Unravelling and exploitation of diversity for resistance to *Mycosphaerella graminicola* and *Fusarium graminearum* in wheat and its progenitors

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Résumé

La fusariose et la septoriose sont deux maladies très dommageables (chute de rendement, toxicité) pour le blé. Il est important d'identifier de nouvelles sources de résistance efficaces contre des isolats de septoriose très virulents et contre la fusariose *Fusarium graminearum* (majoritairement présente en France). Ainsi, ce projet nous a permis d'identifier de nouvelles sources de résistance à la fusariose et à la septoriose. Parmi celles-ci, les plus intéressantes ont été retenues pour être les parents de nouvelles populations (HD) d'étude.

Parallèlement, une population de 235 lignées HD Apache/Balance (ApBa) a été étudiée pour identifier des QTL de résistance à la fusariose qui offrent la possibilité au sélectionneur d'identifier plus efficacement, dans leur matériel, les caractères de résistance issus d'Apache.

Identification des nouvelles sources de résistance à la fusariose et à la septoriose.

Fusariose

Les tests de résistance à la fusariose ont été menés sur une soixantaine de lignées avec 3 méthodes d'inoculation sur 3 ans. L'inoculation par cannes de maïs en périmètre irrigué avait pour objectif de reproduire les conditions naturelles présentes chez l'agriculteur, mais avec des conditions très favorables au développement du champignon. L'inoculation par pulvérisation de spores de *F. graminearum* a permis d'évaluer la résistance du matériel avec une pression parasitaire maximale. La méthode (spot) par injection de spores dans les épillets a permis d'observer la résistance à la propagation du champignon dans l'épi (résistance de type II). La teneur en DON a également été mesurée dans le grain des lignées.

Des lignées comme 04CYBHFU12, 04CYBHFU17, C10SRSN165, C10SRSN104, C10SRSN215, C10SRSN108, C10SRSN315, A39.9.2.1, IFA.136.6.1.1, 20828 se sont révélées avoir un excellent niveau de résistance à la fusariose, avec très peu de symptômes (quelle que soit la méthode d'inoculation et avec de faibles teneurs en DON dans le grain).

Septoriose

Les tests de résistance au stade adulte ont été menés sur deux lieux avec un ou 2 isolats de *M. graminicola* différents (IPO232 et IPO98047) selon les années. Le matériel a également été testé au stade jeune plante avec 31 isolats choisis pour maximiser les virulences. Ces isolats ont été prélevés dans plusieurs lieux de l'Hexagone.

Nous avons identifié les lignées (FD02112 et FHD2054.3) les plus résistantes au stade jeune plante ainsi que les lignées (Bio719, Bio5019) avec un excellent niveau de résistance à la septoriose au champ. Ce matériel a également été testé pour vérifier l'absence de gènes majeurs connus par marquage moléculaire et comparaison avec une gamme d'hôtes différentiels.

Production à partir des sources de résistances de 30 nouvelles populations HD d'étude

A partir des résultats obtenus sur la fusariose et la septoriose, le développement de 30 populations HD a été initiée et quatre sont maintenant obtenues (FD12/SE11, Mercato/Bio2000, Robigus/Soissons et Chine 94.4/Guadalupe). Les 3 premières présentent un intérêt pour l'étude de la résistance à la septoriose, alors que la dernière a été développée pour l'étude de la résistance à la fusariose. Les autres populations, en cours d'obtention, ont des géniteurs comme 04CYBHFU12, A39.9.2.1…

Étude la résistance de la population HD Apache/Balance (ApBa) et identification de QTL

Les 235 lignées HD qui composent la population ApBa ont été testées au champ avec des inoculations par pulvérisation et injection de spores de *F. graminearum* durant les 3 ans du projet.

Un travail de cartographie a été effectué avec 231 marqueurs DArT et 184 marqueurs SSR afin de détecter des QTL de résistance. Cinq QTL ont été identifiés sur les chromosomes 2D, 3B, 4D, 7A et 7B. Le QTL identifié sur le chromosome 4D colocalise avec le gène *Rht2* (présent chez Balance) confirmant l'effet négatif de ce gène de nanisme sur la résistance à la fusariose.

1. Introduction

Septoria tritici blotch (STB) and *Fusarium* head blight (FHB) are major threats to European wheat production. STB is caused by the ascomycete *Mycosphaerella graminicola* and is one of the most devastating diseases in wheat throughout Europe. Initial inoculum usually originates from airborne ascospores that infect newly sown wheat crops from autumn until spring, depending on the weather conditions. Although, it was found that ascospores can be produced year-round, observations and simulations (Hunter et al., 1999; Kema et al. 1996; Eriksen et al., 2001) indicate that the asexual splash dispersed pycnidiospores are the major drivers of the epidemic development in the growing season. *M. graminicola* on wheat can be controlled by protective fungicides. It is estimated that approximately 600M€ are spent on fungicide input to control this disease in Western Europe annually. Significantly increased costs of disease management, environmental pollution and the evolution of fungicide resistant strains are major negative aspect of fungicide applications (Fisher and Griffing, 1984; Felsentein, 1999). *M. graminicola* has a heterothallic bipolar mating system, which is equivalent with out breeding in plants, resulting in extremely diverse pathogen populations that are responsible for the aforementioned observations (Kema et al., 1996).

