Theor Appl Genet (2008) 117:813–828
DOI 10.1007/s00122-008-0822-3

ORIGINAL PAPER

Evaluation of genetic diversity of Fusarium head blight resistance in European winter wheat

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Received: 19 December 2007 / Accepted: 8 June 2008 / Published online: 28 June 2008

Abstract Genetic diversity in relation to Fusarium head blight (FHB) resistance was investigated among 295 European winter wheat cultivars and advanced breeding lines using 47 wheat SSR markers. Twelve additional wheat lines with known FHB resistance were included as reference material. At least one SSR marker per chromosome arm, including SSR markers reported in the literature with putative associations with QTLs for FHB resistance, were assayed to give an even distribution of SSR markers across the wheat genome. A total of 404 SSR alleles were detected. The number of alleles per locus ranged from 2 to 21, with an average of 8.6 alleles. The polymorphism information content of the SSR markers ranged from 0.13 (*Xwmc483*) to 0.87 (*Xwmc607*), with an average of 0.54. Cluster analysis was performed by both genetic distance-based and model-based methods. In general, the dendrogram based on unweighted pair-group method with arithmetic averages showed similar groupings to the model-based analysis. Seven clusters were identified by the model-based method, which did not strictly correspond to geographical origin. The FHB resistance level of the wheat lines was evaluated in field trials conducted over multiple years or locations by assessing the following traits: % FHB severity, % FHB incidence, % diseased kernels, in spray inoculation trials, and % FHB spread and % wilted tips, in point inoculation trials. Association analysis between SSR markers and the FHB disease traits detected markers significantly associated with FHB resistance, including some that have not been previously reported. The percentage of variance explained by each individual marker was, however, rather low. Haplotype analysis revealed that the FHB-resistant European wheat lines do not contain the 3BS locus derived from Sumai 3. The information generated in this study will assist in the selection of parental lines in order to increase the efficiency of breeding efforts for FHB resistance.

Communicated by F. Ordon.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-008-0822-3) contains supplementary material, which is available to authorized users.

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Introduction

Fusarium head blight (FHB) is a devastating disease of wheat (*Triticum aestivum* L.) that causes significant quality losses, yield losses and accumulation of hazardous mycotoxins in the grain. Numerous species of *Fusarium* have been associated with FHB in wheat, with the predominate species present in western Europe being *Fusarium graminearum* Schwabe [telemorph = *Gibberella zeae* (Schwein.) Pech] and *F. culmorum* (W.G. Smith) Sacc. (Parry et al. 1995; Snijders 1990). While crop management practices and chemical applications may reduce the damage, the deployment of resistant cultivars is the most effective, economical and environmentally friendly means to control the disease (Bai and Shaner 2004). However, breeding for

FHB resistance is difficult for various reasons: (1) the most resistant germplasm is of exotic origin and has poor agronomic traits, (2) the inheritance is oligogenic to polygenic, and (3) screening for FHB resistance is environmentally biased, tedious and expensive (Buerstmayr et al. 2002). In addition, there are at least two main components of resistance: type I, resistance to initial infection and type II, resistance to spread of FHB symptoms (Schroeder and Christensen 1963).

Molecular markers associated with major and minor QTL for FHB resistance from different sources have been detected on almost all of the 21 wheat chromosomes. QTL have been extensively studied and identified in Asian spring wheat germplasm and derivatives: Sumai 3, major QTL on chromosomes 3BS, 6BS, and 5AS, plus minor QTL on 2AS and 6AS (Waldron et al. 1999; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Shen et al. 2003b; Yang et al. 2003); Wangshuibai, a major QTL on chromosome 3BS and minor QTL on 1BS, 2AS, 2AL, 2BL, 2DS, 2DL, 3A, 3D, 4BL, 5A, 5BL, 5D, 6BS, 7AL and 7DS (Lin et al. 2004; Zhang et al. 2004; Zhou et al. 2004; Jia et al. 2005; Mardi et al. 2005; Li et al. 2007); Wuhan-1, on chromosomes 2DL and 4BS (Somers et al. 2003) and Chokwang, major QTL on chromosome 5DL and minor QTL on 3BS and 4BL (Yang et al. 2005). QTL for FHB resistance have been identified in moderately resistant European winter cultivars: Arina, major QTL on chromosomes 4AL, 5BL and 6DL, plus minor QTL on 1BL, 2AL, 2B, 2DL, 3BL, 4DS, 5AL, 6BL and 7AL (Paillard et al. 2004; Draeger et al. 2007; Semagn et al. 2007); Renan, major QTL on chromosomes 2BS and 5AL, plus minor QTL on 2AL, 3BL, 5AS, 5DL and 6DS (Gervais et al. 2003); Dream, on chromosomes 2BL, 6AL and 7BS (Schmolke et al. 2005); Cansas, on chromosomes 1BS, 3DL, 5BL and 7BS (Klahr et al. 2007); Fundulea 201R, on chromosomes 1B, 3AS and 5AS (Shen et al. 2003a); NK93604, on chromosomes 1AL and 7AL (Semagn et al. 2007). QTL have been mapped in the FHB resistant South American spring wheat cultivar, Frontana, major QTL on chromosome 3AL and minor QTL on 5AS and 7AS (Steiner et al. 2004; Mardi et al. 2006) and in North American winter wheat cultivars: Ernie, major QTL on chromosomes 3B, 4BL and 5A, plus minor QTL on 2B (Liu et al. 2007); Freedom, on chromosome 2AS (Sneller et al. 2004). Furthermore, studies have reported minor QTL with favourable contributions to FHB resistance from susceptible varieties: Forno, on chromosomes 3AL, 3DS, 5BL and 6AL (Paillard et al. 2004); Récital, on chromosome 3A (Gervais et al. 2003); Lynx, on chromosome 1B (Schmolke et al. 2005); Ritmo, on chromosomes 1DS, 3B and 7AL (Klahr et al. 2007); Riband, on chromosomes 3DL, 5AS, 7BL and 7DL (Draeger et al. 2007); Patterson, on chromosome 3D and 5BL (Bourdoncle and Ohm 2003; Shen et al. 2003a); Seri82, on chromosome 1BL (Mardi et al. 2006); Alondra, on chromosomes 1B and 2DS (Shen et al. 2003b; Zhang et al. 2004).

Of this large number of QTL detected, the major QTL, which have been confirmed and validated in several mapping populations and environments are considered of immediate interest to breeders. Moreover, despite the considerable progress in the search for alternative sources of FHB resistance, wheat breeding programs globally have, to date, relied heavily on the stable and well characterised resistance derived from the Asian spring wheat, Sumai 3. A major FHB resistance gene, *Fhb1*, has been fine mapped to the QTL peak of *Qfhs.ndsu-3BS*, derived from Sumai 3 (Cuthbert et al. 2006; Liu et al. 2006). Other sources of resistance appear to have a smaller effect on FHB resistance than this major gene (Bai and Shaner 2004). However, the extensive use of a single source of resistance may introduce a selection pressure on the pathogens to erode the effectiveness of the resistance genes involved (Gervais et al. 2003). In addition, exotic sources of FHB resistance, such as Sumai 3, have many undesirable agronomic features (low yield, low quality, susceptibility to other diseases) that hamper breeding strategies. The accumulation of resistance genes from different sources that are better adapted to European conditions may be a more effective strategy for increasing the FHB resistance level of wheat cultivars. Genotypes resistant to FHB, but genetically divergent, and carrying alternative sources of FHB resistance could be used as potential parents in FHB resistance breeding programs. Several studies have investigated the genetic diversity of FHB resistance in wheat lines originating from Asia (Wei et al. 2005; Yang et al. 2005; Yu et al. 2006), Europe (Gosman et al. 2007) or diverse material (Bai et al. 2003; Sun et al. 2003; McCartney et al. 2004). Knowledge of the levels and distribution of genetic diversity in existing gene pools, particularly the genetic relationship between exotic sources of resistance and adapted cultivars, is an essential requirement for developing efficient and effective strategies for exploitation of useful genes in plant breeding and genetic improvement programs.

