FUSARIUM HEAD BLIGHT RESISTANCE OF WHEAT GENOTYPES
AND THE GENETIC BACKGROUND OF RESISTANCE

Main points of the PhD thesis

Katalin Puskás

Martonvásár
2013
Postgraduate school

Name: Postgraduate School for Plant Sciences

Branch of science: Crop Production and Horticultural Sciences

Head: Prof. László Heszky
Member of the Hungarian Academy of Sciences
Institute of Genetics and Biotechnology
Faculty of Agricultural and Environmental Sciences
Szent István University

Supervisors: Gyula Vida, PhD
Senior Research Associate
Department of Cereal Resistance Breeding
Agricultural Institute, Centre for Agricultural Research
Hungarian Academy of Sciences

Prof. Ferenc Virányi, DSc
Plant Protection Institute
Faculty of Agricultural and Environmental Sciences
Szent István University

--------------------------------------------------------
Dr. Gyula Vida
Supervisor
--------------------------------------------------------
Dr. Ferenc Virányi
Supervisor

--------------------------------------------------------
Dr. László Heszky
Head of Postgraduate School

--------------------------------------------------------
BACKGROUND AND AIMS

Fusarium head blight (FHB) is one of the most serious diseases of wheat, causing economic and yield losses all over the world. When the environmental conditions are favourable for the disease, *Fusarium* species are undoubtedly the most important and most dangerous pathogens of winter wheat in Hungary.

As facultative parasites, *Fusarium* species are present throughout the life cycle of wheat and may thus infect the plants. Their yield-damaging activities begin in the early phases of grain development. Unlike biotrophic pathogens, they do not require live plant tissues to survive. Their toxin and enzyme production is designed to ensure that they colonise the host organism as quickly as possible in order to exploit its nutrients. They are able to synthesise large concentrations of mycotoxins during the grain ripening period, and this activity continues during grain storage. Wheat lots contaminated with mycotoxins cannot be sold for either human or animal consumption.

FHB resistance is a quantitative trait in wheat, regulated by a number of genes. This has the advantage that if a wheat variety with outstanding resistance is developed, its resistance is likely to be durable. A good example of this is the Chinese spring wheat variety Sumai 3, registered in the 1970s, which is still one of the most resistant genotypes to FHB. It is not easy to trace quantitative genetic traits in progeny generations. Consequently, although a great deal of research has been performed all over the world in recent decades on the host plant–pathogen relationship, it has still not proved possible to breed winter wheat varieties with FHB resistance approaching that of the Far Eastern resistance sources. The cultivation of resistant varieties would lead to a reduction in the chemical pollution of the environment, in the pesticide residue content of the grain and in production costs. Wheat breeders thus give priority to the development of varieties whose resistance level makes them capable of avoiding severe spike infection even under weather conditions favourable for the pathogen.

Experiments on FHB resistance were set up under artificially inoculated conditions to achieve the following aims:

1. To make a survey of the FHB resistance of the Martonvásár winter wheat collection using several inoculation and evaluation methods, the comparison of which will make it possible to obtain more detailed knowledge on the resistance of the varieties.
2. To search for potential resistance sources among foreign and Hungarian wheat genotypes, which could serve as initial material for crossing programmes aimed at breeding for FHB resistance.
3. To assess the advantages and limitations of the inoculation methods used to test FHB resistance (spray or single spikelet inoculation) in field experiments.
4. To carry out phenotyping and genotyping on recombinant inbred lines from the mapping population created by crossing the FHB-resistant variety Ning 8331 with the moderately susceptible variety Martonvásári 17, and to identify significant QTLs for resistance.
MATERIALS AND METHODS

Production of inoculum

Isolates of two Fusarium species, F. graminearum ‘IFA-65’ and F. culmorum ‘IFA-104’, were used in the experiments. F. graminearum was multiplied on mung bean liquid medium, and F. culmorum on a mixture of wheat and oat kernels. The concentration of the conidium suspension was adjusted to $5 \times 10^4$ macroconidia/ml for spray inoculation and to $5 \times 10^5$ for single spikelet inoculation.

Field experiments on FHB resistance

The field testing of wheat varieties and lines was carried out in the artificially inoculated FHB nursery of the Agricultural Institute (Centre for Agricultural Research, Hungarian Academy of Sciences). The high relative humidity favourable for infection was ensured using automatic micro-nozzle irrigation equipment.

