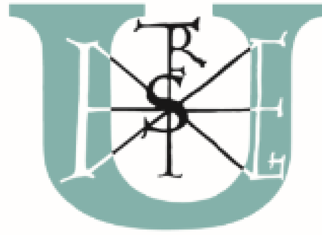

SZENT ISTVÁN EGYETEM
GÖDÖLLŐ

# PLANT SCIENCE DOCTORAL SCHOOL

## ANALYSIS OF GENETIC FACTORS DEFINING HEAD BLIGHT RESISTANCE IN OLD HUNGARIAN WHEAT CULTIVARS

**TOPICS OF THE DOCTORAL (PhD) THESIS**

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Description: Szent István University, Plant Science Doctoral School

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# Preliminaries to the work, the objectives set

The territory of the Carpathian Basin, owing to its climatic endowments, favours high quality grain production and the exports of high grade grains provide sources of revenue for our country, important even at the level of the national economy, therefore, special attention must be devoted to each factor causing both quantitative and qualitative damage. Among these, particularly significant are the weak seedling emergence, root and stem rot in wheat as well as the various *Fusarium* species causing infection in the head and seeds. The most striking condition is the fusarium head blight. As a result of the head infection, the crop volume may drastically decline, the quality for the bakery industry and the germination vigour may deteriorate. In addition to the qualitative and quantitative losses, the *Fusarium* species produce, as secondary metabolites, micotoxins (dezoxinivalenol, nivalenol, zearalenon, T-2 toxin etc.) presenting risks both for human and veterinary hygiene, the occurrence of which above the specific limit value, makes the crop unsuitable for processing. Their presence is particularly harmful and dangerous as no solution is available for their subsequent removal from the infected crop, and as they are thermostable molecules, their decomposition cannot be expected during processing either.

Fusarium head blight is a disease known in Hungary already since the 1920s, however, the first nationwide epidemics occurred only in 1970, which can be explained also by the susceptibility of the varieties in addition to the increasingly intensive production processes and the weather conditions favouring the infection, at the same time the genetic background of the varieties in public production might have also contributed, with great probability, to the insignificant level of infection observed in the period of 1920-1970, free of nationwide epidemics.

The objective of this research is to assess the resistance to fusarium head blight and to identify the genetic factors and chromosome regions encoding the properties, through the analysis of a population established by crossing an old Hungarian wheat variety and a modern wheat variety improved in Martonvásár.

# Material and method

## Description of the plant material analysed

'BKT9086-95'
The field fusarium head blight resistance of several lines of Bánkúti origin has been analysed earlier in Martonvásár. In this experiment, known sources of resistance and susceptible control varieties were also tested, which were treated with a method identical with those applied to the 'Bánkúti 1201' lines. Of the lines analysed, the line 'BKT9086-95' proved to be resistant to fusarium head blight consistently at a level equal to the resistant control variety ('Sumai 3'), therefore, this line was assigned as the resistant parent of the mapping population to be used for the identification of the genetic region, which determined resistance to fusarium.

'Mv Magvas'
However, the variety Mv Magvas proved to be more sensitive than the average to the disease on the basis of the routine determination of fusarium head blight resistance carried out as a routine in Martonvásár. Being aware of this property, it was selected to be the susceptible cross-breeding partner in the course of establishing the population.

Production of the mapping population
250 lines were established by the SSD method (in Martonvásár, from 2001), using these lines the chromosome regions related to the 'BKT9086-95' FHB resistance can be identified (the tests were started from generation $F_5$).

## Resistance tests

Production of the infection substance
IFA66 *F. graminearum* and IFA104 *F. culmorum* isolates were used for infecting. The isolates were sustained in permanent cultivations, in sterilised earth/sand mixture. The monoconidia cultivation was started from SNA (synthetischer nährstoffarmer Agar) medium.

The amount necessary for artificial infection was multiplied in a mungo bean liquid medium in case of *F. graminearum* and in wheat-oats seed mixture sterilised in autoclave in case of the *F. culmorum* inoculate. The mass of mycelia produced was removed by vacuum pump from the medium solution *Fusarium graminearum* and after filtration, the concentration of conidia was assessed. In case of *Fusarium culmorum,* the macroconidia was washed by distilled

water from the wheat-oats mixture and the concentration of the suspension was analysed.

Assessment of artificial inoculation and level of infection

The conidia concentration was set to $5*10^5$ pc/ml in case of both greenhouse and field infection, followed by the injection of 5 µl suspension in 1-1 basal floret of upper 1/3rd of flowering head. Experiments were conducted over 3 years (2007-2009) under controlled conditions in the experimental greenhouse of the Centre for Agricultural Research, Agricultural Institute, Hungarian Academy of Sciences, in the course of which we artificially infected the plants by the spike injection method with *F. culmorum* isolate (Figure No. 1). The tests were conducted in four repetitions in each experiment.

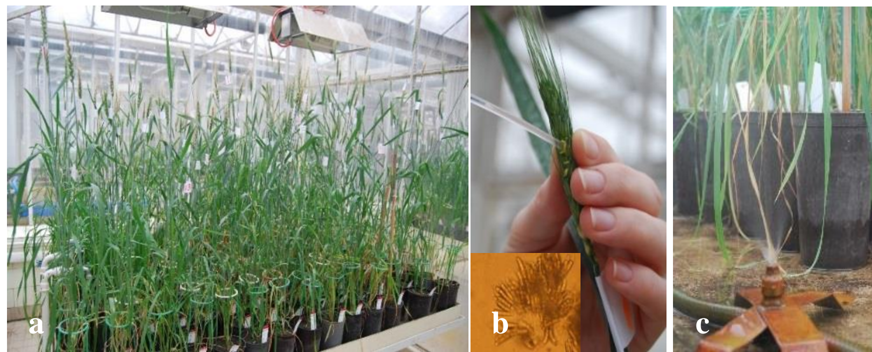


**Figure No. 1:** Plant growing chamber (a – mapping population, b – process of artificial infection, c – with supplementary humidification) (Martonvásár, 2007).

