

## Multi-environment evaluation of level and stability of FHB resistance among parental lines and selected offspring derived from several European winter wheat mapping populations

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### Abstract

During 2 years and at five locations in Europe, 56 winter wheat genotypes were evaluated for resistance to Fusarium head blight (FHB). The genotypes were both parents and selected recombinants taken from the following populations previously tested for FHB resistance: 'Arina'/Forno', 'Arina'/Riband', 'Dream'/Lynx', G16-92/'Hussar', 'Renan'/Récital', SVP-72017 × 'Capo' and 'Capo'/Sumai-3'. In addition, a few control lines were included. FHB resistance was evaluated in replicated experiments under artificial inoculation, disease severity was assessed by repeated visual scorings. The highest level of FHB resistance was found in lines selected from crosses of FHB-resistant winter wheat × 'Sumai-3'. The best lines selected from crosses of moderately resistant winter wheat with susceptible winter wheat were similar in their resistance response to the resistant parent. The level of FHB resistance was correlated with stability of resistance. Susceptible wheat lines tended to exhibit severe symptoms under high disease pressure. The symptoms on resistant lines remained comparatively low even under high disease pressure.

**Key words:** *Fusarium culmorum* — *Triticum aestivum* — resistance breeding — stability

Fusarium head blight (FHB) is a serious fungal disease of wheat and other small grain cereals almost worldwide. Apart from yield losses the contamination of the cereal crop with toxic fungal secondary metabolites (mycotoxins) has raised considerable concern in the agriculture and food sector. The cultivation of resistant varieties plays a key role in controlling Fusarium infection and the associated mycotoxin contamination of cereals and cereal products (Placinta et al. 1999). Improving the FHB resistance of regionally adapted cultivars is therefore an important breeding goal. In the wheat gene pool, large quantitative genetic variation for Fusarium resistance has been detected (e.g. Snijders 1990a, Mesterhazy 1995, Buerstmayr et al. 1996, Gosman et al. 2007). In general, Fusarium resistance is influenced by many factors, these may be active physiological resistance factors (gene products of resistance genes) and also passive (e.g. morphological or developmental) factors which influence infection and/or disease development (Mesterhazy 1995).

Wheat breeders may use a range of different genetic resources in their crossing and selection programmes to introduce or enhance the Fusarium resistance of their breeding material. In recent years, several different resistant wheat sources have been genetically analysed using molecular mapping techniques. So far, no orthogonal comparison of the different resistance sources used in these mapping projects has been available. Therefore, this study was undertaken to evaluate and compare the level and stability of Fusarium resistance of the parental lines and the selected offspring among the different populations. The relative efficacies of different Fusarium resistant resources for European wheat breeding were assessed in an orthogonal series of trials across diverse European environments.

### Materials and Methods

**Plant material and resistance testing:** A set of 56 winter wheat genotypes was evaluated for FHB resistance in inoculated field experiments at five sites in Europe during two seasons (2004 and 2005). The locations were: Rennes (FR), Norwich (UK), Freising (DE), Changins (CH) and Tulln (AT) (Table 1). The experiments are abbreviated in the following text with the ISO country codes (see above) followed by the abbreviated year, e.g. AT\_04 stands for the experiment conducted in Tulln (Austria) in 2004.

A list of all evaluated lines is shown in Table 2. The plant material was composed of both parents and five offspring lines showing a highly resistant phenotype from the following mapping populations: 'Dream'/Lynx' (Schmolke et al. 2005), G16-92/'Hussar' (Schmolke et al. 2008), 'Renan'/Récital' (Gervais et al. 2003), 'Arina'/Forno' (Paillard et al. 2004), 'Arina'/Riband' (Draeger et al. 2007) and WEK0609/'Hobbit-sib' (Gosman et al. 2005). Generally, the resistant offspring lines were chosen based on the resistance evaluations used in the above mentioned mapping studies. For the 'Arina'/Riband' population, the recombinant lines were selected based on one inoculation experiment only (P. Nicholson, unpublished data). The Swiss cultivar 'Arina' was included twice, the duplicated entries were identified with the extensions CH or UK for the seed taken from the stock used for the 'Arina'/Forno' and the 'Arina'/Riband' cross respectively. In addition, trials included five breeding lines developed for a quantitative trait loci (QTL) validation population of crosses of 'Sumai-3', or its derivative

Table 1: Experimental conditions at five trial locations

Location	Tulln	Freising	Rennes	Changins	Norwich
Country	AT	DE	FR	CH	UK
Coordinates	16°03'E, 48°19'N	11°43'E, 48°23'N	1°43'W, 48°06'N	6°13'E, 46°23'N	1°16'E, 52°40'E
Elevation (m)	174	444	36	460	36
Plot size (m <sup>2</sup> )	0.4	0.6	0.75	0.2	0.2
No. reps	2	2	2	3	3
Inoculum	<i>Fusarium culmorum</i> single spore isolate	<i>F. culmorum</i> mix of two isolates	<i>F. culmorum</i> single spore isolate	<i>F. culmorum</i> mix of 20 isolates	<i>F. culmorum</i> single spore isolate
Inoculation method	Spray at flowering 2x	Spray at flowering 2x	Spray at flowering 1x	Spray at flowering 3x	Spray at flowering 2x
Spore concentration	50 000 ml <sup>-1</sup>	500 000 ml <sup>-1</sup>	200 000 ml <sup>-1</sup> (04) 50 000 ml <sup>-1</sup> (05)	500 000 ml <sup>-1</sup>	50 000 ml <sup>-1</sup>
Humidity control	Mist irrigation	No irrigation	Irrigation	Mist irrigation	Mist irrigation

