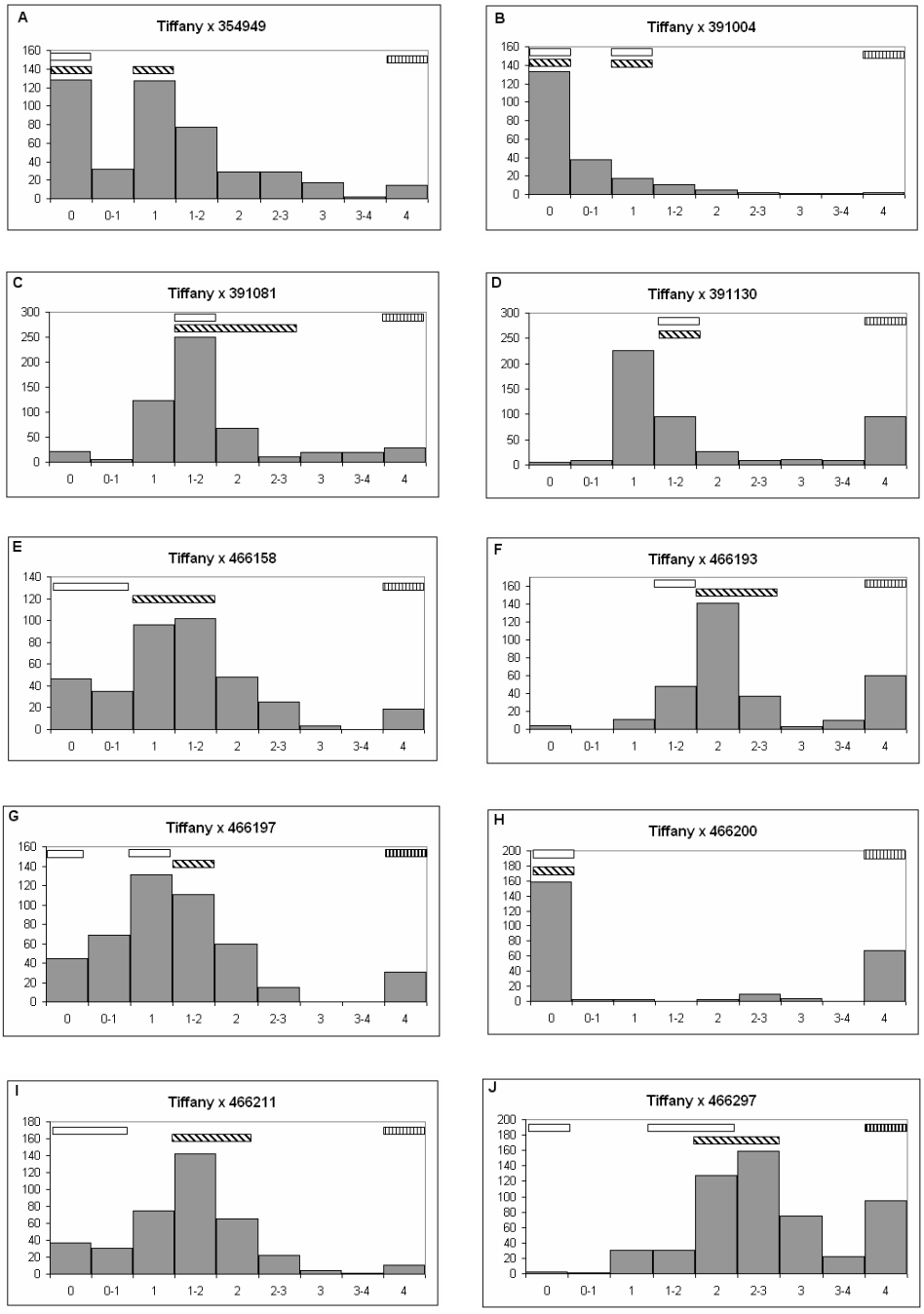
[a] Reaction types 0 to 2-3

[b] Reaction types 3 to 4

[c] The reaction type 3 is considered resistant

* Significance of the tested segregation ratio was confirmed at $P > 0.05$

tween 1-2 and 2-3 reflected a semi-dominant mode of inheritance (Figure 1C). In PI391130, only one dominant resistance allele was estimated ($P = 0.46$) and the allelism test confirmed its location at the *Mla* locus. RT1-2 was found in the parental generation and also in the F_1 plants (Figure 1D). In PI466158, two independent resistance genes ($P = 0.76$) were found; one of them was allelic with the *Mla* locus. The two genes exhibited semi-dominant inheritance (Figure 1E). In PI466193, one gene conferring resistance ($P = 0.48$) was detected. Screening with the avirulent pathotype revealed both resistant and susceptible plants, which indicated that the gene was not tightly linked with the *Mla* locus. RT1-2 of the paternal plants and RTs 2 and 2-3 of the F_1 plants were in agreement with a semi-dominant mode of inheritance (Figure 1F). In PI466197, two resistance genes ($P = 0.68$) were estimated, one allelic with the *Mla* locus). Their inheritance was semi-dominant because the RTs were 0 and 1 in the resistant accession and 1-2 in the F_1 plants (Figure 1G). In PI466200, one resistance gene