**SZENT ISTVÁN UNIVERSITY**

# INVESTIGATION OF THE MOLECULAR BACKGROUND OF THE FUSARIUM HEAD BLIGHT RESISTANCE IN FRONTANA DERIVED WHEAT MAPPING POPULATIONS

**PhD thesis**

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**Gödöllő**

**2013**

**The doctoral school**

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## 1. BACKGROUND AND AIMS

Fusarium head blight (FHB) is one of the most devastating diseases of wheat (*Triticum aestivum* L.) worldwide. Serious epidemics appeared in Canada, in the USA and in South-America in Asia as well as in Europe. Epidemics, causing heavy damages, occurred several times in the last decades in Hungary (for example in 1970, 1975, 1999 and in 2010). The head symptoms might attend Fusarium damaged kernels (FDK), which is important, because the damaged seeds are the main toxin bearers. Beyond the yield loss caused, the danger of toxins is in their harmful effect to human and animal organism.

*Fusarium* species can be found on cereal seeds, on cereal stem, in the soil and on weeds, however most of the infection agent (inoculum) is produced by the infected plant debris left on the field. The toxins, produced by the pathogens might appear also in haulmy feed, in straw as well as in cereals on stock (Parry et al. 1995).

The most outstanding ecological effect influencing Fusarium head blight is the weather. Rain or high relative humidity and relatively warm weather is necessary for the infection, therefore the amount of rain during the flowering period is important. Following the infection, warm and humid conditions are beneficial for the fungus to spread (Atanasoff 1920; Mesterházy 1995).

There are several methods for protection. The use of proper crop rotation, soil development and nutrient-supply as well as fungicide treatment can efficiently reduce the injury caused by *Fusarium*. The spread of infection can be prohibited by professional treatment of the harvested cereal and by proper storage conditions. However, only the production of resistant varieties would mean a real solution.

Resistance breeding against Fusarium head blight aims to find plant stocks worldwide, from which chromosome section or sections could be transferred into new breeding lines. Asian spring wheat lines, like Sumai 3, Nobeoka Bozu, Wangshuibai and Chokwang are the most often investigated sources with high resistance. The former resistance sources are usually sensitive against other diseases (e.g.: powdery mildew, leaf rust), moreover their yield capacity and fitness is low and their technical quality is bad. The Chinese originated Sumai 3 is one of the most often used resistance sources in the breeding programs worldwide (Rudd et al. 2001; McCartney et al. 2004; Kosová et al. 2009). The low number of resistance sources and the similar genetic background of their resistance can cause selection pressure to the pathogen, which makes the breeding process more labor-intensive. Despite these theoretical considerations, these traces have not provided any problems in practice yet. At the same time, involvement of new resistance sources would be necessary to improve the level – more preferably the duration – of the resistance (Ruckenbauer et al. 2001; Gervais et al. 2003).

A good candidate for this could be the Brazilian moderately resistant Frontana, which originates from the cross of Fronteira/Mentana (Schroeder and Christensen 1963; Van Ginkel et al. 1996). Steiner et al. (2004) mapped Frontana originated Fusarium head blight resistance (Type I.) QTL on chromosomes 3A, 5A, 2B, 4B and 6B, and Remus originated ones on chromosomes 1B, 2A and 3B. Mardi et al. (2006) confirmed the existence of the Fusarium head blight resistance QTL on chromosome 3AL and identified two additional QTL on chromosomes 1BL and 7AS during the mapping of the Frontana/Falat population. Srinivasachary et al. (2008) ascribed the Fusarium head blight resistance QTL on chromosomes 1B, 2B, 3A, 6A, 7A and 7D as well as the plant height QTL on chromosomes 2B and 5B to the Frontana originated RL4137 parent, during the mapping of RL4137/Timgalen RIL population. Berzonsky et al. (2007) identified Frontana originated Fusarium head blight resistance QTL on chromosomes 3A, 6A and 4D. The importance of Frontana as a resistance source was affirmed by Yang et al. (2006), who claimed that the

Frontana does not possess identical QTL with the Sumai 3 on chromosomes 3B and 6B. This was verified by the experiments of McCartney et al. (2004), who stated that the fragment sizes gained by markers on chromosomes 3BS, 3BSc and 5A, were not identical between Frontana and Sumai 3 (together with the most Chinese originated resistance sources). This pointed to the fact that the Asian originated resistance sources have different QTL than the Frontana has.