After the artificial infection, the high humidity (80-90%) favourable for the fungus was insured for 3 days in a special mist chamber. The infected heads were identified by means of a self-adhesive label affixed on the head support stem segment, also stating the date of infection.

The field experiments were carried out in the fusarium test nursery of our institute (47°18'47'' north, 18°46'24'' east) between 2006-2011. The plants were planted in double-row parcels with a length of 2 m and 20 cm distance between the rows. Humidity necessary for the infection was provided by mist irrigation. The heads to be infected were marked by self-adhesive labels, stating in each case the number of repetitions, the pathogen used for infection and the date of infection. 5 plants were inoculated for each pathogen. In the field experiments, the flowering date, the height of plant (from

ground level to the thick of the head) and the length of the heads were also recorded.

Evaluation was carried out according to an identical system both under greenhouse and field conditions. Simultaneously with the infection, the position of the inoculated spike calculated from the tip of the head, the number of all the spikes were recorded, stating the proportion of the infected spikes in %. The emergence of the symptoms was assessed on days 7, 14 and 21 after inoculation (Figure No. 2), stating the position of the infected spikes calculated from the tip.

**Figure No. 2**: Symptoms of infection on day 21 in the greenhouse experiment (a – resistant genotype, b – moderately resistant, c – susceptible) (Martonvásár, 2007).

The area under the disease progress curve was also calculated from the results of counting the infected spikes every 7 days (AUDPC, area under the disease progress curve), from these data conclusions can be drawn concerning the progress of infection over time.

## Statistical assessment of the phenotype data

The statistical assessment of the data was carried out by means of the programme package R, in accordance with the requirements of the parameter tests the distribution of the samples was tested by the Shapiro-Wilk method and the homogeneity of deviation was checked by the Levene test. Variance analysis and correlation calculation were applied for testing the correlations.

## Molecular methods of inspection

Extraction of DNA from plant samples
In the tests, DNA extraction was made from the plant samples of 250 lines and the parents using the Qiagen DNeasy Plant Mini Kit, in accordance with the instructions of the manufacturer (from generation $F_6$). The DNA amount of the samples was determined by means of the spectrophotometer type NanoDrop 1000 (Thermo Scientific, USA). The templates were stored at -20 °C until the PCR reaction was completed.

DNA-based markers (SSR, AFLP, SNP)
As a starting step, the differences among the parents were analysed with the available SSR and AFLP primers. The separation of the reaction products and the detection of the samples were performed in case of the SSR markers by means of the Li-Cor 4200 (Li-Cor Biosciences, USA) instrument on 6% polyacrylamide gel.
The AFLP fragments were separated on 7% polyacrylamide gel, the patterns were analysed by means of the Typhoon Trio™ (GE Healthcare, UK) system (at 570 nm, 670 nm and 520 nm wave length).
The genotypes, regarding which we had the full data series in the greenhouse each year and in each repetition, were also analysed by means of the Illumina Infinium (TraitGenetics, Germany) 20k wheat chip.

## QTL identification

Market property analysis was performed on a genetic database based upon AFLP and SSR markers. The possibility of relationship between the markers and the head fusariosis was analysed through the use of the programme package GAPIT – Genome Association and Prediction Integrated Tool on the basis of position of the SNP markers occupied in the pseudo-reference genome. The individual lines were characterised by the average values of the greenhouse and field data series as well as the BLUP (best linear unbiased prediction) values applied to the total test. The BLUP values were calculated with the programme package lme4.

In the other experimental locations and year of production BLAST (basic local alignment search tool) characterisation was performed on the basis of the known sequence of the Affimetrix markers with significant effect.

# Results

## Review of resistance type II of the offspring lines originating from the ’BKT9086-95/Mv Magvas’ experimental corss under greenhouse conditions

With respect to head infection on day 21 (%), the values of the wheat lines constituting the population covered the entire scale of infection. The average infection was the greatest in 2009 (65.20%), however, the average of 2007 was also similar (53.59%). In contrast, the value of the average infection was as low as 24.12% in 2008 (1).

1: Annual average infection of lines and parental genotypes under greenhouse conditions (n=175, Martonvásár, 2007-2009).

| | Head infection (%) | | | | |
|---|---|---|---|---|---|
| | Parents | | Lines (n=173) | | |
| | ’BKT9086-95’ | ’Mv Magvas’ | Average | Range | Deviation |
| 2007 | 32.25 | 95.24 | 52.95 | 4.09-95.91 | 24.75 |
| 2008 | 12.00 | 94.43 | 24.17 | 5.15-96.45 | 17.99 |
| 2009 | 38.06 | 97.52 | 65.20 | 3.85-100.00 | 25.44 |

It was found that the average infection values of the lines each year exceeded the infection values of the resistant parent (’BKT9086-95’) by a factor of almost two, moreover, the pathogen could reach almost each spike and trigger symptoms therein, in the head of the susceptive parent (’Mv Magvas’)

The two-factor variance analysis of the infection values at the $p<0.001$ level also presented significant correlation among the genotypes and the data of infection, with respect to both head infection and AUDPC values (2). At the same time, it can be determined that the year of production had an impact on the rate of fusarium infection with statistical proof.

Our greenhouse results support the presence of the genetically determined fusarium resistance in the population tested. As an information also useful for the practical improvement, the QTL of appropriate effect is also expressed under different environmental conditions, therefore, the population was subject to detailed field tests to determine this feature in the remaining part of the process.

