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Back-cross reciprocal monosomic analysis of Fusarium head blight resistance in wheat (*Triticum aestivum* L.)

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Abstract Fusarium head blight (FHB or scab) caused by *Fusarium spp.* is a widespread disease of cereals causing yield and quality losses and contaminating cereal products with mycotoxins. The breeding of resistant varieties is the method of choice for controlling the disease. Unfortunately, the genetic basis of scab resistance is still poorly understood. We present the results of a back-cross reciprocal monosomic analysis of FHB resistance using the highly resistant Hungarian winter wheat line 'U-136.1' and the highly susceptible cultivar 'Hobbit-sib'. Resistance testing was performed in a field trial artificially inoculated with a *Fusarium culmorum* conidial suspension. Five hemizygous families containing 'U-136.1' chromosomes 6B, 5A, 6D, 1B, and 4B had a visually reduced spread of infection compared to lines having the 'Hobbit-sib' chromosome. Chromosome 2B from 'U-136.1' had an increased spread of infection. The critical chromosomes controlling seed weight were 6D, 3B, 5A, and 6B while those controlling deoxynivalenol (DON) content were homoeologous groups 2 and 6, although the latter effects were not significant due to a high coefficient of variation. Results from this and other studies show that chromosomes 6D, 6B, 5A, 4D, and 7A have frequently been associated with scab resistance in a number of wheat cultivars. Research groups now attempting to map scab resistance in wheat using markers should pay special attention to the above-mentioned chromosomes.

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Introduction

Fusarium head blight (FHB or scab) is a widespread disease of wheat and other small grain cereals. For recent reviews on FHB see Mesterhazy (1995), Parry et al. (1995), and Miedaner (1997). Fungi of the group *Fusarium* spp. infect spikes at anthesis causing heavy losses, and grain harvested from infected wheat may show reduced grain mass, test weight, baking quality and seed quality (Diehl 1984; Bechtel et al. 1985; Wiersma et al. 1996). Moreover, besides yield losses, the most serious concern associated with FHB infection is the contamination of the harvested crop with a range of mycotoxins, such as deoxynivalenol, zearalenone and moniliformin, produced by different *Fusarium* spp. The contamination of agricultural products with Fusarium mycotoxins is considered a worldwide problem (Tanaka et al. 1988; Gareis et al. 1989).

Agronomic and chemical measures are only partly effective in controlling FHB. The cultivation of genetically resistant varieties together with appropriate crop management practices is the best method for controlling the disease (Bai and Shaner 1994; Parry et al. 1995). Genetic variation for FHB resistance was first reported by Arthur (1891) and has been well documented since then (e.g. Mesterhazy 1983, 1995; Snijders 1990; Saur 1991; Wilcoxson et al. 1992; Lemmens et al. 1993; Buerstmayr et al. 1996). However, the genetic basis of FHB resistance in wheat is rather poorly understood. Depending on the materials and methods used the inheritance of resistance was described as being monogenic (Chen 1989), oligogenic (Bai et al. 1989) and polygenic (Liu and Wang 1991). It was frequently concluded that in FHB-resistant genotypes from China and South-America less than five genes are involved in

resistance (Yu 1982; Bai et al. 1989; Singh et al. 1995; Van Ginkel et al. 1996). The present study was initiated to determine the chromosomal location of scab resistance genes in a resistant winter wheat breeding line having the well known resistant cultivars 'Sumei #3' (from China) and 'Nobeokabozu' (from Japan) in its pedigree.

Material and methods

Plant material

The monosomic series of the winter wheat cultivar 'Hobbit-sib' as well as euploid 'Hobbit-sib' were kindly provided by the John Innes Center, Norwich, UK. The monosomic series in this cultivar was produced by back-crossing to the monosomic series of the cultivar 'Capelle-Desprez'. At least eight back-crosses were carried out (Worland et al. 1993). 'Hobbit-sib' which was selected at the Plant Breeding Institute in Cambridge is a highly productive, semi-dwarf winter wheat cultivar. It is closely related to the cultivar 'Hobbit', which was commercially grown in the UK during the 1970s. A series of genotypes in which cytogenetic tester stocks are available have been evaluated for scab resistance over several years (Buerstmayr et al. 1996). 'Hobbit-sib' has been identified as being very susceptible to FHB. This confirms results by Saur (1991) who also found that 'Hobbit' is highly susceptible. Based on these findings and the availability of a monosomic series, 'Hobbit-sib' was chosen as the recipient genotype for the back-cross reciprocal monosomic analysis in the present study. The resistant wheat genotype was kindly provided by the Cereal Research Institute, Szeged, Hungary. The line 'U-136.1' was among the most-promising winter wheat genotypes in their FHB resistance breeding program at the time when this study was started. It has the pedigree Sagvari/Nobeokabozu// Mini-Mano/Sumei #3; it is a tall plant type and is awned. 'U-136.1' probably carries resistance genes from both resistant progenitors: 'Sumei #3' and 'Nobeokabozu' (A. Mesterhazy, personal communication).