Fusarium head blight resistance might be influenced by different morphological traits, like plant height and heading date (Parry et al. 1995; Mesterházy 1987, 1995). The accurate QTL analysis requires the differentiation between the QTL responsible for resistance and other morphological traits. These are important in order to avoid that the breeders transfer an undesired morphological trait with resistance QTL (in case of pleiotropy, linkage drag), or instead of it (in case of false QTL detection) into an elite, productive variety during crossing.

The validation of molecular markers is necessary for breeders to mark constantly the presence of a locus responsible for the investigated trait. The validation of marker effectiveness linked to a given trait in different populations and in diverse environments is expedient. All these considerations were taken into account during the investigation of the molecular background of the Frontana resistance so as the wheat breeders will be able to use our results later.

**The aims of the work were as follows:**

1. Validation of the Fusarium head blight resistance QTL in the Brazilian originated resistance source, Frontana across several environments, with diverse inoculation methods and in different genetic backgrounds.
2. Identification of chromosome regions, linked not only to Fusarium head blight severity, but Fusarium damaged kernels, which is rarely investigated in the literature.
3. Investigation of the molecular background of the Fusarium head blight resistance and its influential traits (e.g.: plant height, heading date, awnedness) as well as interactions between them.
4. To study the possibility of marker assisted selection, using the Frontana wheat variety in a breeding process.

# 4. MATERIALS AND METHODS

## 4.1. Development of the mapping populations

The two investigated DH mapping populations were developed from the cross of 'Frontana' and 'Remus' as well as from the cross of 'GK Mini Manó' and 'Frontana'. Frontana (Fronteira/Mentana) is a Brazilian spring wheat variety moderately resistant to Fusarium head blight. The Austrian (IFA-Tulln) Frontana/Remus population (210 lines) was generated in the research group of Hermann Buerstmayr using the wheat by maize pollination technique; in the cross the sensitive partner was the German (Bavarian State Institute for Agronomy, Freising) Remus. The GK Mini Manó/Frontana population (168 lines) was generated at the Department of Biotechnology, Cereal Research Nonprofit Ltd., Szeged using anther culture method (Pauk et al. 2003); in the cross the sensitive parent was a former, Fusarium head blight susceptible breeding line, GK Mini Manó.

## 4.2. Field experiments

The Frontana/Remus population was evaluated over 2002, 2004, 2005 and 2006, while the field test of GK Mini Manó/Frontana population was settled up in 2008 and 2009. Seed was sown in the nursery of Cereal Research Nonprofit Ltd. (Szeged, Kecskés telep), in autumn (mid-October) using Wintersteiger Plot Spider seedling-machine (Wintersteiger GmbH, Ried, Austria). The plots were planted in a randomized complete block design, with 1.5 m long rows, and the width of plots were set according to the number of isolates, used for inoculation. Each isolate was used in two replications meaning bunches consisting about 20 heads.

## 4.3. Inoculation procedure

In the experiment, the inoculum production, the in vitro test of aggressiveness of the isolates and the inoculation procedure were performed at full flowering (Feekes growth stage 10.5.1) according to the method of Mesterházy (1985, 1987, 1995). In order to inoculate the spikes uniformly, bunches of 15-25 spikes were sprayed from all sides with a hand-held sprayer, using about 15-20 ml fungal suspension for each sample. In each year, the plant material was inoculated individually with 2-6 isolates of either *F. graminearum* or *F. culmorum*. Only one bunch was placed on each row of a single genotype. In the different rows 30-40 cm distance was kept between bunches. After inoculation, bunches were covered with a transparent polyethylene bag for 48 hours. After removing the bags, the plants were loosely bound with a label for identification to allow the leaves to photosynthesize freely.

## 4.3. Fusarium head blight severity assessment

Observations of Fusarium head blight severity was started on the $10^{th}$ day after inoculation and were repeated on every fourth day until the heads turned yellow, which meant 4-5 observations in a vegetation period. The Fusarium head blight severity was estimated

according to the percentage rate of infected spikelets. Fusarium head blight severity rates mentioned in the dissertation are mean values of all observations.

### 4.3. Fusarium damaged kernels assessment

Following the manual harvest of infected bunches, the samples were threshed using a stationary thresher (Wintersteiger LD 180, Austria) at low wind, than the chaff was removed using Ets Plaut-Aubry (41290 Conan-Oucques, France) air separator in order to retain the shriveled, low thousand kernel weight (TKW) Fusarium-damaged kernels. After that, the percentage of Fusarium damaged kernels was estimated on a 0-100 linear scale.