allelic with the *Mla* locus might be expected ($P = 0.16$). The paternal and the F_1 plants exhibited the same RT0, which was indicative of dominant inheritance (Figure 1H). In PI466211, two independent resistance genes ($P = 0.08$), one allelic with the *Mla* locus, were confirmed. Semi-dominance was concluded from the fact that paren-


A
Tiffany x 354949
B
Tiffany x 391004
C
Tiffany x 391081
D
Tiffany x 391130
E
Tiffany x 466158
F
Tiffany x 466193
G
Tiffany x 466197
H
Tiffany x 466200
I
Tiffany x 466211
J
Tiffany x 466297
0 0-1 1 1-2 2 2-3 3 3-4 4

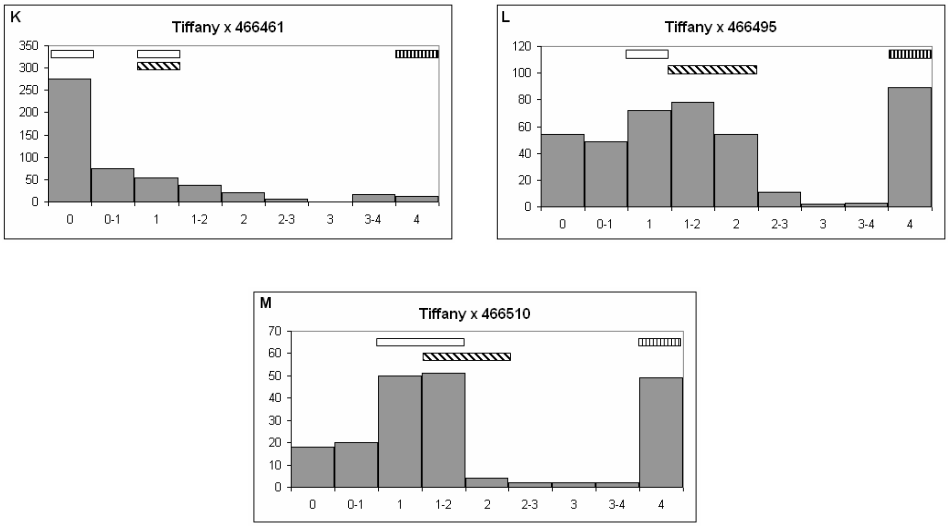


Figure 1. Distribution of reaction types of plants in the F_2 populations after screening with the *Va7* pathotype of *Blumeria graminis* f. sp. *hordei* and comparison with the parental (resistant accessions, variety 'Tiffany') and the F_1 generations. F_2 populations were obtained after crossing variety 'Tiffany' and individual resistant accessions of *Hordeum vulgare* ssp. *spontaneum* (A – PI354949, B – PI391004, C – PI391081, D – PI391130, E – PI466158, F – PI466193, G – PI466197, H – PI466200, I – PI466211, J -PI466297, K – PI466461, L – PI466495, M – PI466510)
X – scored reaction types (RTs) of leaf segments, Y – the number of plants for individual RTs
Resistance accession, Variety 'Tiffany', F_1 generation, F_2 population