2: Result of the variance analysis on the basis of the infection values on 21st day after inoculation and the average of the AUDPC data, under greenhouse conditions (n= 173+2 parent, Martonvásár, 2007 and 2009).

| 21st DAI | Df | SQ | MQ | F-value | Pr (>F) |
|---|---|---|---|---|---|
| Genotype | 174 | 448802 | 2579 | 4.001 | $1.45e^{-10}$ *** |
| Year | 1 | 5751 | 5751 | 2.461 | 0.035 * |
| Genotype×Year | 174 | 123863 | 712 | 0.552 | 1.000 |
| Residual value | 796 | 1026146 | 1289 | | |
| **AUDPC** | **Df** | **SQ** | **MQ** | **F-value** | **Pr (>F)** |
| Genotype | 174 | 51294274 | 294795 | 2.361 | 1.2e-15 *** |
| Year | 1 | 50592 | 50592 | 0.405 | 0.525 |
| Genotype×Year | 174 | 16902477 | 97141 | 0.778 | 0.979 |
| Residual value | 796 | 99403034 | 124878 | | |

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Significance levels: '***' 0.001 '*' 0.05

Df: degree of freedom, SQ: total square, MQ: mean square, Pr (>F): probability

## Review of resistance type II of the offspring lines 'BKT9086-95/Mv Magvas' under field conditions

With respect to head infection (%), the values of the wheat lines constituting the population had a variability on a wide scale also under field conditions (3), covering in each year the total interval of infection. The strongest infection was experienced in 2011 in the case of both *F. culmorum* (53.16%) and *F. graminearum* isolate (65.95%) in the average of the lines, while the lowest average infection was observed in 2006 (25.27%). The resistant and susceptible parent was infected in all the three years to the rate matching their resistance level.

It was found that the parental genotypes occupied the position corresponding to their known resistance each year. The genotypes less infected than the more resistant parent were identified each year, while the susceptible 'Mv Magvas' was located at the endpoint of the scale, with the exception of the year 2011 characterised by very intensive average infection. However, the most important point of the observation is that in the course of our tests - in accordance with our observations of the greenhouse - such genotypes were identified in which the disease could progress from the location of infection to a minimum extent only or not at all.

3**:** Average field infection values of the lines and parental genotypes (n=223, Martonvásár, 2006,2009,2011).

| | | Head infection (%) | | | | |
|---|---|---|---|---|---|---|
| | | Parents | | Lines (n=221) | | |
| | Factor | 'BKT 9086-95' | 'Mv Magvas' | Average | Range | Dev. |
| 2006 | Fc | 6.67 | 100.0 | 30.90 | 4.35-76.90 | 18.14 |
| | Fg | 8.51 | 92.86 | 25.27 | 4.35-92.56 | 14.14 |
| 2009 | Fg | 26.19 | 100.0 | 47.10 | 7.01-91.67 | 17.49 |
| 2011 | Fc | 25.53 | 72.75 | 53.16 | 8.95-100.00 | 18.83 |
| | Fg | 23.99 | 85.99 | 65.95 | 9.93-100.00 | 20.62 |
| **Ave.** | Fc | 16.10 | 86.38 | 42.03 | 4.37-100.00 | 18.22 |
| | Fg | 19.56 | 92.95 | 46.10 | 9.93-100.00 | 20.74 |
| | Main average | 18.18 | 89.67 | 44.48 | 11.19-90.83 | 12.99 |
| | PH | 125.0 | 85.00 | 115.6 | 85.00-142.50 | 11.74 |
| | Heading | 19.0 | 21.00 | 21.60 | 14.00-30.00 | 2.51 |
| | Ear comp. | 1.67 | 2.50 | 2.10 | 1.53-2.78 | 0.28 |

Fc: *F. culmorum*, Fg: *F. graminearum*, PH: plant height (cm), Heading: number of days from May 1, Ear comp.: ear compactness (number of spikes/cm), Dev.: deviation, Ave.: average

The most exact picture about resistance to fusarium head blight can be obtained by analysing multi-year data series. Therefore, with respect to the total experiment, the impact of genotype, year of production, infection time, plant height and genotype x year of production interaction on the values of infection was also tested. As in the various years different numbers of repetitions were used, we applied the unbalanced ANOVA test for the statistical evaluation of the 3-year data series (4).

It was found that the genotype, the year of production, the time of infection has an impact on both the values of infection experienced on day 21 and the area under the disease progress curve, with statistical proof. The plant height and the ear compactness, with respect to the entire test, has an impact on the values of infection expressed in % with statistical proof, however, not on the AUDPC values. This difference shows that despite the close interrelationship between the two indicator numbers, they may provide

supplementary information in the course of characterising the level of resistance. Moreover, knowing the related p and F values, it can be stated that the effect of year of production has a smaller influence on the AUDPC value than on the values of infection on day 21. Based upon the result of ANOVA, no statistical evidence could be found to prove the genotype × year of production interaction in case of any of the properties analysed. This shows that while the year of production also has an impact on the rate of resistance to head fusariosis, however, the sequence of genotypes among the specific years did not change in a statistical sense. Consequently, the specimens of the population tested may indeed carry a genetically defined fusarium head blight resistance, with the variability experienced in the population being adequate for identifying those with molecular methods.

4**:** The results of the unbalanced ANOVA test, on the basis of the Bon21 and ANOVA values (21$^{st}$ DAI: values of head infection on 21$^{st}$ day after inoculation, AUDPC: area below the epidemic curve, n=221, Martonvásár, the average of years 2006, 2009 and 2011).

Table Anova

Variable: **21$^{st}$ DAI**

| | SQ | Df | F-value | Pr (>F) |
|---|---|---|---|---|
| Genotype | 609534 | 220 | 2.9057 | $< 2.2e^{-16}$ *** |
| Year | 55986 | 2 | 5.4406 | $6.829e^{-7}$ *** |
| Date of infection | 37636 | 12 | 3.3491 | 0.019742 * |
| Height of plant | 5095 | 16 | 3.7365 | $7.247e^{-5}$ *** |
| Ear compactness | 12525 | 25 | 3.3436 | 0.009695 ** |
| Genotype×Year | 190936 | 192 | 1.0619 | 0.272454 |
| Residual value | 685810 | 2868 | | |