The objectives of this study were to (1) investigate the patterns of genetic diversity and population structure within the western-European winter wheat gene pool and (2) identify genotypes from the western-European winter wheat gene pool with putatively novel FHB resistance genes.

Materials and methods

Plant material

A set of 295 wheat genotypes consisting of 144 European winter wheat cultivars and 151 advanced breeding lines

developed by various breeding companies in Belgium (103), France (53), Germany (77), Netherlands (16), UK (38), Denmark (4), Czech Republic (1) and Switzerland (3), were investigated in this study. A list of the wheat lines and their respective breeder and country of origin is provided as electronic supplementary material (S1). Included in this set of germplasm were four European winter wheat cultivars known to contain QTL for FHB resistance (Arina, Forno, Dream and Renan). An additional 12 characterised sources of FHB resistance were included in the study as reference lines: eight Asian spring wheat genotypes (Sumai 3 plus four derivatives, Chokwang, Wuhan-1 and Wangshuibai), two spring wheat cultivars from the Americas (Frontana and Alondra), two winter wheat cultivars from the Americas (Ernie and Patterson).

FHB resistance evaluation

Field trials were conducted to evaluate type I resistance (resistance to initial infection), type II resistance (resistance to spread) and overall resistance (combined type I and type II resistance). Two hundred and eighty-six of the total 307 wheat lines were assessed for overall and type I resistance in artificial spray-inoculation trials conducted in 2005 and 2006 by Clovis Matton N.V. in fields located at Tiegem, Belgium (Experiment 1). These trials were conducted as part of the normal operations of the breeding company. A nested design was used with one plot per genotype and each plot divided into two replications that were independently evaluated. Plots consisted of three double rows 2.5 m in length. The centre double rows were spray-inoculated with a mixed conidial suspension of *F. graminearum* and *F. culmorum*. A manual spray tank was used for inoculation. To account for variation in anthesis dates between the genotypes, all plots were inoculated three times at three day intervals around the time of heading. During the inoculation period the plots were irrigated every evening. The plots were rated 22 days after the heading date for (a) % FHB severity, as a measure of overall resistance, by evaluating the % diseased spikelets in a random sample of 20–30 heads per plot and (b) % FHB incidence, as a measure of type I resistance, by evaluating the % heads showing disease symptoms in a random sample of 20–30 heads. At maturity, thirty stems were randomly selected within each plot and the heads were hand harvested and threshed. The number of diseased kernels in a representative sub-sample of 100 seeds was counted by hand to determine the % diseased kernels, as an additional measure of overall resistance. Plant height at maturity and heading date (recorded as days from first January) were also recorded. Plant height at maturity, heading date and % diseased kernels were only recorded in the 2006 trial.

The full set of 307 wheat genotypes were assessed for type II resistance in point-inoculation trials conducted in 2006 at two field locations in Merelbeke, Belgium, with two replications at each location (Experiment 2). Plots consisted of single rows sown at a density of 3 g seed/2 m row. Two highly aggressive *Fusarium* isolates, confirmed by Petri-dish infection tests (Lemmens et al. 1993), were used as inoculum: *F. graminearum* IFA 65 and *F. culmorum* IFA 104. A split plot design was used, with wheat genotypes as the main plots and *Fusarium* species as the sub-plots. Ten heads per sub-plot were inoculated at anthesis by injecting 10 µl of a 100,000 conidia/ml suspension into a single spikelet located one-quarter of the length down the spike. FHB disease symptoms were assessed on days 10, 14, 18, 22 and 26 after inoculation and area under the disease progress curves (AUDPC) were calculated for (a) % FHB spread, by evaluating the % diseased spikelets from the point of inoculation down the spike and (b) the % wilted tips, by evaluating the % heads showing bleaching and wilting symptoms above the point of inoculation.

Molecular marker analysis

DNA was extracted from young leaf material using a modified CTAB method (Saghai-Maroof et al. 1984). A set of 50 SSR markers were selected on the basis of their chromosomal location. Five SSR markers were located in the *QFhs.ndsu-3BS* region, 31 SSR markers were located in chromosome regions associated with other putative QTL for FHB resistance and the remaining 14 SSR markers were selected to give an even coverage of markers across the wheat genome with the aim of assaying at least one SSR marker per chromosome arm. Primer sequences were obtained from the Graingenes website (http://wheat.pw.usda.gov/GG2/index.shtml), Röder et al. (1998), Pestsova et al. (2000) and Song et al. (2005). All forward primers were modified during synthesis with the addition of the M13(-21) universal sequence (5′ TGT AAA ACG ACG GCC AGT 3′) at the 5′ end (Schuelke 2000). PCR conditions were optimised for M13-tailing and fluorescent capillary electrophoresis on an Applied Biosystems 3130 Genetic Analyser. PCR amplifications were performed in a total volume of 25 µl and contained: 0.04 µM forward primer, 0.16 µM M13 primer (5′ TGT AAA ACG ACG GCC AGT 3′) fluorescently labelled with HEX, FAM (MWG Biotech), or NED (Applied Biosystems), 0.2 µM reverse primer, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 1U *Taq* DNA polymerase (Promega) and 50 ng template DNA. PCR was performed on a GeneAmp PCR system 9700 (Applied Biosystems) using the following thermal cycling conditions: 94°C for 5 min, then 8 cycles at 94°C for 1 min, 65–51°C dropping 2°C/cycle for 30 s, 72°C for 1 min, followed by 27 cycles at 94°C for 1 min,

annealing temperature (specific for primer pair, Table 1) for 30 s, 72°C for 1 min and a final extension at 72°C for 10 min. Amplified fragments were sized using the internal molecular weight standard GeneScan-500 ROX and GeneMapper v 3.7 software (Applied Biosystems). Polymorphic information content (PIC) values (Botstein et al. 1980) were calculated for the SSR markers using PowerMarker v3.25 (Liu and Muse 2005).

Phenotypic data analysis

Phenotypic data was analysed by analysis of variance (ANOVA) across years or locations, using the software package SPSS v11.5 for Windows (SPSS Inc.). All traits displayed a normal distribution, except for % FHB incidence, which was skewed towards susceptibility. ANOVA was performed considering genotypes as fixed and replications and environments (years or locations) as random factors. Heading date, plant height and % diseased kernels, were recorded in one replication (Experiment 1, 2006) and were not included in the ANOVA. Correlations between the FHB disease traits were identified using Pearson's coefficient. Genotype means across years for % FHB severity, % FHB incidence, % diseased kernels and genotype means across locations for % FHB spread and % wilted tips, were used to correlate the traits with the genotypic data. Broad-sense heritabilities were estimated according to Nyquist (1991).

Genetic distance-based cluster analysis

CS Chord distance (Cavalli-Sforza and Edwards 1967) was used to calculate pairwise genetic distances among all of the wheat genotypes using PowerMarker software. This distance method produces true tree topology irrespective of the microsatellite model used (Takezaki and Nei 1996). An unweighted pair-group method with arithmetic average (UPGMA) tree with bootstrap values (1,000 permutations performed over all loci) was reconstructed using the majority rule setting of the Consensus program of Phylip v3.63 (Felsenstein 1989) and displayed using the program TreeView (Page 1996).