Resistance breeding is an important alternative and hence resistance genes will have to be available for commercial breeding programs. The interaction between wheat and *M. graminicola* was studied in a complementary genetic study of host resistance and pathogen avirulence, leading to a formal conclusion that both organisms interact in a gene-for-gene manner (Brading et al., 2002). In recent years 15 major genes for resistance to *M. graminicola, stb1 – stb15*, have been identified based on interactions between wheat cultivars and *M. graminicola* isolates (Somasco et al.,1996; Arraiano et al., 2001; Brading et al., 2002; Adhikari et al ., 2003; McCartney et al., 2003; Adhikari et al., 2004a,b,c; Chartrain, 2004; Chartrain et al., 2005a,b; Arraiano et al., 2007). This gene pool, however, is too narrow and many of these genes have only partial functionality to the natural populations in Europe. Thus new sources of resistance need to be identified and characterized in order to support resistance breeding.

FHB or scab is caused by a suite of Fusarium fungi, with *Fusarium graminearum* as a major representative. FHB is a devastating disease in humid and semi humid areas worldwide (Bai and Shaner, 1994). The pathogen is capable to infect wheat and other small grain cereals like barley, rice, oat and maize (Goswami et al., 2004). This destructive disease has the capacity to destroy a potentially high-yielding crop within a few weeks (McMullen et al., 1997). Direct and secondary economic losses due to FHB for all crops in the central and Northern great plains in the United States were estimated to be 2.7 billion$ from 1998 to 2000 alone (Nganje et al., 2002). Moreover, accumulation of mycotoxins, especially deoxinonvalenol (DON), in the infected grain can significantly reduce food quality and feed safety (McMullen et al., 1997; Benett and Klich, 2003). DON also was reported as a virulence factor to aid colonization of the pathogen in the host (Jansen et al., 2005; Desjardins et al., 1996).

The principal mode of fungal spread in wheat from floret to floret inside the spikelet and from spikelet to spikelet is through the vascular bundles in the rachis and rachilla (Ribichich et al., 2000). The fungus may not be able to move into rachis in the absence of DON because strong cell wall fortifications may develop in rachis nodes (Jansen et al., 2005). Collectively, resistance to FBH is controlled by one or few major genes and several minor genes (Bai et al., 1999; Waldron et al., 1999). Fungicide treatments and agronomical management only reduce the yield damage to some extent, but cannot prevent quality reduction (Mielke,1988; Teich, 1989; Milus and Parsone, 1994; Jiang et al., 2007). Hence, the development of resistant cultivars is the most effective approach to control FHB. Resistance mechanisms to FHB in wheat were classified as either passive or active (Mesterhazy, 1995). Passive mechanisms are associated with phenotypic traits such as plant height, presence of awns, spikelet density and time of flowering. Active mechanisms includes five different components; (i) Type I resistance to initial infection, (ii) Type II resistance to spread of infection, (iii) Type III resistance to kernel infection, (iv) Type IV characterizes tolerance and (v) Type V resistance to mycotoxins accumulation

Despite the wide genetic variation for resistance to STB and FHB in wheat and its progenitors, the number of genes that has been characterized and mapped is limited. Hence, a few major sources of resistance are intensively deployed by breeders. This project aims at unlocking the vast genetic variation for resistance to STB and FHB in synthetic wheat hexapoloids (derivatives) and under evaluated wheat cultivars, which reduces the vulnerability of wheat to these diseases.

The specific objectives of this project are: (i) Identify and characterize new resistance genes to *M. graminicola* and *F. graminearum*; (ii) Determine the map positions of these new resistance genes; and (iii) Develop molecular markers for these genes, aiming at molecularly assisted breeding to effectively improve resistance levels STB and FHB.

2. Septoria in France

France is a major wheat producer in Western Europe, contributes with 6 % to the total global production and has STB as the major threat to is national production. As stated above resistant cultivars provide the most environmentally sound and sustainable approach to control STB. Fifteen resistant genes to STB have been identified but this is truly scarce compared to the characterized 38 genes for stem rust resistance, 34 genes for stripe rust resistance, 52 genes for leaf rust resistance and 32 genes for powdery mildew resistance. Hence this project calls for a comprehensive explorative approach to find new genes for resistance to STB. Existence of differential series and molecular markers for stem, leaf and stripe rust as well as powdery mildew facilitate gene postulation to identify new resistant genes among the wheat cultivars. However, gene postulation for STB is only in its infancy. According to the gene – for –gene model in the wheat – STB interaction, resistance is expressed when the host and pathogen have matching resistance and avirulence genes (Brading et al., 2002). One of the first required studies, therefore, is to describe the virulence spectrum of the French *M. graminicola* population in order to determine the utility of the Stb genes in a French context.

▶ Material and Methods

Pathogen isolates

We collected single pycnidium isolates from leaves that were hierarchically sampled from five locations in France (Villaines la Gonais, St. Pol de Léon, Cappelle en Pévèle, Beauce and Aire d'Havrincourt), and included for comparison two Dutch tester isolates, IPO323 and IPO94269.