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Variable: **AUDPC**

| | SQ | Df | F-value | Pr (>F) |
|---|---|---|---|---|
| Genotype | 39177695 | 220 | 2.8577 | $< 2e^{-16}$ *** |
| Year | 1875384 | 2 | 1.9151 | 0.01534 * |
| Date of infection | 1610250 | 12 | 2.1925 | 0.0994 * |
| Height of plant | 28482 | 16 | 0.4654 | 0.49518 |
| Ear compactness | 385892 | 25 | 1.5763 | 0.17782 |
| Genotype×Year | 12957508 | 192 | 1.1027 | 0.16585 |
| Residual value | 75532613 | 2868 | | |

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Significance levels: ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05

Df: degree of freedom, SQ: total square, Pr (>F): probability

**Molecular tests in the 'BKT9086-95/Mv Magvas' offspring population**

Microsatellite and AFLP marker tests

Based upon data of the literature primarily markers connected to fusarium resistance and markers providing signals of minimum medium intensity were selected for testing the entire population, therefore, the detailed analysis of the lines was performed with 33 SSR markers. The total population was also tested with 32 AFLP primer combinations as well in total, as a result of which 286 different patterns were identified. As the genetic map that could be created by means of the 319 polymorph markers available to us in total, would not cover all the chromosomes of the entire wheat genome, therefore, the ANOVA test was applied for analysing the relationship between fusarium head blight resistance and the markers. Parallel tests were conducted under greenhouse and field conditions of the relationships among the other phenotype properties related to resistance using the AFLP and SSR markers.

As the QTL analysis performed under the genetic map provides a statistically more reliable result than the test of association of the marker-properties, therefore, only those markers were considered really linked which manifested significant effect within the specific experimental systems in each testing year and also with respect to the average. Altogether 30 such markers were identified (29 AFLP, 1 SSR), which satisfied the criteria noted. As in the course of assessing the phenotype data, a significant correlation was shown by the plant height, the ear compactness and the flowering time with the expression of the HFB-resistance, therefore, only those markers can be considered indeed related to fusarium resistance, which are not influenced by the properties mentioned.

Altogether 6 such markers were identified, which had a significant impact on the infection values experienced on day 21 in both experimental systems. On the basis of the results, the agat17, or gtac2 and gtac3 markers may be related exclusively to the region encoding the resistance to the progress of fusarium in the head.

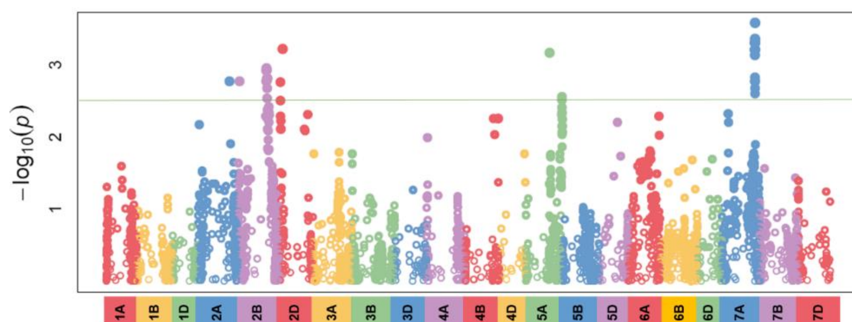
The ANOVA test was also performed in case of the AUDPC values, as a result of which 25 such markers were identified which were related to the AUDPC values of the lines under greenhouse or field conditions, proven by statistical evidence. Partial overlapping can be detected among the markers in significant connection with the values of infection and the rate of progress of the infection, markers were identified in 14 cases connected to both values. However, the

markers exclusively connected to the AUDPC values may also be related in each case to other phenotype properties.

However, the markers type agat17, gtac2, gtac3 also have an impact in case of the AUDPC proven by statistical evidence, under which it can be determined that the 'BKT9086-95/Mv Magvas' offspring lines may carry with high probability a genetically determined fusarium resistance.

<u>Identification of the genetic background related to the infection percentage</u>

We have used the average values of the greenhouse and field data series to characterise the lines. Based upon the K model of the GAPIT programme package, the presence of significant QTL's was confirmed under greenhouse conditions with respect to the infection values on day 21 (1). The connection of 32 markers was identified with the tested property altogether on 5 chromosomes. The lowest p-values were calculated in case of the chromosome 7A p= 0.00026 (Ra_c8394_1381) and p=0.00442 (BobWhite_c30461_131) in the nucleotide positions 645092185 and 647933749, respectively, on the physical map of the wheat. A total of 14 markers in significant relationship with the infection values of day 21 were identified on the chromosome 7A under greenhouse conditions. On the short arm of the chromosome 2B (Tdurum_contig29563_257, pos: 28339816) 1, while on the long arm 10 markers manifesting close correlation with the property tested were identified (the region between markers AX-94393508 and BobWhite_c16130_362). In case of the chromosomes 2D and 5A, additional 3 connected markers are located in each case in the nucleotide regions 15967374 – 62023977 and 466617397 – 702461388, respectively. A single SNP marker of significant effect was identified on the chromosome 2A (RAC875_c29716_871, pos: 607997395).

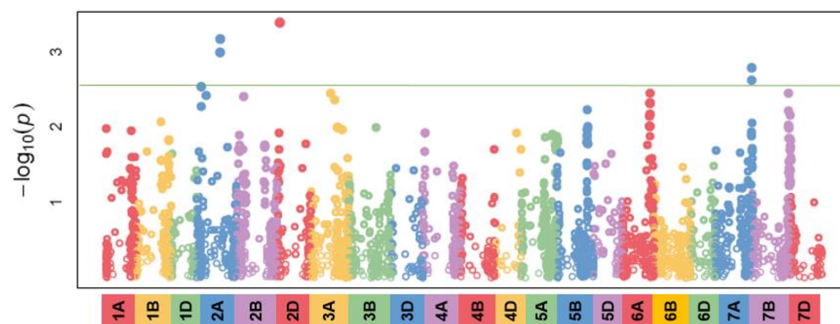



1: Manhattan plot, average infection values on 21st day after inoculation under greenhouse conditions, according to the chromosomal localisation of

the markers (level of significance: $-\log_{10}(p)$ 2,5; Martonvásár 2007, 2009 average).