Model-based cluster analysis

The genetic structure among the 307 wheat genotypes was explored using a model-based method implemented in the software Structure v2.2 (Pritchard et al. 2000). The cluster analysis was based on 1,000,000 iterations, following an initial burn-in of 100,000 iterations and performed using the admixture model and correlated allele frequencies (Falush et al. 2003). For the model to be valid for a self-pollinating species (in which loci are largely homozygous), a haploid dataset was created by deleting one allele at random at each locus (Semon et al. 2005; Ronfort et al. 2006). No prior population information was used to infer the number of clusters and three independent runs were performed at each K value for K ranging from 1 to 10. The optimal number of clusters was predicted when the estimate of ln Pr($X|K$) reached a minimum stable value. Genotypes were assigned to the cluster with the highest probability of membership from each of the K inferred clusters. Clusters of genotypes associated with FHB resistance were identified through correlation of the assigned membership with each of the FHB disease traits using SPSS software.

Genetic variation and differentiation

The average number of alleles per locus and genetic diversity (calculated using an unbiased estimator of average gene diversity, often called expected heterozygosity, (Weir 1996), within geographic origins and within the clusters identified by the Structure analysis were computed using PowerMarker software. Pairwise F_{st} values (Weir and Cockerham 1984) were computed using SPAGeDi v1.2 (Hardy and Vekemans 2002) on the inferred clusters to estimate the between populations component of variation. The statistical significance of the F_{st} values were tested through 1,000 permutations of individuals across groups.

Marker-trait associations

A mixed model association analysis (Yu et al. 2006) for the 295 western European wheat genotypes was implemented in TASSEL using both the large-scale population structure and pairwise kinship coefficients derived from the SSR marker data to correct for population stratification. Rare alleles with an allele frequency <5%, null alleles and residual heterozygosity were treated as missing data (Thornsberry et al. 2001). This reduced the number of marker alleles to 162. The population structure matrix was obtained from Structure software as described above. SPAGeDi software was used to estimate the Loiselle kinship coefficient (Loiselle et al. 1995). Negative values in the kinship matrix were set to zero, implying no relationship. The extent of linkage disequilibrium (LD) across all 47 marker loci was calculated using TASSEL (http://www.maizegenetics.net/) for all pairwise comparisons of SSR loci genome-wide. The significance of LD for SSR pairs was determined by 1,000 permutations. Locus positions of linked markers were determined using the wheat consensus map of Somers et al. (2004).

Table 1 Chromosomal location, associated QTL for FHB resistance, annealing temperature (T_m), number of alleles amplified, number of alleles with a frequency above 0.05 (excluding rare alleles), size range of alleles (bp) and polymorphism information content (PIC) value for the 47 SSR marker loci used in the genetic diversity study of 307 wheat genotypes

Locus	Chr	Associated QTL for FHB resistance	T_m (°C)	No. alleles[s]	No. alleles freq. > 0.05[s]	Allele size (bp)	PIC[s]
Xbarc83	1AS	–	60	3 (3)	3 (3)	255–270	0.19 (0.19)
Xwmc312	1AL	–	60	19 (18)	4 (5)	216–257	0.70 (0.68)
Xgwm413	1BS	*QFhs.nau-1B*[a,b]	60	7 (7)	3 (3)	87–109	0.49 (0.49)
Xwmc44	1BL	*QFhs.fal-1BL*[c]	60	14 (12)	5 (5)	208–267	0.66 (0.64)
Xgwm337	1DS	*QFhs.whs-1DS*[d]	55	9 (9)	5 (5)	153–187	0.64 (0.62)
Xwmc36	1DL	–	60	12 (9)	3 (3)	148–175	0.64 (0.62)
Xwmc407	2AS	Undesignated[e]	60	6 (6)	5 (5)	111–130	0.74 (0.73)
Xgwm148	2BS	*QFhs.inra-2B*[f]	55	7 (7)	5 (5)	140–164	0.55 (0.54)
Xgwm388	2BL	*QFhs.inra-2B*[f]	60	6 (6)	3 (3)	162–172	0.60 (0.59)
Xgwm261	2DS	*QFhs.pur-2D*[g]	60	5 (5)	2 (2)	164–202	0.42 (0.40)
Xgwm539	2DL	*QFhs.fal-2DL*[c]	60	11 (9)	5 (5)	125–154	0.74 (0.73)
Xgwm674	3AS	*QFhs.ndsu-3AS*[h]	60	4 (3)	2 (2)	132–152	0.26 (0.26)
Xgwm155	3AL	*QFhs.fal-3AL*[c]	60	8 (7)	2 (2)	124–152	0.42 (0.39)
Xgwm389	3BS	*QFhs.ndsu-3BS*[i]	55	12 (11)	4 (4)	113–150	0.72 (0.69)
Xbarc075	3BS	*QFhs.ndsu-3BS*[i]	49	4 (4)	3 (3)	107–110	0.49 (0.48)
Xbarc133	3BS	*QFhs.ndsu-3BS*[j]	50	6 (6)	3 (3)	113–126	0.52 (0.48)
Xbarc147	3BS	*QFhs.ndsu-3BS*[k]	50	4 (4)	3 (3)	103–152	0.39 (0.40)
Xgwm493	3BS	*QFhs.ndsu-3BS*[i]	60	12 (9)	2 (2)	110–196	0.43 (0.40)
Xwmc754.2	3BS	*QFhs.crc-3B.1*[l]	58	15 (15)	6 (6)	134–179	0.85 (0.85)
Xwmc754.1	5AS	–	58	3 (3)	3 (3)	127–138	0.52 (0.51)
Xgwm566	3BSc	*QFhs.crc-3B.2*[l]	60	8 (8)	3 (3)	118–140	0.52 (0.51)
Xgwm131	3BL	*QFhs.inra-3B*[f]	60	9 (9)	4 (4)	129–159	0.65 (0.64)
Xgwm161	3DS	*QFhs.fal-3DS*[c]	60	8 (8)	5 (5)	151–229	0.68 (0.67)
Xgdm008	3DL	Undesignated[a]	60	13 (13)	4 (4)	143–177	0.78 (0.78)
Xgwm160	4AL	*QFhs.fal-4AL*[c]	60	8 (7)	2 (2)	175–208	0.23 (0.22)
Xwmc238	4BS	*QFhs.crc-4B*[l]	60	11 (11)	4 (4)	217–237	0.75 (0.74)
Xbarc1096	4BL	*QFhb.ksu-4BL.1*[m]	52	2 (2)	2 (2)	147–157	0.37 (0.37)
Xgwm495	4BL	*QFhs.umc-4BL*[n]	55	6 (5)	3 (3)	155–176	0.34 (0.32)
Xwmc285	4DS	–	60	6 (6)	3 (3)	274–300	0.56 (0.55)
Xcfd84	4DL	–	60	5 (5)	2 (2)	181–187	0.31 (0.29)
Xgwm293	5AS	*QFhs.ifa-5A*[o]	60	7 (6)	3 (3)	166–200	0.48 (0.44)
Xgwm304	5AS	*QFhs.ifa-5A*[o]	60	13 (12)	4 (4)	196–220	0.52 (0.49)
Xwmc754.1	5AS	–	58	3 (3)	3 (3)	127–138	0.52 (0.51)
Xgwm291	5AL	*QFhs.fal-5AL.1*[c]	60	11 (10)	5 (5)	109–168	0.73 (0.72)
Xwmc616	5BS	*QFhs.nau-5B*[b]	60	17 (16)	4 (4)	139–175	0.72 (0.70)
Xgwm371	5BL	*QFhs.fal-5BL*[c]	61	8 (8)	2 (2)	167–186	0.45 (0.44)
Xbarc143	5DS	–	55	5 (5)	4 (4)	227–235	0.62 (0.61)
Xcfd29	5DL	*QFhs.inra-5D*[f]	60	16 (15)	5 (5)	161–206	0.76 (0.74)
Xgwm334	6AS	–	55	6 (6)	3 (3)	111–121	0.56 (0.53)
Xwmc580	6AL	*QFhs.fal-6AL*[c]	55	9 (9)	6 (6)	293–324	0.76 (0.75)
Xgwm361	6BS	*QFhs.nau-6B*[b]	60	4 (4)	3 (3)	131–137	0.43 (0.43)
Xbarc24	6BL	*QFhs.jic-6B*[p]	50	6 (5)	2 (2)	171–189	0.24 (0.20)
Xcfd47	6DL	*QFhs.fal-6DL*[c]	60	5 (5)	2 (2)	182–196	0.37 (0.35)
Xwmc168	7AS	–	60	9 (9)	3 (3)	280–322	0.54 (0.54)
Xwmc607	7AL	Undesignated[q]	60	21 (19)	7 (7)	109–174	0.87 (0.86)