Tests on the field resistance of wheat varieties and breeding lines

Martonvásár winter wheat varieties, prospective varieties, advanced breeding lines (F7 and later generations), and foreign winter or spring wheat genotypes described as being resistant to FHB were included in the experiments. The varieties and lines were sown in 6-row plots measuring 1×2 m. At flowering, spikes were tied in bunches and the whole surface of the spikes was sprayed with inoculum prepared from F. graminearum or F. culmorum isolates, in three replications. An evaluation was made of the degree of spike infection and later of the kernel infection of harvested samples. In addition, the kernel weight per spike, the test weight and the thousand-kernel weight were compared with those of the untreated control.

Search for FHB resistance sources

The investigations involved foreign wheat varieties and lines, lines from D experiments originating from the Martonvásár wheat breeding programme (F7–F9 generations) and selected homogeneous lines of old Hungarian varieties, all of which were sown in 2-row plots without replication. An isolate of F. culmorum was used to spray the plots at flowering. Spike and kernel infection were evaluated, and yield losses were recorded for the lines of old Hungarian varieties.

Tests on type II FHB resistance

Wheat varieties and lines from Martonvásár and abroad were tested in the experiment. The wheat genotypes were sown in one-row plots in each of which five spikes were treated with F. graminearum and five with F. culmorum by means of single spikelet inoculation. The level of spike infection was evaluated using the 1–5 scale suggested by Xu and Fang.
Analysis of the genetic background of FHB resistance

Plant material used for the genetic analysis

A total of 228 recombinant inbred lines (RILs) were produced from the progeny population of a cross between the resistant line Ning 8331 (pedigree: Yangmai 4/3/Aurora/Anhui 11//Sumai 3) and the moderately susceptible variety Martonvásári 17.

Phenotypic analysis of FHB resistance

Field resistance (types I+II) was assessed in the FHB nursery by spraying the whole stand with inoculum. The level of spike infection was evaluated in the RIL plots, and after harvest the proportion of kernels with visible symptoms of infection was recorded, together with the yield loss (relative kernel weight per spike, test weight and thousand-kernel weight).

Type II resistance was determined in the greenhouse by means of single spikelet inoculation. The main spike of 4–8 plants per RIL was treated at flowering and the level of infection was evaluated 21 days later using the 1–5 scale suggested by Xu and Fang.

Genetic analysis of the mapping population

DNA samples from the Ning 8331 line and the Martonvásári 17 variety were analysed using 166 microsatellite (SSR) primer pairs, after which 73 SSR primer pairs exhibiting polymorphism between the parental genotypes and 17 randomly chosen AFLP (Sse and Mse) primer combinations were designated for the analysis of the Ning 8331/Martonvásári 17 (NM) population. Sample separation and signal detection were performed using polyacrylamide gel electrophoresis in a Li-Cor DNA Analyzer.

Construction of a linkage map, and QTL analysis

The SSR and AFLP marker data for the NM lines were analysed with JoinMap 4 software to detect linkage groups. The program settings were: grouping parameter – independence LOD, algorithm – regression mapping, Haldane’s mapping function.

After the construction of the linkage map, all three data series (marker data, information from the linkage map, and the results of phenotyping for FHB resistance) were imported into the MapQTL 5 program file. The analysis involved interval mapping, after which cofactors were selected and QTLs responsible for FHB resistance were detected using the multiple-QTL model mapping method.
RESULTS

Resistance of Martonvásár wheat varieties and breeding lines

The results proved that the FHB resistance of the high quality, high-yielding Martonvásár wheats was considerably poorer than that of the Far Eastern spring resistance sources. However, a number of Martonvásár winter wheats were identified as having FHB resistance equal to or better than that of known European winter wheat resistance sources (e.g. Arina, Praag 8, F201R). Among these, Mv Emese proved to have reliable moderate field resistance over a period of many years. The yield losses suffered by the varieties Mv Palotás, Mv Csárdás and Mv Táltos were also significantly lower than the experimental mean. In the single spikelet inoculation tests two Martonvásár varieties, Mv Marsall and Mv Kolo were classified in the moderately resistant category on the basis of mean level of infection over the years, but these results were greatly influenced by the year.

