STUDIES ON POWDERY MILDEW RESISTANCE TYPES AND THE HOST PLANT–PATHOGEN RELATIONSHIP IN WHEAT

Main points of the PhD thesis

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BACKGROUND AND AIMS

In all the wheat-growing areas of the world, the leaf disease wheat powdery mildew, caused by the fungus *Blumeria graminis* f. sp. *tritici*, attacks the crops every year, resulting in substantial economic losses. It was first observed on an epidemic scale in Hungary in 1961, and has been present on Hungarian wheat fields ever since. Several types of powdery mildew resistance can be detected in wheat. One special type of resistance is adult plant resistance, which is only manifested in fully developed plants. In many cases this is a quantitative type of genetic trait, developing due to the influence of multiple genes, making it more durable than the resistance conferred by major genes, since major genes for powdery mildew resistance only provide protection against certain powdery mildew pathotypes. As the pathogen population is constantly changing, within a short time new pathotypes appear, against which the previously effective major resistance genes are no longer able to provide adequate protection.

Research on wheat powdery mildew resistance and the practical utilisation of the results in breeding will promote the development of new, disease-resistant cultivars that can be cultivated using less fungicide. This is not only of outstanding importance due to the reduction in production costs, but is also in the long-term interests of mankind in terms of sustainability and environment protection.

In the course of the work the aim was to carry out the following tasks:

1. identification of sources of special adult plant resistance to powdery mildew;
2. analysis of the genetic background of the extremely efficient powdery mildew resistance observed in the winter wheat cultivar Mv Hombár;
3. description of the manifestation of powdery mildew resistance in the Mv Hombár cultivar in field and greenhouse experiments, including the microscope monitoring of the host plant–pathogen relationship;
4. detailed studies on how ascospores of *Blumeria graminis* f. sp. *tritici* cause infection.

**MATERIALS AND METHODS**

*Identification of special adult plant resistance to powdery mildew in winter wheat*

When 24 winter wheat cultivars and breeding lines were examined in field and greenhouse experiments in Martonvásár in two consecutive years (2004 and 2005), winter wheat genotypes with a special type of powdery mildew resistance were identified. The aim was to find wheat cultivars or lines which, although they became infected with powdery mildew in the seedling stage, proved to be resistant to the disease in the adult stage. In a parallel experiment, winter wheat cultivars and breeding lines found to be completely resistant or only slightly infected by powdery mildew in previous years were tested in the field in three replications and in the greenhouse at two different times, again in three replications. The aim of these experiments was to determine whether the given genotypes possessed special adult plant resistance to powdery mildew. Winter wheat genotypes developed in Martonvásár were included in the experiments, together with the cultivars tested in international trials organised by CIMMYT. The cultivars Carsten V
and Vermillion were sown as susceptible standards and the cultivar Massey, reported in the literature as having adult plant resistance to powdery mildew, as the resistant standard.

In the field experiments the powdery mildew infection of the genotypes was determined on the Saari-Prescott scale and as the severity of infection, after which the data were used to calculate the area under the disease progress curve (AUDPC). The data were analysed using analysis of variance. The data from the replicated greenhouse experiments were also evaluated using analysis of variance. Correlations between the field and greenhouse data were determined by means of correlation analysis.

**Analysis of the Ukrainka/Mv Hombár mapping population**

In order to identify genetic factors linked with the powdery mildew resistance of the winter wheat cultivar Mv Hombár, a mapping population consisting of 178 lines was developed in 2003 by crossing the wheat cultivars Ukrainka (pedigree: Selena/Mayak/Promin) and Mv Hombár (pedigree: Fleming/Mv Matador). Mv Hombár is completely resistant to powdery mildew, while Ukrainka is susceptible to this disease. The natural powdery mildew infection of the progeny lines and the parental genotypes was recorded at 7-day intervals on five occasions from early May to mid-June in all the years examined (2006, 2008, 2010, 2012, 2013 and 2014) using two scoring methods (the Saari-Prescott scale and disease severity).

In the greenhouse, seedling powdery mildew resistance was tested on four occasions, in 2006 (F3), 2007 (F4), 2008 (F4) and 2010 (F5).