Table 1 continued

Locus	Chr	Associated QTL for FHB resistance	T_m (°C)	No. alleles[s]	No. alleles freq. > 0.05[s]	Allele size (bp)	PIC[s]
Xgwm046	7BS	Undesignated[r]	60	13 (10)	4 (4)	128–168	0.51 (0.49)
Xwmc438	7DS	–	60	6 (5)	1 (1)	241–258	0.13 (0.08)
Xgwm428	7DL	–	60	4 (4)	2 (2)	137–143	0.34 (0.34)
Mean				8.6 (8.0)	3.3 (3.3)	173	0.54 (0.52)

[a] Shen et al. (2003a)
[b] Lin et al. (2004)
[c] Paillard et al. (2004)
[d] Mohler et al. (2002)
[e] Sneller et al. (2004)
[f] Gervais et al. (2003)
[g] Shen et al. (2003b)
[h] Otto et al. (2002)
[i] Anderson et al. (2001)
[j] Liu and Anderson (2003)
[k] Zhou et al. (2002)
[l] Somers et al. (2003)
[m] Yang et al. (2005)
[n] Liu et al. (2007)
[o] Buerstmayr et al. (2002)
[p] Draeger et al. (2007)
[q] Zhou et al. (2004)
[r] Schmolke et al. (2005)
[s] Values for the set of 295 European winter wheat genotypes, excluding the FHB resistant reference lines are given in parenthesis

Results

FHB resistance evaluation

For all FHB disease traits evaluated, ANOVA revealed significant variation ($P < 0.001$) for FHB resistance among the wheat genotypes and genotype-by-environment (year or location) interactions (Table 2). In the point inoculation trials (Experiment 2), where the wheat genotypes were inoculated separately with both *F. graminearum* and *F. culmorum*, ANOVA revealed non-significant genotype-by-isolate and genotype-by-isolate-by-environment effects. The correlation coefficient between *F. graminearum* and *F. culmorum* was $r = 0.85$ ($P < 0.001$) for % FHB spread AUDPC and $r = 0.77$ ($P < 0.001$) for % wilted tips AUDPC. The factor isolates was merged with replications for further statistical analysis. Type II FHB resistance evaluations recorded on day 22 after point inoculation were most strongly correlated with AUDPC ($r = 0.99$, $P < 0.001$ for % FHB spread; $r = 0.97$, $P < 0.001$ for % wilted tips). The heritabilities were good for all traits (Heading date, 0.92; plant height, 0.87; % FHB severity, 0.67; % FHB incidence, 0.75; % diseased kernels, 0.73; % FHB spread, 0.84; % wilted tips, 0.81).

Correlation coefficients for associations between the phenotypic traits are shown in Table 3. As expected, heading date and plant height were not correlated with the traits evaluated in the point inoculation trials (% FHB spread and % wilted tips) where the inoculum was applied directly into a spikelet. All FHB disease traits were correlated to some degree with each other. There was a highly significant correlation between % FHB severity and % FHB incidence ($r = 0.76$, $P < 0.001$). The two measures of type II FHB resistance, % FHB spread and % wilted tips, were also highly correlated ($r = 0.80$, $P < 0.001$). The postharvest assessment of % diseased kernels showed a higher correlation to % FHB spread AUDPC and % wilted tips AUDPC than with % FHB severity and % FHB incidence, which were evaluated 22 days after inoculation. The significant, but low correlations between the FHB disease resistance traits evaluated in Experiment 1 and those evaluated in Experiment 2 could be expected. The spray-inoculation trial (Experiment 1) was designed to measure overall FHB resistance (combined type I and II) and separately type I FHB resistance, whereas the point inoculation trial (Experiment 2) was designed to measure only the type II component of FHB resistance.

Table 2 Analysis of variance for (a) % FHB severity and % FHB incidence on day 22 after inoculation across two years (Experiment 1), and (b) % FHB spread and % wilted tips area under the disease progress curve across two locations (Experiment 2)

Source of variation	% FHB severity			% FHB incidence		
	df	Mean square	*F*	*df*	Mean square	*F*
(a) Experiment 1						
Replications (in years)	1	22	0.95	1	3,759	57.25**
Genotypes	285	2,457	6.89*	285	900	2.31***
Years	1	588	1.65**	1	1,112	2.86
Genotypes × years	88	357	15.27***	88	389	5.92***
Error	374	23		374	66	
Source of variation	% FHB spread			% Wilted tips		
	df	Mean square	*F*	*df*	Mean square	*F*
(b) Experiment 2						
Replications (in locations)	1	7,305,050	800.72**	1	13,999,984	1,663.86***
Genotypes	304	42,077	3.48***	304	338,612	3.14***
Locations	1	22,149	189.81***	1	20,009,030	186.13***
Isolates	1	319,777	90.29***	1	291,670	7.41**
Genotypes × locations	302	11,714	3.79***	302	107,913	2.78***
Genotypes × isolates	300	3,470	1.12	300	38,806	1.00
Genotypes × isolates × locations	300	3,086	0.34	300	38,796	0.46
Error	1,147	9,123		1,147	83,541	

Table 3 Phenotypic correlation coefficients

Trait	Exp 1				Exp 2	
	Plant height (cm)	% FHB severity	% FHB incidence	% Diseased kernels	% FHB spread AUDPC	% Wilted tips AUDPC
Heading date	ns	0.39**	0.40**	ns	ns	0.10*
Plant height		−0.20**	−0.27**	ns	ns	ns
% FHB severity			0.76**	0.11*	0.13**	0.18**
% FHB incidence				0.17**	0.13*	0.19**
% Diseased kernels					0.50**	0.59**
% FHB spread AUDPC						0.80**

Pearson's correlation coefficient

*, ** Indicate significance at $P < 0.05$ and $P < 0.01$ level, respectively

ns indicates no significant correlation

Allele diversity

From the set of 50 SSR markers selected, markers on each of the chromosome arms, except for 2AL, 4AS, 5BS, 6DS and 7BL were polymorphic over all the wheat genotypes. In total 47 SSR marker loci amplified 404 alleles in the total set of 307 wheat genotypes and 375 alleles in the set of 295 western European wheat lines (Table 1). Considering only the European winter wheat lines, the number of alleles detected per locus ranged from 2 (*Xbarc1096*) to 19 (*Xwmc607*) and in the full set of wheat genotypes, from 2 to 21. The average number of alleles per locus was 8.0 and 8.6, in the European and full datasets, respectively. However this lowered to 3.3 (for both datasets) when rare alleles with a frequency less than 0.05 were excluded. The PIC value of the SSR markers ranged from 0.13 (*Xwmc438*) to 0.87 (*Xwmc607*), with an average PIC value of 0.54 for the full dataset. The average observed heterozygosity of the total set of genotypes across all 47 SSR loci was 1.0% and was mainly due to advanced breeding lines that were not yet fixed. Six advanced breeding lines had an observed heterozygosity of ≥ 10% across all loci. However, no locus

had an observed heterozygosity ≥ 10% across all the 307 genotypes. SSR marker diversity for the different geographical origins are summarised in Table 4.