Based upon the estimation from the effects of the specific alleles, the QTL region on chromosome 7A originates from the susceptible parent (’Mv Magvas’), while the regions on chromosome 2A, 2B, 2D and 5A can be connected to the resistant parent (’BKT9086-95’).

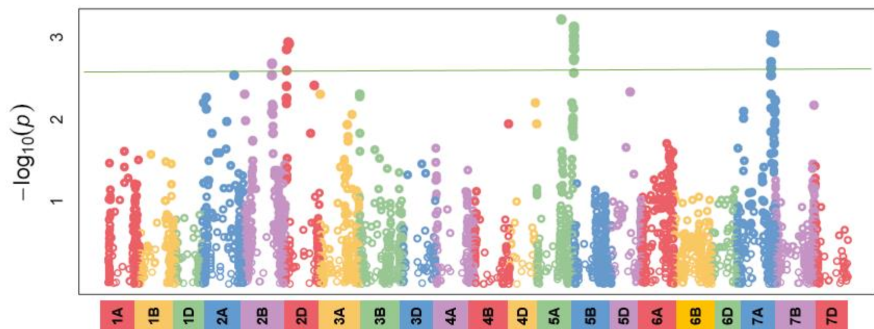
However, genetic regions of significant effect could be identified in a smaller number in case of the infection percentage projected to the entire field trial. Connection could be identified with 6 markers in total, on the chromosomes 2A, 2D and 7A (2). 3 significantly connected markers were identified on chromosome 2A, of which RAC875_c29716_871 indicated the presence of genetically coded resistance even under controlled circumstances. 1 region of significant effect was identified on chromosome 2D and 2 in case of chromosome 7A. Deviating from the greenhouse tests no linkage could be confirmed to the encoded resistance on the chromosomes 2B and 5A under field conditions.




2**:** Manhattan plot, average infection values on 21st day after inoculation, under field conditions, according to the chromosomal localisation of the markers (level of significance: $-\log_{10}(p)$ 2,5; Martonvásár 2009, 2011 average).

In the course of analysing quantitative properties, the characterisation of the lines by average values may hide the presence of low-effect QTLs, therefore, the analysis was also performed with the BLUP values of the lines projected to the entire trial (3), as a result of which 37 connected markers were identified on 5 chromosomes. The marker group of the greatest number was identified on chromosome 5A, 16 markers in the region between the markers wsnp_Ku_c38543_47157828 and wsnp_Ex_c2171_4074003, while the marker AX-94978476 (pos: 466617397) was identified in position deviating from the earlier locations. A group of 11 markers was identified on chromosome 7A

in the position between markers AX-94976788 and Kukri_rep_c98227_390. The presence of a group consisting of 5 markers was detected on chromosome 2B in the position between markers AX-94393508 and AX-94507617. A group of 4 markers was identified on chromosome 2D and we could detect connection on chromosome 2A in case of a single marker (RAC875_c29716_871, pos: 607997395).



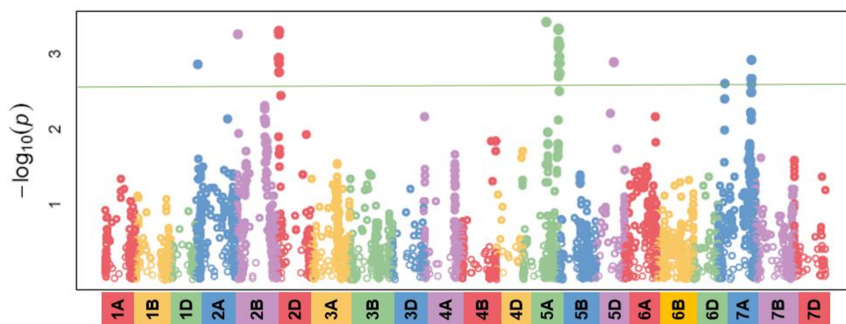

3: Manhattan plot, BLUP values of infection on 21st day after inoculation, across years and study sites, according to the chromosomal localisation of the markers (significance level: $-\log_{10}(p)$ 2,5; Martonvásár 2006, 2007, 2009, 2011)

A special feature of the loci determining the quantitative character of small and medium effect, respectively, was that they could not be detected in each case under different environmental conditions. Therefore, the correlations of the resistance to head fusariosis and genotypes was analysed also in an annual breakdown. With respect to the entire experiment, the presence of genetic region with impact on progress of fusarium in the head was confirmed on chromosome 12. However, the loci identified on chromosomes 1B, 3A and 4D exclusively indicated correlation in a specific year and within that, with a single marker each.

**Identification of the genetic background in correlation with the size of area under the disease progress curve**

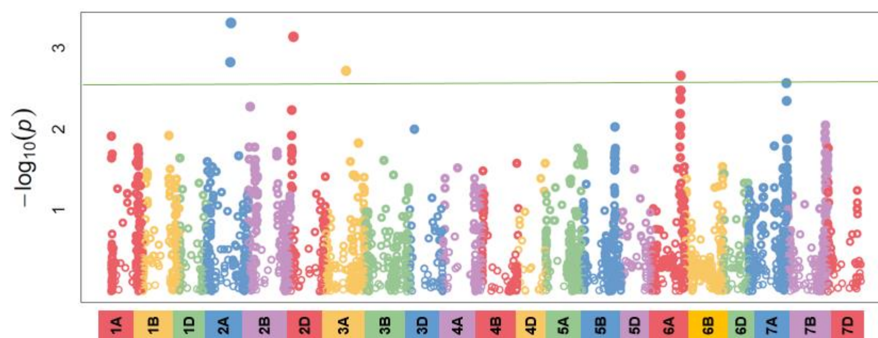
A total of 37 markers were identified in 6 chromosomal regions under greenhouse conditions in significant correlation with average size of the area under the disease progress curve (4). Correlations could be detected on chromosomes 2A, 2B and 5D in the case of one marker each. Compared to the infection values on day 21, a difference is represented by the significant QTL presence in the 349866506 nucleotide position of chromosome 5D, which, however, is also of BKT9086-95 origin, similarly to the QTLs of chromosomes 2A, 2B and 2D. A group of 7 markers was identified

