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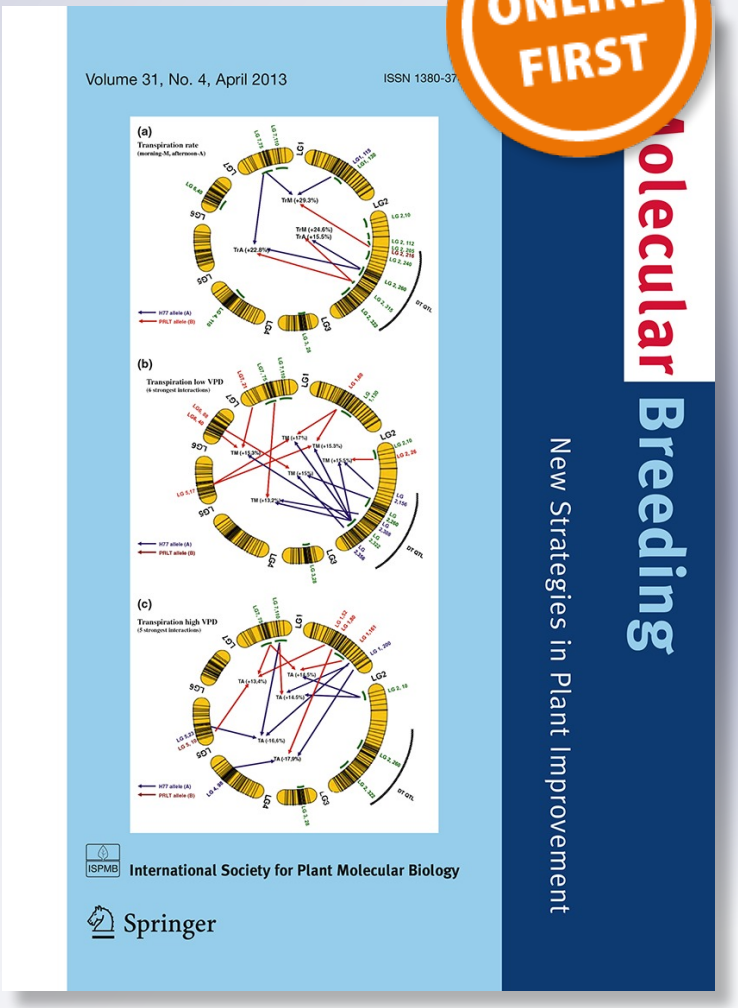

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Association mapping and meta-analysis: two complementary approaches for the detection of reliable Septoria tritici blotch quantitative resistance in bread wheat (*Triticum aestivum* L.)

Ellen Goudemand · Valérie Laurent · Laure Duchalais · Seyed Mahmod Tabib Ghaffary · Gert H. J. Kema · Philippe Lonnet · Eric Margalé · Olivier Robert

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Abstract Septoria tritici blotch (STB), caused by the ascomycete *Mycosphaerella graminicola*, is one of the most ubiquitous and important diseases of bread wheat worldwide. The aim of this study was to identify markers linked to loci conferring resistance to STB from seven biparental populations. Linkage analysis, meta-analysis and association mapping were combined to identify robust quantitative trait loci (QTLs) for resistance. Linkage analysis led to the detection of 115 QTLs for resistance to STB and 66 QTLs linked to plant height and/or earliness. Meta-analysis clustered these 115 QTLs into 27 Meta-QTLs (MQTLs) of pathogen resistance, of which 14 were found to be linked to plant height and/or earliness. Both the relationship between dwarfing and susceptibility to STB and the significant negative correlation between earliness and STB symptoms were confirmed. Eleven loci were linked to STB resistance by association mapping using a general linear model and/or a mixed linear model, of which eight co-located with STB MQTLs and two co-located with individual QTLs. Associated markers located in MQTL regions enhanced the relevance of the results and validated the potential of an association mapping approach. With several biparental populations, meta-analysis is the most relevant form of genetic analysis study, but association mapping can be used as a validation method. Regions linked to resistance in both methods should be relevant for use in breeding programs for improving resistance to STB in wheat varieties. The main interest in comparing both approaches is to detect robust loci that will be functional in many genetic backgrounds rather than just in one or a few specific backgrounds.