### 4.4. Assessment of phenotypic traits influencing Fusarium head blight

In each year, plant height was measured (as the distance from the soil surface to the top of heads excluding awns) as well as lodging (percentage of lodged plant in a plot), date of heading (as the number of days from 1st January to heading) and awnedness were recorded.

### 4.5. Statistical analysis of the phenotypic traits

Statistical analyses were made by SPSS 15.0 software (SPSS Inc., Chicago, IL, USA) using the "Descriptive statistics", „Compare means", „General Linear Model" and „Correlate" functions. All data of each fungal isolate (*F. graminearum*, or *F. culmorum*) from different years were analyzed as single experiments (epidemic situations), because there are no races within the *Fusarium* spp.

### 4.6. Molecular markers

To map the 'Frontana/Remus' population, a database of 583 marker data (135 microsatellite markers, 416 AFLP and 32 RFLP markers) was provided by Steiner *et al*. (2004) from their previous experiments. On this population 45 SSR markers, on the GK Mini Manó/Frontana population 40 SSR (24 polymorphic) markers were tested at the Cereal Research Ltd. Most of the primers used in Hungary were selected from the literature (Röder et al. 1998; Steiner et al. 2004; Somers et al. 2004; Mardi et al. 2006). According to our aim to validate QTL, in the Mini Manó/Frontana population, SSR markers were used which were tightly linked to Fusarium head blight resistance in the Frontana/Remus population. In the Mini Manó/Frontana population the SSR marker database was extended with 619 polymorphic DArT markers, accordingly altogether a database of 643 molecular markers was available to use for mapping. The DArT marker data were provided by the Australian Diversity Arrays Technology Pty Limited (Yarralumla, Australia), following the „Wheat *Pst*I(*Taq*I)" genetic analysis, for what the genomic DNA of each lines were sent in the conditions requested by the institute.

### 4.7. Molecular mapping and QTL analysis

Linkage groups were constructed using JoinMap® 3.0 software (Van Ooijen & Voorrips, 2001) and interval mapping was done using MapQTL® 5 software (Van Ooijen, 2004). The order of markers was set according to the molecular map published on the GrainGenes (http://wheat.pw.usda.gov/ggpages/SSRclub/ GeneticPhysical/) and by Somers et al. (2004). Order of DArT markers was determined according to the Triticarte database published on the Diversity Arrays Technology Pty Ltd homepage (http://www.triticarte.com.au/content/further_development.html). Interval mapping (IM) was made with the phenotypic data of single experiments, with the mean values of *F. graminearum* and *F. culmorum* inoculations and overall means for Fusarium head blight severity and Fusarium damaged kernel rates of all the selected isolates, as well as with lodging, plant height and heading date data. The minimum LOD score was set to 2.0 during the mapping, however in case of several experiments the permutation tests indicated LOD> 1.2 as significance level of linkage (Van Ooijen 1999).

# 5. RESULTS

## 5.1. Molecular mapping and QTL analysis in a Frontana/Remus population

### 5.1.1. Results of the Fusarium head blight severity and Fusarium damaged kernels analysis

Overall mean of Fusarium head blight severity (mean value of all observations) and Fusarium damaged kernels rate showed normal distribution. The Fusarium head blight severity values were high: between 6.6 and 65.4%, while the Fusarium damaged kernels rate were higher: between 8.3 and 85.0%. The reproducibility of the experiments is indicated by the heritability ($H^2$) value, which was 0.64 for Fusarium head blight severity and 0.74 for Fusarium damaged kernels.

### 5.1.2. Results of assessing other phenotypic traits

No influential effect was observed in differences between the lines of the population in lodging, plant height or heading date, which would confuse the mapping significantly. The differences between the lines in plant height were not significant either. No significant difference was observed in Fusarium head blight between the awnless and awned lines.

### 5.1.3. Molecular map construction

Linkage map was constructed by mapping of 504 polymorphic molecular markers covering a genetic distance of 1779 cM (average marker distance 3.53 cM). Markers were mapped to 31 linkage groups, among them 20 groups were mapped on defined chromosome position. Linkage groups were mapped on each chromosome except for 4D, 5B, 5D and 6D. Among the phenotypic traits, gene coding red glume color (*Rg1*) on chromosome 1B and gene of awnedness (*B1*) on chromosome 5A were also mapped.