Normality tests on the progeny lines indicated that the population investigated did not have normal distribution, so further
statistical analysis was based on the non-parametric Kruskal-Wallis test. The data series for powdery mildew infection recorded in different years at different locations were compared using the pairwise Mann-Whitney test.

Genetic analysis on DNA samples from Ukrainka/Mv Hombár lines and the parental genotypes was performed using DArT and microsatellite markers. The 178 recombinant lines and the parental genotypes were tested with a total of 1230 binomial dominant DArT markers and 66 microsatellite primer pairs.

The DArT and SSR marker data of the Ukrainka/Mv Hombár lines were analysed with JoinMap 4 software and linkage groups were formed. After the map was constructed, all three data series (the marker data, information on the linkage groups and the results of phenotypic tests on powdery mildew resistance) were imported into a file created using the MapQTL 5 program. The analysis, involving interval mapping and cofactor selection followed by the multiple QTL model mapping method, was designed to detect QTLs responsible for powdery mildew resistance.

Microscope analysis of the wheat–wheat powdery mildew host plant–pathogen relationship

The stages of fungal development and plant responses to the fungus were examined in the wheat cultivar Mv Hombár using light and confocal laser scanning microscopes (CLSM) after artificial inoculation with three different wheat powdery mildew pathotypes. Races 76 and 51, which were detected most frequently in Hungary in recent years, were used for inoculation, together with the only pathotype to which Mv Hombár is susceptible. Experiments on the tester set of cultivars showed that this pathotype belonged to race 51, but its virulence on the Mv Hombár cultivar differed from that of the
original race, so it was designated as 51-Ho. In addition to Mv Hombár, the cultivars Ukrainka and Carsten V were included in the experiment as susceptible standards, a barley cultivar (Orca) as a non-host resistant standard, and the wheat line Nannong02Y23, which carries the resistance gene Pm21 conferring complete resistance under Hungarian conditions, as a host resistant standard.

**Analysis of infection caused by ascospores of Blumeria graminis f. sp. tritici, the pathogen responsible for wheat powdery mildew**

The wheat line MVMA/BIPE, which is exceptionally susceptible to powdery mildew, was used as host plant for the analysis of infection caused by the ascospores of *Blumeria graminis* f. sp. *tritici*. Ascospore differentiation and dispersal were induced under laboratory conditions using two methods:

1. Chasmothecia removed from dry leaves using a glass needle were placed in indentations on depression slides into which water had previously been dripped. The slides were then placed on moist filter paper in Petri dishes.

2. The chasmothecia were placed on depression slides on moist filter paper in Petri dishes, as described for the previous method, but no water was dripped onto the slides.

The Petri dishes were stored at 20°C in a controlled environment in a climatic chamber with 16-h illumination. The filter paper was moistened whenever necessary to ensure a constantly moist atmosphere for the chasmothecia. Two replications, each involving five Petri dishes, were used for each method. Over the course of a week, 20 chasmothecia were removed from each dish every day and squashed on a slide. The ascospore differentiation, dispersal and germination patterns were checked under a light
microscope with or without staining with lactophenol cotton blue stain.

A new method was devised to study infection by *Blumeria graminis* f. sp. *tritici* ascospores, which allowed the full process of chasmothecium maturity and ascospore infection to be observed directly, from the differentiation of the ascospores to the formation of colonies of ascospore origin. Winter wheat was grown under controlled conditions at 20°C with 16-h illumination. The plants were isolated in transparent plastic boxes covered with cling film. Dry leaves collected from wheat stands left in the field and containing chasmothecia were used as a source of inoculum. Approximately 100 seedlings were raised in each box to a height of 3 cm. At this stage the chasmothecia were placed in the boxes so that ascospores spontaneously dispersed from the chasmothecia would infect the plants. In order to observe the ascospore infection, five seedlings a day were harvested from each box and slides were prepared for light microscope analysis.

CLSM studies were performed in order to make a detailed examination of the fungal structures formed after ascospore infection and of the plant responses (papilla formation) characterising the host plant–pathogen relationship.
RESULTS

The main aim of the research described in the thesis was to identify efficient sources of powdery mildew resistance in the wheat genotypes used in the Martonvásár breeding programme. The following conclusions were drawn from the results for each task outlined in the aims.

1. Wheat genotypes with special adult plant resistance to powdery mildew were identified

   Due to the variability of the powdery mildew fungus population and to the reproduction and spreading strategy of the fungus, major powdery mildew resistance genes can only usually be used in resistance breeding for a short period and with poor efficiency. The quantitative type of resistance provides more durable protection than that coded by major genes. During a survey of the powdery mildew resistance of the breeding materials used in the wheat breeding programme of the Agricultural Institute (MTA ATK), wheat genotypes with a special type of powdery mildew resistance were identified; these were resistant to the disease in the adult stage, but became infected in the seedling stage. This type of resistance is known as adult plant resistance. Among the winter wheat genotypes bred in Martonvásár, the cultivars Mv Magdaléna and Mv Táltos and line Mv07-03 were found to have excellent adult plant resistance, as did the cultivar Lona and the line ONWPM-13, among the foreign genotypes tested.

2. Investigation of the genetic background of the outstandingly effective powdery mildew resistance observed in the winter wheat cultivar Mv Hombár
Every year for more than a decade the winter wheat cultivar Mv Hombár was found to have excellent powdery mildew resistance both in field experiments and in seedling tests in the greenhouse, in all the vegetation seasons tested. In order to identify the genetic factors behind this outstanding powdery mildew resistance, a mapping population was created by crossing Mv Hombár with the susceptible winter wheat cultivar Ukrainka. In the course of QTL analysis, four QTLs linked to powdery mildew resistance were identified on chromosomes 2A, 1A, 2B and 2D. The QTL identified on the long arm of chromosome 2A was found to have the greatest influence on the powdery mildew resistance of Mv Hombár, as manifested by a LOD peak far exceeding the significance limit in all the years for all the traits studied in all the locations. This explained 11–33% of the phenotypic variance, suggesting that the QTL identified in the 2AL chromosome region was a new major powdery mildew resistance gene, which was designated as \( PmHo \). The race-specific, i.e. major gene nature of the powdery mildew resistance in Mv Hombár was confirmed by the fact that a new powdery mildew fungus pathotype was identified, which was capable of infecting this wheat cultivar. Based on the internationally accepted tester set of cultivars the new powdery mildew fungus pathotype identified in the present work belonged to pathogen race 51, from which it differed only for its virulence on Mv Hombár. It was thus designated as 51-Ho. Microscope studies revealed that although the newly identified pathotype was able to cause visible symptoms on Mv Hombár, the development of the fungus was delayed and approx. 30–40% fewer colonies were formed than on plants of the susceptible standard. This suggests that the much weaker QTLs for powdery mildew resistance identified on chromosomes 1A, 2B and 2D may provide partial resistance against this pathotype, and this may also be true of
the newly identified powdery mildew resistance gene, although its resistance has been broken by the pathogen. The use of \textit{PmHo} in wheat breeding could lead to an improvement in the powdery mildew resistance of new cultivars.

3. \textit{Microscope analysis of the host plant–pathogen relationship between Mv Hombár and Blumeria graminis f. sp. tritici}

In microscope studies on host plant–pathogen interactions, the powdery mildew resistance of Mv Hombár was found to be manifested in the form of a hypersensitive reaction at the time when the fungus penetrated the epidermis cell wall or shortly afterwards. This was the most characteristic plant response, but not the only one. In about 30\% of the interactions the development of infection was prevented by the papillae. In most cases fungal development ceased either around the time of penetration or during the early stage of haustorium and colony formation.

4. \textit{The process of infection by the ascospores of Blumeria graminis f. sp. tritici, the causal agent of wheat powdery mildew, was documented for the first time}

Although \textit{Blumeria graminis} is one of the most intensively researched powdery mildew fungus species, an important phase in its life cycle, the primary infection by ascospores, has not yet been studied in detail. In the present work this process was observed and described for the first time in \textit{B. graminis f. sp. tritici} using light and confocal laser scanning microscope techniques. Unlike other powdery mildew fungus species, the germination pattern of the ascospores of \textit{B. graminis f. sp. tritici} was found to be quite different from that of the conidia under both \textit{in vitro} and \textit{in vivo} conditions. The ascospores form only a single type of germ tube, from which the
penetrating hypha develops. In other words, the primary germ tubes characteristic of the germination of the conidia of *B. graminis* could not be observed. After penetrating into the epidermis cell, the germinating ascospores developed haustoria, after which colony formation began, in agreement with the process observed in the case of conidial infection.

**NEW SCIENTIFIC RESULTS**

1. During a survey of the powdery mildew resistance of the breeding materials used in the wheat breeding programme of the Agricultural Institute (MTA ATK) wheat genotypes were identified that had a special type of powdery mildew resistance making them resistant to the disease in the adult stage, although they were infected in the seedling stage (adult plant resistance). The cultivars Mv Magdaléna and Mv Táltos and the line Mv07-03, all bred in Martonvásár, and the cultivar Lona and line ONWPM-13, both of foreign origin, were found to possess excellent adult plant resistance.

2. Four QTLs linked to powdery mildew resistance were identified on chromosomes 2A, 1A, 2B and 2D in the winter wheat cultivar Mv Hombár, bred in Martonvásár. The QTL identified on the long arm of chromosome 2A was found to have the greatest effect, being manifested significantly in all the years, with both scoring methods and at all locations. The effects of the other three QTLs could not be consistently detected.

3. The QTL identified on the long arm of the 2A chromosome was found to be a new major powdery
mildew resistance gene and was designated as *PmHo*. The major gene nature of the powdery mildew resistance of Mv Hombár was confirmed by the fact that the effect of *PmHo* was manifested under all conditions and was race-specific, as proved by the appearance of a newly identified powdery mildew fungus pathotype that was virulent on this cultivar (point 5).

4. Microscope studies on host–pathogen interactions revealed that the powdery mildew resistance of Mv Hombár was primarily due to programmed cell death caused by a hypersensitive reaction taking place within a short time of the penetration of the fungus into the epidermis cell wall.

5. A new powdery mildew fungus pathotype was identified, which could be classified as pathotype 51 on the basis of the internationally recognised tester set, and which only differed from this in the virulent response on Mv Hombár. It was thus designated 51-Ho.

6. The process of infection by the ascospores of *Blumeria graminis* f. sp. *tritici*, the causal agent of wheat powdery mildew, was documented for the first time. A description is given of ascospore germination, penetration into the epidermis cell, haustorium formation and the process of colony formation. Ascospore germination was found to be variable and to differ from that of the conidia, which have a distinctive germination pattern characteristic only of the *Blumeria* genus.
CONCLUSIONS AND RECOMMENDATIONS

In experiments designed to identify wheat genotypes with special adult plant resistance to powdery mildew, genotypes were detected that were resistant to powdery mildew both in field experiments in the adult stage and in greenhouse tests on seedlings (Mv08-03, Mv Hombár, Mv Regiment and HT02-03). The presence of major resistance genes was hypothesised in these genotypes, and this was proved in the case of Mv Hombár. As no \( Pm \) gene coding for such a high level of resistance has been identified in greenhouse experiments in recent decades, it seemed likely that a previously unknown resistance gene, or an exceptionally efficient gene combination was to be found in these genotypes. Line HT02-03, which also carried the 2BS/2RL wheat–rye translocation, could be a useful source of resistance in the future. Adult plant resistance was detected in the cultivar Mv Táltos and in lines Mv07-03 and ONWPM-13, so these genotypes could become valuable sources for improving resistance to powdery mildew. The durable disease resistance that could be achieved in this way will hopefully contribute to a cost-saving, environment-friendly technology for wheat production.