Development of the test stocks

The back-cross reciprocal monosomic method as described by Law et al. (1986) was applied in this study (Fig. 1). Two reciprocally derived series of monosomic families were developed which, on average, have genetically identical backgrounds but their hemizygous chromosomes are different. This permits the direct comparison of identical chromosomes of the resistant and susceptible parents. The basis of this technique is the different transmission of the monosomic chromosome through pollen and egg cells. On average 96% of the successful pollen cells from a monosomic plant carry all 21 chromosomes whereas about 75% of the egg cells carry only 20 chromosomes (Law et al. 1986). By pollinating a monosomic plant with pollen from another monosomic plant the majority of the monosomic progeny will possess the hemizygous chromosome from the pollen parent. The selection of monosomic plants was done by counting the somatic chromosomes in metaphase root tip cells after standard Feulgen staining. Approximately 1500 plants were characterized by chromosome counting and 300 manual emasculations and pollinations were necessary to yield the desired material.

Head scab resistance testing

Field trial

The site of the field trial was the Institute for Agrobiotechnology (IFA) in Tulln, Austria. The elevation of the test site is 180 m above sea level. The soil type is a meadow-chernozem; the preceding crop in the field was corn. The average annual precipitation in this region is 620 mm and the average annual temperature is 9.2°C.

The experimental layout was a split-plot design with six replications. The main-plot had the designation of the hemizygous chromosome (1A, 1B, … , 7D) and the sub-plot carried the respective chromosome either from the susceptible recipient 'Hobbit-sib' or from the resistant line 'U-136.1'. The two euploid parental genotypes were included in the experiments as checks. A field plot consisted of two rows, 1-m long and 17-cm apart. The sowing density was 50 seeds per plot. The replications were sown at time intervals: replication one on Oct. 16, two on Oct. 23, three on Oct. 27, four on Oct. 31 five and six on Nov. 14, 1995. The seed was treated with 'Sibutol' (a.i.'Bitertanol' plus 'Fuberidazol') seed dressing at a rate of 2 ml/kg of seed to control seed-borne diseases. The trial survived the winter in good condition. In early spring fertilizer was applied at a rate of 90 kg/ha N, 55 kg/ha P_2O_5, 55 kg/ha K_2O and 20 kg/ha MgO. At the late tillering stage weeds were controlled by spraying with 15 g/ha of 'Express' (a.i.'Tribenurometyl') and 0.5 l/ha of 'Starane 250' (a.i.'Fluroxypyr') in 300 l/ha of water.

Inoculum and inoculation procedure

The inoculum was prepared as described by Snijders and Van Eeuwijk (1991). A mixture of oat and wheat kernels (3 : 1) was autoclaved in 250-ml glass bottles. Then each bottle was inoculated with the *Fusarium culmorum* strain 'IPO 39–01' (Snijders and Van Eeuwijk 1991). The bottles were incubated in a growth chamber for 2 weeks at 25°C followed by 3 weeks at 5°C in the dark. This led to a massive production of macroconidia. Macroconidia were washed off the colonized grains with de-ionized water, the spore concentration determined by counting in a Bürker-Türk counting chamber, and the desired spore concentration of 10 000 spores per ml adjusted with de-ionized water. The aggressiveness of the inoculum was monitored with a Petri-dish infection test at the beginning and at the end of the inoculation period as described by Lemmens et al. (1993). This test proved that the aggressiveness of the inoculum remained constant during the inoculation period (data not shown).

At anthesis, each plot was inoculated with the *F. culmorum* macroconidial suspension. Fifty microliters of inoculum were sprayed on each plot using a motor-driven back-packer sprayer (Agritop KS 11 E). Inoculations were performed at the beginning of anthesis and repeated on the same plots 4-days later (full flowering) to inoculate the majority of the heads in each plot at anthesis. Neighboring sub-plots were inoculated on the same days. Inoculations were begun on June 2 and completed on June 16. Inoculations were carried out every 2nd day after 4 p.m. After inoculations were completed in the late evenings the plants were kept wet by mist-irrigating until 12 noon the next day. When plant wetness dropped to a preset level, the irrigation system was activated, by applying 10-s pulses of moisture.

Disease assessment

AUDPC as a measure for visual FHB symptoms on the heads

In each plot the percentage of infected spikelets was scored according to a 0 (no disease symptoms) to 4 (100% infected spikelets) scale, by visually averaging the entire plot. Between 0 and 1 scoring was done in 0.1-scale increments and between 1 and 4 in 0.5-scale steps. Disease symptoms were scored on days 10, 14, 18, 22, 26, and 30 after inoculation. An area under the disease progress curve (AUDPC) was calculated for each entry and was the basis for further statistical analysis:

$$\mathrm{AUDPC} = \sum_{i=1}^{n} \{[(y_i + y_{i-1})/2](x_i - x_{i-1})\} \qquad \text{(Eq. 1)}$$