### 5.1.4. QTL detection

QTL analysis was performed using the data (Fusarium head blight severity and Fusarium damaged kernels) of each epidemic situation, with mean values of the used *Fusarium* species and with overall means. Among other phenotypic traits plant height and heading date were involved in the analysis. The result of the QTL analysis made with the Fusarium head blight severity and Fusarium damaged kernels rate of *F. culmorum*, *F. graminearum* mean and allover mean is detailed in Table 1.

## 5.2. Molecular mapping and QTL analysis in a GK Mini Manó/Frontana population

### 5.2.1. Results of the Fusarium head blight severity and Fusarium damaged kernels analysis

The Fusarium head blight severity (mean of all observations) and Fusarium damaged kernels rate of the GK Mini Manó/Frontana population (168 lines) showed normal distribution in the mean of all isolates. The Fusarium head blight severity was high: between 16.0 and 62.3%, while the Fusarium damaged kernels rate was between 22.3 and 94.4%. The heritability ($H^2$) value between epidemic situations was 0.89 with Fusarium head blight severity and 0.82 with Fusarium damaged kernels rate, which points to a very good reproducibility of the experiments.

### 5.2.2. Results of assessing other phenotypic traits

Wide differences were observed between the plant heights of the lines (from 58 to 133 cm). All lines of the population were awned, which made the QTL analysis more exact, avoiding its influential effect on Fusarium head blight. Further advantage of the GK Mini Manó/Frontana population is that the differences in heading date between the lines were far tighter than those observed in the Frontana/Remus population. Therefore the number of inoculations in the plant material was able to be reduced to two.

### 5.2.3. Molecular mapping

From the database of 643 polymorphic markers, 527 markers were mapped to 28 groups covering a genetic distance of 1381 cM (average marker distance: 2.62 cM). Chromosome positions of 26 linkage groups were defined among the mapped groups. Linkage groups were mapped on each chromosome except for 3D, 4D and 6D.

### 5.2.4. QTL detection

QTL analysis were made with the data (Fusarium head blight severity and Fusarium damaged kernels) of each epidemic situation, with mean values of the used *Fusarium* species and with overall means, as well as with heading date and plant height observed in the two experimental year – similarly to the process used in the analysis of the Frontana/Remus population. The overall means and mean data of the infection provided by the two *Fusarium* species showed stable linkage with the identified chromosome regions (Table 2.).

Table 1.: Locations of the QTL detected and chromosome regions with LOD values, percentage of explained phenotypic variance (VE) for Fusarium head blight (FHB) severity, Fusarium damaged kernels (FDK) levels (**A**) and heading date (**B**) in the Frontana/Remus population. The highlighted values were significant according to the genome wide permutation tests.

**A**

| | | FHB | | | | | | FDK | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chromo- | *F. culmorum* | | *F. graminearum* | | Mean | | *F. culmorum* | | *F. graminearum* | | Mean | |
| Marker interval | some | LOD | VE | LOD | VE | LOD | VE | LOD | VE | LOD | VE | LOD | VE |
| Xgwm526A - Xgwm120 | 2B | **2.43** | *8.3* | **2.39** | *10.2* | **2.30** | *7.8* | **4.50** | *15.9* | **2.09** | *7.2* | **4.22** | *14.6* |
| Xs12m15_4 | 2D | 0.56 | *1.6* | 0.40 | *1.6* | 0.40 | *1.1* | **2.65** | *10.3* | 1.37 | *3.8* | **2.62** | *8.7* |
| Xgwm1121 - Xgwm779 | 3A | **2.27** | *6.2* | 0.92 | *2.6* | **2.08** | *5.7* | 1.64 | *4.6* | 0.72 | *2.2* | 1.54 | *4.3* |
| Xs12m19_5 - Xgwm341 | 3D | 0.96 | *2.9* | 0.08 | *0.2* | 0.60 | *1.8* | **3.24** | *9.7* | 1.08 | *3.3* | **2.88** | *8.7* |
| Xwg232 | 4A | **2.69** | *9.5* | 1.25 | *4.5* | **2.60** | *9.0* | 0.33 | *1.6* | 0.09 | *0.4* | 0.24 | *1.2* |
| Xs13m26_7 - Xs13m18_9 | 4B | **3.30** | *8.8* | **2.16** | *5.9* | **3.49** | *9.4* | 1.40 | *3.8* | **2.82** | *7.6* | **2.26** | *6.1* |
| Xgwm293 - Xs24m19_5 | 5A | 1.82 | *5.2* | 1.57 | *4.3* | 1.62 | *4.7* | **2.50** | *7.2* | 1.87 | *5.4* | **2.43** | *7.0* |
| Xs13m14_10 - Xs23m14_4 | 6B | **2.05** | *6.1* | 1.89 | *5.4* | **2.33** | *6.8* | 0.07 | *0.2* | 0.01 | *0.0* | 0.04 | *0.1* |
| Xs12m25_2 | 7B | **4.57** | *14.2* | **3.01** | *9.6* | **4.96** | *15.5* | **4.92** | *14.0* | **2.07** | *6.0* | **4.50** | *12.7* |