Plant materials

Thirteen wheat cultivars comprising 11 cultivars that carry different *Stb* genes and two checks were tested. Ten seeds per pot were linearly sown in 5 by 5 cm plastic pot with a steamed sterilized peat/sand mixture. Plants were grown in a controlled greenhouse chamber with similar pre/post inoculation conditions with day length 16 hour /days. Pre/post inoculation temperature and related humidity (RH) conditions were 18/16 °C (day/night rhythm) and 70 % RH, and 22/21 °C and ≥ 85 % RH, respectively.

Inoculum preparation, inoculation and data collection

Fifty ml yeast-glucose liquid medium (30gr Glucose, 10 gr yeast per 1 litter of demineralised water) was prepared in 100 ml Erlenmeyer flasks that were individually inoculated with the abovementioned isolates and were subsequently incubated in a shaker with 125 RPM and 18 °C for 5-6 days. Spores concentration were determined using a coulter counter and was adjusted to a 10^7 spores/ml in a total volume of 30 ml supplemented by two drops of Tween 20 for inoculation. Plants were inoculated 7-10 days after sowing on the first leaf appearance, using a turntable, adjusted at 13.5 rpm, in a closed inoculation cabinet equipped with interchangeable atomizer and a water cleaning device to avoid contamination.

Incubation was conducted under polyethylene covered aluminum frames in transparent plastic bag for every isolate, providing suitable leaf wetness for 48 h at a light intensity of approximately 3µE sec^{-1} m^{-2}. Newly emerged second and topper leaves were clipped 10-14 days after inoculation in order to facilitate light penetration to primary leaves and disease assessment.

Disease severity was evaluated 21 days after inoculation by scoring, the percentage of necrosis (N) and pycnidia (P), estimated as percentage of the total primary leaf area.

Design and data analysis

An alpha lattice layout with three replications was used to accommodate all replications over time. All collected percentage data were transformed according to an empirical logistic model. Subsequently a weighted mixed model REML analysis was performed assuming that variation among and within individual trays in an experiment is normally distributed with zero means and variances.

▶ Results

The symptom development in all experiments was excellent and we used percentage of pycnidia as the evaluation criterion. Statistical analysis showed a highly significant interaction component, indicating strong genetic differences between the tested cultivars and isolates (data not shown).

Table 1 provides a summary of the data. Clearly, none of the *Stb* gene cultivars is effective to all French isolates. Some genes such as *Stb9* are largely ineffective, whereas *Stb5* is resistant to the majority of the French *M.graminicola* isolates. We also observed a range of specific resistances such as for cvs. Estanzuela Federal (*Stb7*), M6 synthetic (w7984) (*Stb8*). The most virulent isolates that circumvent the majority of Stb resistance genes are isolates IPO323, IPO98022, IPO98046, IPO98047 and IPO98094.

▶ Discussion

Our current data accord with previous data that *M.graminicola* interacts in a gene-for-gene manner with its host. This implies that testing with natural populations or isolate mixtures will not reveal the potential resistance phenotypes breeders are looking for. Hence, experiments with individual isolates will provide more insight in differences in resistance and possibly in stability of resistance. Breeding for resistance to STB is a major endeavor, which requires continuous phytopathological support.

Not in the last place to select the relevant isolates for screening breeding material. We have identified isolates that are highly valuable for discriminative testing of breeder's germplasm. In addition we suggest to screen with an

		***M. graminicola* isolates**																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Wheat cultivar	Stb Gene	323	94269	98001	98021	98022	98028	98031	98032	98033	98034	98035	98038	98042	98046	98047	98050	98051	98057	98072	98075	98078	98094	98097	98099	98113	99018	99031	99032	99038	99048
Bulgaria	Stb1, 5BL , stb6															37	0														
Veranopolis	Stb2, 3Bs stb11, Stb6			0	0	26											0										0				0
Israel 493	Stb3, 7As , Stb6			0	0											36															0
Tadinia	Stb4, 7Ds, Stb6	0		0	0																		34								0
CS/synthetic (6x)	Stb5, 7Ds			0	0							0			13												0				0
Shafir	Stb6, 3As			0												54															0
Estanzuela Federal	Stb7, 4AL																						64								
M6 Synthetic (w7984)	Stb8, 7BL																						74								
Courtot	Stb9, 2B	81																													
Kavkaz - K4500	Stb6(3As),7(4AL), 10(1D),12(4AL)			0												31	0														0
TE9111	Stb11, 1Bs , stb7	0			0																		67								0

0 = No pycnidia P% < 20 20 < P%< 40 P% > 40

Table 1: Responses of differential cultivars with mapped Stb genes to 30 M. graminicola isolates.

allopatric isolate set comprising isolates from Algeria, Canada, Ethiopia, Iran, Mexico, Peru, Portugal, Syria, Turkey, Uruguay and USA to test the stability of resistance.

When breeding material is selected for resistance to diseases such as STB, it is generally assumed that good sources of resistance with very different pedigree have different resistance gene. Breeders often seek to combine several such genes in new cultivars to reduce the rate of evolution of the pathogen population to virulence. This approach is termed gene stacking or gene pyramiding. If, however, one major resistance gene is present in a large proportion of the available breeding material, breeders may repeatedly select this gene in their material. We have used the collected information for the analyses of previous data sets and determined that the *Stb4* pattern is predominantly present in French germplasm, which was confirmed by *Stb4* specific PCR markers based on the SSR marker *Xgwm111* (Adhikari et al., 2004) (not shown).