on chromosome 2D in the position between markers AX-94908406 and AX-95124335. 16 significant marker-property links were identified on chromosome 5A, the closest correlation was observed in the case of marker AX-94978476 (p=0.0004), which is located in the position of the 466617397 nucleotide of the pseudo-chromosome. On the chromosome additional 15 markers were classified in a single group in significant correlation with the size of the area under the disease progress curve, in a different region with respect to the chromosomal localisation. In case of chromosome 7A, 11 markers in significant correlation with the property analysed were identified, in view of the localisation of the markers it is assumed that two areas are affected. The region identified on chromosome 7A originates from the genetic background of the susceptible parent also in case of the AUDPC values.




4: Manhattan plot, average AUDPC values under greenhouse conditions, according to the chromosomal localisation of the markers (level of significance: -$\log_{10}$(p) 2,5; Martonvásár 2007, 2009 average).

Analysing the size of the average area under the disease progress curve under field conditions (5), similarly to the ratio of infection, significant correlation existed in case of fewer markers. A total of 6 markers could be detected on five chromosomes with detectable correlation with the property analysed.

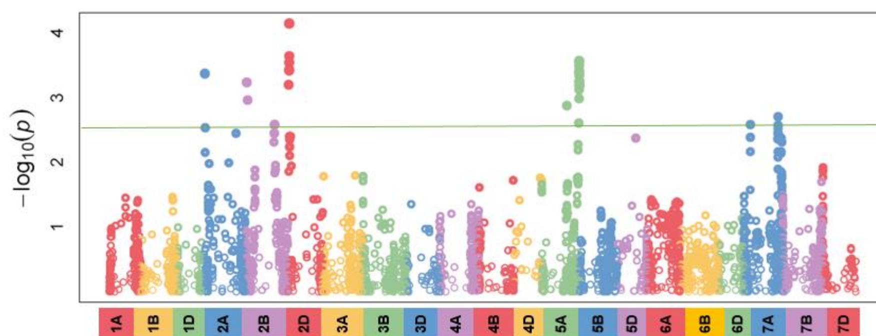


5: Manhattan plot, average AUDPC values under field conditions, according to the chromosomal localisation of the markers (level of significance: $-\log_{10}(p)$ 2,5; Martonvásár 2006, 2009, 2011 average).

However, the chromosomal localisation of the Tdurum_contig915119_224 and AX-94551829 markers identified on the 2A chromosome deviates from the QTL position identified under greenhouse conditions. Significant QTL effect could be observed also under field conditions on the chromosomes 2D and 7A, respectively. At the same time, no QTL could be confirmed under field conditions on the chromosomes 2B and 5A contrary to the greenhouse observations. However, the QTL region identified on chromosomes 3A and 6A could only be detected under field conditions.

Also in the case of the area under the disease progress curve, the chromosomes were analysed for the presence of QTL in case of the BLUP values with respect to the total experimental system (6).

A total of 35 linked markers were identified on the chromosomes 2A, 2B, 2D, 5A and 7A. The presence of locus defining the quantitative property was confirmed in the greatest number on the chromosomes 5A and 7A, respectively. The region coding the resistance originated from the genetic background of the parent of moderate resistance in case of chromosome 5A.




6: Manhattan plot, AUDPC BLUP values, across all study years and sites, according to the chromosomal localisation of the markers (significance level: $-\log_{10}(p)$ 2,5; Martonvásár 2006, 2007, 2009, 2011).

Similarly to the values of infection on day 21, the analysis of the marker-property linkages in an annual breakdown was also conducted in case of the area under the disease progress curve. Significant market-property linkage could be detected at least in a single testing year in the case of a total of 11 chromosomes, however, in one half of the cases, this correlation could not be

consistently detected among the testing years and systems. Marker-property linkage could be detected in specific single testing year on chromosomes 5B, 5D, 6A and 6B.

**Comparison of the genetic regions related to the infection percentage on day 21 and the AUDPC values**

In case of both infection and the AUDPC values, such genetic regions were identified in case of 5 chromosomes, the impact of which proven to be of significant effect in several years and experimental systems (2A, 2B, 2D, 5A, 7A). The QTLs concerned appeared in each year and on each production field, in case of the infection percentage on day 21 and the AUDPC values as well. On the basis of the ANOVA results, the environmental factors had a smaller impact on the AUDPC values of the lines, therefore, that can be considered as a more reliable indicator. All these points are also supported by the results of the marker-property correlation analysis, as the size of the genetic region linked to the ANOVA values was restricted to a narrower region in each case. The only exception was the region found on chromosome 5A, where a slight deviation was detected in the number of markers linked to two resistance metrics for the benefit of AUDPC. From among the identified QTLs, the region detected on chromosome 7A originated from the genetic background of the susceptible parent.

**Linkage of resistance to head blight and other phenotype properties**

The QTLs linked to resistance to head blight are frequently localised in a position equivalent to the chains responsible for other phenotype properties. The height of plant, the time of heading as well as head compactness had a significant impact on the disease symptoms recorded on day 21 after the infection also on the basis of the ANOVA tests. At the same time, the AUDPC values were influenced only by the flowering time. Therefore, the chromosomes were analysed in the specimens of the ’BKT9086-95/Mv Magvas’ population on which the regions defining the properties noted before could be identified.