**B**

| | Chromo- | Heading date | |
|---|---|---|---|
| Marker interval | some | LOD | VE |
| Xwg983 - Xs13m14_6 | 1A | **3.12** | *10.1* |
| Xs25m19_16 - Xgwm608 | 2D | **2.57** | *8.7* |
| Xgwm46 | 7B | **2.87** | *9.5* |

Table 2.: Locations of the QTL detected and chromosome regions with LOD values, percentage of explained phenotypic variance (VE) for Fusarium head blight (FHB) severity, Fusarium damaged kernels (FDK) levels **(A)**, heading date **(B)** and plant height **(C)** in the GK Mini Manó/Frontana population. The highlighted values were significant according to the genome wide permutation tests; chromosome regions painted in the table with grey color overlapped with chromosome regions responsible for Fusarium head blight resistance.

**A**

| | | FHB | | | | | | FDK | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chromo- | *F. culmorum* | | *F. graminearum* | | Mean | | *F. culmorum* | | *F. graminearum* | | Mean | |
| Marker interval | some | LOD | VE | LOD | VE | LOD | VE | LOD | VE | LOD | VE | LOD | VE |
| wPt-734078 - wPt-731843 | 1A | **2.30** | *5.9* | **3.67** | *9.5* | **3.08** | *8.0* | 1.79 | *4.6* | **2.18** | *5.8* | **2.10** | *5.6* |
| wPt-5347 - wPt-2315 | 1B | **4.03** | *13.8* | **5.33** | *18.0* | **5.06** | *17.0* | **4.50** | *11.9* | **4.71** | *12.1* | **5.02** | *13.0* |
| wPt-732882 - wPt-667765 | 2D | **5.52** | *18.7* | **6.67** | *23.0* | **6.61** | *23.0* | **5.81** | *14.3* | **6.60** | *22.2* | **6.34** | *20.6* |
| wPt-3812 - wPt-732411 | 2D | 0.47 | *1.3* | 0.99 | *2.6* | 0.77 | *2.0* | **3.54** | *8.9* | **5.01** | *12.4* | **4.73** | *11.8* |
| Xgwm533 - wPt-3921 | 3B | **4.25** | *10.6* | **3.71** | *9.3* | **3.97** | *10.0* | **2.13** | *5.5* | **2.83** | *7.2* | **2.54** | *6.6* |
| wPt-800509 - wPt-2780 | 4A | **5.10** | *12.6* | **6.07** | *14.8* | **5.73** | *14.6* | 1.80 | *4.7* | 1.39 | *3.6* | 1.71 | *4.4* |
| wPt-5334 - wPt-4243 | 4B | **3.23** | *8.2* | **3.43** | *8.7* | **3.60** | *9.1* | 1.55 | *4.0* | 0.94 | *2.5* | 1.31 | *3.4* |
| Xgwm205 - Xgwm156 | 5A | **3.58** | *9.0* | **5.26** | *13.7* | **4.73** | *12.2* | **4.97** | *13.4* | **3.79** | *10.3* | **4.71** | *12.7* |
| wPt-741134 - wPt-5896 | 5B | **2.45** | *8.2* | **2.36** | *8.1* | **2.54** | *8.7* | **4.34** | *14.1* | **5.43** | *15.0* | **5.34** | *15.0* |
| wPt-7204 - wPt-744786 | 6A | **3.99** | *10.0* | **3.34** | *8.5* | **3.97** | *10.5* | **5.19** | *12.9* | **5.57** | *13.7* | **5.79** | *14.2* |
| wPt-6039 - Xgwm88 | 6B | **7.46** | *18.9* | **7.44** | *18.7* | **8.14** | *20.5* | **5.44** | *13.6* | **4.42** | *11.3* | **5.31** | *13.3* |
| wPt-9925 - wPt-5922 | 7B | **3.13** | *7.9* | **3.55** | *9.6* | **3.52** | *10.8* | **6.28** | *15.3* | **4.32** | *10.8* | **5.67** | *13.9* |
| wPt-0934 - wPt-743601 | 7D | 0.48 | *1.3* | 0.74 | *1.9* | 0.65 | *1.7* | **2.79** | *7.1* | **2.84** | *7.3* | **3.12** | *7.9* |
| Xgwm44 - wPt-744219 | NA1 | **2.51** | *6.5* | **3.54** | *9.0* | **3.33** | *8.5* | **2.71** | *7.0* | 1.45 | *3.8* | **2.16** | *5.6* |
| wPt-666593 - wPt-664682 | NA2 | **2.08** | *5.6* | **2.01** | *5.4* | **2.21** | *7.6* | **2.84** | *10.1* | **2.96** | *10.4* | **3.18** | *11.1* |