The current data clearly show that seedling experiments are ideal for the evaluation of genetic variation in the pathogen population and as such they are very helpful in the selection of the appropriate isolates for resistance evaluation. We recommend using individual isolates instead of bulks for field screening to test for specificity and complementarity in the adult plant stage.

Finally, these data clearly indicate the necessity of exploring new sources of resistance as the majority of the characterized *Stb* genes are not effective to the majority of French *M.graminicola* strains although marker assisted pyramiding may results in some effective combinations of *Stb* genes.

3. Identification QTLs for resistance to Fusarium graminearum in the Apache/Balance mapping population

▶ Material and Methods

Plant materials and (QTL) mapping

In order to obtain phenotypic data for the QTL analysis, we obtained a 235 double haploid lines population produced from the cross between the French bread wheat cultivars Apache and Balance that were tested during several years in the field (Table II). DNA samples of the population were sent to DArT Pty in Australia for genotyping. We subsequently used QTL version 5.0 (Van Ooijen and Jansen, 2007a,b) for QTL mapping.

Year	Location	Spray inoculation	Spot inoculation
2005	Serasem	235 lines in 1 replication	
2006	Serasem	235 lines in 1 replication + 5 plots per parent	
2006	Florimond Desprez		233 lines 1 replication + 9 plots per parent
2007	Florimond Desprez		92 lines in 1 replication

Table II: Locations for the DH Apache/Balance in the field during several years.

Inoculum preparation and inoculation methods

Inoculum was prepared from 40 grams Mung beans that were added to one liter boiling demineralized water until the first seed coats loosened. Then 500 ml was immediately filtered through cheese cloth in 1 liter Erlenmeyer flask, which was subsequently autoclaved for 20 min. at 121 0C. This Mung bean medium was inoculated with a Mung bean pre-culture of *F. graminearum* isolate Fg820. The inoculated flasks were incubated at 26-27 0C in a reciprocal shaker adjusted at 150 rpm for three days. The inoculum (105 conidia/ml) was applied in two ways: (i) spray inoculation by atomizing a fine mist of inoculum over entire plots and (ii) spot inoculation by placing a pice of soaked inoculated cotton in the florets of individual spikelets of a wheat head (Figures. 2 and 3).

Data collection and statistical analyses

FHB symptoms were visually evaluated three weeks after spot inoculations by recording the total number of spikelets and the number of infected spikelets per spike (Figure 4). Evaluation of spray inoculation was recorded in four categories:

- (i) 350 Gs, the percentage of infected spikes per plot at 350 degree days after flowering (3 weeks),
- (ii) 350 GST, the percentage of infected spikelets per spike at 350 degree days after flowering (3 weeks),
- (iii) 450 Gs, the percentage of infected spikes per plot at 450 degree days after flowering (4 weeks)
- (iv) 450 GST, the percentage of infected spikelets per spikes at 450 degree days after flowering (4 weeks).

Preliminary data analyses were performed using a generalized linear model (GLM) procedure, which was complicated by the fact that in 2005/6 only one replication was investigated. A final analysis will take the most comparable data set (450 GST) over all years for a multiple year analysis.

Finally quantitative PCR (Waalwijk et al., 2004) was performed on material that was collected six weeks after inoculation to study the correlation between FHB symptom expression and fungal biomass as well as DON content in the harvested grain (not yet determined). The sampling in spot and spray inoculated plots was different. In spot inoculated plots 10 labeled spikes were harvested. For the parents a total of 180 spikes were harvested. In spray inoculated plots, 10 spikes were randomly collected. All samples were transferred to Plant Research International (PRI) and stored at -20 °C in a freezer.

Genomic DNA was isolated from 50 mg powder using the PROMEGA KIT protocol (Wizard Magnetic DNA Purification System for Food) in three technical replications per sample. The DNA quality and quantity were evaluated in 1 % agarose gels. Several dilution series were run to optimize the quantitative PCR procedure.

Mapping and QTL analyses

A genetic linkage map of the Apache/Balance population was constructed using 231 DArT and 169 SSR markers on a subset of 92 DH lines using Joinmap version 4.0 (Van Ooijen and Jansen, 2007) with the Kosambi mapping function. A minimum logarithm of odds (LOD) threshold of 3 was used to determine linkage groups. In addition, a separate map for each chromosome was constructed using a minimum LOD threshold of 2, similar to reported marker positions for DArT and the Grain gene database and at the Génoplante-INRA websites.

For resistance Type I (spray inoculation) phenotypic data obtained in 2005 and 2006 as the proportion of infected spikes per plot at three weeks (350 GS) and four weeks (450 Gs) after inoculation were analyzed. For resistance Type II (spot inoculation), the number of symptomatic spikelets (NSS) in 2006 and 2007, as well as the number of completely (NCSS) and the number of partially symptomatic spikelets (NPSS) were used for QTL analysis, using MQM (1000 permutation, LOD thresholds were set to declare significant QTL).

▶ Results

Spot and spray inoculations

The analyses of the Type I data (Table III) resulted in eight significantly different groups (P=0.05) of DH lines with predicted values (PV) ranging from 0.2496 to 0.8502. The difference between Apache and Balance was not significant, but there was substantial transgressive segregation.