The locus related to the plant height was identified on the chromosomes 2A, 2D, 4A, 4B, 4D and 6A, of which the regions 2A and 2D indicated a partial overlapping with the QTLs defining the resistance property. In case of the markers indicating the overlapping, the favourable allele was carried by the resistant parent. As a result of the correlation test, it was found that less significant

symptoms were manifest on the taller plants and the disease progressed by a slower rate, respectively. The dwarfing gene *Rht8* is found on chromosome 2D, therefore, in this case, it can be stated that its presence had an unfavourable impact on the head blight resistance. As the pathogen was directly injected into the floret, therefore, presumably the effect of the plant height influencing the microclimate could prevail to a lesser extent, therefore, correlation can be detected between the plant height and the HB-resistivity, at the same time it is assumed that the linkage is based not only the effect of the phenotype. In case of head compactness statistically proven correlation could be identified on chromosomes 2A, 2B and 2D, the positions of which were to a great extent identical with the those of the loci which determine the plant height.

The genetic region linked to the flowering time was also identified on the chromosomes 2A, 2B and 2D, which, however, are located on an area deviating from the regions which define plant height and head compactness. Different chains which influence plant growth according to the reference data are localised in the regions we identified. The *Ppd-A1* genes are located on the short arm of the chromosome 2A, the genes *Ppd-B1* are located on the short arm of chromosome 2B and genes *Ppd-D1* on the short arm of 2D. The moderately resistant parent was heading and flowering 2 days earlier on the average. Based upon the ANOVA results and the correlation analysis, the early development represented an advantage in the population under our analysis in the area of head blight resistance. When the plant encounters the pathogen when it is no longer in its susceptible phenophase, that is the case of passive resistance.

In case of the chromosome 2D, there is a complete overlapping among the indicators of the other phenotype properties and the resistivity, while the overlapping is complete only in case of one of the groups among the 2 marker groups identified on chromosomes 2A and 2B each, and only in case of only one group, among the genetic regions. That is, such genetic regions can be identified on both chromosomes, which are exclusively linked to head blight resistance from among the properties we analysed. The 5A QTL linked to the moderately resistant parental genotype is not correlated with any of the phenotype parameters analysed, therefore, in this case, a region encoding real resistance was identified.

**New scientific results**

1. The head blight resistance of lines originating from 'BKT9086-95/Mv Magvas' was surveyed under greenhouse and field conditions. Based upon the ANOVA test, it was determined that the genotype has a significant effect both on the infection values recorded on day 21 and the size of the area under the disease progress curve.
2. According to our results, the other plant morphological and phenological characteristics have a smaller effect on the evolution of the AUDPC values, therefore, in the course of our mapping works, we find it more suitable for application to compare the resistance levels of the lines. It was found that the sequence of the genotypes has not changed in the individual repetitions with statistical proof, therefore, it was thereby certified that the lines may carry genetically encoded resistivity.
3. The genetic variability of the population was analysed by 33 SSR markers and 32 AFLP combinations. The correlation between the genetic background and the head blight resistivity was confirmed by variance analysis in the case of 3 markers, of which the co-dominant pattern of the gtac2 and gtac3 markers may indicate a continuing marker development possibility.
4. By means of the marker fusarium resistance association tests the presence of the genetic region linked to the infection values on day 21 was confirmed on 12 chromosomes. Consistently emerging QTL was identified on the chromosomes 2A, 2B, 2D, 5A and 7A in several years and in both testing systems (greenhouse and field).
5. In connection with the AUDPC values, the locus related to resistance was identified on 11 chromosomes. The QTLs with the steady manifestation were identical with the ones identified in case of the infection values registered on day 21, however, with the exception of chromosome 5A, they were restricted to a narrower region.
6. In the course of investigating the other properties influencing the head blight resistance (plant height, flowering time, head compactness) and the molecular background of the resistance QTLs, it was found that total overlapping is experienced in case of the region identified on chromosome 2D and partial overlapping on chromosomes 2A and 2B, respectively.

7. In case of the ’BKT9086-95’ line, the presence of a DNA section encoding a protein linked to the plant protection mechanism was confirmed on chromosome 5A. On the basis of the analysis of the allele type, it was found that a rare version was identified.

# Conclusions and proposals

## Phenotype test of the resistance to head blight

The success and precision of QTL determination are fundamentally defined by the reliability of pheno-typing. As the resistance to head blight can be divided into several types, the component to be analysed will also define the selection of the artificial infection method. A generally applied inoculation method is the spraying of the entire head with conidium suspension and spike injection. In case of spraying the heads, the effect of resistance to injection in the head or propagation in the head cannot be distinguished, therefore, the initial tests mainly focused on the resistance type II (to propagation in the head). The amount of the material to be tested also influences the methods to be applied, in the initial years, there is only a small amount of seeds available from the SSD lines, therefore, the spraying of the heads would not even be accomplished on a small parcel scale. Moreover, controlled greenhouse conditions could be provided only in case of a test material of restricted volume, therefore, we focused on resistance type II in case of analysing the lines.

The field resistance of parent 'BKT9086-95' (types I + II) was not significantly different from the results of the 'Sumai3' applied as the resistance control variety, based upon the results of the 3 years of tests. This observation could not be verified on the basis of the resistance tests type II, the resistant parent was assessed to belong to the moderately resistant category on the basis of the greenhouse and field tests.

Infection values higher on the average were recorded under greenhouse conditions compared to the field tests, with respect to both parents of moderate resistance and the lines. In case of the susceptible parent ('Mv Magvas'), no deviation was detected among the greenhouse and field tests, at the same time the sequence of the lines did not change with statistical proof in view of the testing years and locations, therefore, the genetically encoded resistivity of the lines can be considered confirmed. The deviation can be explained that the properties linked to HB resistance under field conditions (flowering time, plant height, compactness of head as well as the length of spike and anther extrusion not tested by us) may have an enhanced impact on the progress of the disease.

We have identified such lines both under greenhouse and field conditions, which were infected to a lesser extent than the resistant

parent, these lines were designated as crossing partners for improvement. The field resistance of lines should also be an important subject of analysis, as the joint impact of resistance type I and II can also be in the background of difference. It is also proposed to analyse the other resistance types, as the actual composition of the genetic fusarium resistance of the population available can only be fully understood in this way.