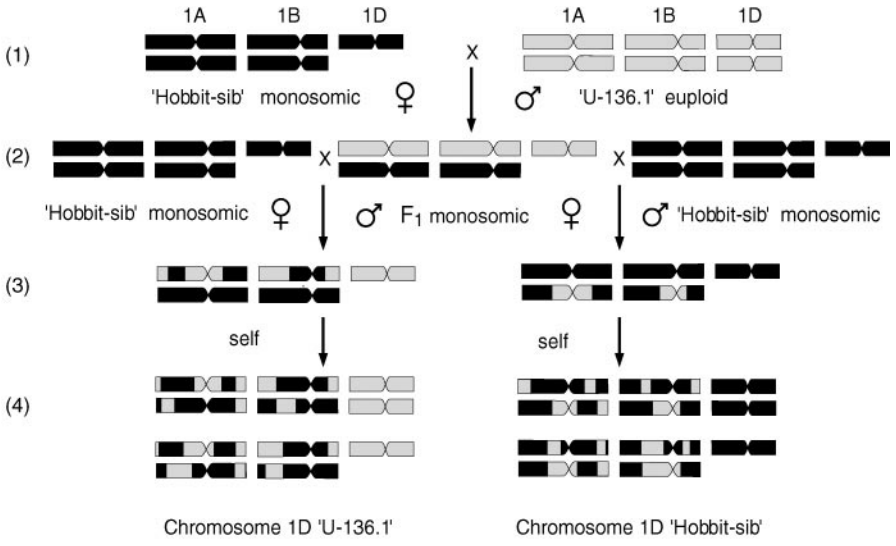

Fig. 1 Scheme of the back-cross reciprocal monosomic method, as applied in this study. (1) Monosomic plants were selected for all chromosomes in 'Hobbit-sib'. The monosomic series of Fusarium head blight susceptible 'Hobbit-sib' was crossed with the resistant genotype 'U-136.1'. (2) The monosomic F_1 was back-crossed reciprocally with the 'Hobbit-sib' monosomics. (3) Monosomic back-cross plants were selected and multiplied. (4) The seeds harvested from several (6 to 15) monosomic back-cross plants were bulked in equal amounts and used for sowing the field trials. The scheme is simplified to show only one set of homoeologous chromosomes and the development of back-cross reciprocal monosomics for chromosome 1D (modified from Law et al. 1986)

where y_i is the visual score of FHB symptoms at the ith observation date and x_i is the day of the ith observation after inoculation, n being the total number of observations (modified from Shaner and Finny 1977).

50-ml-weight as a measure for grain damage

After ripening 60 randomly chosen heads were harvested manually from each plot. The heads were threshed using a 'Wintersteiger SEED BOY' ear thresher at low speed, in order not to destroy infected grains. The threshed grains were then manually cleaned from the remaining chaff. As a measure for grain damage due to FHB, the weight of 50 ml of seed was recorded. The grains were poured into a plastic tube of 2.7-cm diameter and 8.8-cm depth and the seed weight recorded. For each sample the measurement was repeated four times and the means of such repeated measurements were used for further statistical analysis.

DON-content as a measure for mycotoxin contamination

For measuring the content of the mycotoxin deoxynivalenol (DON) approximately 10 g of threshed and cleaned seeds were ground on a 'Culatti' sample mill (1 mm mesh). From this flour 1 g was used for toxin extraction. Extraction and DON determination by quantitative ELISA were carried out at Agriculture and Agri-Food Canada, Ottawa, using the methods described by Sinha et al. (1995).

Statistical analysis

Analyses of variance were calculated with SAS/STAT version 6.09 (SAS Institute Inc. 1990) running on a HP-9000 computer. For comparing the means of corresponding entries possessing their hemizygous chromosome either from 'Hobbit-sib' or from 'U-136.1' the Bonferroni-Holm procedure (Holm 1979) was applied. Other than a plain t-test comparison, the more conservative Bonferroni-Holm procedure keeps the multiple error of the first order smaller than, or equal to, the global error of the first order. Phenotypic and genotypic correlation coefficients between the three measured traits were calculated using the PLABSTAT program (Utz 1991).

Results

Figure 2 shows the development of the disease on the euploid parental lines. On both genotypes initial infection was observed and symptoms were similar at the first and second observation dates. However, for 'U-136.1' disease progress was slow and followed an almost linear mode until the end of the observation period, whereas on 'Hobbit-sib' the disease seemed to progress in an exponential way. On day 30 after inoculation 'Hobbit-sib' showed an average of 70% infected spikelets whereas 'U-136.1' had only 13% infected spikelets. Figure 3 shows the disease progress curves for the populations with chromosome 6B from both 'Hobbit-sib' and 'U-136.1'. During the first 18 days after inoculation the two groups were similar. Towards the end of the observation period the differences in disease progress became obvious, with 6B from 'U-136.1' showing lower disease symptoms than the populations with chromosome 6B from 'Hobbit-sib'.

An analysis of variance revealed highly significant effects caused by the chromosome origin for all three traits: AUDPC, 50 ml-weight and DON-content (data not shown). The parental genotypes showed the largest differences. Although resistant 'U-136.1' had some disease symptoms and DON content, these were greatly reduced compared to susceptible 'Hobbit-sib' (Tables 1–3). 'U-136.1' had only about one-third of the AUDPC and only one-tenth of the DON content compared to 'Hobbit-sib' and its 50 ml-weight was 44% higher.