**B**

| | Chromo- | Heading date | |
|---|---|---|---|
| Marker interval | some | LOD | VE |
| wPt-4664 - wPt-7715 | 2B | **4.28** | *11.1* |
| wPt-741026 - wPt-744786 | 6A | **2.17** | *5.7* |
| wPt-5283 - wPt-7318 | 7B | **3.37** | *12.3* |

**C**

| Marker interval | Chromo-some | Plant height LOD | VE |
|---|---|---|---|
| wPt-666607 | 1A | **3.05** | *7.9* |
| wPt-734078 - wPt-731843 | 1A | **2.67** | *7.0* |
| wPt-0325 - wPt-2315 | 1B | **6.07** | *14.9* |
| wPt-4664 - wPt-3132 | 2B | **5.91** | *14.5* |
| wPt-8916 - wPt-5736 | 2B | **3.57** | *9.0* |
| wPt-732882 - wPt-667765 | 2D | **2.14** | *7.7* |
| wPt-3812 - wPt-732411 | 2D | **3.97** | *10.3* |
| wPt-9268 - wPt-1694 | 3A | **3.44** | *8.8* |
| Xgwm533 - wPt-3921 | 3B | **4.31** | *19.3* |
| wPt-7280 | 4A | **2.08** | *5.3* |
| wPt-8892 - wPt-5303 | 4B | **3.03** | *7.7* |
| Xgwm205 - Xgwm156 | 5A | **5.31** | *13.3* |
| wPt-1409 - wPt-5896 | 5B | **3.39** | *9.3* |
| wPt-7204 - wPt-744786 | 6A | **3.65** | *10.8* |
| wPt-6039 - Xgwm88 | 6B | **4.03** | *12.9* |
| wPt-9925 - wPt-1266 | 7B | **2.99** | *7.7* |
| wPt-0934 - wPt-743601 | 7D | **4.52** | *11.3* |

## 6. CONCLUSIONS AND RECOMMENDATIONS

It is expedient during the marker assisted selection to use molecular markers, which are not linked to any negative agronomic or technological quality trait. The importance of validation of QTL and linked markers have been proved earlier by other research groups also (Li et al. 2008; Xu és Crouch 2008). For example the Swiss resistance source, Arina was investigated in mapping populations generated with different crossing partners by several research groups (Paillard et al. 2004; Draeger et al. 2007; Semagn et al. 2007). The only common Arina derived Fusarium head blight resistance QTL which identically found in the publications mentioned above was on chromosome 1BL. The environmental dependence of small and medium effective QTL is clearly shown through the example of Arina.

The investigated Frontana/Remus population, heterogenic for heading date and awnedness, was tested earlier by an Austrian research group (IFA-Tulln) in diverse environments, using different methods (Steiner et al. 2004). The effect of Frontana derived QTL identified in this last population was investigated in another mapping population, in which the crossing partner was a dwarf genotype (GK Mini Manó), so that the population was quite heterogenic in plant height. All these are important because the fact, that a given infection level can be caused by the influence of morphological traits, and this should be considered during the investigation of the molecular background of Fusarium head blight resistance. Emrich et al. (2008) claimed that breeding for Fusarium head blight resistance will attend unfavorable late heading. Based on our experiences, the possible reason is that the weather is warmer and drier for the genotypes that are inoculated later, and this can reduce the disease development which can be considered as resistance.