Genetic distance-based cluster analysis

The genetic distance between pairs of wheat genotypes ranged from 0.00 (Excellenz and Opus) to 0.88 (Soissons and Raspail), with an average of 0.54. Distance-based UPGMA cluster analysis divided the set of 307 wheat genotypes into 5 main groups. The dendrogram derived from UPGMA cluster analysis is presented in the electronic supplementary material (S2). The majority of the European lines clustered together in one group, which was further divided into 39 sub-groups of very closely related lines. Breeding lines from the same company formed clear clusters in the dendrogram. Four clusters were divergent from this large group. One group consisted of lines originating from France. The remaining three divergent groups contained the twelve exotic FHB resistant reference lines from Asia and the Americas, with the Asian spring wheat lines forming one cluster.

Model-based cluster analysis

The model-based analysis identified an optimal number of sub-populations when K was set at 7. Independent runs produced highly consistent results and the highest probability run observed at $K = 7$ was used to define cluster membership. The number of wheat genotypes assigned to each of the seven inferred clusters ranged from 12 (Cluster 2) to 83 (Cluster 1). The mean and range of the percentage contribution of individuals assigned to each cluster and the level of genetic diversity in the seven clusters is summarised in Table 5. Graphical representation of the membership of the wheat genotypes in the seven sub-populations is presented as electronic supplementary material (S3). Each cluster comprised of wheat genotypes originating from two (Cluster 3) to eight (Cluster 2) geographical regions. A minimum of two Belgian genotypes were assigned to each of the seven clusters, although Cluster 1, the largest group, contained the majority of the Belgian cultivars and advanced breeding lines (69%). All spring wheat genotypes were assigned to Cluster 2, along with some winter wheat genotypes, mostly from France. Cluster 2 displayed the highest levels of genetic diversity, contained 14 of 15 wheat lines with known QTL for FHB resistance and included all genotypes from Asia, the Americas and Switzerland. The remaining winter wheat line with known FHB resistance, Dream, was assigned to Cluster 7. Cluster 3 contained a similar number of Belgian and German genotypes. Clusters 4 and 6 were a mix of genotypes from Belgium, Germany, France, UK and Denmark. Clusters 5 and 7 were comprised of over 50% German genotypes, plus genotypes from at least three other western European countries. The majority of the genotypes from UK (54%) and Denmark (75%) were assigned to Cluster 4 and the majority (56%) of the genotypes from the Netherlands were assigned to Cluster 5. In general, the wheat lines within the sub-groups identified by the genetic distance-based cluster analysis were assigned to the same sub-populations using the model-based analysis (S2).

The Structure clusters were more genetically differentiated than random assemblages of genotypes, as determined by the permutation tests, and the differentiation among all clusters was significant ($F_{st} = 0.17$, $P < 0.0001$). Between clusters pairwise F_{st} estimates varied between 0.08, Clusters 5 and 7) and 0.34 (Clusters 3 and 6). A low level of differentiation was present between Clusters 2, 5 and 7, which were the only clusters with significant associations with increased FHB resistance (Table 6). Genotypes in Cluster 2 had significant associations with increased FHB resistance for all of the five FHB disease traits evaluated. Genotypes in Cluster 5 had significant associations with increased FHB resistance for % diseased kernels, % FHB spread and % wilted tips, indicating that resistant wheat lines in this cluster possess mainly type II FHB resistance. Genotypes in Cluster 7 had significant associations with % diseased kernels only.

Table 4 Statistical parameters of genetic diversity based on geographical origin of the 307 wheat genotypes

Origin	No. genotypes	Mean no. alleles per locus	Gene diversity
Belgium	103	4.9	0.43
Netherland	16	4.1	0.50
Germany	77	5.5	0.58
France	53	5.4	0.56
UK	38	4.8	0.50
Switzerland	3	2.1	0.28
Denmark	4	2.2	0.30
Asia	8	3.3	0.48
Americas	4	2.5	0.37
Total	306[a]	3.9	0.58

Number of wheat genotypes analysed from each country, mean number of alleles per locus and gene diversity estimated using an unbiased estimator implemented in PowerMarker following Weir (1996)

[a] The one genotype originated from Czech Republic not included in statistics

Haplotyping of 3BS region

A set of five SSR loci (*Xgwm389, Xbarc075, Xbarc133, Xbarc147* and *Xgwm493)* spanning a 10 cM region of the *QFhs.ndsu-3BS* QTL identified in Sumai 3, were used to

Table 5 The number of genotypes assigned to each cluster, the mean and range of the percentage contribution of individuals (*Q*) to each of the clusters, mean number of alleles per locus and gene diversity estimated using an unbiased estimator implemented in PowerMarker

Cluster	Origin[a]	No. genotypes	Mean *Q* (range *Q*)	No. alleles/locus	Gene diversity
1	BEL(71) NLD(5) FRA(7)	83	0.621 (0.330–0.972)	4.5	0.38
2	BEL(3) NLD (1) DEU(2) FRA(12) GBR(3) CHE(3) Asia(8) Americas(4)	36	0.769 (0.309–0.964)	6.6	0.66
3	BEL(7) DEU(5)	12	0.595 (0.441–0.972)	2.2	0.27
4	BEL(10) DEU(11) FRA(18) GBR(20) DNK(3)	62	0.624 (0.251–0.923)	4.9	0.49
5	BEL(7) NDL (9) DEU(26) FRA(5) GBR(4)	51	0.642 (0.353–0.929)	5.3	0.54
6	BEL(2) NLD(1) DEU(12) FRA(7) GBR(11) DNK(1)	34	0.626 (0.392–0.968)	3.7	0.40
7	BEL(3) DEU(21) GBR(4) CZE(1)	29	0.617 (0.361–0.887)	4.4	0.52
All genotypes		307		4.5	0.47

BEL Belgium, *NLD* Netherlands, *FRA* France, *DEU* Germany, *GBR* Great Britain, *CHE* Switzerland, *DNK* Denmark, *CZE* Czech Republic

[a] Number of genotypes for each geographical origin shown in parenthesis

Table 6 Mean disease ratings (±standard error) of the seven inferred clusters for each of the five FHB disease traits (% FHB severity, % FHB incidence, % diseased kernels, % FHB spread, % wilted tips)

Cluster	Exp. 1			Exp. 2	
	% FHB severity	% FHB incidence	% Diseased kernels	% FHB spread	% Wilted tips
1	15.85 ± 0.92	73.05 ± 1.60	65.89 ± 1.60	157.25 ± 8.07	763.43 ± 20.59
2	10.15 ± 1.52**	47.69 ± 2.65**	33.70 ± 2.65**	82.70 ± 11.51**	518.77 ± 29.37**
3	11.36 ± 2.24	56.88 ± 3.90	64.12 ± 3.90	184.21 ± 19.67	862.30 ± 50.15
4	18.23 ± 1.01	71.74 ± 1.76	68.35 ± 1.76	187.21 ± 8.65	868.15 ± 22.06
5	17.31 ± 1.10	72.33 ± 1.92	52.91 ± 1.92**	121.74 ± 9.40**	706.05 ± 23.98*
6	19.25 ± 1.35	76.36 ± 2.35	63.23 ± 2.35	163.04 ± 11.68	784.66 ± 29.80
7	17.33 ± 1.54	75.05 ± 2.67	48.75 ± 2.67**	146.67 ± 12.76	776.85 ± 32.54

*, ** Significant correlation (Pearson coefficient) for resistance between the FHB disease traits and clusters at 0.05 and 0.01 level, respectively

investigate haplotype diversity in the 295 western-European wheat genotypes. Marker order of the SSR markers was determined from the wheat composite map (http://wheat.pw.usda.gov/GG2/index.shtml) and is as listed above. None of the western-European wheat genotypes contained the same alleles as Sumai 3 for SSR loci *Xgwm389* and *Xgwm493*, the flanking markers of the 3BS QTL. The Sumai 3 type allele for *Xbarc147* was common in the European wheat genotypes (54%), while *Xbarc075* and *Xbarc133* Sumai 3 type alleles were rare (13 and 1%, respectively).