Search for potential resistance sources

The results confirmed that, almost without exception, the best FHB resistance was detected in wheats from the Far East, or in crosses involving them. The resistance of the wheat variety Sumai 3 was outstanding in every year, regardless of the inoculation method. When testing spring genotypes, however, adaptation problems often resulted in substantial differences both in the extent to which the genotypes were infected in different years, and in the evaluation of spikes and kernels in the same year.

European winter wheat varieties and lines were tested to search for FHB resistance sources. A few foreign genotypes proved to have above-average field resistance over the years. In experiments involving either the spraying of bunches of spikes or single spikelet inoculation, however, the majority of these genotypes proved to be more susceptible than expected based on the results of other research teams. Among the breeding lines developed in Martonvásár the proportion of materials with good FHB resistance was much lower.

Among the genotypes found to have good FHB resistance in investigations on spring and winter wheats, several have been used as parental partners in crossing programmes in the Cereal Resistance Breeding Department over the last 10 years, leading to the development of over a hundred progeny populations.

Experiments on the variety Bánkúti 1201 confirmed that its field resistance is generally better than that of modern winter wheat varieties. A number of lines developed from the population of this variety have, however, proved to be even better: the FHB resistance of BKT9086-95 and BKT9158-95 was comparable with that of several known sources from every point of view. This outstanding resistance was characteristic not only of Bánkúti 1201, but also of other old Hungarian varieties (Bánkúti 1205, Bánkúti 5, Béta-Bánkúti, Fertődi 293, Lovászpatonai 407), and of numerous lines developed from the heterogeneous populations of old Hungarian varieties.
Application of various FHB inoculation methods and their limitations

Two spraying techniques and the single spikelet inoculation method were used in this work to test the FHB resistance of the plant material.

Both spraying methods were suitable for the estimation of field resistance. The advantage of spraying bunches of spikes is that the inoculation can be performed in replicated experiments, using isolates from two Fusarium species, thus greatly increasing the reliability and accuracy of the results. Spraying did not have to be repeated on more than two occasions, and no spikes from untreated side-tillers could be unwittingly included in the samples. The disadvantage of this technique, however, is that it is very time-consuming and labour-intensive. In the irrigated nursery the level of infection often proved to be too severe, which meant that differences between genotypes with moderate resistance were more difficult to distinguish. Spraying the whole canopy was extremely useful for the rapid estimation of the FHB resistance of several hundred genotypes, but due to the lack of replications it was less reliable.

A combination of spraying and single spikelet inoculation allowed more detailed knowledge to be acquired on the FHB resistance of Martonvásár wheat varieties and lines and of other genotypes of interest for breeding.

Effect of environmental factors on FHB infection

The statistical analysis of the replicated experiments performed in the Martonvásár FHB nursery revealed a significant genotype × year interaction in the case of both spraying and single spikelet inoculation, indicating that weather factors are not alone in influencing the reproducibility of resistance tests, but form a complex system which also involves the physiological and morphological traits of wheat. Observations show that under the conditions experienced in Martonvásár the level of FHB infection was substantially influenced by the following traits of the wheat genotypes:

a. flowering date (which became decisive as the spring temperatures in one of the years deviated considerably from those in the other years); this had an effect primarily on type II resistance.

b. plant height; in some cases taller wheat plants lodged, leading to much higher rates of infection.

c. poor winter hardiness; particularly in the case of spring wheats (including known resistance sources) secondary pathogens and Fusarium species were able to multiply with ease on the spikes of less vigorous plants.
**Analysis of the genetic background of FHB resistance**

*Phenotypic analysis*

A reliably high level of field resistance was detected in the case of spray inoculation for the Ning 8331 line, one of the parental genotypes of the population developed for the purposes of genetic analysis. When single spikelet inoculation was applied, this line exhibited various levels of infection over the years, in some cases only achieving the moderately susceptible category. The other parental genotype, the variety Martonvásári 17, was not infected to a much greater extent, exhibiting moderate infection in all three years after single spikelet inoculation in the greenhouse. In tests of field resistance, only the level of spike infection proved to be a satisfactory trait for distinguishing between the resistance levels of the parental genotypes.

For all the traits examined transgressive segregation was observed within the population. Numerous RILs were detected as having better resistance than Ning 8331, while many also exhibited a greater level of infection and yield loss than Martonvásári 17.