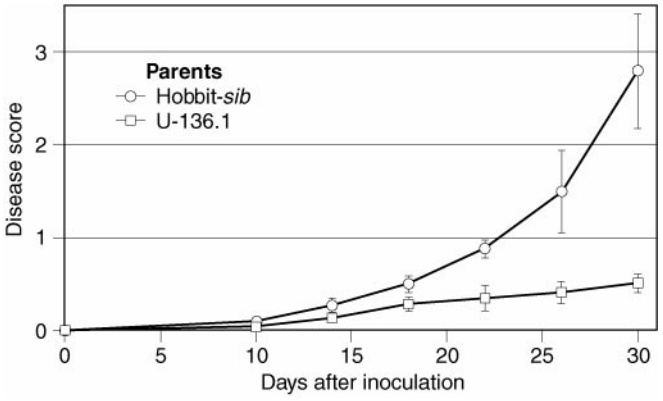

Fig. 2 Disease progress curves of the euploid parental genotypes. Means and standard deviations for the disease scores are plotted against the observation dates

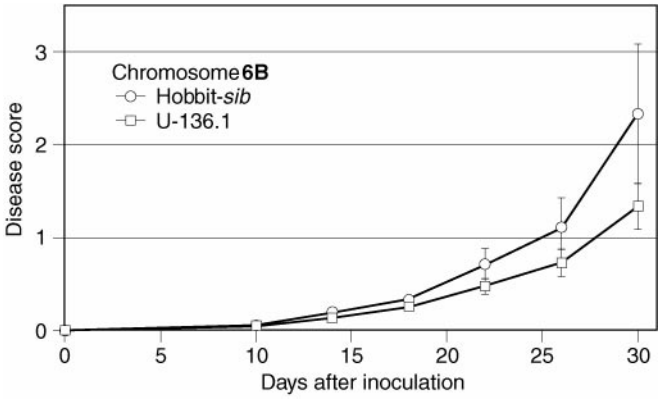

Fig. 3 Disease progress curves of the families for chromosome 6B. Means and standard deviations for the disease scores are plotted against the observation dates

The effects of specific hemizygous chromosomes originating from the two parental lines were much less pronounced. For AUDPC (Table 1) several chromosomes of 'U-136.1' exhibited a significant reduction, with 6B, 5A, 6D, 1B and 4B showing the largest effects. Plant populations with chromosome 2B originating from 'U-136.1', however, had significantly more head blight compared to those with 2B from 'Hobbit-sib'. An additional five chromosomes of 'U-136.1' had non-significantly greater AUDPC means than their 'Hobbit-sib' counterparts but to a lesser degree than chromosome 2B.

In the harvested seed of 'U-136.1' only a few scabby grains were present whereas 'Hobbit-sib' had almost 100% diseased grains leading to a drastic reduction in 50 ml-weight (Table 2). The most pronounced effect of a specific chromosome on 50 ml-weight was for 6D followed by 3B, 5A and 6B. In all of these cases the hemizygous chromosome from 'U-136.1' led to a higher 50 ml-weight (indicating less grain damage) compared to the corresponding 'Hobbit-sib' chromosome.

DON content showed rather high standard deviations of the means compared to AUDPC and 50 ml-weight (Table 3). The difference between the parents was again clear, with 'U-136.1' containing less than 10% the amount of DON than 'Hobbit-sib'. Several chromosomes from 'U-136.1' led to a reduced DON content by 30, or more, percent and especially in the case of the group-6 and group-2 chromosomes. However, none of these differences was statistically significant when applying the rather conservative Bonferroni-Holm test.

When considering several traits simultaneously, chromosomes 6D, 6B and 5A influenced 50 ml-weight and AUDPC and, of these, 6D and 6B also showed a tendency to a reduced DON content. Table 4 displays the phenotypic and genotypic correlation coefficients between the three measured traits. All three traits were highly correlated. The highest correlation coefficients were found between 50 ml-weight and DON-content.

Discussion

Inoculation procedure and FHB infection

Apart from plant resistance, a number of factors influence the FHB development on wheat heads, e.g. type and amount of inoculum, developmental stage of the plant, temperature and humidity (Parry et al. 1995). These sources of variation have to be controlled as carefully as possible in breeding experiments. A macroconidial inoculum originating from a single-spore isolate of *F. culmorum* was used for artificial inoculations. Because there is no evidence that resistance to FHB is race-specific or species-specific, inoculation with only one pathogenic Fusarium isolate of high aggressiveness is appropriate to test genotypes for resistance (Van Eeuwijk et al. 1995). The most sensitive plant stage for Fusarium ear infection is anthesis because infection of wheat florets tends to begin in retained wheat anthers before spreading to other parts of the spikelet (Pough et al. 1933). Inoculations for resistance screening were therefore made at this stage. Humidity and plant wetness were controlled by mist-irrigating. The influence of temperature on disease development cannot be controlled artificially in field experiments. By replicating trials over environments the temperature effect can be covered. Although the FHB data in this study were obtained from 1 year's experiments, it is felt that results are reliable because many variables were controlled:

(1) Inoculations were made at the beginning of anthesis and repeated 4 days later (full flowering). Repeated inoculations buffer the environmental (temperature) effects on infection as well as the variation in flowering time from ear to ear within a plot.