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E. Goudemand · V. Laurent · L. Duchalais · P. Lonnet · E. Margalé · O. Robert
Bioplante, 3 rue Florimond Desprez, BP 41, 59242 Cappelle-en-Pévèle, France

E. Goudemand (✉) · P. Lonnet · O. Robert
Florimond Desprez, 3 rue Florimond Desprez, BP 41, 59242 Cappelle-en-Pévèle, France
e-mail: ellen.goudemand@florimond-desprez.fr

L. Duchalais · E. Margalé
RAGT, 60 rue Léon Beauchamp, 59930 La Chapelle d'Armentières, France

S. M. Tabib Ghaffary · G. H. J. Kema
Plant Research International, Biointeractions and Plant Health, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Present Address:
S. M. Tabib Ghaffary
Safiabad Agricultural Research Center, P.O. Box 333, Dezfoul, Iran

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Introduction

Septoria tritici blotch (STB) is a severe foliar disease of wheat (*Triticum aestivum* L.) caused by the ascomycete fungus *Mycosphaerella graminicola* (Fuckel) J. Schröt in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.). In wheat-producing areas, STB not only causes significant yield losses that can reach 50 % under weather conditions conducive to its development (Goodwin et al. 2003), but it also degrades grain quality (Arabi et al. 2007). Consequently, STB can cause a major reduction in both crop quantity and quality and thereby to significant economic losses.

The fungicides strobilurin and triazole are the main agents used to control STB, but they are expensive to apply and are becoming less effective (Fraaije et al. 2005, 2007; Cools and Fraaije 2008). A more recent development is the pesticide reduction programs which have been established in several European countries (e.g. "Ecophyto 2018" in France, with the aim of reducing the quantity of pesticides used by 50 %; Paillotin 2008). Taking both of these factors into account, the utilization of genetic resistance remains the most cost-effective and environmentally friendly approach to control STB disease in wheat.

Genetic resistance to fungal disease can broadly be divided into two types: race-specific major resistance genes and non-specific genes for partial resistance. Eighteen major resistance genes to STB (*Stb1–18*) have been identified and characterized (Brading et al. 2002; Arraiano et al. 2007; Goodwin 2007; Chartrain et al. 2009; Tabib Ghaffary et al. 2011a, b). However, partial or quantitative resistance is isolate non-specific and polygenic and is generally more durable than race-specific resistance and, therefore, more relevant in breeding programs.

Many quantitative trait loci (QTLs) have been identified in classical mapping populations developed from a cross of two parents [recombinant inbred lines (RILs), doubled haploids (DHs), backcross populations (BCs)] by the linkage analysis approach. These populations are used to map the QTLs (Kearsey and Farquhar 1998), with the precision of the mapping mainly limited by the number of recombination events and the reliability of the trait measurement. Moreover, QTLs detected in one population in a specific environment may not always be effective if transferred to other genetic backgrounds due to the importance of environmental effects. Meta-analysis is a method of combining data from different sources in a single study to reveal co-locations between QTLs (Goffinet and Gerber 2000) with the ultimate aim of achieving a more accurate identification of the QTLs. Meta-analysis is mainly used in medical, social and behavioral sciences, but its relevance in genetics and evolution had also been pointed out by several authors (Allison and Heo 1998; Lohmueller et al. 2003). The first algorithms used in meta-analysis were developed by Goffinet and Gerber (2000) and Etzel and Guerra (2003), but the procedures were limited to a small number of underlying QTL positions (1–4), also called meta-QTLs (MQTLs). To eliminate this drawback, Veyrieras et al. (2007) developed a new meta-analysis procedure that integrates multiple independent QTL mapping experiments and implemented this into the Java package MetaQTL.

Association mapping or linkage disequilibrium (LD) mapping is an alternative method of QTL detection which has the aim to identify direct associations between genotype and phenotype on the basis of linkage disequilibrium. This method and its derivatives were originally developed for human genetics (Bodmer 1987), but they are now being applied to crops due to the availability of markers that are cheaper and available at higher densities (Thornsberry et al. 2001; Zhu et al. 2008). This approach does not require the development of a mapping population and can be applied to a range of populations. In the general linear model (GLM), a set of random markers is used to estimate the population structure (Q) before associations are tested (Yu and Buckler 2006) in order to reduce the number of false positive associations. The approach has been widened to a mixed linear model (MLM), including the kinship relations of the population, as this provides an improved control of both type I and type II error rates (Yu et al. 2006). The suitability of this new MLM method for association mapping has been demonstrated in humans and maize (Yu et al. 2006) as well as in *Arabidopsis thaliana* (Zhao et al. 2007), rapeseed, potato and sugar beet (Stich and Melchinger 2009). Association mapping has a higher probability of QTL detection due to the increased allelic diversity in the mapping population utilized, as compared with the limited diversity

between two specific parental lines in traditional linkage analysis (Buckler and Thornsberry 2002). However, association mapping will fail to identify rare alleles because of limited statistical power (Lewis 2002).