*Genotypic analysis and the identification of linkage groups*

A total of 97 polymorphisms were identified with the SSR primers and 366 with the AFLP primers. Of these, the data of 441 polymorphic markers were used for the construction of the linkage map, which consisted of 44 linkage groups. Altogether the linkage map covered almost 1400 cM of the hexaploid wheat genome.

*Mapping of QTLs for field resistance*

In the analysis of field resistance, eight linkage groups and one isolated SSR marker exhibited correlations with various traits.

In the case of Ning 8331, the most effective field resistance QTL was found on the short arm of chromosome 3B, the effect of which could be detected in every year, though to varying extents, and for all the traits tested. Averaged over the years, the 3BS QTL explained 10.2–19.5% of the phenotypic variation for the individual traits.

The connection between the QTL identified on chromosome 4B of the resistance source and FHB resistance could only be detected by analysing yield components, especially kernel infection and test weight loss. Further QTLs were identified on the 1AS, 7BS and 7BL chromosome arms of the Ning 8331 parental line, but these only influenced a single trait in a single year.

QTLs linked to resistance were also identified in the variety Martonvásári 17. The locus found on the 5AL chromosome arm was correlated with the mean level of spike infection ($R^2$=5.6%).
Mapping of QTLs for type II resistance

QTL analysis revealed four chromosome segments linked with type II resistance in the Ning 8331 line. Major QTLs were identified on the long arm of chromosome 2D and the short arm of chromosome 3B, which proved to be effective in several years: the 2DL locus in all three years and the 3BS locus in two. Based on the mean level of infection over the years, these QTLs were responsible for 22% and 14.2%, respectively, of the phenotypic variation in type II resistance. In single years, further QTLs were mapped to the long arm of chromosome 3B and the short arm of chromosome 5A. The latter was also identified when analysing the mean results over the years.
CONCLUSIONS AND RECOMMENDATIONS

It is recommended that in the future an experimental design that combines the advantages of the spray inoculation methods presented in the thesis should be used for the testing of field (types I+II) resistance. These include the use of isolates of various *Fusarium* species, replications, rapid testing and moderate pathogen pressure. It may be possible to exploit these advantages if the inoculation is performed exclusively by canopy spraying on two-row plots, allowing each genotype to be tested in three replications on the same unit area previously used for spraying bunches of spikes. The plants in each plot can also be inoculated with two *Fusarium* isolates, but this can only be done at the expense of the untreated control plot, which means that relative yields cannot be determined.

Five traits were recorded in the spray inoculation field experiments for the determination of the level of FHB infection. It was concluded from the results that although a clearer picture of the resistance of wheat genotypes can be obtained from the detailed evaluation of the yield, in the case of large sample numbers the resistance of varieties and lines can be reliably estimated from the analysis of spike and kernel infection. This was confirmed by the close relationship revealed by genetic analysis between relative yields and the level of kernel infection. Relative yield data did not detect any major QTL effects which were not also identified from the spike and kernel infection data.

Spray inoculation is an efficient method for the study of wheat breeding materials, allowing simultaneous selection for both main types of resistance. It is considered necessary, however, to continue tests involving single spikelet inoculation. The parallel application of the two inoculation methods provides more accurate information on the FHB resistance of the varieties. It became clear, for example, that type I resistance plays a greater role in the field resistance of the wheat variety Mv Emese, as this variety proved to be moderately susceptible in the type II resistance tests.

From the point of view of breeding for FHB resistance in Hungary, advanced breeding lines from the Martonvásár breeding programme, identified as having good resistance, could be of much greater importance than foreign genotypes, as these lines have been selected under Hungarian cultivation conditions, and their quality, agronomic traits and disease resistance have also been tested in replicated trials. When investigating their FHB resistance there will be no adaptation problems that could lead to the aggravation of spike infection. In addition, the number of genotypes that can be included in the experiments is also far greater than the number of designated resistance sources. There is a greater chance of selecting progeny with both satisfactory agronomic and technological quality traits and above-average FHB resistance from combinations developed using adaptable genotypes.
The results of several years of experimentation revealed that the FHB resistance of Bánkúti 1201 is genetically coded, suggesting that the heterogeneous nature of its population, similar to those of landraces, could have played an important role in preventing the outbreak of widespread epidemics before the introduction of modern wheat varieties. In the present experiments numerous lines of old Hungarian wheat varieties were only moderately infected. It would definitely be worth subjecting these lines to more detailed examination to determine both their field and type II resistance. When planning future experiments, however, special attention should be given to preventing lodging. One partial solution could be not to sown the lines immediately next to each other. Although old Hungarian varieties have several disadvantages (danger of lodging, susceptibility to leaf diseases), they could nevertheless be better crossing partners than foreign resistance sources for the purposes of resistance breeding, since they are adapted to Hungarian conditions, while Bánkúti 1201 also has excellent technological quality.