Marker-trait association

The association of SSR markers with heading date, plant height and FHB disease resistance traits in the presence of population structure identified marker-trait associations ($P < 0.05$) for all traits evaluated (Table 7). Ten of the 25 SSR markers associated with FHB disease resistance were significant for more than one FHB disease trait. Eight markers were associated with each of the traits, plant height, % FHB incidence and % diseased kernels, nine markers were associated with heading date and % wilted tip, 11 markers were associated with % FHB severity and six markers were associated with % FHB spread. The maximum variance in the disease resistance traits accounted for by these markers was 8%. The maximum variance explained for both heading date and plant height was 5.6%. Twenty-one of the 25 significant marker-trait associations involved markers located in QTL regions previously identified for FHB resistance. All of the cultivars shown in the last column of Table 7 were assigned to Cluster 2 in the Structure analysis,

except Ritmo and Dream, which were assigned to Clusters 5 and 7, respectively, and Freedom and Maringa, which were not included in this study. FHB resistance of Maringa is likely to be inherited from Frontana (Somers et al. 2003), which was included in this study and also assigned to Cluster 2. Marker-trait associations with putative chromosome regions involved in FHB resistance not previously reported in the literature were identified on chromosome arms 1AS, 5DS, 6AS and 7AS. In Table 8, the allelic form of these markers positively associated with FHB resistance traits is shown, together with a number of representative cultivars carrying the allele.

LD analysis showed 23% of the 1,081 possible genome-wide pairwise comparisons had significant LD ($P < 0.0001$) (Electronic supplementary material S4). Of these significant pairwise comparisons, only four had $r^2 > 0.2$. The maximum LD ($r^2 = 0.74$) observed extended for 7 cM for linked locus pair *Xgwm304* and *Xgwm293* on chromosome 5A associated with *QFhs.ifa-5A* (Buerstmayr et al. 2002). Linked markers on chromosome 3B, *Xwmc754.2* and *Xgwm493* (3 cM), associated with *QFhs.ndsu-3BS* (Anderson et al. 2001) showed LD of 0.24 and linked markers on chromosome 4B, *Xbarc1096* and *Xwmc238,* associated with *QFhb.ksu-4BL.1* (Yang et al. 2005) and *QFhs.crc-4B* (Somers et al. 2003), respectively, showed LD of 0.21. Independent markers *Xgwm046*, linked to an undesignated QTL for FHB resistance on chromosome 7BS (Schmolke et al. 2005) and *Xwmc616,* associated with *QFhs.nau-5B* (Lin et al. 2004), located on chromosomes 7BS and 5BS, respectively, exhibited LD of $r^2 = 0.23$.

Discussion

FHB resistance evaluation

Multiple field trials were conducted using two different inoculation techniques (spray and point) in order to evaluate the different components of resistance in the complex FHB disease system. Numerous traits (% FHB severity, % FHB incidence, % diseased kernels, % FHB spread and % wilted tips) were assessed to provide a reliable and thorough characterisation of the level of FHB resistance expressed by each of the wheat genotypes. The measurement of type II resistance in point inoculation experiments is subject to fewer environment influences, such as temperature, humidity, plant development stage, inoculum dose, than type I and overall resistance measured in spray inoculation trials (Bai and Shaner 2004). The postharvest assessment of the % diseased kernels accounted for the continued development of the disease after visual evaluations could no longer be recorded in the field. Despite significant genotype-by-environment interactions, heritabilities of the FHB disease traits were high and comparable to those reported in other studies (Buerstmayr et al. 2002; Gervais et al. 2003; Yang et al. 2003, 2005; Draeger et al. 2007; Klahr et al. 2007; Liu et al. 2007), indicating that the measurements of FHB resistance were reliable. In accordance with the common report that the portion of the head distal to the infection site dies prematurely (Buerstmayr et al. 2002; Lemmens et al. 2005), a high correlation between % FHB spread and % wilted tips was observed. Wilting due to blockage of water and nutrient supplies to the tip of the head could be a downstream effect of the trait % FHB spread. Alternatively, these two traits for type II resistance could be under similar genetic control or under the control of independent but tightly linked genes. The strong correlation between AUDPC and FHB disease traits recorded at day 22 after inoculation is also confirmed by numerous previous reports (Bai et al. 1999; Buerstmayr et al. 2002; Yang et al. 2005). Further evidence of the non-specific nature of FHB resistance (Van Eeuwijk et al. 1995) was also obtained in the point inoculation experiments, where wheat genotypes inoculated with both *F. graminearum* and *F. culmorum* showed no significant difference in resistance level. The inoculum used for the spray inoculation trials consisted of a mixture of *F. graminearum* and *F. culmorum* isolates in order to ensure an appropriate level of aggressiveness in variable environmental conditions. In accordance with Gosman et al. (2007), most of the cultivars on National lists were highly susceptible to FHB. Nevertheless, several wheat lines with good overall resistance to FHB, as well as lines with good type I or type II resistance, under Belgian field conditions, were identified.

Genetic diversity and structure

Although the level of SSR allele diversity found in the 295 western-European wheat lines (8.0 alleles/locus) was lower than that from other studies involving a greater number of European wheat cultivars, including 10.5 alleles at 19 loci on 502 cultivars (Röder et al. 2002) and 16.4 alleles at 39 loci on 480 cultivars (Roussel et al. 2005), allele diversity was greater than the mean allele number per locus for studies involving a smaller number of European wheat lines, including 4.8 alleles at 42 loci on 60 eastern-European cultivars (Stachel et al. 2000) and 6.5 alleles at 52 loci on 56 wheat accessions (Hai et al. 2007). The number of rare alleles observed in this study was 56%. In comparison with other studies on European germplasm, which also considered the number of alleles occurring with a frequency less than 5%, Hai et al. (2007) reported 31% of alleles were rare, whereas Roussel et al. (2005) reported rare alleles at 73%. A twofold variation in genetic diversity was observed in wheat from different European countries. Denmark showed the least variation (gene diversity 0.30) and Germany

Table 7 Association (r^2) of SSR markers with heading date, plant height and five FHB resistance traits (% FHB severity, % FHB incidence, % diseased kernels, % FHB spread and % wilted tips) at $P < 0.05$ and previously reported QTL for FHB resistance in the same regions