**Identification of the SSR and AFLP markers linked to the progress of fusarium in the head**

No experiments have been conducted on the study of genetic background of the fusarium head blight resistance in case of a population of old Hungarian variety before this research. Therefore, in case of the 'BKT9086-95/Mv Magvas' lines, the SSR markers correlating with the QTLs linked to head blight resistance described in the literature were selected. When planning the experiment, we envisaged to accomplish the marker coverage of the genome to the fullest possible extent by AFLP tests in addition to the microsatellite markers of known chromosomal localisation. However, from the data available, only an extremely fragmented map could be created, which would have been suitable for determining the position of potential QTLs with restrictions only, therefore, association tests were conducted on the results of the AFLP and SSR tests. Linkage could be detected exclusively to the HB resistivity in case of 3 AFLP markers. Based upon the co-dominant pattern of the gtac2 and gtac3 markers, the PCR product can be suitable for the development of diagnostic markers, however, this would require by all means the testing with a broader range of varieties.

**Identification of the QTLs linked to the progress of fusarium in the head**

The QTLs of 'BKT9086-95' origin located on chromosomes 2A, 2B, 2D and 5A manifested linkage with the progress of fusarium in the head and the size of the area under the disease progress curve. In the comparison with the QTLs described in the literature, the results recorded in the wheat gene catalogue were taken into consideration.

QTL of slight effect was described first on the long arm of the chromosome 2A as a result of the greenhouse test of recombinant in-breed lines 'Sumai-3/Stoa' applying RFLP markers. The region responsible for the resistance originated from the pollinating variety of medium susceptibility. QTL of low effect was also identified on

the long arm of chromosome 2A by the analysis of RILs of 'Arina/Forno' (resistant/susceptible) origin ($R^2$=6.8%), which had a significant effect on the field resistance only projected to the entire trial and not among the testing years and locations. We have observed similar results in our tests, as we could not find such a marker related to either the level of infection on day 21 or the AUDPC values, which could have led to a consistent identification among the testing years and conditions. Based upon the marker AX-94551829, the allele type of 'Arina' can be identified among the known resistance sources, which has the more frequent version deviating from the 'BKT9086-95' line in the tested position (96.7 in contrast with the 3.3% frequency).

Based upon the McIntosh gene catalogue, 2 QTLs can be found on chromosome 2B. The Qfhs.crc-2BL resistance locus originates from the 'Strongfield' durum variety ($R^2$=26%) and is responsible for the resistance type II. By testing the offsprings of 'Renan/Recital' crossing under field conditions, a region linked to head blight resistance was also identified on the short arm of chromosome 2B, which was responsible for 12% of the phenotype variance. According to our own observations, we have also identified regions linked to the progress of fusarium in the head also on the long and short arms of chromosome 2B. The comparison of the sequence data could not be completed in case of markers identified by us as linked, as we have searchable sequence exclusively about the long arm, however, a major part of these are not available in the database.

By analysing the RILs originating from the 'Ning894037/Alondra' cross on chromosome 2D, the presence of low-effect QTL accounting for 12.1% of the phenotype variance was confirmed, which originated from the susceptible parent (*QFhs.pur-2D*). However, the method of evaluation deviated from the one we applied, as only the spikes perished below the infection point were considered as a real infection symptom, while in the survey, we conducted the head tip decay was not treated separately. Based upon the other plant properties brought into correlation with the head blight resistance it can be concluded that the QTL identified by us on chromosome 2D can be connected to QTL passive resistance.

One of the head blight resistance regions analysed most frequently is located on chromosome 5A. QTLs were also detected in this chromosomal region on genotypes of Asian, South and north American as well as European origin. The gene *Fhb5* described in the 'Wangshuibai' variety is located close to the centromere, however, the resistivity is inherited linked to several properties of

agronomic importance, regarding which the resistant parent has an unfavourable allele. However, the QTL we identified deviates from the *Fhb5* gene with respect to both position and resistance level. The resistance locus of *Qfhs.ifa-5A* ’CM-82036’ origin on chromosome 5A accounted for 20% of the phenotype variance. The effect of QTL was more pronounced in the head spraying trials, on that basis it was assumed that the resistance provides protection rather against the penetration of fusarium in the head. QTLs linked to fusarium resistance was also detected in the ’Frontana’ spring wheat variety, which indicated an overlapping with the QTL which determined the plant height. At the same time, in case of ’BKT9086-95’, there was no correlation between the resistance level and the other plant properties tested. Based upon the sequence data, the AX-94387470 SNP marker is linked to a region encoding a protein responsible for protection. The function of the protein was defined as a fungicide protein rich in *Arabidopsis* cysteine. Analysing the allele frequency, it was found that parent of old Hungarian variety origin with moderate resistance has an extremely rare allele type in the specific position, which occurs only in 15.6% of the reference genotypes. On that basis it is assumed that we have identified a new resistance locus not yet determined in literature.

Connected to the susceptible parental genotype, we identified a genetic region linked to head blight resistance on chromosome 7A. According to data in the reference literature, QTL was identified mainly from resistance sources of Chinese origin and in the ’Ritmo’ variety (Buerstmayr *et al*. 2009). However, the presence of *Qfhs.fcu-7AL* QTL was confirmed reliably only in case of the ’Langdon’ (durum) – *T. turgidum* ssp. *dicoccoides* substitution lines. Knowing the origin of ’Mv Magvas’ and QTL localisation, it is assumed that once again we identified a resistance locus in the case of the susceptible parent, which has not yet been described.

Regarding their intrinsic features and other agronomic properties, the old Hungarian wheat varieties are more heterogeneous than the modern varieties currently in public production, therefore, we find it necessary to carry out the analysis of genetic background of the head blight resistance also involving a wider range of varieties, as a result of which the QTLs identified in this research can be validated, moreover, the identification of other low-frequency allele types is also expected. Moreover, we find it necessary to test the ’BKT9086-95/Mv Magvas’ offspring population also with respect to the other types of head blight resistance.

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