CM-82036, by winter wheat (Angerer et al. 2003) and three lines from a phenotyped, but unmapped, population of SVP-72017 × 'Capo' (Buerstmayr et al. 2000). Four lines were included as controls: F201-R [a *Fusarium* resistant breeding line from Romania (Shen et al. 2003)], the German cultivar 'Petrus' and two breeding lines derived from the cross 'Sagvari'/'Nobeokabozi'/'Minimano'/'Sumai-3' which were designated H136-6 and H136-16. These two lines were derived from breeding material developed by A. Mesterhazy, Szeged, Hungary.

Table 1 shows the experimental conditions at the five testing sites. At all locations, small plots were sown in two or three replications. Generally, sowing, crop husbandry and inoculation methods were performed as described previously for Tulln (Buerstmayr et al. 2003), Norwich (Gosman et al. 2007), Changins (Haller-Gartner et al. 2008), Rennes (Gervais et al. 2003) and Freising (Haberle et al. 2007). Briefly, spray inoculations were applied using local inoculation methods with local *Fusarium culmorum* isolates. FHB severity was scored by visually averaging the percentage of infected spikelets per plot at four to five time points after inoculation, depending on disease development at the respective sites. We present here results on FHB severity (% infected spikelets per plot) at the end of the observation period of each trial, which was between 26 and 33 dpi (days postinoculation), depending on the experiment. As an integrated measure for disease severity the relative area under the disease progress curve (R-AUDPC) was calculated. The R-AUDPC was obtained by expressing the AUDPC value of each entry in per cent of the maximum possible AUDPC. The maximum possible AUDPC is the theoretical AUDPC value when a plot would reach 100% diseased spikelets per plot already on the first observation date after inoculation (Lemmens et al. 1993). The R-AUDPC is thus a standardized AUDPC value that represents a comparable measure for disease severity across experiments with varying scoring dates, disease pressure and observation periods.

At nine experiments (all but CH\_05), flowering date was recorded and expressed in days after 1 January. At five experiments (AT\_04, AT\_05, DE\_04, DE\_05 and UK\_04), plant height was measured from the soil surface to the tip of the head excluding awns.

**Statistical analysis:** For statistical analysis, SAS/STAT 9.1 was applied (SAS Institute Inc. 2004). Analysis of variance using the procedure GLM and analysis of correlation using the procedure CORR were employed. Mean values and standard deviations of mean values were calculated using the MEANS procedure. Regression analysis using the procedure REG was applied to assess stability of the disease response of the tested genotypes as suggested by Finlay and Wilkinson (1963). Across the 10 environments the R-AUDPC mean values of a specific genotype in each environment were regressed against the average disease level at these environments. As a measure for the average disease level at an environment, the R-AUDPC mean values of all genotypes at this environment were used. The slope of the linear regression and the deviation from the regression allow conclusions on the stability of the disease response of a genotype (Finlay and Wilkinson 1963, Becker and Leon 1988).

## Results

At all locations FHB inoculations were successful, the highest average disease severity as measured by R-AUDPC was obtained in DE\_05 followed by DE\_04. The lowest average disease severity occurred at FR\_05 and AT\_05 (Table 3). Both measures for FHB severity (%FHB severity and R-AUDPC) were highly correlated with each other:  $r = 0.97$  for overall mean values.

Correlation coefficients for disease severity measured by R-AUDPC between experiments were positive and significant apart from the experiments CH\_04 with FR\_05 (Table 4). The correlation coefficients for R-AUDPC values obtained in two consecutive years at the same location varied between  $r = 0.92$  for the UK experiments and  $r = 0.34$  for the FR experiments (Table 4). The analysis of variance for the traits R-AUDPC and %FHB severity is shown in Table 5. All sources of variation were highly significant. The variation due to genotypes was by far the largest source of variation compared to that due to genotype-by-environment interactions leading to a broad sense heritability estimate of  $H = 0.89$  for both R-AUDPC and %FHB severity at the end of the scoring period.

The highly resistant Hungarian control lines H136.6 and H136.16 had an average disease severity of 7.0% and 7.8% respectively. The moderately resistant German cultivar 'Petrus' reached on average 20.5% infected spikelets and the Romanian breeding line F201-R appeared to be moderately susceptible with an average FHB severity of 34.7% at the end of the scoring period.

Within each population the susceptible parent was the most diseased line. In the same way, the resistant parent was at the lower end of the distribution and in all cases not significantly different from the best recombinant line within each population (Fig. 1, Table 6). The FHB resistance level of the best selected recombinant lines within each population was similar or slightly better than the resistant parent used in the respective crosses (Fig. 1, Table 6).

The lines with the lowest average FHB severity were those selected from the winter wheat × 'Sumai-3' (or its derivative CM-82036) crosses. Across the 10 experiments, these recombinant lines had an average of 5.5–11.0% infected spikelets per plot at the end of the observation period.