When a complete genome-wide association mapping approach is not possible, association mapping can be used as a validation method, using markers linked to QTLs previously detected by classical methods (Thornsberry et al. 2001). The relationship between QTLs detected by association mapping and QTLs/MQTLs detected by linkage analysis needs to be considered, along with the degree of overlap between the two types of QTLs. The use of both methods on the same dataset would enable QTL locations to be compared and demonstrate the power of each approach.

In the study reported here, we investigated LD between markers and performed association mapping, linkage analysis and meta-analysis of STB resistance in seven DH biparental populations with the aim of identifying reliable markers linked to STB quantitative resistance that would be useful for breeding.

Materials and methods

Plant material

Seven F_1 DH populations were obtained from the following biparental crosses: Apache/Balance (referred as A × B; 91 lines); Robigus/Soissons (R × S; 92 lines); FD3/Robigus (F3 × R; 87 lines); Bermude/Timber (Be × T; 82 lines); Cordiale/Nuage (C × Nu; 97 lines); Bio110/Nogal (Bi × No; 63 lines); FD12/SE11 (F12 × S11; 88 lines). DH lines were produced by the maize pollination method (Laurie and Bennett 1986; Kisana et al. 1993).

Disease evaluation

Trials

Field trials involving the seven biparental populations were carried out in two locations in the north of France (Cappelle-en-Pévèle and Prémesques) between 2004 and 2010. Thirty seeds per line and per replicate were sown in three 1.5-m-long rows in a randomized complete block design with two replicates. Moisture was artificially controlled with an irrigation system (5 mm/h each night). Experimental details are shown in Table 1.

Greenhouse trials for two populations (A × B and R × F3) were conducted at the Plant Research International (PRI) Wageningen (The Netherlands). Five seeds per line and per replicate were sown in 5.5 × 5-cm round Jiffy® pots using a steam-sterilized peat/stand mixture.

Plants were grown in an alpha lattice experimental design with three replications, with pots as experimental units that were randomly arranged for each replication on separate parallel tables in the greenhouse compartment. Before inoculation, seedlings were kept in a controlled greenhouse compartment at 18 °C/day and 16 °C/night; a photoperiodicity of 16/8 (light/dark) was maintained, and relative humidity (RH) was kept at 70 %.

Isolates

A total of 14 different European isolates of *Mycosphaerella graminicola* were produced (Hun2, Hun3, IPO323, IPO87016, IPO89011, IPO9007, IPO94269, IPO98022, IPO98042, IPO98046, IPO98047, IPO98094, IPO98099 and IPO98113) and inoculated individually on plant material; these 14 isolates cover a large part of the European pathogenicity. Some of the isolates had been tested earlier for their virulence by Tabib Ghaffary (2011c) who concluded that IPO323 possesses at least virulence V6 and V7; IPO94269, V7, V8, V9; IPO98022, V6, V7, V9; IPO98047, V6, V7, V8, V9, V11; IPO98094, V7, V8, V9, V11.

Pre-cultures of each isolate were prepared in a 100-ml Erlenmeyer flask containing 50 ml yeast-glucose (YG) liquid medium (30 g glucose and 10 g yeast in 1 l of demineralized water). Each flask was inoculated with a small piece of isolate mycelium and placed in an incubated rotary shaker (Innova 4430; New Brunswick Scientific, Edison, NJ) set at 125 rpm and 18 °C for 5–6 days. The pre-cultures of each isolate were used to inoculate three 250-ml Erlenmeyer flasks each containing 100 ml YG media, and the flasks were then incubated under the same conditions as the pre-cultures.