Table 1 Mean values (mean) and standard deviations of means (+/− SD) for the AUDPC are displayed in columns 2 to 5. Column 6 (%) shows the relative percentage of the AUDPC of populations with their hemizygous chromosome from 'U-136.1' compared to the corresponding 'Hobbit-sib' populations. Column 7 (α_t) contains probability values α for means comparison based on a plain *t*-test and column 8 (α_{BH}) based on a Bonferroni-Holm test. The bottom row displays the overall mean value for AUDPC and the coefficient of variation of the subplot error

Chromosome designation	Hemizygous chromosome from				%	Significance value	
	'Hobbit-sib'		'U-136.1'			α_t	α_{BH}
	Mean	+/−SD	Mean	+/−SD			
Euploid Parents	18.9	3.5	6.0	1.7	32	< 0.001	< 0.001
1A	14.4	3.5	15.9	3.1	111	0.173	> 0.999
1B	16.7	2.2	12.7	2.6	76	0.001	0.010
1D	14.6	4.5	15.1	2.3	104	0.630	> 0.999
2A	16.8	1.7	17.1	2.0	102	0.763	> 0.999
2B	13.6	1.6	17.2	3.8	127	0.001	0.025
2D	15.5	3.5	15.1	2.5	98	0.763	> 0.999
3A	14.0	2.0	13.7	2.2	98	0.763	> 0.999
3B	13.2	2.4	14.4	3.2	109	0.286	> 0.999
3D	15.7	3.3	13.3	3.1	85	0.034	0.447
4A	18.4	4.8	15.7	3.6	85	0.017	0.235
4B	15.5	1.9	12.3	3.0	80	0.005	0.080
4D	12.8	2.0	12.4	2.6	97	0.718	> 0.999
5A	11.1	2.1	6.3	1.6	57	< 0.001	0.001
5BL7BL	13.2	3.6	12.0	1.9	91	0.286	> 0.999
5BS7BS	16.4	3.8	16.2	3.6	99	0.845	0.845
5D	13.5	2.7	11.2	2.0	83	0.040	0.476
6A	14.1	2.4	13.2	2.3	94	0.417	> 0.999
6B	14.3	3.7	9.3	1.3	65	< 0.001	< 0.001
6D	15.6	4.0	11.5	3.2	74	< 0.001	0.007
7A	13.0	3.5	10.3	3.6	79	0.015	0.232
7D	12.0	3.5	12.2	2.1	102	0.833	> 0.999
Overall mean:	13.8						
Coefficient of variation of subplot error: 13.9%							

(2) As the genotypes under investigation differed genetically in their flowering date, not all genotypes could be inoculated on the same day, thus leading to a possible bias in resistance evaluation in a single trial. To overcome this problem, the replications were sown at staggered time intervals. This resulted in a few days intervals in flowering between the replications. Therefore the same genotype was inoculated on several dates across the trial. This also means different microenvironmental conditions at inoculation (mainly temperature) for the same genotype. The overall mean FHB value for a genotype obtained from such a design will, therefore, be less biased since each was exposed to a variety of weather conditions during the inoculation period.
(3) Furthermore, the corresponding genotypes, with their respective chromosome either from the susceptible or from the resistant parent, did not differ much in anthesis date and could therefore be inoculated on the same days. The estimation of the effect of a specific chromosome by comparing the two corresponding plant populations is thus not biased by environmental effects.

The infection procedure resulted in FHB symptoms on all strains, which allowed a quantitative differentiation between the genotypes. None of the genotypes had 100% infection at the end of the observation period, not even the highly susceptible 'Hobbit-sib'. On the other hand no genotype exhibited an immune reaction, even the highly resistant 'U-136.1' showed at least some FHB. This is in agreement with other reports on FHB resistance in wheat (e.g. Mesterhazy 1983, 1995; Snijders 1990; Saur 1991; Wilcoxson et al. 1992; Lemmens et al. 1993; Buerstmayr et al. 1996). None of the aneuploid lines had a lower AUDPC value than the resistant parent.

Scab symptoms on the heads, grain damage and mycotoxin content

Assessment of the percentage of infected spikelets in a uniformly inoculated plot permits the evaluation of Type-I (resistance to initial infection) and Type-II (resistance to spread of disease after initial infection) resistance simultaneously (Schroeder and Christensen 1963; Wilcoxson et al. 1992). Initial infection took place on all tested genotypes. Generally up to day 14 after inoculation most genotypes showed almost equal infection levels (e.g. Figs. 2 and 3). This is an indication that the genotypes did not differ in Type-I resistance. However, towards the end of the observation period, the disease development was slow on resistant genotypes and faster on susceptible genotypes. This means that the observed differences in FHB resistance can be attributed

Table 2 50 ml-weight. Mean values (mean) and standard deviations of means (+/−SD) for 50 ml-weight in g are displayed in columns 2 to 5. Column 6 (%) shows the relative percentage of the 50 ml-weight of populations with their hemizygous chromosome from 'U-136.1' compared to the corresponding 'Hobbit-sib' populations. Column 7 (α_t) contains probability values α for a comparison of means based on a plain *t*-test and column 8 (α_{BH}) based on a Bonferroni-Holm test. The bottom row displays the overall mean value for 50 ml-weight and the coefficient of variation of the subplot error