SSR locus	Chr	Trait	Exp	Type of FHB resistance	r^2	Previously reported FHB QTL[a]	
						QTL designation	Source of FHB resistance
Xbarc83	1AS	Heading date	1		0.0.316**		
		Plant height	1		0.0215*		
		% FHB severity	1	I + II	0.0294**	–	
		% FHB spread	2		0.0595***		
		% Wilted tips	2		0.0278**		
Xwmc44	1BL	Heading date	1		0.0329*		
		Plant height	1		0.0375*		
		% Wilted tips	2	II	0.0778***	*QFhs.fal-1BL*	Arina
Xgwm337	1DS	Heading date	1		0.0387**		
		% FHB severity	1	I + II	0.0409**	*QFhs.whs-1DS*	Ritmo
		% FHB spread	2		0.0393**		
Xwmc407	2AS	Plant height	1		0.0487*		
		% Diseased kernels	1	I + II	0.0339*	Undesignated	Freedom[b]
Xgwm148	2BS	Heading date	1		0.0313*		
		Plant height	1		0.0363*		
		% FHB Severity	1	I + II	0.0342*	*QFhs.inra-2B*	Renan
		% Diseased kernels	1		0.0371**		
Xgwm388	2BL	% FHB incidence	1	I	0.0195*	*QFhs.inra-2B*	Renan
Xbarc133	3BS	% FHB incidence	1	I	0.0301**	*QFhs.ndsu-3BS*	Sumai 3
Xgwm493	3BS	% Wilted tips	2	II	0.0177*	*QFhs.ndsu-3BS*	Sumai 3
Xwmc754.2	3BS	Plant height	1		0.0556*		
		% FHB spread	2	II	0.0678**	*QFhs.ndsu-3BS*	Sumai 3
		% Wilted tips	2		0.0541**		
Xgwm566	3BSc	% FHB severity	1	I + II	0.0259*	*QFhs.crc-3B.2*	Maringa[b]
		% FHB incidence	1		0.0221*		
		% Diseased kernels	1		0.0336**		
Xgwm131	3BL	Heading date	1		0.0250*		
		% FHB incidence	1		0.0815***		
		% Diseased kernels	1	I + II	0.0244*	*QFhs.inra-3B*	Renan
		% Wilted tips	2		0.0355**		
Xgdm008	3DL	Plant height	1		0.0288*		
Xgwm160	4AL	% FHB severity	1	I + II	0.0148*	*QFhs.fal-4AL*	Arina
		% Diseased kernels	1		0.0123*		
Xbarc1096	4BL	% FHB spread	2	II	0.0130*	*QFhb.ksu-4BL.1*	Chokwang
Xgwm495	4BL	Heading date	1		0.0558***		
		% FHB severity	1	I + II	0.0336**	*QFhs.umc-4BL*	Ernie
		% FHB incidence	1		0.0226*		
		% Wilted tips	2		0.0323**		
Xgwm304	5AS	% Diseased kernels	1	I + II	0.0260*	*QFhs.ifa-5A*	Sumai 3
Xgwm291	5AL	Heading date	1		0.0538**		
		Plant height	1		0.0393*		
Xwmc616	5BS	% FHB severity	1	I + II	0.0367*	*QFhs.nau-5B*	Wangshuibai
Xgwm371	5BL	% FHB spread	2	II	0.0176*	*QFhs.fal-5BL*	Forno
Xbarc143	5DS	% FHB spread	2	II	0.0227*	–	

Table 7 continued

SSR locus	Chr	Trait	Exp	Type of FHB resistance	r^2	Previously reported FHB QTL[a]	
						QTL designation	Source of FHB resistance
Xgwm334	6AS	Heading date	1		0.0254*		
		% FHB severity	1	I + II	0.0381**	–	
		% Diseased kernels	1		0.0206*		
		% Wilted tips	2		0.0415***		
Xwmc580	6AL	% FHB incidence	1	I	0.0390*	*QFhs.fal-6AL*	Forno
Xgwm361	6BS	% Wilted tips	2	II	0.0240*	*QFhs.nau-6B*	Wangshuibai
Xbarc24	6BL	% Diseased kernels	1	I + II	0.0156*	*QFhs.jic.6B*	Arina
Xcfd47	6DL	Heading date	1		0.0175*		
		Plant height	1		0.0269**		
Xwmc168	7AS	% Wilted tips	2	II	0.0218*	–	
Xwmc607	7AL	% FHB severity	1	I + II	0.0531*	Undesignated	Wangshuibai
		% FHB incidence	1	I	0.0449*		
Xgwm046	7BS	% FHB incidence	1	I	0.0366*	Undesignated	Dream

*, **, *** Indicate significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level, respectively

[a] QTL for which the marker has been directly reported, or markers within 15 cM (according to the consensus wheat map of Somers et al. 2004) of markers reported to be linked to FHB resistance in published mapping populations

[b] Cultivar not included in the present study

showed the highest level of variation (gene diversity 0.58). Diversity in European wheat lines can be explained both by temporal and geographical variation trends linked to breeding practices and agricultural policies in different countries (Roussel et al. 2005). The higher genetic diversity observed in wheat lines originating from Germany and France may have resulted from the more frequent use of exotic germplam in the breeding programs of these countries compared with the other western-European countries.

Distance-based cluster analysis showed the genetic relationships among the wheat lines at high levels of similarity. Characterisation of population structure is critical for identifying and correctly interpreting associations between functional and molecular diversity (Prichard and Rosenberg 1999). Model-based methods have recently been used in wheat genetics to identify population structure in diversity studies (Maccaferri et al. 2005; Breseghello and Sorrells 2006; Chao et al. 2007; Hai et al. 2007; Somers et al. 2007; Tommasini et al. 2007). A good consensus between genetic distance-based cluster methods and the model-based cluster methods has been consistently reported in these studies and this was also observed in the present study. The seven clusters identified by the model-based method did not strictly correspond to geographical origin. This lack of correspondence between the genetic clusters and geographical origin could possibly be due to germplasm exchange among breeding programs. Additionally, many breeding companies target markets in different European countries associated with regional agro-ecological conditions. To our knowledge, lines with available pedigree information were observed in the same cluster. All of the wheat lines in the four groups divergent from the large grouping of European wheat lines in the distance-based method were assigned to Cluster 2 in the model-based method. The high level of gene diversity for Cluster 2 (0.66) reflects the heterogeneous origin of this group. It contains all spring wheat and winter wheat reference lines, except Dream, which was assigned to Cluster 7. Most other winter wheats assigned to Cluster 2 are of French origin, but bred by six different companies (S1).

Combining wheat cultivars with good type I FHB resistance from Cluster 2 (such as, Arina, Cadenza, Farandole, Frodo, Hurley, Kansas, Renan, Segor, SWTopper, Tybalt) with cultivars with good type II resistance from Cluster 5 (such as, Captor, Centenaire, Certo, Drees, Ephoros, Herrmann, Koch, Plectrum, Sokrates, Solitär) maybe an efficient and effective strategy for pyramiding different sources of FHB resistance to enhance the overall level of FHB resistance within the western-European winter wheat gene pool. Importantly, clusters of wheat lines with increased susceptibility to FHB were also identified. Exclusion of these lines in future breeding is likely to decrease the risk of FHB epidemics through the removal of sources for inoculum build-up.

Haplotyping of 3BS region

SSR marker *Xgwm389*, the flanking marker at the distal end of the major QTL on chromosome 3BS conferring type II