Score	Variable	df	Deviance	Mean deviance	Deviance ratio
Data (7-7-2005 450 Gs 2006)	Year	1	7.3	7.3	36.17 ***
	DH Line	237	223.3	0.9	4.68 **
	Year.line	236	169.5	0.7	3.57 **
	Residual	13	2.6	0.2	

Table III: GLM analysis of spray inoculation of the Apache/ Balance DH population (2005 and 2006 data collected at Serasem).

All factors in the analysis of the spot inoculation (Table IV) showed strong significances. Thus the day of inoculation and the year of inoculation had a significant effect on the expression of resistance in the individual DH lines. As the day of inoculation had such a strong effect on the expression of resistance we transformed the PV to adjusted predicted value (APV) using the date of inoculation as a co-factor in the analysis. The DH lines APVs ranged from 0.0576 to 0.8010 and were separated in 31 homogeneous significantly different groups (P-0.05). The APVs (based on nine replications) of Apache and Balance were significantly different at the P=0.01 level indicating that Balance is more susceptible than Apache for Type II resistance (see also Figure 1).

Apache/Balance DH Lines 2006[1]				Common Apache/Balance DH Lines in 2006 and 2007				
Variables	d.f.	Mean deviance	Deviance ratio	Fixed term	d.f.	Wald	Wald/ df	Chi-value
Inoculation day	4	75.6	24.30***	Year	1	91.0	91.0	<0.001
DH Lines	246	9.0	2.90***	DH Line	91	354.7	3.9	<0.001
Residual	2249	3.1		Year Line	91	160.1	1.8	<0.001
[1]*Four DH lines were removed from the analysis.*								

Table IV. Type II GLM analysis of the Apache/Balance DH population (collected at Florimond Desprez in 2006 and 2007).

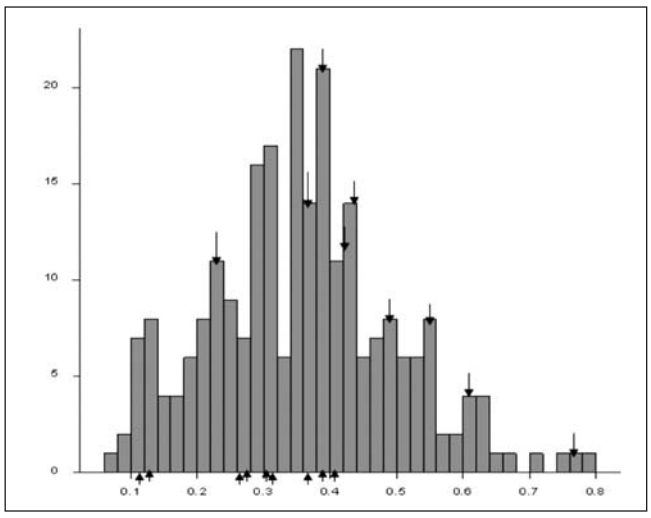

Figure 1: Histogram of distribution of APV in Apache/ Balance DH mapping population for Type II spot inoculation in 2006. Nine replicated parents indicated by top arrows for cv. Balance and bottom arrows for cv. Apache.

TaqMan Analysis

1. Parents spot inoculation

The average *F. graminearum* biomass in Apache was 3449 pg DNA/mg dried tissue and for Balance 5448 pg DNA/mg dried tissue based, which was significantly different (based on three replications, t = 3.9, d.f.= 459.38 corrected for not equal variance using Levene's test, p < 0.001). The Pearson coefficient of correlation showed a positive significant relationship between *F. graminearum* biomass and APV at 0.05 probability.

2. Parents spray inoculation

The average of *F. graminearum* biomass in cv. Apache was 2793 pg DNA/mg dried tissue and in cv. Balance it was 893 pg DNA/mg dried tissue, which is significantly different at the P=0.01 level (t = 4.27, d.f.= 17.3 corrected for not equal variance using Levene's test, p < 0.001). The Pearson correlation coefficient showed that Fusarium biomass and spray scoring criteria were not significantly correlated.

Mapping analysis

The Apache/Balance DArT map resulted in 318 placed DArT markers (Figure 2).

