IDENTIFICATION OF WHEAT GENOTYPES WITH ADULT PLANT RESISTANCE TO POWDERY MILDEW

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Summary

As in the case of other wheat diseases, adult plant resistance (APR) to powdery mildew remains effective longer than monogenic hypersensitive resistance, so the objective was to identify winter wheat genotypes with this type of resistance. Field and greenhouse tests conducted on 41 varieties and breeding lines indicated that 36 were susceptible in the seedling stage, and only five were resistant in all stages of development. It is probable that these latter genotypes contain major resistance genes. The area under the disease progress curve was the same for most of the wheat genotypes as for the APR control variety Massey, but varieties and lines with significantly better resistance were also identified. Among the genotypes in the Martonvásár breeding stock, Mv Táltos and the line Mv07-03 were found to have excellent adult plant resistance.

Key words: winter wheat, wheat powdery mildew, adult plant resistance (APR)

Introduction and literary review

Powdery mildew [*Blumeria graminis* (DC.) E. O. Speer f. sp. *tritici* Em. Marchal] is a frequent pathogen of Hungary's major cereal, bread wheat (*Triticum aestivum* L.). A majority of the wheat varieties currently grown are susceptible to this pathogen, so one of the main tasks facing resistance breeding is to improve the powdery mildew resistance of varieties. The use of the known major resistance genes (*Pm* genes) is of limited effect. None of the major *Pm* genes or gene combinations found or deployed in *T. aestivum* and tested over the last few decades have proved to offer durable protection against powdery mildew (Szunics et al. 2001). New *Pm* genes are constantly being incorporated into the wheat genome from related species, but these only provide effective protection for a limited length of time. The effectiveness of resistance genes can be maintained for a longer period by gene pyramiding, when crosses are carried out to combine several different resistance genes into a single wheat genotype. Experience shows, however, that it is still only a matter of time before the pathogen overcomes this protection. The virulence of the powdery mildew population was examined in Hungary by Vida et al. (2002) using a seedling test. All the isolates tested were found to infect varieties carrying the Pm4a, Pm5 and Pm8 resistance genes. A small (< 20%) number of isolates was found to be virulent to the Pm3b and Pm3d genes and to the gene combination Pm1,2,9. Among the *aestivum* varieties carrying known resistance genes or gene combinations none were completely resistant to powdery mildew.
McDonald and Linde (2002) suggested that in the course of resistance breeding the best breeding strategy should be chosen after consideration of the major traits of the pathogen. One

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of the main criteria for choosing the best strategy is the genetic diversity of the pathogen, the virulence gene frequency and rate of gene flow. From this point of view, powdery mildew can be classified as a genetically diverse pathogen, as it reproduces both sexually and asexually and has a high gene flow rate, as its conidia are spread over wide areas by the wind. For this reason, these authors recommend the exploitation of quantitative resistance and/or the use of major genes in variety mixtures for this pathogen.

The special form of resistance that does not give complete protection in most cases, but which retards the growth and reproduction of the pathogen, has been described under various names in the literature. The terms horizontal resistance (Van der Plank 1963), slow mildewing (Shaner 1973) and partial resistance (Hautea et al. 1987) all refer to the fact that protective mechanisms exist in the host plant that provide a certain level of protection against all pathotypes.

The expression of this type of resistance to powdery mildew can be observed as early as the tillering stage in wheat, but may only be fully expressed with the initiation of the reproductive stage. This adult plant resistance (APR) has been recognised in the interaction between wheat and biotrophic pathogens for several decades (Samborski and Ostapyk 1959; Mares and Cousen 1977; Sunderwirth and Roelfs 1980; Gustafson and Shaner 1982). One example of the successful utilisation of APR in breeding for resistance to wheat powdery mildew is the winter wheat variety Massey, derived from Knox62, where effective resistance has been maintained for over 20 years (Griffey and Das 1994). According to studies made by Johnson et al. (1998) regions coding for powdery mildew APR are found on seven chromosomes in the variety Knox. Other investigations have shown that adult plant resistance to powdery mildew is governed by two or three genes in the varieties Knox62, Massey, Redcoat and Houser (Griffey and Das 1994; Das and Griffey 1994). Hautea et al. (1987) and Das and Griffey (1995) reported that additive gene effects were dominant in the case of wheat powdery mildew APR. Using the molecular marker technique, three quantitative trait loci (QTL) associated with APR were found in Massey on chromosomes 1B, 2A and 2B, and these explained 11–29% of the phenotypic variance (Liu et al. 2001). Chantret et al. (2000) localised the M1RE gene on chromosome 6AL using bulk segregant analysis (BSA). The residual effect of this gene on adult resistance was later detected in combinations developed using line RE714 (Mingeot et al. 2002).

The types of resistance listed above can be expected to remain effective longer than monogenic hypersensitive resistance. The aim is definitely to achieve durable resistance, which, according to Johnson (1984), is said to be present if resistance is retained even when a variety is grown on a large area for a long time under environmental conditions favourable for the pathogen.

The present paper reports on the results of experiments carried out to detect adult plant resistance to powdery mildew in a diverse set of winter wheat genotypes.

Materials and methods

A total of 41 winter wheat varieties and advanced lines were tested in Martonvásár for powdery mildew resistance under field and greenhouse conditions in two consecutive years (2004 and 2005). The experimental material included winter wheat genotypes of Martonvásár origin and varieties from an international experiment [ONWPM (observation nursery winter

powdery mildew), specially designed by CIMMYT (Mexico) to test for adult plant resistance to powdery mildew.
Of the 41 varieties, 24 were obtained from the CIMMYT-ONWPM observation experiment. Due to the small seed quantity available, these genotypes were only sown in non-replicated field and greenhouse experiments in 2004 and 2005.

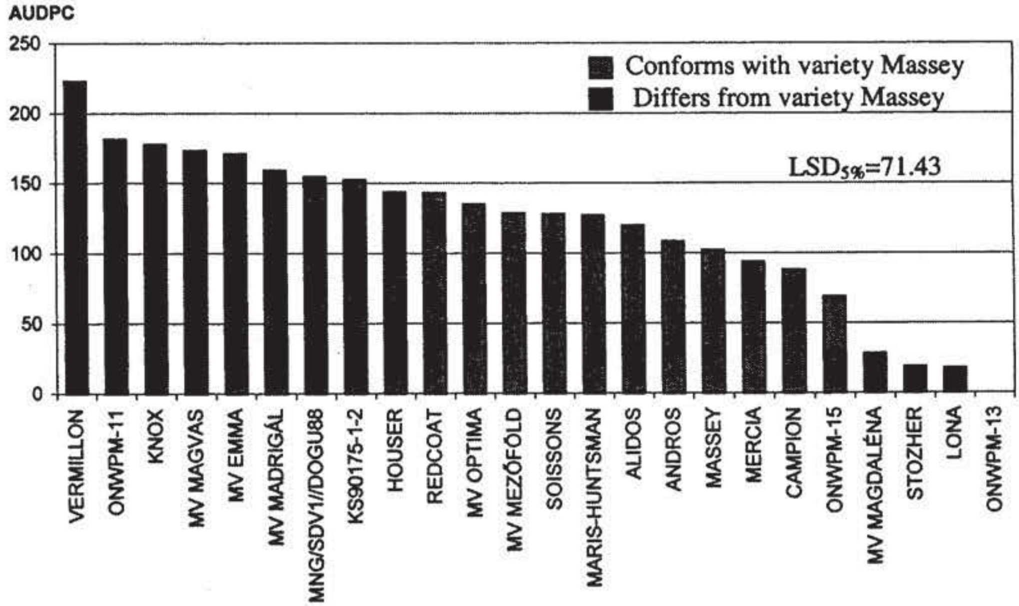
The remaining varieties, most of which were bred in Martonvásár, have been tested for a number of years in the field and were found to exhibit outstanding resistance to powdery mildew in previous years. These were tested in the field in a randomised block experiment with three replications in 2005, and in the greenhouse on two occasions, each with three replications.
In the field experiments the reaction of wheat genotypes to natural *Blumeria graminis* infection was examined in a nursery with a microclimate favourable for the spread of the pathogen. In both years sowing was carried out on the optimum date, in mid-October. The field was treated with 180 kg NPK (1:1:1) mineral fertiliser in autumn and a further 60 kg/ha nitrogen top dressing in spring. Chemical weed control was carried out on one occasion (Logran 75 WG, 15 g/ha), and the leaf surface was protected by two applications of insecticide (Karate 2.5 WG, 0,2 l/ha). From the end of May the ground was irrigated a few metres from the plots to ensure the high relative humidity needed by the pathogen for successful infection. The experiment was separated from the irrigated area by a border of plants including susceptible genotypes. The level of infection with powdery mildew was scored on five occasions at 7-day intervals from early May to mid-June using the internationally accepted 0–9 scale (Saari and Prescott 1975). The susceptible control was Vermillon and the APR standard Massey.
Seedling resistance to powdery mildew was tested in the greenhouse. The genotypes tested were inoculated under an isolator on the 8th day after sowing (GS11) using a mixture of pathotypes representing the natural powdery mildew population. The isolates used in the greenhouse test were collected in Martonvásár from a number of varieties currently or previously grown on large areas in Hungary. Prior to infection the powdery mildew population was multiplied on the variety Carsten V (Pm0). The level of seedling infection was scored on a 0–4 scale (0–2=resistant, 3–4=susceptible) after ten days.
Field data were used to determine the area under the disease progress curve (AUDPC) (Shaner and Finney 1977), which was then evaluated using analysis of variance (ANOVA). The greenhouse experiments, carried out in three replications, were also evaluated with ANOVA, using Microsoft Excel 2000 and the statistical program from the Breeder Program Package developed in Martonvásár (Kuti et al. 2004).

Results and discussion

In the first experiment, 24 winter wheat varieties and advanced lines were evaluated for their reaction to *Blumeria graminis* in the field in two consecutive years (2004 and 2005). The results of ANOVA revealed significant differences between the AUDPC data of the tested varieties, but the year did not have a statistically significant effect. The powdery mildew resistance of individual varieties, averaged over the two years, is illustrated in Figure 1.

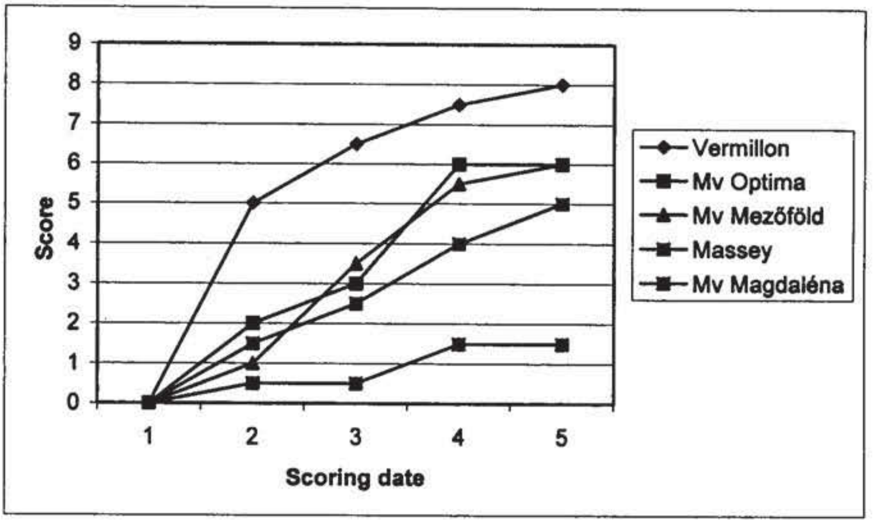
As expected, the susceptible control variety Vermillon exhibited the greatest extent of infection of all the varieties (AUDPC=223). The next most infected line had a value more than 40 lower than this, but a total of seven lines or varieties had a level of infection that did not differ significantly from that of Vermillon. These included the variety Knox, reported in the literature to have adult plant resistance to the pathogen. The AUDPC value of the variety Massey (102), used most frequently as a control in studies on adult plant resistance to powdery mildew, differed significantly from that of the susceptible control. When this variety was used as the standard, only three genotypes, Vermillon, the line ONWPM-11 and Knox, had AUDPC values that were significantly higher, while four varieties (Mv Magdaléna, Stozher, Lona and ONWPM-13) exhibited significantly less infection than Massey. Of these four genotypes, the line ONWPM-13 proved to be completely resistant to powdery mildew in both years. Further tests will be required to determine whether the complete resistance of this genotype was caused by a major resistance gene.

Figure 1. Mean AUDPC values calculated for the powdery mildew infection of winter wheat varieties (Martonvásár, 2004–2005)



The disease progress curves of the varieties Vermillon (susceptible) and Massey (APR control) are illustrated in Figure 2, together with those of some Martonvásár varieties with values significantly different from those of the susceptible control. These curves provide a good illustration of the different types of infection. In the susceptible variety Vermillon there was already a high level of powdery mildew infection even in early stages of the epidemic, after which the leaf area open to attack by the pathogen declined. For the resistant control, Massey, the ratio of plant parts attacked by powdery mildew continuously increased, but the rate of disease spread was much slower. In Mv Optima the final level of infection was reached by the fourth date of scoring, after which no further spread of the disease was observed. The steepness of the curve for Mv Mezőföld was similar to that of the susceptible control, but infection took place later than in Vermillon. In Mv Magdaléna the mildew developed slowly and the curve was flatter than for either the susceptible or the APR control.

Figure 2. Disease progress curves for the powdery mildew infection of representative winter wheat varieties
(Martonvásár, 2004–2005)



Some of the varieties included in the experiment possess known *Pm* genes. For example, genes *Pm*2 and *Pm*6 are present in Maris Huntsman (Wolfe and Wright 1972) and gene *Pm*3g in Soissons (Bouget et al. 2002). Several Martonvásár varieties were found to contain the *Pm*8 resistance gene (Kőszegi et al. 2000). In the case of the Martonvásár varieties it can be confidently stated that the increased resistance to powdery mildew is not the residual effect of the *Pm*8 resistance gene, whose effect has been overcome by the pathogen, since examinations have shown 100% virulence to this gene in the pathogen population (Szunics et al. 1999). In addition, although the presence of the 1B/1R translocation can be detected in line KS90175-1-2 and in the variety Mv Madrigál, their field infection with powdery mildew did not differ significantly from that of Vermillon. For the other two varieties, Maris Huntsman and Soissons, however, this possibility cannot be excluded. Data recorded over the last four years showed that 38–76% of the powdery mildew isolates infected Maris Huntsman. Although no data are available for Soissons, genotypes carrying other alleles of the *Pm*3 gene (*Pm*3b, *Pm*3d) have proved resistant to most isolates.

All the varieties included in the experiment were also tested in the greenhouse to determine their seedling reaction to powdery mildew. All the wheat varieties and lines became infected with powdery mildew, though two varieties (Knox and Stozher) exhibited a moderately resistant type of reaction. It can be concluded from the data that two of the varieties tested satisfied the criterion for adult plant resistance, i.e. they were susceptible in the seedling stage, but resistant in the adult stage (Mv Magdaléna and Lona).

In the second experiment, winter wheat varieties and lines identified in previous years as having complete resistance to powdery mildew in the field, or exhibiting only slight infection, were tested for infection and reaction to powdery mildew in the field in three replications and in the greenhouse in two independent experiments, each with three replications. The objective of this experiment was to determine whether these varieties possess adult plant resistance. A significant difference was observed between the AUDPC values of the varieties in the field, while in the greenhouse both the variety and the date of testing were found to have a significant effect on powdery mildew infection and host reaction, though the interaction between these two factors was not significant. The mean AUDPC values of the varieties and the seedling infection data are presented in Table 1.

Table 1. Field AUDPC values and seedling infection of the tested varieties (Martonvásár, 2005)

Variety	**Field AUDPC**	**Seedling infection rate**
Vermillon	196.50	3.67
Mv Süveges	129.33	4.00
Mv Csárdás	77.17	4.00
Mv Pálma	68.17	4.00
Mv Verbunkos	59.50	4.00
Zenthos	58.33	3.83
Mv Béres	54.83	3.83
Mv Ködmön	46.83	4.00
Mv Kemence	37.17	3.67
Re9801	34.17	3.33
Mv Táltos	6.67	3.33
Mv08-03	6.67	0.00
Mv Hombár	6.67	0.00
Mv07-03	0.00	3.83
ONWPM-13	0.00	3.83
Mv Regiment	0.00	0.33
HT02-03	0.00	0.00
$LSD_{5\%}$	50.51	0.36

The field data indicated that all the varieties tested were less infected with powdery mildew than the susceptible control variety Vermillon. Four genotypes (Mv07-03, ONWPM-13, Mv Regiment and HT02-03) were completely resistant in the field, while two other varieties and one line (Mv Táltos, Mv Hombár and Mv08-03) were almost completely resistant to powdery mildew. Comparing field and seedling data, it could be seen, however, that four of these genotypes were also resistant to the pathogen in the seedling stage, so they probably carry major resistance genes. As no known *Pm* genes conferring such a high level of resistance have been identified in greenhouse tests over the last few decades, these genotypes probably contain either an as yet unknown resistance gene, or an exceptionally effective gene combination.

Line HT02-03, which carries the 2BS.2RL wheat-rye translocation, could prove to be an interesting new source of resistance in the future (Merker and Forsström 2000). The variety Mv Táltos and the lines Mv07-03 and ONWPM-13 are interesting examples of adult plant resistance, since they had excellent powdery mildew resistance in the field experiment, but became severely infected in the seedling tests.

Winter wheat genotypes with adult plant resistance to powdery mildew were successfully identified in field and greenhouse experiments. These varieties could be a valuable asset to breeders in the future, as they could lead to the development of durable resistance, contributing to the achievement of low-cost, environment-friendly wheat production.

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