Table 8 SSR markers not previously reported to be associated with FHB resistance

SSR locus	Allele (bp)	Trait	Genotypes with marker[a] (mean ± SE)	Genotypes without marker[a] (mean ± SE)	Difference of means
Xbarc83	273	% FHB severity	18.82 ± 3.74 (13)	16.29 ± 0.63 (273)	−2.53
		% FHB spread	160.76 ± 15.22 (16)	149.47 ± 4.43 (289)	−11.29
		% Wilted tips	833.52 ± 44.35 (16)	753.92 ± 13.03 (289)	−79.60
	285[b]	%FHB severity	15.94 ± 0.63 (261)	21.18 ± 2.82 (25)	5.23
		% FHB spread	145.81 ± 4.25 (273)	186.34 ± 17.71 (32)	40.52*
		% Wilted tips	749.37 ± 12.84 (273)	832.56 ± 47.82 (32)	83.19*
	288	% FHB severity	23.72 ± 4.3 (12)	16.09 ± 0.62 (274)	−7.63
		% FHB spread	211.91 ± 31.27 (16)	146.64 ± 4.10 (289)	−65.27
		% Wilted tips	831.6 ± 86.51 (16)	754.03 ± 12.41 (289)	−77.58
Xbarc143	245[c]	% FHB spread	131.42 ± 11.48 (33)	152.54 ± 4.53 (275)	21.12
	247[d]	% FHB spread	82.40 ± 61.07 (4)	157.17 ± 4.21 (304)	68.78
	249[e]	% FHB spread	138.09 ± 15.14 (33)	151.74 ± 4.39 (275)	13.65
	251[f]	% FHB spread	143.74 ± 7.48 (115)	154.18 ± 5.09 (193)	10.44
	253	% FHB spread	166.93 ± 5.57 (123)	139.21 ± 5.88 (185)	−27.72***
Xgwm334	129	% FHB severity	16.98 ± 2.17 (32)	16.23 ± 0.66 (253)	−0.75
		% Diseased kernels	59.80 ± 3.66 (32)	59.46 ± 1.27 (253)	−0.34
		% Wilted tips	775.60 ± 44.23 (35)	756.22 ± 13.08 (269)	−19.38
	131	% FHB severity	19.85 ± 3.50 (10)	16.19 ± 0.64 (275)	−3.66
		% Diseased kernels	65.00 ± 8.35 (10)	59.30 ± 1.20 (275)	−5.70
		% Wilted tips	640.95 ± 93.27 (12)	763.28 ± 12.54 (292)	122.33
	133	% FHB severity	18.19 ± 1.32 (79)	15.60 ± 0.71 (206)	−2.59
		% Diseased kernels	60.26 ± 2.50 (79)	59.21 ± 1.35 (206)	−1.06
		% wilted tips	792.0 ± 22.93 (84)	745.65 ± 15.03 (220)	−46.35
	135[g]	% FHB severity	6.20 ± 3.18 (2)	16.39 ± 0.64 (283)	10.19
		% Diseased kernels	23.54 ± 13.54 (2)	59.75 ± 1.19 (283)	36.21*
		% Wilted tips	376.74 ± 115.91 (2)	760.98 ± 12.57 (302)	384.24**
	137[h]	% FHB severity	3.01 ± 1.90 (3)	16.46 ± 0.63 (282)	13.44*
		% Diseased kernels	15.32 ± 7.00 (3)	59.97 ± 1.18 (282)	44.64***
		% Wilted tips	435.10 ± 200.7 (5)	763.86 ± 12.24 (299)	328.77
	139[i]	% FHB severity	15.41 ± 0.76 (159)	17.46 ± 1.06 (126)	2.05
		% Diseased kernels	60.00 ± 1.38 (159)	58.87 ± 2.07 (126)	−1.13
		% Wilted tips	760.70 ± 14.33 (166)	755.76 ± 21.89 (138)	−4.94
Xwmc168	305[j]	% Wilted tips	745.11 ± 16.98 (160)	780.72 ± 18.74 (128)	35.61
	307	% Wilted tips	795.64 ± 25.48 (58)	752.18 ± 14.39 (230)	−43.46
	309	% Wilted tips	778.31 ± 27.47 (64)	755.97 ± 14.20 (224)	−22.34
	328[k]	% Wilted tips	744.32 ± 251.68 (2)	761.05 ± 12.63 (286)	16.73
	332[l]	% Wilted tips	577.08 ± 310.14 (2)	762.22 ± 12.57 (286)	185.14

The mean FHB disease score with and without the marker allele are shown for all alleles (frequency > 0.05) of each of the markers, along with the difference of the two marker class means. Moderately resistant western-European wheat cultivars with the allele positively associated with FHB resistance are listed

*, **, *** Indicate significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$ level, respectively

[a] Number of wheat genotypes in each of the marker classes is shown in parenthesis

[b] Capnor, Ephoros, Herrmann, Hurley, Milvus, Nirvana, Paroli, Plectrum, Quebon, Renan

[c] Alchemy, Alonso, Bagou, Centenaire, Claire, Mulan, Nirvana, Perfector, Piranha, Zebedee

[d] Parador, Renan, Segor

[e] Akratos, Buteo, Drees, Ephoros, Hurley, Jenga, Savoy, Striker, SWTopper

[f] Albatros, Alitis, Apache, Capnor, Cardos, Farandole, Herrmann, Milvus, Pytagor, Tybalt

[g] Capnor, Hurley

[h] Enorm, Segor

[i] Attlass, Boisseau, Drees, Herrmann, Mulan, Romanus, Sokrates, Solitär, Tommi, Tybalt

[j] Bagou, Buteo, Claire, Ephoros, Ernie, Mulan, Nirvana, Pytagor, Quebon, Soissons

[k] Hurley

[l] Renan

FHB resistance, was highly polymorphic among the set of European winter wheat germplasm (PIC values 0.69), while all the other markers within the 10 cM QTL region had lower PIC values (0.40–0.48). Haplotyping of the 3BS region associated with type II FHB resistance in the Asian cultivar Sumai 3, conferred with the results of Gosman et al. (2007) that the 3BS locus is absent among continental European cultivars. The Sumai 3 haplotype at the 3BS region was also rare in a worldwide collection of 54 FHB resistant and moderately resistant lines (Liu and Anderson 2003). Advanced breeding lines derived from Sumai 3 have been reported to contain the same haplotype at the 3BS locus (McCartney et al. 2004; Yang et al. 2006; Yu et al. 2006). Wheat lines with resistance to FHB, yet lacking SSR alleles similar to those of any characterised FHB resistant cultivar are likely to be carrying potentially novel resistance.

Marker-trait associations

Taking population structure into account, we found significant associations between FHB disease resistance traits and SSR markers in western-European germplasm. At a low marker density, markers linked to QTL for all of the FHB resistance traits evaluated (% FHB severity, % FHB incidence, % diseased kernels, % FHB spread and % wilted tips) were found. The genome-wide association analysis had a low resolution accounting for the number of assessed loci per chromosome. Increasing the marker density could lead to markers more closely linked to the QTL regions, explaining a greater proportion of the variation than detected in this study. SSR markers associated with both major and minor QTL for FHB resistance, explaining from 2 to 41% of the genetic variation in original mapping populations for QTL regions spanning from 5 to 39 cM were used in this study. Thus, it is important to note that for these SSR markers, similar haplotypes do not necessarily represent similar resistance loci. Nevertheless, most of the associations found represented previously reported QTL for FHB resistance. Also putative associations between traits and marker regions were identified that have not been previously implicated to influence FHB resistance. Thus, at least part of the variation for FHB resistance in the western-European cultivars and advanced breeding lines is likely to be due to novel loci that have not previously been detected in QTL mapping studies. Mapping populations could be developed to confirm the marker-trait associations and investigate in greater detail the novel FHB resistance genes identified in this study.

LD among the set of European winter wheat lines was observed around QTL regions for FHB resistance, although, it is possible that the extent of LD observed among unlinked loci was influenced by the close relatedness of the wheat lines studied (Breseghello and Sorrells 2006). In the presence of LD extending for the distance of several centiMorgans, it is possible to identify genetic regions associated with a particular trait of interest by genome-wide scans. The extent of genome-wide LD patterns have been investigated in durum wheat (Maccaferri et al. 2005; Somers et al. 2007) and common wheat (Breseghello and Sorrells 2006; Chao et al. 2007; Tommasini et al. 2007) to determine the implications of applying association mapping in wheat. These studies have indicated that there is extensive variation in the extent of LD throughout the wheat genome, with closely linked markers frequently not in LD and the possibility of LD existing between distant markers. The initial assessment of LD in our study provided an indication that the LD block in the centrometric region of chromosome 5A, observed by Breseghello and Sorrells (2006) in a set of winter wheat germplasm from the United States, also exists in this comprehensive set of European winter wheat. Future association studies should focus on the identified QTL regions and saturate those regions with markers or candidate genes for disease resistance.

Knowledge of the presence or absence of markers linked to QTL for FHB resistance will assist in the selection of parental lines in order to combine different sources of FHB resistance and increase the efficiency of breeding efforts. The discovery of new sources of resistance and the development of molecular markers is of great interest to effectively introgress and pyramid resistance genes into adapted western-European winter wheat cultivars.

Acknowledgments We wish to thank J. Dermaut and L. Vandenabeele (Clovis Matton N.V., Belgium) for conducting the spray-inoculation field trials and for supplying seed, and M. Lemmens (IFA-Tulln, Austria) for advice and for supplying the *Fusarium* isolates. Thanks also to C. Merckaert, N. Mergan and K. Succaet for excellent technical assistance. Financial support was provided by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Vlaanderen).

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