For the greenhouse trials, the inoculum solution was adjusted to a concentration of 10^7 spores/ml in a total volume of 40 ml for a set of 24 pots and supplemented with two drops of surfactant (Tween 20; Polyoxyethylene-sorbitan monolaurate; MERCK®,

Table 1 Populations used for the detection of Septoria tritici resistance quantitative trait loci, stages, environments and isolates tested

Population	Individuals	Stage	Location	Year/isolate[a]
A × B	91 DH	Ad	CAP	05/IPO323, 07/IPO323, 08/IPO323
			PRE	05/IPO323, 06/IPO323, 07/IPO323, 08/IPO323
		Se	WAG	07/IPO98046, 07/IPO98022, 07/IPO87016, 07/IPO89011, 07/IPO323
F3 × R	87 DH	Ad	CAP	04/IPO323, 08/NAT, 10/IPO98099
			PRE	04/IPO323, 05/IPO323, 06/IPO323, 09/IPO323
		Se	WAG	08/IPO94269, 08/IPO323, 08/IPO98094, 08/IPO98042
R × S	92 DH	Ad	CAP	09/NAT, 10/IPO98046, 10/Hun2
F_{12} × S11	88 DH	Ad	CAP	05/IPO323, 08/IPO98113, 09/IPO98113, 09/IPO323, 10/IPO98022, 10/IPO9007
C × Nu	97 DH	Ad	CAP	09/NAT
			PRE	10/IPO98047
Be × T	82 DH	Ad	CAP	09/NAT
			PRE	10/IPO94269
No × Bi	63 DH	Ad	CAP	10/Hun3

DH doubled haploid, *Ad* adult stage, *Se* seedling stage, *CAP* Cappelle-en-Pévèle (FR), *PRE* Presmeques (FR), *WAG* Wageningen (NL), *NAT* natural infection

[a] Numerical suffixes show the years in which each experiment was carried out

Whitehouse Station, NJ). The plants were inoculated 7–10 days after the sowing date (first leaf stage). After inoculation, the greenhouse temperature was kept at 22 °C and RH at more than 85 %.

For the field trials, the spore concentration was adjusted to 10^6 spores/ml. Tween 20 (Polysorbate 20) was added at 0.5 ml/l. The plants were first inoculated shortly after flag leaf emergence and then twice in the following 2 weeks with a 10-l sprayer, which was calibrated at a rate of 10 l/100 m^2.

Scoring

In the field trials, STB resistance was assessed 4 weeks after inoculation either as the area under the disease progress curve (AUDPC), the percentage of symptoms on flag leaf (PS) or a breeder's notation (BN) ranging from one (resistant) to nine (susceptible). Five flag leaves per plot were analyzed and their scores averaged. Height was measured as the mean size of the plants in a plot. Heading date was recorded as the date when half of the ears in the plot were fully emerged.

Disease severity was assessed 21 days after inoculation in seedling experiments by estimating the percentage of necrosis and pycnidia on the inoculated first leaves.

Marker analysis

Young leaves were harvested from the parents and the DH populations 14 days after sowing. Genomic DNA was extracted from 50 mg of lyophilized leaves with the NucleoSpin®96Plant kit (Macherey–Nagel, Düren, Germany). Simple Sequence Repeat (SSR) and Diversity Arrays Technology (DArT) markers (Diversity Arrays Technology, Pty Ltd., Yarralumla, Australia) were applied on DH lines and parents.

SSR amplification and sequencing

Microsatellite markers gwm (Röder et al. 1998) or wmc (Gupta et al. 2002), gpw (Sourdille et al. 2001) cfa, cfe, cfd (Guyomarc'h et al. 2002) and barc (USDA-ARS Beltsville Agriculture Research Station, Beltsville, MD) were used.

SSRs were genotyped by PCR using M13-marked primers as described in Saintenac et al. (2009). DNA fragments were visualized using capillary electrophoresis on an ABI PRISM® 3130xl automatic DNA sequencer (Applied Biosystems, Foster City, CA; http://www.appliedbiosystems.com). The alleles were analyzed and scored with GENEMAPPER® 4.0 (Applied Biosystems®) software.

DArT

DNA samples of DH populations were analyzed by Triticarte Pty Ltd. (Yarralumla, Australia; www.triticarte.com.au) for the wheat DArT® service. DArT markers are named using the prefixes "wPt", "rPt" and "tPt" followed by a unique numerical

identifier. The populations A × B and F3 × R were genotyped first with DArT chip v2.3 (2,500 markers) and then with DArT chip v3 (7,000 markers); the population F12 × S11 was analyzed with DArT chip v2.6 (around 6,000 markers); all other populations were analyzed with DArT chip v3. DArT markers of chips v2.3 and v2.6 were all included in chip v3.