McCartney et al. (2007) identified Fusarium head blight resistance QTL on chromosome 5A linked also to negative quality traits (reduced thousand kernel weigh and protein content). The effect of plant height on Fusarium head blight resistance was mentioned in several publications also (Mesterházy 1995; Hilton et al. 1999; Buerstmayr et al. 2000; Gervais et al. 2003; Paillard et al. 2004). Paillard et al. (2004) and Gervais et al. (2003) found identical the chromosome regions responsible for the last mentioned traits, although it was not evident that these loci are linked to each other or have pleiotropic effect. Resolving the function of this kind of QTL or genes is easier if the Fusarium head blight resistance QTL are investigated in a population where the crossing partner is diverse in traits (like plant height, awnedness, head compactness) that can influence the resistance. The fact, that a large number of plant height QTL were detected, was unexpected considering the earlier publications describing 1-2 QTL in other populations. The investigation of the background relations remains an important research area.

The moderately Fusarium head blight resistant wheat genotypes (like Frontana) usually bear with several small and medium effective QTL. The dissertation highlighted the results that the number of these QTL might be different in the populations of the same resistance source. These plant materials are important, because they are better adapted to the local environment compared to the exotic sources; in addition, they possess minor effective QTL, which can improve the level of Fusarium head blight resistance.

The most stable Frontana derived Fusarium head blight resistance QTL were on chromosomes 4B, 5A and 6B, which were also identified in the Frontana/Remus population by us and by Steiner et al. (2004); we also detected these in the GK Mini Manó/Frontana population. We verified the overlap of these QTL with the ones, determining plant height. From the view of breeding, this means that the use of markers, linked to Fusarium head blight resistance QTL on chromosome 4B, 5A and 6B, should be completed with field tests during the selection. In the field, not only plants with appropriate plant height can be selected, but the

effect of QTL which did not seem stable or showed linkage with plant height can be detected as well. Similar consequences were derived by Wilde et al. (2008) who made marker assisted selection using the markers linked to Fusarium head blight resistance on 2BL, 6AL and 7BL, and realized that the selected resistant genotypes are significantly taller. This result is verified by other publications claiming that these QTL are linked to plant height, too (Steiner et al. 2004; Schmolke et al. 2005; Mao et al. 2010). However it should be taken into account that for morphological reasons, higher plants with the same physiological resistance level can be infected less. The optimum height of wheat varieties is 80-100 cm, but pesticides are often used to reduce plant height, destructing the positive effect of this trait.

Our results show that even the stable Fusarium head blight resistance QTL are not constant in the mean of investigated trait (FHB or FDK), as well as in the mean of different epidemic situations. Differences between the QTL linked to Fusarium head blight severity and/or Fusarium damaged kernels were identified even in the validated results of the two populations investigated in Szeged. This verifies that not only the Fusarium head blight severity must be investigated, but the Fusarium damaged kernels should be taken into account during the breeding process. Moreover emphasis should be placed on this last trait, because the statistical analysis (correlation analysis, ANOVA, QTL analysis) suggests that the FDK data are more accurate, in addition it shows better correlation with toxin accumulation according to our earlier experimental results. Certainly the toxin content is cardinal from the view of food safety. Our results indicate that only the Fusarium head blight resistance QTL on 4A and Fusarium head blight and Fusarium damaged kernels resistance QTL on chromosome 5A and 7B were able to be identified unequivocally in both investigated populations. Further verification was gained that the marker assisted selection should be completed with field tests and not only the Fusarium head blight severity but the Fusarium damaged kernels should also be considered during the selection; moreover according to other experimental results, beyond these two resistance components the toxin accumulation should also be investigated. Additionally the markers used should be chosen from QTL regions linked to more resistance traits and were possibly validated in several resistance sources.

The methodology of Fusarium resistance field tests provokes several questions among the researcher-breeders. In China and in North-America large emphasis was put on one-floret inoculation, which tests only the Type II resistance. The inoculum spraying method, used by us is suitable to test Type I and Type II resistance at the same time, moreover able to test the toxin accumulation (Mesterházy 1995; Mesterházy et al. 2007).

In our experiments the hypothesis that the Fusarium head blight resistance is horizontal was strengthen, since differences between the LOD values of QTL linked to symptoms, caused by *F. culmorum* or *F. graminearum* were observed in the Frontana/Remus population, which can be explained with that we had four epidemic situations with *F. culmorum* and only two epidemic situations with *F. graminearum*. There was no such difference between the numbers of epidemic situations in the GK Mini Manó/Frontana population, therefore smaller variance was observed. Comparing the LOD values of the single epidemic situations several significant differences were found. Between the results of single epidemic situations, maximum moderate correlations could be detected; better correlations were observed between the epidemic situations and species specific mean data, and in addition the best correlations are found with the overall mean data. All these points out the importance of carrying out field tests in many epidemic situations, and this should be considered not only during mapping but in the breeding process as well. Jiang et al. (2007) verified that the results of a QTL analysis, made with mean data of several epidemic situations, are more proper and usually lead to higher LOD values. The results of the GK Mini Manó/Frontana population confirmed that it is advised to use more isolate in a given year to

reduce the effect the environmental effects, because in that case the 2-3 isolates are in the same environment.