The effect of the resistance factor (\( PmHo \)) identified in the Martonvásár-bred winter wheat cultivar Mv Hombár was manifested in all environments, all years and with both scoring methods, suggesting that \( PmHo \) is a dominant major gene. The major gene nature of powdery mildew resistance in Mv Hombár was confirmed by the detection of a new pathotype, 51-Ho, which, unlike the other powdery mildew pathotypes, was capable of forming clearly visible sporulating colonies on Mv Hombár. This finding was indicative of the race-specific nature of the powdery mildew resistance, which is
characteristic of the major genes. Although over 90 powdery mildew resistance genes or alleles have been identified to date on the various chromosomes of wheat, resistance genes that are as effective as the $PmHo$ gene newly identified on the 2AL chromosome arm are rarely found. One such $Pm$ gene that provides complete protection is $Pm21$, derived from *Dasypyrum villosum*. In field experiments on the effectiveness of powdery mildew resistance genes, a further resistance gene providing excellent protection against powdery mildew under Hungarian conditions ($Pm3d$) was identified in addition to $Pm21$. However, in seedling tests in the greenhouse the cultivar Ralle, which carries the $Pm3d$ gene, proved to be susceptible to a number of powdery mildew isolates. As shown by the example of $Pm3d$, the variability of the pathogen means that the use of major genes alone is only a temporary solution in the development of cultivars resistant to powdery mildew. Even winter wheat genotypes carrying resistance genes $PmHo$ or $Pm21$, which have excellent efficiency at present, may be affected by virulent pathotypes within a short time. The successful application of major genes in the long term will therefore only be possible through the joint use of several genes either at genotype (gene pyramiding) or population (multiline) level.

Race-specific powdery mildew resistance (in effect, *host* resistance) is generally manifested after the penetration of the pathogen and the formation of haustoria in the form of a hypersensitive reaction (HR) resulting in the inhibition of fungal development at this stage. After the inoculation of the winter wheat cultivar Mv Hombár with races 51 and 76 of *B. graminis* f. sp. *tritici*, HR after fungus penetration was found to be the most frequent defensive plant response, characteristic of almost 70% of interactions, but not the only one. In the remaining 30% of the
interactions, papillae prevented the penetration of the powdery mildew fungus into the epidermis cell walls of the plant. It is no surprise that papillae are formed in the course of the interactions, as papilla formation is a characteristic form of plant response in host plant–pathogen relationships, irrespective of the type of resistance and of whether the interaction is incompatible or compatible. Papilla formation, however, proved to be an effective defence response primarily in the case of non-host resistance, where it completely prevented the penetration of the fungus into the epidermis cells. This is partially contradicted by the fact that the most important plant defence strategy in the interaction between triticale and the B. graminis form that has recently become specialised for this host plant (host type resistance) is the formation of efficient papillae, while in the interaction between Arabidopsis and its powdery mildew fungus, Golovinomyces orontii the callose accumulated in the papillae plays an active role in the development of resistance. In other words, the role of effective papillae is not irrelevant even in host type resistance. This is confirmed by the 30% ratio of efficient papillae in the case of Mv Hombár. There is more similarity than difference in the manifestation of host and non-host resistance at the cell level, although up till now they have been distinguished on the basis of host plant–pathogen interactions.

In contrast to the conidial infection caused by B. graminis f. sp. tritici, which has been thoroughly studied from many points of view, the process of ascospore infection is relatively unknown, though, as in the case of many other species of powdery mildew fungus, the differentiation and dispersal of the ascospores has already been examined in detail for B. graminis. A comparison of the present results with those of earlier work confirmed that the development of B. graminis ascospores from asci containing undifferentiated
protoplasm is a relatively rapid process for which a moist environment is required. *In vitro* and *in vivo* experiments on chasmothecia collected from the end of July to the end of August 2013 showed that three days were required on average for the maturing of the ascospores. The process took longer in the case of chasmothecia collected in June 2014, approximately a month before harvesting. The ascospores of most powdery mildew fungus species take much longer to mature, possibly weeks or months. In some species even morphologically differentiated ascospores are still physiologically immature, as observed in the case of *E. necator*, or the *Podosphaera aphanis* species that infects strawberries. When physiologically immature ascospores of these two species were artificially dispersed from the chasmothecia, they often burst in water instead of germinating. In the case of *B. graminis* the ascospores are immediately capable of infecting the host plant after their rapid process of morphological differentiation, indicating that they can also be regarded as physiologically mature.
LIST OF SCIENTIFIC PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

Scientific publications

Papers published in international scientific journals


Papers published in Hungarian scientific journals


Other scientific publications:

Conference proceedings


Articles in non-scientific publications