tal plants were of two RTs, 0 and 0-1, and the F_1 plants were of RTs 1-2 and 2 (Figure 1I). In PI466297, two independent genes (P = 0.09), one probably semi-dominant and the other recessive, were considered. Allelism with the *Mla* locus was not supported, owing to the identification of susceptible plants in the F_2 generation after avirulent pathotype testing. The parental plants exhibited up to three different RTs (Figure 1J). In PI466461, two independent resistance genes (P = 0.84) were confirmed; one was allelic with the *Mla* locus. Two RTs, 0 and 1, were detected in the parental plants. In the F_1 generation, only RT1 was observed, which indicated semi-dominant inheritance for one gene and dominant inheritance for the other (Figure 1K). In PI466495, one resistance gene (P = 0.30) allelic with the *Mla* locus was estimated. Semi-dominance of the gene could be assumed on the basis of the RTs' shifting from 1 in the resistant parent to 1-2 and 2 in the F_1 plants (Figure 1L). Also in PI466510, one resistance gene (P = 0.58) allelic with the *Mla* locus was confirmed. Partly overlapping RTs of the resistant accession (1 and 1-2) and

the F_1 plants (1-2 and 2) indicated the semi-dominant inheritance of the resistance allele (Figure 1M).

Discussion

Blumeria graminis f. sp. *hordei* ranks high among cereal pathogens for its adaptability and potential to cause crop losses (McDonald and Linde 2002). It is desirable to combine more effective resistance genes in one variety. Therefore, localisation of such genes in the barley genome is important.

We used 13 genetic resources selected from a large group of wild barley accessions fully resistant to powdery mildew (Dreiseitl and Bockelman 2003, Dreiseitl and Dinoor 2004). Our genetic analyses showed that 10 out of these 13 accessions contained an allele of the *Mla* locus. It confirmed the unique significance of the *Mla* locus among other barley loci conditioning resistance to powdery mildew.

The size of the evaluated F_2 populations was sufficient for individual ratios testing, including differentiation between 13:3 and 3:1. Nevertheless, owing to large differences in RTs of resistant plants in the F_2 generation (particularly those shown in Figures 1A, 1E, 1G, 1I, 1J and 1L), each locus, either the Mla or another, could include additional linked resistance gene(s). This means that the trait might not be determined in a simple manner similarly to the *Mla* locus which is not defined by one multiallelic gene but by three distinct, closely linked resistance-gene homologue families (Wei et al. 1999).

The number of resistance genes was determined and confirmed. In most cases, a correlation was observed between the gene number in the F_2 generation and the number of RTs determined for parental plants of a corresponding resistant accession (two genes in PI466158, PI466197, PI466211, PI466297 and PI466461; one gene in PI391130, PI466193, PI466200 and PI466495). For two crosses (PI354949 and PI391081), the number of genes determined in the F_2 generation was higher than the number of RTs determined for the parental plants.

The identities of resistance genes in the 13 accessions are not known yet and at present, we cannot determine which of the genes are already known or new. Obtaining genetically characterised F_2 populations with a known number of genes determining resistance of the accessions to powdery mildew and knowledge about the alleles' modes of inheritance will enable us to answer the question of gene identity. Recombinant analyses utilising DNA markers will help us to localise these genes on the barley genetic map and to compare their positions with those of known resistance genes.

Current methods of molecular biology enable, among others, development of various types of DNA markers and genetic map construction, thus opening new possibilities for effective and fast breeding of new varieties. The identification of DNA markers tightly linked with individual resistance genes will facilitate the purposeful selection of offspring (marker-assisted selection) and the combination of fully effective resistance genes in one genotype.

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