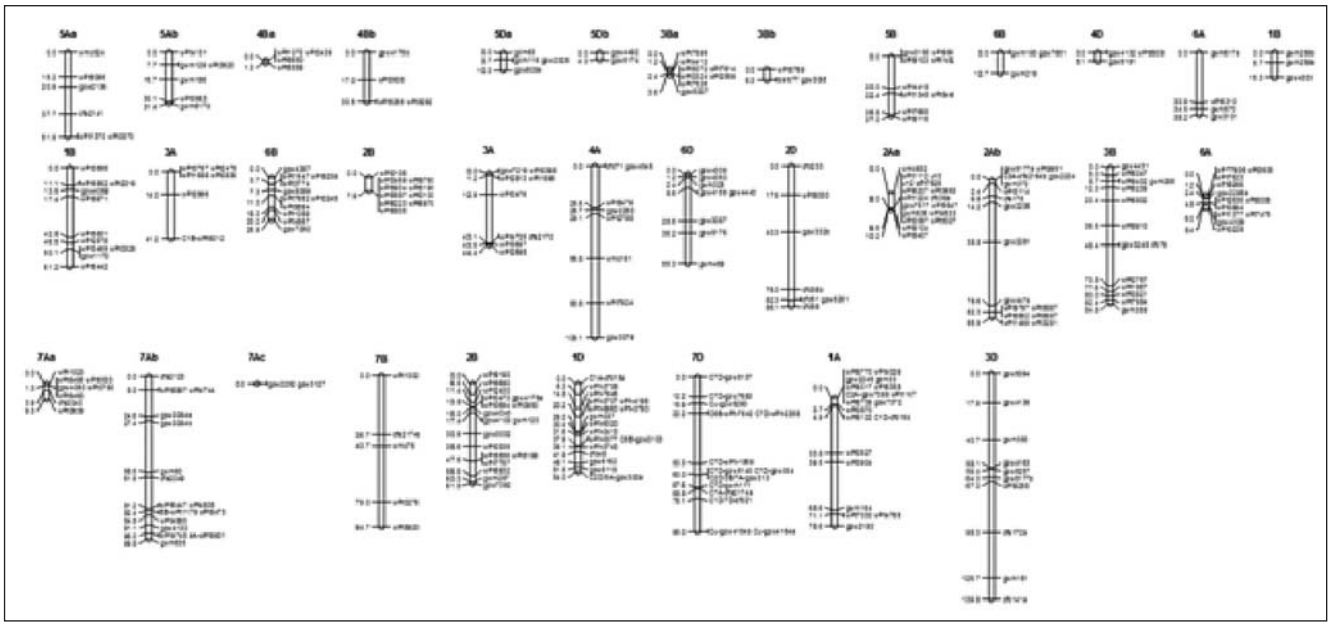

Figure 2: Apache/Balance map.

QTL analysis

We used the collected data to map QTLs for FHB Type I and Type II resistance in the Apache/Balance mapping population (Figure 3), using the individual DArT and SSR marker maps for each chromosome.

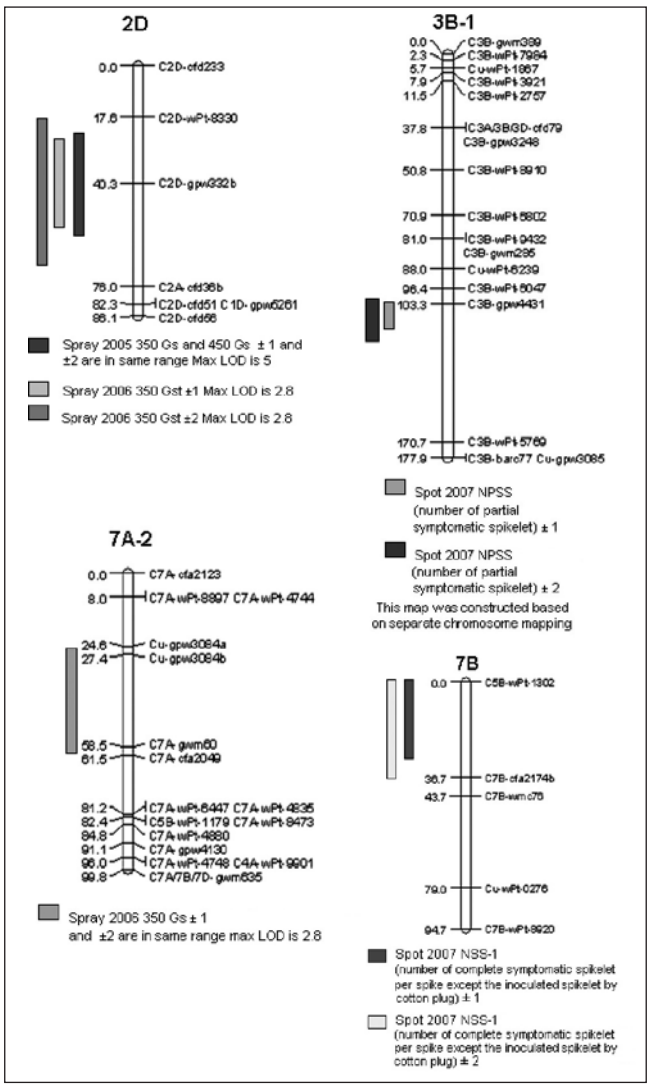

Figure 3: Mapped FHB QTLs for Type I and Type II resistance in the Apache/Balance DH population using the 2005, 2006 and 2007 field score data sets.

► Discussion

Our results showed that the year and location effects in the *Fusarium* experiments were highly significant, which influenced the statistical analyses substantially. Moreover, we identified significant contradictions between the visual FHB field scores and the *F. graminearum* biomass estimated by qPCR. Hence, the QTLs analyses were exclusively based on the visual observations. However, despite the fact that we have focused on these data, indications from qPCR that there are significant differences between Type I and Type II resistance to FHB in the tested lines and parents were confirmed in the QTL mapping. We identified two significant QTLs on chromosome 2D and 4D for Type I resistance and one significant QTL on chromosomes 7B and 3B for Type II resistance in the Apache/Balance population. The QTL on chromosome 4D is linked to the dwarfing gene *Rht2* (data not shown). However, these QTLs for FHB resistance were also reported in other studies (Liu et al., 2007; Zhou et al., 2004). We, therefore, decided to summarize the *Fusarium* experiments in a research paper and not to invest in additional experiments dealing with the Apache/Balance mapping population.