When mapping FHB resistance, two major QTLs were identified in line Ning 8331. These made a great contribution to the phenotypic manifestation of resistance and could thus be useful in marker-assisted selection. Judging from its location on the chromosome, the 3BS QTL is probably identical to the \textit{Fhb1} locus described in the variety Sumai 3. Its effectiveness in improving resistance (particularly type II resistance) has been reported by many authors, but the present experiments indicated that in addition to its effect on spike and kernel infection, it also plays a decisive role in yield losses. The QTL on the 2DL chromosome arm had a primary role in type II resistance, and its effect was found to be more stable over the years than that of the 3BS QTL.

The QTL detected on chromosome 4B was mainly responsible for improving resistance to kernel infection, as a significant effect on the spike infection level was not detected in any of the years. The location of this resistance QTL is very similar to that of the \textit{Fhb4} resistance QTL reported in the Wangshuibai wheat variety. This chromosome segment was also correlated with plant height in the mapping population, so there is a possibility that plant height had a direct effect on the level of infection. The significance of this QTL was smaller, however, than that of the major QTLs, which proved to be independent of plant height.
NEW SCIENTIFIC RESULTS

1. The single spikelet inoculation method was introduced for the first time in Hungary for the routine testing of FHB resistance in wheat breeding materials. Using this form of inoculation in the field, the type II resistance of at least 100 genotypes can be tested in replicated experiments each year, using two *Fusarium* isolates.

The simultaneous application of the spray and single spikelet inoculation methods in field experiments makes it possible to obtain a more precise picture of the components of FHB resistance. Tests on winter wheat varieties and breeding lines from Martonvásár revealed a number of genotypes that were only moderately infected. Several wheat varieties proved to be more resistant than reportedly resistant European winter wheat genotypes; the lowest level of spike infection was detected for variety Mv Emese, which has type I resistance. This demonstrated that resistance to the penetration of *Fusarium* into the spike tissues can be incorporated into wheat varieties adapted to Hungarian growing conditions.

2. The use of artificial inoculation proved that the FHB resistance of the Báňkútí 1201 variety is generally better than that of the Martonvásár wheat varieties that were cultivated in Hungary up to 2006. Tests on other old Hungarian wheat varieties and their lines led to the identification of further genotypes with good field resistance, suggesting that genetically determined resistance may have played an important role in the fact that prior to the 1970s FHB epidemics were rare. Lines with moderate levels of infection could be useful sources for resistance breeding.

3. Microsatellite and AFLP primers were used to construct a linkage map for the Ning 8331/Martonvásári 17 population, involving data from a total of 441 markers. Forty-four linkage groups were identified. Altogether the linkage map covered almost 1400 cM of the hexaploid wheat genome.

4. The genetic background of FHB resistance was analysed in the Ning 8331/Martonvásári 17 population. It was demonstrated that the resistance QTL on the 3BS chromosome arm, previously described in a number of resistant varieties, also played an outstanding role in Ning 8331. When using the spray inoculation method this QTL was found to have an important role in the development of resistance to spike and kernel infection, and its effect was also proved in the analysis of traits related to yield losses (kernel weight per spike, test weight and thousand-kernel weight). In lines derived from Sumai 3 the effect of the 3BS QTL is generally considered to be decisive for type II resistance. In the single spikelet inoculation experiment, however, it was only found to be the second most important locus.

The QTL with the greatest effect on type II resistance in Ning 8331 was mapped to the 2DL chromosome arm. The importance of this QTL for resistance to the spread of *Fusarium* within the spike was detected every year in the single spikelet inoculation experiment. Averaged over the years, this QTL explained 22% of the phenotypic variation.
PUBLICATION ACTIVITIES OF THE AUTHOR

Scientific publications:

Publications in international scientific journals:


Publications in Hungarian scientific journals:


Other scientific works:

Scientific book chapter:


Conference proceedings:


Conference abstracts:


