Evaluation of genetic diversity of Fusarium Head Blight resistance in European winter wheat

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INTRODUCTION

Molecular markers associated with major and minor QTLs for Fusarium Head Blight (FHB) resistance from different sources have been detected on almost all of the 21 wheat chromosomes. Despite the considerable progress in the search for alternative sources of FHB resistance, a limited number of sources of FHB resistance have been identified in adapted European wheat cultivars and breeding programs globally have, to date, relied heavily on the stable and well characterised resistance derived from the Asian spring wheat, Sumai 3. 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. In addition, exotic sources of FHB resistance 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.

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. Included in this set of germplasm were four European winter wheat cultivars known to contain QTLs for FHB resistance (Arina, Forno, Dream and Renan). An additional 12 characterised sources of FHB resistance were included as reference lines: 8 Asian spring wheat genotypes (Sumai 3 plus four derivatives, Chokwang, Wuhan-1 and Wangshuibai), two spring wheat cultivars (Frontana and Alondra) and two winter wheat cultivars (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). Artificial spray-inoculation trials were conducted in 2005 and 2006 by the breeding company Clovis Matton N.V. in fields located at Tiegem, Belgium. To account for variation in anthesis dates between the genotypes, all plots were inoculated with a mixed suspension of *F. graminearum* and *F. culmorum*, three times at three day intervals around the time of ear emergence. The plots were rated 22 days after the date of ear emergence for (a) % FHB severity (overall resistance), by evaluating the % diseased spikelets in a random sample of 20-30 heads per plot and (b) % FHB incidence (type I resistance), by evaluating the % heads showing disease symptoms in a random sample of 20-30 heads. Grains were harvested at maturity and assessed for % diseased kernels (an additional measure of overall resistance). Plant height at maturity and date of ear emergence were also recorded.

Point-inoculation trials to assess type II resistance were conducted in 2006 at two field locations in Merelbeke, Belgium, with two replications at each location. Resistance to *F. graminearum* and *F. culmorum* was assessed separately using a split plot design, in which main plots (wheat genotypes) were split into two sub-plots (*Fusarium* isolate). Ten heads per sub-plot were inoculated at anthesis by injecting a pure spore suspension into a single spikelet. 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

A set of 50 SSR markers were selected for analysis. Five SSR markers were located in the *QFhs.ndsu-3BS*[1] 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. PCR assays were performed using M13-tailing and fluorescent capillary electrophoresis on an Applied Biosystems 3130 Genetic Analyzer.

Cluster analysis

CS Chord distance was used to calculate pairwise genetic distances among all of the wheat genotypes using PowerMarker software. 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.

The genetic structure among the wheat genotypes was also explored using a model-based method implemented in the software Structure v2.2. Pairwise Fst values were computed using SPAGeDi v1.2 on the inferred clusters to estimate the between populations component of variation. The statistical significance of the Fst values was tested through 1,000 permutations of individuals across groups. Clusters of genotypes associated with FHB resistance were identified through correlation of the assigned membership with each of the FHB disease traits using SPSS.

Marker-trait associations

A mixed model association analysis 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. This reduced the number of marker alleles to 162. SPAGeDi software was used to estimate the Loiselle kinship coefficient. The extent of linkage disequilibrium (LD) across all 47 marker loci was calculated using TASSEL 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[2].

RESULTS AND DISCUSSION

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. In the point inoculation trials, ANOVA revealed non-significant genotype-by-isolate and genotype-by-isolate-by-environment effects. Thus, 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 (Ear emergence, 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). 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).

Allele diversity

From the set of 50 SSR markers analysed, 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 full set of 307 wheat genotypes and 375 alleles in the set of 295 western European wheat lines. Considering only the European winter wheat lines, the number of alleles detected per locus ranged from 2 (*Xbarc1096*) to 19 (*Xwmc607*). The average number of alleles per locus was 8.0 and 8.6, in the European and full datasets, respectively. The PIC value of the SSR markers ranged from 0.13 (*Xwmc438*) to 0.87 (*Xwmc607*), with an average PIC value of 0.54.

Genetic distance-based cluster analysis

Distance-based UPGMA cluster analysis divided the set of 307 wheat genotypes into 5 main groups. 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, with the Asian spring wheat lines forming one cluster.

Model-based cluster analysis

The model-based analysis identified an optimal number of subpopulations when K was set at 7. The number of wheat genotypes assigned to each of the 7 inferred clusters ranged from 12 (Cluster 2) to 83 (Cluster 1). Each cluster comprised of wheat genotypes originating from two (Cluster 3) to 8 (Cluster 2) geographical regions. 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 QTLs for FHB resistance. 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 was 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.

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 (Fst = 0.17, P<0.0001). Between clusters pairwise Fst 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 1). 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 1: Mean disease ratings (± standard error) of the 7 inferred clusters for each of the five FHB disease traits.

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

*, ** indicate significance at P<0.05 and P<0.01, respectively.

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

Analysis of a set of five SSR loci (*Xgwm389, Xbarc075, Xbarc133, Xbarc147* and *Xgwm493)* spanning a 10cM region of the *QFhs.ndsu-3BS* revealed that none of the western-European wheat genotypes contained the same alleles as Sumai 3 for SSR loci flanking the QTL (*Xgwm389* and *Xgwm493*). 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 associations

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, 9 markers were associated with ear emergence date and % wilted tip, 11 markers were associated with % FHB severity and 6 markers were associated with % FHB spread. The maximum variance in the disease resistance traits accounted for by these markers was 8%. Twenty-one of the 25 significant marker-trait associations involved markers located in QTL regions previously identified for FHB resistance. 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.

LD analysis showed 23% of the 1081 possible genome-wide pairwise comparisons had significant LD (P<0.0001). Of these significant pairwise comparisons, only four had R^2 values > 0.2. The maximum LD (R^2 = 0.74) observed extended for 7cM for linked locus pair *Xgwm304* and *Xgwm293* on chromosome 5A associated with *QFhs.ifa-5A*. This initial assessment of LD in our study provided an indication that the LD block in the centrometric region of chromosome 5A, observed in a set of winter wheat germplasm from the United States[3], 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.

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