4. Screening for resistance to *Septoria tritici* blotch and *Fusarium* head blight in a broad germplasm collection

In the general introduction we indicated that the number of genes available for effective resistance breeding to STB and FHB is limited. One of the main purposes of this project is to identify new sources of resistance. In a previous chapter we have focused on analyzing the *M. graminicola* virulence spectrum in France as well as on an analysis of an existing mapping population for FHB QTL mapping. Here, we will focus on the screening of germplasm collections using the developed techniques and data to identify potential new sources of resistance to STB and FHB that will be used to develop new DH populations for QTL mapping and marker development.

► Material and Methods

Resistance to STB

We used six highly virulent French and Dutch *M. graminicola* isolates (IPO323, IPO94269, IPO98022, IPO98046, IPO98047, IPO98064) as well as four new French isolates (S-117, S-181, S-182, and S-183) that were received from Biogemma. Seventy-three lines, including susceptible checks and *Stb* differentials, were tested according to an alpha-lattice statistical design with three replications and percentage necrosis and pycnidia development were scored at 21 days after inoculation in the seedling tests.

In the field we tested two different cultivar sets in 2005, 2006 and 2007 that were inoculated with one and two *M. graminicola* isolates, respectively (2006-IPO323, 2007-IPO323 and IPO98047). Inoculation dates were based on flag leaf appearance in early flowering cultivars and were repeated three times with a one-week interval. Inoculum (10^6 spores/ml) was prepared as described above in Chapter 2.1.3. In both years we scored the STB severity by symptom expression on the flag leaf (F) and the second leaf (F-1). The field trial in 2007 was severely contaminated with natural yellow rust and leaf rust infections, and these observations were used as cofactor in the covariance analysis.

Resistance to FHB

We screened for type I and type II resistance at Florimond Desprez and Serasem in 2006, 2007 and only type I resistance in 2005. At Arvalis, type I resistance was scored during the 3 years. Inoculum preparation and inoculation procedures were as described in Chapter 3.1.2. In Arvalis fields, natural infection was improved by infected maize stalks that were spread in the rows. Resistance was assessed by counting the number of infected kernels per spike, for 50 spikes per line. DON levels in harvested kernels were also quantified.

► Results

Resistance to STB

All factors in the analysis were significant, confirming the strong isolate x cultivar interaction in the *M. graminicola*-wheat pathosystem (Table V). We selected FHD2054.3 and FD02112 as two highly resistant lines. The four additional *M. graminicola* isolates that were obtained through Biogemma were not highly virulent, particularly isolates S-117 and S-181 did not produce any pycnidia, even on the susceptible check cv. Taichung 29.

Fixed term	Wald statistic	d.f.	Wald/d.f.	Probability
Isolate	100.6	9	11.2	<0.001
Cultivar	755.3	72	10.5	<0.001
Isolate.Cultivar	863.4	648	1.3	<0.001

Table V: GLM analysis of STB seedling data (73 cultivars and 10 M. graminicola isolates) based on percentage pycnidia.

Subsequent field analyses showed significant isolate x cultivar interactions that were also tied to significant location (Florimond Desprez and Serasem) effects (Table VI). Nevertheless, we identified according to the flag leaf data that Bio.719 and Bio. 5019 performed very well at both locations in 2007. In addition cv. Robigus had a very low necrosis percentage at both locations, but it is a late cultivar and we therefore cannot exclude escape effects.

		Fixed term	Wald statistic	d.f.	Wald/ d.f.	Chi pr
2006	Flag leaf	line	136.61	20	6.83	<0.001
	F-1	line	68.88	20	3.44	<0.001
2007	Flag leaf	cultivar	240.65	26	9.26	<0.001
		Loc iso	56.31	3	18.77	<0.001
		Cultivar-lociso	251.22	63	3.99	<0.001
		cov	0.22	1	0.22	<0.638
	F-1	cultivar	205.58	26	7.91	<0.001
		Loc iso	26.39	3	8.8	<0.001
		Cultivar-lociso	334.06	63	5.3	<0.001
		cov	7.96	1	7.96	0.005

Table VI: GLM analysis of STB field data at two locations in 2006 and 2007 based on percentage necrosis.

Type I resistance to FHB

Despite the significant year.location and location.cultivar effects (data not shown), we identified highly resistant and susceptible germplasm both in 2006 and 2007. Based on Gs 450 and Gst 450 in 2006 we identified ND2710 and Sumai 3 as the most resistant lines in the spring wheat set; C10 SRSN 165 in the CIMMYT set; and IFA.136.6.1.1, 20828 and 04 CY BH FU 12 in the winter wheat set. The most susceptible line in the spring wheat set was Guadalupe.

In 2007, pair wise testing at 0.05 probabilities showed that 04 CY BH FU 12, C10 SRSN 315, C10 SRSN 215, C10 SRSN 108, C10 SRSN 204 and C10 SRSN 165 were highly resistant to FHB and Guadalupe, Bio2000 were again in the most susceptible set of cultivars (Table VII). In 2007, Royssac got fewer symptoms than the two years before, because natural infection did not occur during flowering, but it is usually highly infected after spray inoculations.