Important consequences can be drawn to methodology and resistance breeding aspects. Regarding methodology, the hypothesis was verified that to validate Fusarium head blight QTL, plant materials should be tested in more epidemic situations than usually published (2-3), in addition they should be tested in several populations and environments. This is even more important in the case of small and medium effective QTL, which are influenced by the environment more easily. The breeders should make phenotypic selection - involving more resistance traits - following the marker assisted selection with markers linked to stable QTL.

**New scientific results**

1. During our work the marker map of the Frontana/Remus population was extended with new markers and a completely new maker map was constructed in the GK Mini Manó/Frontana population. Therefore new markers linked to Fusarium head blight resistance QTL were detected and validated on chromosomes 2D, 4A and 7B.

2. Beyond the chromosome regions influencing Fusarium head blight severity, QTL linked to Fusarium damaged kernels were also identified, which is a unique discovery not only for the Frontana, but in the case of other donors also. Based on our results it is ascertainable that the investigation of more traits correlating with Fusarium head blight is necessary, because their genetic regulation can be different.

3. We clarified during the investigation of molecular background of the traits influencing Fusarium head blight resistance, that the morphological homogeneity of a population is cardinally important. In our experiments QTL influencing these traits were detached from real Fusarium head blight resistance QTL. However in the GK Mini Manó/Frontana population several plant height QTL overlapped with chromosome regions, influencing Fusarium head blight resistance.

4. Through the investigation and validation of Frontana derived Fusarium head blight resistance QTL in several environments, using different inoculation methods, in diverse genetic background we drew the conclusion that the most stable QTL are on chromosome 4B, 5A and 6B. Therefore the markers in these chromosome regions are the most suitable for marker assisted selection with the integration of phenotypic selection.

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## SCIENTIFIC ACTIVITY OF ÁGNES SZABÓ-HEVÉR

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## ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to Professor László Heszky – the head of the Doctoral School of Plant Sciences, SZIU – and to Professor Erzsébet Kiss – the head of Department of Genetics and Biotechnology, SZIU – for their mentorship during my MSc and PhD study.

I gratefully acknowledge to my supervisor Professor Ákos Mesterházy all the support he gave me. I appreciate the support of Dr. János Matuz previous and László Szilágyi present head of the Cereal Research Nonprofit Company (CRC). Many thanks to the colleges on the Department of Resistance Research for their conscientious assistance. I am grateful to Dr. László Purnhauser, Dr. Lászlóné Csősz, Dr. János Matuz, Dr. Mónika Varga, Szabolcs Lehoczki-Krsjak for reviewing the thesis and for their thoughtful and detailed comments. Thank you to all employees of the CRC: researchers, technicians and manual workers.

A lot of thanks for the research groups of Dr. János Pauk and Dr. Hermann Buerstmayr who provided the wheat populations investigated in the dissertation. I would like to say a very big thank to Dr. Barbara Steiner for her molecular marker database.

To my family and friends, thank you for being there to listen, console and love!

This research was realized in the frames of TÁMOP 4.2.4. A/1-11-1-2012-0001 „National Excellence Program – Elaborating and operating an inland student and researcher personal support system" The project was subsidized by the European Union and co-financed by the European Social Fund.

„I have serious reason to believe that the planet from which the little prince came is the asteroid known as B-612. This asteroid has only once been seen through the telescope. That was by a Turkish astronomer, in 1909.
On making his discovery, the astronomer had presented it to the International Astronomical Congress, in a great demonstration. But he was in Turkish costume, and so nobody would believe what he said. Grown-ups are like that. . .
Fortunately, however, for the reputation of Asteroid B-612, a Turkish dictator made a law that his subjects, under pain of death, should change to European costume. So in 1920 the astronomer gave his demonstration all over again, dressed with impressive style and elegance. And this time everybody accepted his report.
If I have told you these details about the asteroid, and made a note of its number for you, it is on account of the grown-ups and their ways."

(Antoine se Saint-Exupéry: The little prince)