Lines selected from the cross-combination of SVP-72017 × 'Capo' achieved on average 9.2–14.6% disease severity at the end of the scoring period, and were thus the second best population.

Two independent populations involving the moderately resistant Swiss cultivar 'Arina' were used. The duplicated

Table 2: List of 56 winter wheat genotypes evaluated for resistance to Fusarium head blight at five locations in 2 years

No.	Name	Pedigree	Reference
1	<b>Capo</b>	Martin/Pokal	Angerer et al. (2003)
2	20476	Aurus/CM-82036	Angerer et al. (2003)
3	20378	Capo/Sumai-3	Angerer et al. (2003)
4	20828	Capo/Sumai-3	Angerer et al. (2003)
5	20816	Capo/Sumai-3	Angerer et al. (2003)
6	20818	Capo/Sumai-3	Angerer et al. (2003)
7	<b>SVP-72017</b>	Marzotto//Dippes-Triumph/Mironovskaja-808	Snijders (1990b)
8	A40-22-1-2	Capo/SVP-72017	Buerstmayr et al. (2000)
9	A39-9-2-1	SVP-72017/Capo	Buerstmayr et al. (2000)
10	A40-19-1-2	Capo/SVP-72017	Buerstmayr et al. (2000)
11	<b>Forno</b>	NR72837/Kormoran	- <sup>1</sup>
12	<b>Arina-CH</b>	Moisson//Can3842/HeineVII	- <sup>1</sup>
13	Ar_Fo-177	Arina/Forno	Paillard et al. (2004)
14	Ar_Fo-338	Arina/Forno	Paillard et al. (2004)
15	Ar_Fo-240	Arina/Forno	Paillard et al. (2004)
16	Ar_Fo-314	Arina/Forno	Paillard et al. (2004)
17	Ar_Fo-341	Arina/Forno	Paillard et al. (2004)
18	<b>Riband</b>	Norman//Maris-Huntsman/TW261	- <sup>1</sup>
19	<b>Arina-UK</b>	Moisson//Can3842/HeineVII	- <sup>1</sup>
20	Ar_Rib-27	Arina/Riband	Draeger et al. (2007)
21	Ar_Rib-17	Arina/Riband	Draeger et al. (2007)
22	Ar_Rib-05	Arina/Riband	Draeger et al. (2007)
23	Ar_Rib-52	Arina/Riband	Draeger et al. (2007)
24	Ar_Rib-37	Arina/Riband	Draeger et al. (2007)
25	<b>Lynx</b>	CWW-4442-64/Rendezvous	Schmolke et al. (2005)
26	<b>Dream</b>	Disponent/Kronjuwel//Monopol/3/Orestis	Schmolke et al. (2005)
27	Dr_Ly-266	Dream/Lynx	
28	Dr_Ly-342	Dream/Lynx	Schmolke et al. (2005)
29	Dr_Ly-311	Dream/Lynx	Schmolke et al. (2005)
30	Dr_Ly-334	Dream/Lynx	Schmolke et al. (2005)
31	Dr_Ly-330	Dream/Lynx	Schmolke et al. (2005)
32	<b>Hussar</b>	Squadron/Rendezvous	- <sup>1</sup>
33	<b>G16-92</b>	Pedigree includes 'Arina', 'Cariplus', Töring 5 and Mexican line	Schmolke et al. (2008)
34	Hus_G-715	G16-92/Hussar	Schmolke et al. (2008)
35	Hus_G-692	G16-92/Hussar	Schmolke et al. (2008)
36	Hus_G-700	G16-92/Hussar	Schmolke et al. (2008)
37	Hus_G-702	G16-92/Hussar	Schmolke et al. (2008)
38	Hus_G-690	G16-92/Hussar	Schmolke et al. (2008)
39	<b>Hobbit-sib</b>	Professeur-Marchal//Marne-Desprez/VG-9144/4/CI-12633/4* Capelle-Desprez//Heines-110/Capelle-Desprez/3/Nord-Desprez	- <sup>1</sup>
40	<b>Wek0609</b>	not disclosed	
41	Hs_Wek-60	Hobbit-sib/Wek0609	Gosman et al. (2005)
42	Hs_Wek-28	Hobbit-sib/Wek0609	Gosman et al. (2005)
43	Hs_Wek-42	Hobbit-sib/Wek0609	Gosman et al. (2005)
44	Hs_Wek-18	Hobbit-sib/Wek0609	Gosman et al. (2005)
45	Hs_Wek-51	Hobbit-sib/Wek0609	Gosman et al. (2005)
46	<b>Récital</b>	Mexique-267/5/V81.12/Bezostaya//Heine VII/3/Nord-Desprez/4/Tadorna	C. C. Benoist, personal communications
47	<b>Renan</b>	Mironovskaja-808/Maris-Huntsman//VPM/Moisson/3/Courtot	Gervais et al. (2003)
48	Re_R-055	Renan/Récital	Gervais et al. (2003)
49	Re_R-049	Renan/Récital	Gervais et al. (2003)
50	Re_R-141	Renan/Récital	Gervais et al. (2003)
51	Re_R-075	Renan/Récital	Gervais et al. (2003)
52	Re_R-044	Renan/Récital	Gervais et al. (2003)
53	<i>F201-R</i>	F15615-2112/F2076W12-11	Shen et al. (2003)
54	<i>Petrus</i>	Nimbus/Vuka//Falke/4/Benno/Caribo//Kormoran/3/Kronjuwel	- <sup>1</sup>
55	<i>H136.16</i>	Sagvari/Nobeokaboza//Minimano/Sumai-3	A. Mesterhazy, personal communications
56	<i>H136.6</i>	Sagvari/Nobeokaboza//Minimano/Sumai-3	A. Mesterhazy, personal communications

The names of the parents are printed in bold, the names of the control lines in italics.

<sup>1</sup>Pedigree obtained from <http://genbank.vurv.cz/wheat/pedigree/>.

entries of 'Arina' showed essentially the same disease response. Selected lines from the 'Arina'/'Forno' cross showed a moderately resistant phenotype, similar to the parental cultivar 'Arina' and the German cultivar 'Petrus'. The lines selected from the 'Arina'/'Riband' cross displayed more variable disease responses. Even the best line from this cross (Ar\_Rib-37) was inferior in FHB resistance compared to the

lines selected from the 'Arina'/'Forno' cross. The best lines derived from the crosses involving 'Dream', G16-92, 'Renan' and WEK0609 were similar in FHB response as their resistant parents, respectively, but displayed higher average disease severity compared to the most resistant lines within the crosses winter wheat × 'Sumai-3', SVP-72017 × 'Capo' or 'Arina'/'Forno'.

Table 3: Experiment mean, line mean with highest and lowest FHB severity and least significant difference (LSD) at  $\alpha = 0.05$  for R-AUDPC observed on 56 winter wheat lines evaluated at five locations in 2 years

	AT_04	AT_05	CH_04	CH_05	DE_04	DE_05	FR_04	FR_05	UK_04	UK_05
Mean	15.4	5.7	10.0	7.3	22.9	24.9	7.4	4.8	18.7	11.1
Min.	3.3	0.0	0.8	0.2	4.7	2.5	0.0	0.0	3.8	1.5
Max.	41.0	42.7	60.8	41.6	76.2	75.5	48.3	40.2	94.2	69.5
LSD5%	6.5	5.1	8.9	4.4	8.9	7.3	9.1	9.1	7.7	8.3

Table 4: Pearson correlation coefficients for R-AUDPC values observed on 56 winter wheat lines in 10 experiments

	AT_05	CH_04	CH_05	DE_04	DE_05	FR_04	FR_05	UK_04	UK_05
AT_04	0.77***	0.71***	0.89***	0.76***	0.67***	0.75***	0.63***	0.80***	0.83***
AT_05		0.34*	0.84***	0.91***	0.73***	0.38**	0.79***	0.88***	0.90***
CH_04			0.70***	0.30*	0.36**	0.85***	0.25 <sup>ns</sup>	0.56***	0.62***
CH_05				0.78***	0.65***	0.73***	0.64***	0.91***	0.93***
DE_04					0.82***	0.43***	0.81***	0.78***	0.82***
DE_05						0.44***	0.60***	0.56***	0.67***
FR_04							0.34*	0.51***	0.58***
FR_05								0.67***	0.69***
UK_04									0.92***

\*P = 0.05; \*\*P = 0.01; \*\*\*P = 0.001; ns, P > 0.05.

Table 5: Analysis of variance for the traits R-AUDPC and % FHB severity at the end of the scoring period

Source	df	R-AUDPC			% FHB severity		
		Mean squares	F-value	P-value	Mean squares	F-value	P-value
Blocks within (L*Y)	14	404.4	2.1	0.0089	306.1	4.3	< 0.0001
Locations	4	115351.2	608.2	< 0.0001	41470.2	586.0	< 0.0001
Years	1	56385.2	297.3	< 0.0001	10164.2	143.6	< 0.0001
L*Y	4	12912.6	68.1	< 0.0001	14845.2	209.8	< 0.0001
Genotypes	55	22068.4	116.4	< 0.0001	7506.8	106.1	< 0.0001
G*L	220	2056.0	10.8	< 0.0001	463.2	6.6	< 0.0001
G*Y	55	1022.0	5.4	< 0.0001	565.1	8.0	< 0.0001
G*L*Y	220	734.6	3.9	< 0.0001	235.5	3.3	< 0.0001
Residual	770	189.7			70.8		

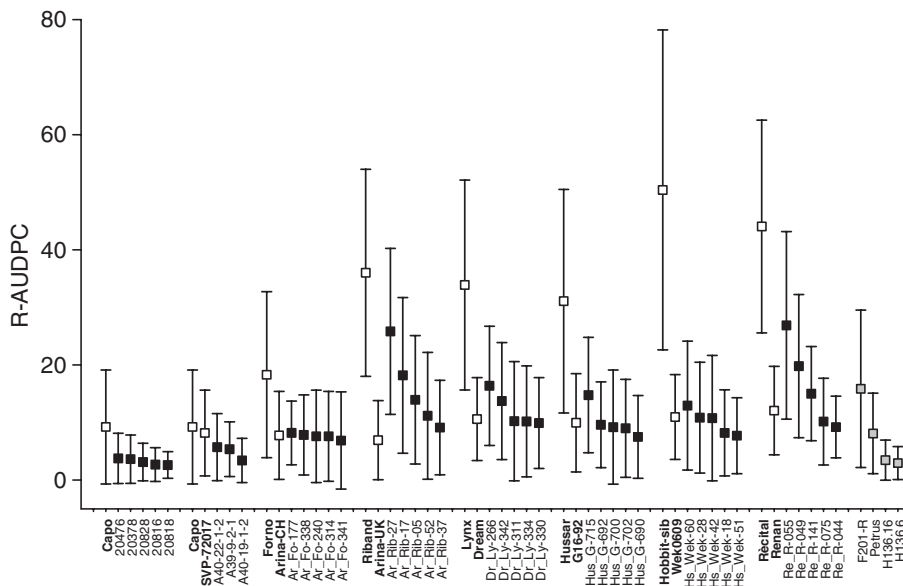


Fig. 1: Mean R-AUDPC values (%) and standard deviations of 56 winter wheat genotypes evaluated for FHB resistance at five locations in 2 years. Empty squares: parents; black squares: recombinant lines; grey squares: control lines

In six of the eight evaluated populations, the selected resistant lines did not differ significantly in their FHB response from each other and from their resistant parent. On the contrary, the disease severity of selected recombinant lines

within the crosses ‘Arina’/‘Riband’ and ‘Renan’/‘Réctal’ varied in a wide range between the values of the parents and some lines were significantly more susceptible than the resistant parent (Table 6, Fig. 1).

Table 6: Overall mean R-AUDPC values and standard errors of mean values across 10 environments, slope and deviation from the regression (SE) of the linear regression of the mean R-AUDPC values of 56 winter wheat genotypes evaluated in 10 environments on the environmental mean values, and mean values and standard errors of plant height measured in five experiments and flowering date recorded in nine experiments

Genotype	R-AUDPC (%)		Regression analysis		Plant height (cm)		Flowering date (days after 1 January)	
	Mean	SE	Slope	SE	Mean	SE	Mean	SE
<b>Capo</b>	9.2	9.9	1.24	0.29	117	17	154	4
20476	3.8	4.4	0.46*	0.16	113	15	152	4
20378	3.6	4.2	0.40*	0.16	111	16	152	4
20828	3.1	3.3	0.39*	0.09	111	13	152	4
20816	2.7	2.9	0.32*	0.08	105	11	155	4
20818	2.6	2.3	0.25*	0.07	111	14	151	3
<b>SVP-72017</b>	8.2	7.4	0.99	0.10	96	10	157	4
A40-22-1-2	5.7	5.8	0.70	0.16	115	15	154	4
A39-9-2-1	5.4	4.7	0.63*	0.08	92	11	158	4
A40-19-1-2	3.4	3.8	0.50*	0.09	114	15	158	4
<b>Forno</b>	18.3	14.4	1.87	0.39	100	9	155	4
<b>Arina-CH</b>	7.7	7.6	0.98	0.20	112	11	156	4
Ar_Fo-177	8.2	5.5	0.70*	0.08	108	6	157	4
Ar_Fo-338	7.8	7.0	0.91	0.17	112	13	157	4
Ar_Fo-240	7.6	8.0	0.97	0.16	113	11	155	4
Ar_Fo-314	7.6	7.8	1.10	0.15	107	10	155	4
Ar_Fo-341	6.9	8.4	1.11	0.21	114	12	155	4
<b>Riband</b>	36.0	18.0	1.91	0.58	83	6	158	4
<b>Arina-UK</b>	6.9	6.9	0.91	0.14	112	12	156	4
Ar_Rib-27	25.8	14.4	1.85*	0.26	86	10	155	3
Ar_Rib-17	18.2	13.5	1.73*	0.27	103	10	154	4
Ar_Rib-05	13.9	11.2	1.44	0.28	124	11	157	4
Ar_Rib-52	11.2	11.0	1.41	0.32	117	13	154	4
Ar_Rib-37	9.1	8.2	1.02	0.21	117	13	157	4
<b>Lynx</b>	33.9	18.2	1.65	0.64	73	6	160	4
<b>Dream</b>	10.6	7.2	0.93	0.14	106	13	161	3
Dr_Ly-266	16.4	10.4	0.79	0.35	113	12	161	3
Dr_Ly-342	13.7	10.2	0.93	0.37	100	11	161	3
Dr_Ly-311	10.2	10.4	1.13	0.29	122	15	160	4
Dr_Ly-334	10.2	9.6	1.28	0.17	118	14	160	4
Dr_Ly-330	9.9	7.9	1.00	0.16	116	11	161	3
<b>Hussar</b>	31.1	19.4	2.15	0.52	82	7	158	3
<b>G16-92</b>	9.9	8.5	0.99	0.25	111	15	156	3
Hus_G-715	14.8	10.0	0.94	0.35	102	9	155	4
Hus_G-692	9.6	7.5	0.95	0.16	108	12	155	3
Hus_G-700	9.2	9.9	1.37	0.22	106	9	156	5
Hus_G-702	9.0	8.5	1.07	0.22	111	12	156	3
Hus_G-690	7.5	7.2	0.98	0.10	122	13	160	3
<b>Hobbit-sib</b>	50.4	27.8	2.92	0.89	82	7	158	3
<b>Wek0609</b>	11.0	7.4	0.53	0.26	93	14	150	4
Hs_Wek-60	12.9	11.2	1.61*	0.13	111	15	152	4
Hs_Wek-28	10.8	9.6	1.22	0.25	101	10	158	4
Hs_Wek-42	10.8	10.9	1.42	0.23	106	12	153	4
Hs_Wek-18	8.2	7.5	0.94	0.10	110	13	152	3
Hs_Wek-51	7.7	6.6	0.84	0.14	95	12	151	3
<b>Récital</b>	44.1	18.5	1.46	0.61	80	10	147	5
<b>Renan</b>	12.1	7.7	0.95	0.15	93	9	152	3
Re_R-055	26.9	16.3	0.46	0.55	71	10	147	4
Re_R-049	19.8	12.4	0.42*	0.18	91	10	147	5
Re_R-141	15.0	8.2	0.55	0.26	97	15	150	4
Re_R-075	10.2	7.5	0.45	0.71	96	11	152	4
Re_R-044	9.2	5.4	0.48	0.32	108	14	149	4
<i>F201-R</i>	15.9	13.7	0.21	0.60	88	8	149	4
<i>Petrus</i>	8.1	7.0	0.96	0.10	113	15	158	8
<i>H136.16</i>	3.5	3.5	0.38*	0.09	113	16	152	5
<i>H136.6</i>	2.9	2.9	0.28*	0.09	105	13	154	4

The names of the parents are printed in bold, and the names of the control lines in italics.

\*The slope of the regression is significantly ( $P = 0.05$ ) different from 1.

## Stability of resistance

The highly resistant genotypes showed little variability in their disease response and remained resistant across all experiments as indicated by their low standard deviation of the mean values. On the other hand, the more susceptible genotypes (parents and recombinant lines) gave much more variable results as indicated by large standard deviations of the mean values (Table 6, Fig. 1). For example, the susceptible cultivar 'Lynx' varied in its FHB severity scores at the end of the observation period between 20% in the FR\_04 experiment and 100% in the DE\_04 experiment. In contrast, the FHB resistant line 20816 ('Capo'/'Sumai-3') varied in a narrow range only from no visible disease symptoms at the AT\_05 trial to 17.5% disease severity at the DE\_04 trial.

Genotype specific regressions of mean R-AUDPC values on environmental mean values revealed significantly different disease response patterns (Table 6, Fig. 2). The slope of the linear regression was significantly  $< 1$  for most of the highly resistant genotypes: all lines derived from the 'Capo'/'Sumai-3' cross, two of the SVP-72017  $\times$  'Capo' crosses and the lines Re\_049 and Ar-Fo\_177. Lines with a slope of regression significantly  $> 1$  were generally the moderately susceptible to susceptible lines. The mean values for R-AUDPC were highly correlated with all three measures of stability calculated herein: the standard error of the mean ( $r = 0.94$ ), the slope of the linear regression ( $r = 0.64$ ) and the deviation from the regression ( $r = 0.82$ ).

## Association of FHB response with flowering date and plant height

Correlations between disease severity measured by R-AUDPC and flowering date did not reveal a general pattern but were highly variable across nine experiments. The extremes were  $r = -0.51$  for the experiment FR\_04 to  $r = 0.31$  for the experiment DE\_04. Across nine experiments the average correlation coefficient was close to zero ( $r = 0.02$ ).

The average plant height of the genotypes varied between 71 cm (Re\_R-055) to 124 cm (Ar\_Rib-17). The parental lines 'Lynx', 'Hussar', 'Récital', 'Riband' and 'Hobbit-sib' were all relatively short types (average plant height  $< 85$  cm) (Table 2). The correlations between plant height and disease severity were rather consistent across the five experiments where plant height was measured. In all cases, the correlation coefficient was negative and significantly different from zero, ranging from  $r = -0.72$  for the AT\_04 trial to  $r = -0.28$  for the DE\_05 experiment. The average correlation coefficient between R-AUDPC and plant height across five trials was  $r = -0.55$ .

## Discussion

Fusarium head blight infection and development on wheat depends on both genetic and environmental factors and their interaction. One of the main problems in testing Fusarium resistance is to reproduce experimental results (Dill-Macky 2003). Genotype-by-environment interactions may account for a large proportion of the variation in such resistance evaluations (Campbell and Lipps 1998) and can significantly influence QTL estimates (Ma et al. 2006). The main reasons are that the pathogen is highly variable, and more importantly,

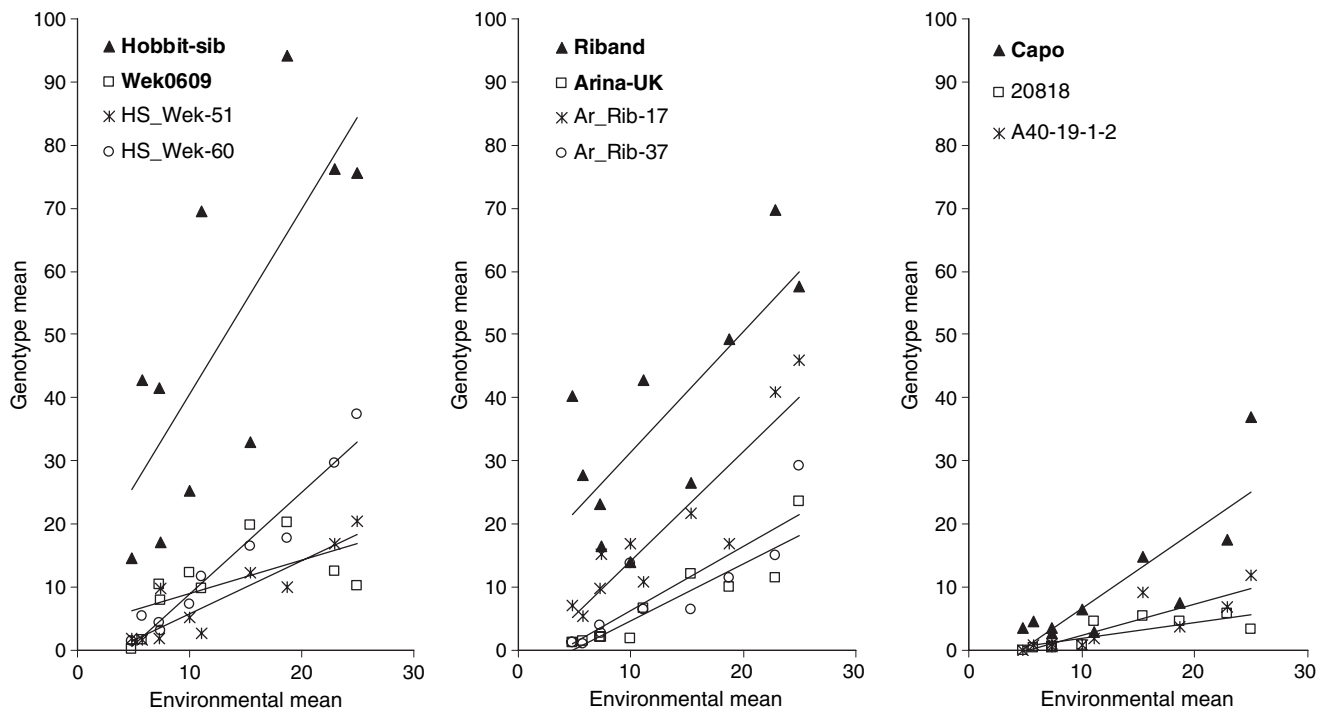


Fig. 2: Regression of mean R-AUDPC (%) of selected parental (bold text) and offspring lines in 10 environments on the environmental mean values

that disease initiation and development are subject to environmental variability. In a plant breeding context, the genetically determined resistance level of the tested genotypes has to be determined as precisely as possible. Measures to take into account genotype-by-environment interactions could include: (i) provide uniform conditions for infection and disease development over space and time; (ii) replicate experiments over environments (e.g. locations, years) (Campbell and Lipps 1998, Ruckebauer et al. 2001); and (iii) use of homozygous lines as opposed to early generation segregates from a breeding programme. All of these measures were taken in this experimental series: similar methods of artificial inoculations were performed at all tests and the experiments were replicated across five sites in 2 years. At all environments, either a single or a mix of aggressive *F. culmorum* isolates was used for inoculations. Snijders and Van Eeuwijk (1991) evaluated 17 winter wheat genotypes with four *F. culmorum* strains over 3 years and found no evidence for strain specific resistance. Interactions were mainly caused by a divergence of genotypical responses with increasing disease pressure. FHB resistance in wheat is thus non-specific or horizontal as later confirmed by several authors (e.g. Van Eeuwijk et al. 1995, Mesterhazy et al. 1999). The use of local isolates for FHB resistance evaluations at different environments is therefore fully justified. The positive and almost always significant correlations between experiments and the very high broad sense heritability for Fusarium severity underline the high reproducibility of the Fusarium resistance estimations of the 56 genotypes in this series of experiments. The mean values across the 10 experiments are therefore considered to be the best estimators of the true FHB resistance of the tested genotypes.

The lines with the lowest average disease severity were derived from the cross 'Capo'/'Sumai-3'. In a similar range were two highly resistant control lines from Hungary, which

also descended from an Asian spring wheat by European winter wheat cross. It is likely that these lines have inherited their high level of Fusarium resistance from their highly resistant Asian parents, their level of resistance matches 'Sumai-3' and CM-82036 (H. Buerstmayr, unpublished results). The second best population was derived from the SVP-72017 × 'Capo' cross which are both moderately resistant winter wheats (Buerstmayr et al. 2000). The selected offspring lines from this cross-combination probably carry resistance factors from both of their parents.

Two crosses with 'Arina' as resistant parent were tested. The parents: 'Arina'-CH and 'Arina'-UK appeared identical in their phenotypes and are therefore most likely genetically identical. The 'Arina'/'Forno' lines showed higher resistance compared to the 'Arina'/'Riband' lines. This fact can be partly explained by different susceptible parents: 'Forno' was moderately susceptible but 'Riband' highly susceptible.

While the selected lines within six cross-combinations showed similar FHB response among each other, the five lines taken from the 'Arina'/'Riband' and the 'Renan'/'Récital' cross were more variable. This is an indication that the resistance data used for selecting the five best lines from the original populations were reliable for most of the populations, apart from 'Arina'/'Riband' and 'Renan'/'Récital'. In the 'Arina'/'Riband' cross, the five lines for this test series were chosen among 116 doubled haploid lines based on one single inoculation test in a polytunnel. Further evaluation of this mapping population revealed that Ar\_Rib-17 and Ar\_Rib-27 were indeed moderately susceptible, whereas Ar\_Rib-37, Ar\_Rib-52 and Ar\_Rib-05 ranked 7th, 11th and 12th, respectively, in subsequent trials at JIC Ar\_Rib-17 and Ar\_Rib-27 ranked 28th and 46th, respectively, among the 116 lines (P. Nicholson, unpublished results).

In the 'Renan'/'Récital' population, the lines were chosen among 194 RILs using adjusted AUDPC mean values across three field tests conducted 1999–2002 (Gervais et al. 2003). The five best lines for which enough seed was available were submitted to this experimental series, all were in the top 10. However, significant variation in disease severity was observed across the three field tests for the lines Re\_R-044, Re\_R-049 and Re\_R-055 (M. Trotter, unpublished results).

Across the 56 genotypes all measures for stability of FHB resistance were highly correlated with the mean values of resistance. As indicated by the regression analysis, susceptible lines obviously developed excessive amounts of disease under high disease pressure (slope > 1) while on resistant lines disease severity remained comparatively low even under severe disease pressure (slope < 1). The fact that highly resistant genotypes are usually also stable in their resistance reaction while susceptible lines tend to exhibit different levels of disease severity in different environmental conditions is in full agreement with Snijders and Van Eeuwijk (1991) and Mesterhazy (1995).

No systematic association between flowering date and FHB severity was found. Obviously, site specific factors, most likely the weather conditions around flowering and inoculation, have influenced these correlations, although no specific analysis has been performed to test this hypothesis. In contrast, plant height was significantly correlated with FHB resistance. This finding is in agreement with several previous studies which have shown that tall lines tend to develop less FHB severity compared to short wheat lines (e.g. Mesterhazy 1995, Buerstmayr et al. 2000, Draeger et al. 2007). In the crosses involving the short straw parents 'Lynx', 'Hussar' and 'Hobbit-sib', none of the selected FHB resistant offspring lines had short straw length. In the crosses with the parents 'Riband' and 'Récital', the relatively susceptible recombinant lines (Re\_R-055 and Ar\_Rib-27) were the shortest ones. The reasons for the association between plant height and Fusarium severity may be genetic and/or environmental. A genetic association between FHB severity and plant height was clearly identified for instance in the mapping population 'Arina'/'Riband' where a large effect QTL was detected at the *Rht-D1* locus on chromosome 4DS that modulates plant height in wheat. The semi-dwarf allele (*Rht-D1b*) contributed by 'Riband' enhanced Fusarium susceptibility (Draeger et al. 2007). The FHB susceptible short straw parents 'Hobbit-sib' (Gale et al. 1975), 'Riband' (Draeger et al. 2007), 'Hussar' (Berry et al. 2007) and 'Lynx' (J. Häberle, personal communications) possess the *Rht-D1b* (syn. *Rht2*) semi-dwarf allele, but the FHB resistant offspring lines most likely inherited the tall (*Rht-D1a*) allele from their resistant parents. Obviously, presence of the *Rht-D1b* semi-dwarf allele impairs FHB resistance significantly. The association between susceptibility to FHB and *Rht-D1b* is supported by the finding that UK winter wheat varieties, the great majority of which carry the *Rht-D1b* semi-dwarfing allele, are generally highly susceptible to FHB (Gosman et al. 2007). Whether or not other dwarfing genes like *Rht-B1* (chromosome 4BS) or *Rht-8* (chromosome 2BS) are also associated with FHB resistance needs further investigations. 'Récital' and 'Renan' both possess the *Rht-B1b* (syn. *Rht1*) semi-dwarf allele (Gervais et al. 2003), but differ significantly in their FHB response; also the lines SVP-72017 and A39-9-2-1 carry the *Rht-B1b* allele (J. Förster, personal communications) and combine moderate straw length with good FHB resistance. Environmental factors like higher humidity and higher inoculum pressure may also contribute

to enhanced FHB development on short wheat lines compared to tall lines, especially under natural infection conditions (Mesterhazy 1995). When using artificial spray-inoculations and measures to provide uniform humidity around inoculation and disease establishment, the effect of plant height as a morphological resistance factor should be small compared to genetic differences in physiological resistance between tall and short lines as reported by Draeger et al. (2007).

## Conclusions

All in all the results showed that:

1. The scale of FHB resistance of the best offspring lines derived from the different winter wheat populations was quite similar.
2. In cross-combinations involving highly resistant spring wheat parents, like 'Sumai-3', recombinant lines with exceptional resistance could be selected.
3. The level of resistance of the best selected lines depended mainly on the resistance level of the resistant parent and to a lesser extent on the susceptible parent. As an example the best line selected from the cross 'Hobbit-sib'/WEK0609 was similar in its FHB resistance performance to the best line from 'Arina'/'Forno', although 'Hobbit-sib' was significantly more susceptible than 'Forno'.
4. The stability of resistance depended largely on the level of resistance. Highly resistant lines developed little disease severity across varying epidemic conditions whereas less resistant lines showed larger variability and susceptible lines were very variable in their response.
5. Presence of the *Rht-D1b* semi dwarfing allele impaired FHB resistance significantly.
6. For European winter wheat breeding, a range of diverse resistance sources can be used in crossing and selection programmes to enhance Fusarium resistance.

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