Rht genes

Perfect markers for *Rht-D1* and *Rht-B1* from Ellis et al. (2002) were used to genotype the parents of the mapping populations according to the conditions described in the article.

Statistical analysis

Significant contribution to variation to main phenotypic traits (PS, BN, plant height and heading date) of each replicate and line was demonstrated by analysis of variance according to the following model: $Pij = \mu + Li + Rj + \varepsilon ij$ where Pij is the phenotypic value of the ith line located in the jth replicate, μ is the mean of all data, Li is the line i effect, Rj is the replicate j effect and εij is the residual. In this model, the line effect was considered as fixed and the replicate effect as random because contrary to the replicate effect, it was assumed that all levels of the individual effect are known and represented. Adjusted line mean values in each environment were used for the detection of QTLs in the association mapping and linkage analysis. Therefore, each population by environment was studied in a separate analysis. Taking the environment factor into account, we calculated Pearson correlation coefficients between traits. Heritability (h^2) was estimated for whole DH populations with the formula: $h^2 = \sigma^2 g/[\sigma^2 g + (\sigma^2 e/n)]$ with $\sigma^2 g$ being the genetic variance, $\sigma^2 e$ the environmental variance and n the number of replicates. Basic statistical analyses were carried out using the software R v2.6.0 (R Foundation for Statistical Computing 2007).

Linkage analysis

For each marker, the allelic marker segregation was compared to the expected 1:1 ratio with a Chi square test (significant threshold 0.05). MapDisto v1.7.0 (Lorieux 2006) was used for the genetic construction of the seven linkage maps, one per population, with the Kosambi mapping function. Linkage groups were formed with a minimum log-likelihood threshold of 3 and a maximum recombination fraction of 0.4.

Classical QTL detection

For each population, trait and environment, QTL effects were estimated by composite interval mapping (CIM) (Zeng 1994) implemented in Windows QTL Cartographer ver. 2.5 (Wang et al. 2007). A forward–backward stepwise regression analysis was used in the CIM procedure, with ten marker cofactors and a 10-cM window size. Optimal LOD thresholds were determined with 500 permutations and a significance level of 0.05. QTL confidence intervals (CI) corresponded to a decrease of 1 LOD score on both sides of the QTL peak, as an estimation of 95 % CI (Lander and Botstein 1989). The percentage of phenotypic variance explained by QTLs was described by the R^2 parameter.

Meta-analysis

Meta-analysis was performed with the MetaQTL software (Veyrieras et al. 2007) that implements a series of programs which can be combined for a complete analysis (consensus map creation, QTL projection, QTL clustering).

The construction of the consensus maker map was based on a weighted least square strategy. As the number of common markers between the individual studies was insufficient, a map of approximately 6,290 markers combining 35 wheat maps (Bordes et al. 2011; Bordes, personal communication) was used as an initial framework. The genetic linkage maps of the seven populations of this study were projected onto this reference map with the MetaQTL software to obtain a new consensus map.

Meta-analysis was carried out separately for all chromosomes. QTLs identified in one population evaluated in a given environment (year/location) were defined as an independent experiment. The QTL projection stage consisted in positioning the QTLs located on a given map onto the reference map with a dynamic algorithm. For QTL clustering, the software implemented two clustering algorithms, and the method used in this study was based on a Gaussian mixture model. The number of MQTLs present was determined as the model which minimized the Akaike

information criterion. QTLs of all traits were projected onto the consensus map but only STB QTLs were clustered as MQTLs.

Graphic representations of linkage groups were generated with MAPCHART 2.2 (Voorrips 2002).

Association mapping

Structure, LD and association mapping analyses were performed in the entire collection (613 individuals).

Population structure was investigated with STRUCTURE v2.3.3 (Pritchard et al. 2000). Models with a putative number of subpopulations (K) varying from two to 14 without admixture and a total of 10,000 burn-in and 10,000 saved Markov-Chain Monte Carlo iterations were applied. The likely number of subpopulations present was estimated following the method of Evanno et al. (2005). CoCoa 1.0 (Maenhout et al. 2009) was used to generate the kinship matrix with an alikeness in state (AIS) estimator.