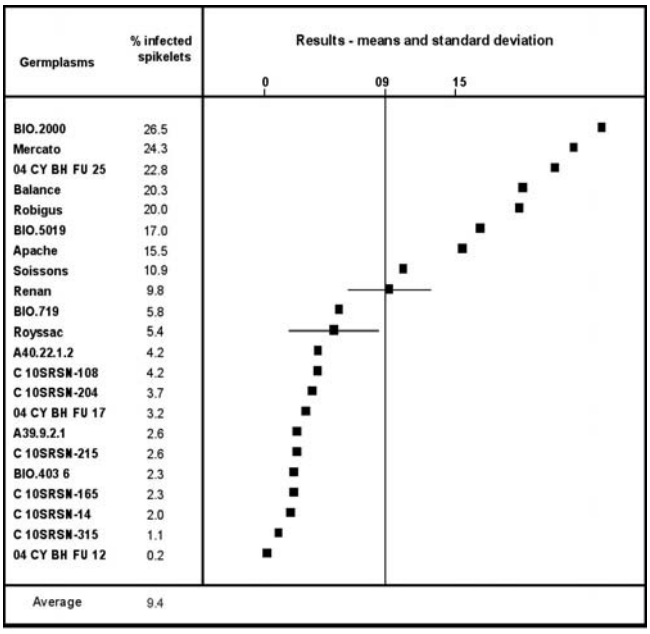

Table VII: Type I resistance to FHB on winter wheat at Arvalis in 2007.

Type II resistance to FHB

Type II analyses were carried out by inoculating 10 spikes per plot. Pair wise testing of predicted values (PV) determined homogenous groups with significantly different disease levels at 0.05 % probability. Despite the significant effect of inoculation day and significant year.location.culivar effects on the expression of Type II resistance we were able to identify highly resistant germplasm (Tables VIII and IX). In 2006, cvs. Sumai 3 and CM 82036 were the best resistant lines in the spring wheat set; cv. Centrum was the most resistant cultivar in the winter wheat set. Interestingly, cv. Charger was not significantly different from cv. Centrum for Type II resistant although it was highly susceptible for Type I resistance. The most susceptible cultivars were cvs. Guadalupe and 04CY BP77.

In 2007, some resistant lines such as Centrum, 20828, IFA.136.6.1.1, CM 82036, ND2710 were missing. At Serasem the most resistant lines were 04 CY BH FU 12, C10 SRSN 165 and 04 CY BH FU 17, and at Florimond Desprez, 04 CY BH FU 17, 04 CY BH FU 12 and C10 SRSN 165 showed the highest FHB resistance.

Over years and locations 04 CY BH FU 12 and 04 CY BH FU 17 showed the highest levels of Type II resistance to FHB at 0.05 probability.

Material type	Variables	df	Mean deviance	Deviance ratio
Spring wheat set	Inoculation day	1	35.1	29.90 ***
	Lines	20	5.8	4.97 ***
	Residual	206	1.18	
Winter wheat set	Inoculation day	4	58.1	19.78 ***
	Lines	60	14.1	4.80***
	Residual	632	2.9	

Table VIII

Fixed Term	Wald Statistic	d.f.	Wald/df	Probability
Year.location	2.8	2	1.41	0.244
Cultivar	211.9	28	7.57	<0.001
Yearloc.cultivar	88.9	36	2.47	<0.001

Table IX. GLM analysis of FHB screening for Type II resistance in 2006 and 2007.

► Discussion

Resistance to STB

The exploitation of the French and global M. graminicola isolates and their virulence spectrum on the known Stb genes enabled the identification and characterization of germplasm with potentially new resistance genes to STB. Sometimes cultivars were highly resistant in the seedling stage but susceptible in the adult plant stage such as cv. C4013 to isolate IPO98047. Based on the collected data the following new DH populations will be developed for STB QTL mapping and marker development that will be studied in the remainder of the project: FD02112 (Nogal) x Bio.719, FD02112 (Nogal) x Soissons, FDH 2054.3 x Soissons and 02CY 399 x Soissons.

Resistance to FHB

We categorized the tested germplasm in the following categories:

1. Lines with Type I and Type II resistance; such as cv. 04 CY BH FU 12
2. Lines with Type I resistance but lower Type II levels; such as cvs. A.39.9.2.1 and A40.22.1.2
3. Lines with Type II resistance but lower Type I levels; such as cvs. Charger and Centrum
4. Lines with no Type I and Type II resistance; such as cv. Royssac

Based on the collected data the following new DH populations will be developed for FHB QTL mapping and marker development that will be studied in the remainder of the project : 04 CY BH FU 12 x Guadalupe and A39.9.2.1 x Royssac.

The data generated so far have shown that identification of resistance to FHB is very complicated and strongly influenced by (micro)climatic factors such as temperature and relative humidity (RH) as well as the physiological state of the wheat head (inoculation day). These factors will be used to carefully plan future experiments for QTL mapping and marker development and will also be supplemented with DON data. In addition we will address the significant differences that we observed between Type I and Type II ranking of cultivars.

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