Chromosome designation	Hemizygous chromosome from				%	Significance value	
	'Hobbit-sib'		'U-136.1'			α_t	α_{BH}
	Mean	+/− SD	Mean	+/− SD			
Euploid parents	25.9	1.7	37.2	0.7	144	< 0.001	< 0.001
1A	27.1	2.1	27.6	1.2	102	0.539	> 0.999
1B	30.7	1.2	30.7	0.6	100	0.948	0.948
1D	29.0	2.2	28.1	1.3	97	0.170	> 0.999
2A	26.9	1.2	27.5	1.1	103	0.328	> 0.999
2B	25.5	1.3	26.9	1.6	105	0.050	0.845
2D	27.8	1.5	28.7	1.8	103	0.221	> 0.999
3A	28.7	0.9	29.2	0.8	102	0.464	> 0.999
3B	26.7	1.4	28.5	1.0	107	0.008	0.159
3D	28.9	2.0	28.8	1.2	100	0.904	> 0.999
4A	28.2	0.9	28.1	1.9	100	0.946	> 0.999
4B	29.5	0.8	30.0	1.2	102	0.451	> 0.999
4D	30.3	1.2	31.1	1.2	102	0.284	> 0.999
5A	29.6	0.5	31.4	1.3	106	0.010	0.181
5BS7BS	30.0	1.6	29.2	0.5	97	0.222	> 0.999
5BL7BL	29.6	1.4	30.2	1.2	102	0.402	> 0.999
5D	29.8	2.0	30.9	0.9	104	0.112	> 0.999
6A	28.5	1.6	28.3	1.1	99	0.715	> 0.999
6B	29.2	1.0	31.0	0.9	106	0.010	0.177
6D	27.9	1.5	30.9	1.0	111	< 0.001	0.001
7A	29.6	0.7	30.3	1.8	102	0.312	> 0.999
7D	29.4	2.7	30.1	1.7	102	0.339	> 0.999
Overall mean:	29.2						
Coefficient of variation for subplot error: 4.1%							

Table 3 DON-content. Mean values (mean) and standard deviations of means (+ / − SD) for DON-content in ppm are displayed in columns 2 to 5. Column 6 (%) shows the relative percentage of the DON-content of populations with their hemizygous chromosome from 'U-136.1' compared to the corresponding 'Hobbit-sib' populations. Column 7 (α_t) contains probability values α for a comparison of means based on a plain *t*-test and column 8 (α_{BH}) based on a Bonferroni-Holm test. The bottom row shows the overall mean value for DON-content and the coefficient of variation of the subplot error

Chromosome designation	Hemizygous chromosome from				%	Significance value	
	'Hobbit-sib'		'U-136.1'			α_t	α_{BH}
	Mean	+/− SD	Mean	+/− SD			
Euploid parents	49.7	14.9	4.4	1.4	9	< 0.000	< 0.001
1A	38.9	24.5	35.0	16.4	90	0.531	> 0.999
1B	15.8	7.5	19.3	5.5	122	0.571	> 0.999
1D	26.7	12.8	30.5	7.5	114	0.539	> 0.999
2A	39.4	23.6	23.9	6.4	61	0.014	0.304
2B	53.0	10.7	38.2	14.7	72	0.019	0.375
2D	37.0	14.6	25.0	11.4	67	0.055	> 0.999
3A	33.3	14.0	28.6	9.7	86	0.449	> 0.999
3B	26.4	12.4	23.6	9.0	89	0.646	> 0.999
3D	32.2	13.6	34.5	12.7	107	0.709	> 0.999
4A	25.8	14.4	24.9	11.5	97	0.885	0.885
4B	21.6	9.5	26.4	6.1	122	0.439	> 0.999
4D	19.8	11.0	14.0	7.5	71	0.351	> 0.999
5A	17.9	6.6	15.2	2.7	85	0.669	> 0.999
5BL7BL	23.3	18.1	19.3	11.5	83	0.518	> 0.999
5BS7BS	25.0	10.3	26.8	6.4	107	0.767	> 0.999
5D	16.6	9.7	11.8	3.2	71	0.442	> 0.999
6A	31.6	12.5	22.3	9.5	71	0.140	> 0.999
6B	20.6	9.8	15.1	7.3	73	0.380	> 0.999
6D	24.7	7.1	13.4	6.8	54	0.071	> 0.999
7A	22.8	8.8	27.0	12.9	119	0.496	> 0.999
7D	23.6	12.6	21.1	11.0	89	0.685	> 0.999
Overall mean:	25.6						
Coefficient of variation for subplot error: 42.2%							

Table 4 Phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficients among AUDPC, 50 ml-weight and DON-content measured in back-cross reciprocal monosomic lines from crosses of 'U-136.1' with 'Hobbit-sib'. Note: All phenotypic correlation coefficients were significantly different from zero with a probability $\alpha < 0.001$, and all genotypic correlation coefficients were larger than twice their standard error

Item	AUDPC	50 ml-weight	DON-content
AUDPC		− 0,72	0.59
50 ml-weight	− 0.78		− 0.82
DON-content	0.67	− 0.90	

mainly to Type-II resistance. It has been reported earlier that resistance to spread of the disease is a more stable component of FHB resistance and less affected by environmental conditions than resistance to initial infection (Bai 1995).

Visual estimates for FHB symptoms measure only whether glumes are bleached or not and do not necessarily identify damaged kernels (Bekele et al. 1994; Mesterhazy 1995). Mesterhazy (1995) has reported genotypic differences in FHB resistance as well as genotypic differences in tolerance. That means that genotypes showing similar visual FHB symptoms on the heads can differ significantly in the percentage of infected grains or in mycotoxin content.

A modified test weight (50 ml-weight) was used for estimating Fusarium kernel damage. Although genotypic differences between cultivars in test weight are common in the absence of FHB, these are not comparable with differences created by FHB infection. Without artificial FHB infection, test weight among Austrian breeding lines varies typically between 76 and 82 kg per 100 l (Werteker 1995), this means a variation of about 8%. In the present study much larger differences in 50 ml-weight were found among the genotypes, ranging from 25 g to more than 37 g, demonstrating a variation of 48%. In samples with a high 50 ml-weight only a few scabby grains were present whereas in samples with a low 50 ml-weight a high percentage of the grains was shrivelled and showed typical discoloration.

The ultimate goal of resistance breeding against scab is to reduce not only yield losses but also the quality loss due to mycotoxin contamination. The quantitative ELISA method applied here for DON measurement allows mass screening at reasonable cost and gives similar results to the more-expensive gas chromatography measurement (Sinha and Savard 1996). The correlation coefficients between the three measured traits in this study are in good agreement with other reports (Wiersma et al. 1996; Lemmens et al. 1997). The easy and cheap to measure 50 ml-weight is possibly a good indicator for DON content. Test weight has also been found, for instance by Wiersma et al. (1996), to be highly correlated with the percentage of infected grains ($r = -0.94$) and DON content ($r = -0.89$) in FHB-infected wheat.

The suitability of the back-cross reciprocal monosomic method

The back-cross reciprocal monosomic method was used in this study. By this low-cost and low-tech approach it was hoped that candidate chromosomes could be identified in a promising breeding line for later molecular mapping work with the chromosomes of interest. The back-cross reciprocal method is more flexible than the reciprocal monosomic method because it requires a monosomic series in only one of the genotypes under investigation (Law et al. 1983 a, b). The method is faster than the development of a full intervarietal substitution series, because it needs only one generation of back-crossing (Law et al. 1986). The disadvantage is, that the method is laborious, because several monosomic back-cross plants have to be developed in parallel for each hemizygous chromosome in order to balance genetic background effects.

Furthermore, the effect of a specific chromosome may be masked by the effects of the heterozygous genetic background. Only those chromosomes with major effects can therefore be identified. If a trait under investigation is governed by many genes on many chromosomes, the back-cross reciprocal monosomic method will be less effective. For example, the method of back-cross reciprocal monosomic analysis has been successfully applied for studies of the genetic control of plant height (Snape and Law 1980), plant height and yield components (Snape et al. 1983), earliness, plant height, spike length, spikelet number and grain weight (Tao et al. 1991).

In the present analysis, the progeny of at least six independently developed monosomic plants have been bulked for the field trials. This appears to be an appropriate plant number to balance for the heterozygous genetic background (A. J. Worland, personal communication).

None of the families possessing one specific chromosome from the resistant genotype reached the resistance level of 'U-136.1'. This is not surprising and for several reasons:

(1) FHB resistance is not a monogenic, but an oligogenic to polygenic, trait. One single chromosome from the resistant genotype will therefore not have sufficient effect to retain the full resistance level of the original parental genotype.
(2) The genetic background is 25% heterozygous in the first back-cross generation.
(3) Progeny of monosomic plants have been tested for resistance. On average, therefore, 75% of the plants possess only a hemizygous chromosome with the possible resistance gene(s). In case of a dosage effect, the expression of resistance will be lower than in a normal euploid genotype.

Furthermore, monosomy itself influences plant morphology (Kleijer and Fossati 1977; Law et al. 1986); for instance 5A monosomics have a spelt ear type. This may also have an influence on FHB infection. The effect of monosomy could have been ruled out by selecting disomic substitutions and then to test those for resistance, as described by Stein et al. (1992 a). However, we would have needed a further generation of selection to achieve this and because of the limited time schedule of our program we were not able to establish the disomics. Further possible sources of inaccuracy in our approach are that a monosomic-shift may have occurred resulting in heterogeneity among the monosomic lines of the parental genotype (Stein et al. 1992 b). Because of the above mentioned reasons only chromosomes determining a rather large effect can be identified. In order to quantify the effects of each whole chromosome on FHB resistance precisely, the development of a disomic substitution series is necessary and to locate genes of interest to particular regions within a chromosome, the application of molecular marker technology is indispensable.

Comparing results on the chromosomal location of FHB resistance genes

Several chromosomes were identified to have a significant influence on FHB resistance. This confirms its oligogenic inheritance as also reported by others (e.g. Yu 1982; Liao and Yu 1985; Yu et al. 1986; Zhou et al. 1987; Bai et al. 1989; Yu 1991; Bai 1995; Singh et al. 1995; Van Ginkel et al. 1996). For instance Zhou et al. (1987) estimated two, and Bai et al. (1989) three, major resistance genes in the cultivar 'Sumei #3' using quantitative genetic methods. Similarly in 'Ning 7840', a cultivar descended from 'Sumei #3', two (Van Ginkel et al. 1996) and three (Bai 1995) major resistance genes have been estimated.

In 'U-136.1', compared to 'Hobbit-sib', five chromosomes (6B, 5A, 6D, 1B and 4B) had a significant effect on reducing head-blight symptoms. Based on visual observations chromosome 2B of 'U-136.1' even enhanced FHB on wheat. Also in the highly resistant cultivar 'Sumei #3' a negative allele(s) for FHB resistance has been reported on chromosome 2D in comparison to the moderately susceptible cultivar 'Chinese Spring' (Yu 1982; Y. Ge, personal communication). The occurrence of chromosomes (carrying alleles) with a negative effect in otherwise resistant genotypes is therefore not unusual.

Chromosome 3B in 'U-136.1' resulted in a significantly increased 50 ml-weight, but had no significant effect on visual symptoms, compared to 3B from 'Hobbit-sib'. One may speculate that this chromosome carries genes for tolerance against FHB. This is the first monosomic analysis of FHB resistance which includes mycotoxin measurements. Group-2 and especially group-6 chromosomes show a tendency to influence DON content. Both homoeologous groups appear to carry genes for resistance to DON.

Table 5 compares the results obtained herein to reports from the literature. Y. Ge (personal communication) tested an intervarietal substitution series of 'Sumei #3' in 'Chinese Spring'. He found that chromosomes 7A, 2B and 4D of 'Sumei #3' had positive effects on resistance and 2D had a negative effect. Yu (1982, 1991) and Yu et al. (1986) carried out F_2 monosomic analysis by crossing 'Chinese Spring' monosomics with a range of resistant genotypes. In 'Sumei #3' Yu (1982) reported that 2A, 5A, 1B, 6D, and 7D determined positive effects while 2D resulted in a negative effect on resistance. The only agreement between the results of Yu (1982) and Y. Ge (personal communication) is the negative effect of 2D. It is likely that 'Chinese Spring', a moderately susceptible cultivar, carries a resistance gene on 2D, which is not present in 'Sumei #3'. However, the striking differences between these two studies on the same genotype could be due to several reasons. (1) The genotype with the label 'Sumei #3' in one

Table 5 Comparison between the results on the chromosomal location of Fusarium head blight resistance genes and reports from the literature (see text for details)

Tested genotype	Chromosomes with positive effect on resistance	Chromosomes with negative effect	Author
U-136.1	5A 1B 3B 4B 6B 6D	2B	This study
Sumei #3	7A 2B 4D	2D	Y Ge, personal communication
Sumei #3	2A 5A 1B 6D 7D	2D	Yu 1982
Wangshiubai	4A 5A 7A 7B 4D		Yu et al. 1986
Wenzhouhongheshang	2A 5B 6B 3D 4D 7D		Yu et al. 1986
Pinghujianzimai	7A 3B 5B 6B 6D		Yu 1991
Yangangfangzhou[a]	3A	5D	Yu 1991
Wannian 2	4D		Yu 1991
Triticum macha	4A 5A 6B		Grausgruber 1998

[a] Japanese synonym = Nobeokabozu (T. Ban, personal communication)

study might be genetically different from the genotype with the same name in the other study. (2) Misclassification of monosomic lines and univalent-shift (Law et al. 1986) may have occurred. (3) The methods used in the two studies were different. Grausgruber (1998) screened the substitution series of a *Triticum macha* Dek. and Men. line in 'Hobbit-sib' (obtained from John Innes Centre, Norwich, UK) for scab resistance. He found effects of chromosomes 4A, 5A, and 6B. Considerable agreement between the results of our study and those of Yu (1982) were evident especially concerning chromosomes 6D, 5A, and 1B.

On examining our data on the identity of the chromosomes controlling the three factors associated with FHB resistance, and comparing these results with other studies listed in Table 5, it is obvious that all three traits are quantitatively inherited. Secondly, the effects of only two chromosomes, 6D and 6B, go across all traits. Some chromosomes, such as 2D, have an enhancing effect on DON reduction but a depressing effect on FHB symptoms. In breeding for resistance it will probably be necessary to score for at least two traits simultaneously, e.g. FHB symptoms and DON content, while for effective resistance it will probably be necessary to pyramid genes for at least two traits.

In conclusion, to-date a number of different chromosomes have been described to carry FHB resistance genes in wheat. Furthermore, it is also obvious that a few wheat chromosomes, especially chromosomes 6D, 6B, 5A, 4D and 7A, have been found across several different genotypes to be involved in resistance. These chromosomes could carry genes for FHB resistance which are present in a range of scab-resistant genotypes. Several research groups, including our own, are at present attempting to map scab resistance in wheat by molecular tools. Only preliminary results have been reported so far (e.g. Bai 1995; Buerstmayr et al. 1997; Gilbert et al. 1997; Moreno-Sevilla et al. 1997). For further work on molecular mapping we recommend paying special attention to the above mentioned chromosomes.

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