LD was estimated between each pair of markers located on the same chromosome (intrachromosomal pairs) using the squared allele frequency correlation (r^2). Considering two bi-allelic loci on the same chromosome with alleles A and a at the first locus and with alleles B and b at the second locus, the allele frequencies as π_A, π_a, π_B and π_b and the four haplotype frequencies as π_{AB}, π_{Ab}, π_{aB}, and π_{ab}, then the r^2 parameter (Hill and Robertson 1968) was estimated as: $[(\pi_{AB} - \pi_A\,\pi_B)^2]/[\pi_A\,\pi_a\,\pi_B\,\pi_b]$. LD analysis was realized with the software HAPLOVIEW 4.1 (Barrett et al. 2005). All markers with a minor allele frequency (<0.05) were excluded because of large variance of r^2 with rare alleles. The *P* value for LD was calculated to determine the proportion of gamete distributions that is less probable than the observed gamete distribution. Loci were considered to be in significant LD if the *P* value was <0.01.

Two traits (PS and BN), represented by their adjusted means of the different environments, were investigated by association mapping. Alleles occurring at a low frequency ($F < 0.05$) were excluded from the analysis to avoid false marker/trait associations. TASSEL 2.1 (Bradbury et al. 2007) was used to calculate associations between markers and traits, employing the GLM and the MLM. The GLM was derived from the model of Pritchard et al. (2000) where population membership estimates, integrated in the selected Q-matrix of STRUCTURE, served as covariates in the model. The MLM, adapted from Yu et al. (2006) using Q-matrix and the kinship matrix, was implemented as described in Henderson's notation (Henderson 1975): $y = Xb + Zu + e$, where *y* is the vector of phenotypic observations; *b* is a vector containing fixed effects including genetic marker and population structure; u is a vector of random additive genetic effects from multiple background QTL; X and Z are the incidences matrices of 1 and 0 s; e is the unobserved vector of random residuals. The variances of the random effects are assumed to be $Var(u) = KVg$, where K is the kinship matrix and Vg is the genetic variance. The EMMA method (Kang et al. 2008) was applied to fit the MLM instead of solving the mixed-model equations via Henderson's iterative procedure. In the MLM approach, heritability was calculated separately for each marker. The association of a marker with a trait is represented by its R^2 value, an estimate of the percentage of variance, estimated as: $[(F \times df\,\text{marker})/df\,\text{error}]/[1 + (F \times df\,\text{marker}/df\,\text{error})]$ with *F* corresponding to the *F* value from the *F* test on the marker (Bradbury, personal communication). In order to control the type I error rate when multiple tests were carried out, *P* value thresholds of 0.001 were adopted as a criterion for significant marker–trait associations (MTAs).

For a given trait, markers detected as significantly linked by association mapping that were in close vicinity and in high LD were assumed to be linked to the same QTL. These QTLs derived from association mapping analyses are referred to as A-QTLs.

Results

Descriptive statistics

Wheat genotypes demonstrated large phenotypic differences [Electronic Supplementary Material (ESM) Table S1] both within and between biparental populations. Medium to high heritability values were observed for all traits. The highest heritability values were observed for heading date ($0.63 \leq h^2 \leq 0.97$) and plant height ($0.63 \leq h^2 \leq 0.91$) and the lowest values were found for STB traits ($0.39 \leq h^2 \leq 0.71$).

A high significant correlation coefficient was observed between the PS and the BN (0.85). Moderate negative correlations were found between the heading date and the PS (−0.43) or the BN (−0.47) and

between plant height and the PS (−0.43) or the BN (−0.45). No significant correlation was found between the heading date and plant height.

Individual maps and QTL linkage analyses

The number of markers mapped in the seven populations varied from 572 to 824. A total of 181 QTLs were identified (ESM Table S2), of which 115 were linked to STB resistance (STB QTL) and 66 were linked to plant development (earliness/height) (Table 2). In this study, 25 height QTLs were found, of which 18 were located in the same interval as STB QTLs. Forty-one QTLs for earliness were detected, and 24 of these overlapped with STB QTLs. The number of individual STB QTLs per population ranged from four for F12 × S11 and C × Nu to 43 for population A × B. STB QTLs were identified on all chromosomes except 1D, 5D and 7B. The percentage of *M. graminicola* resistance variation explained by each QTL ranged from 5 to 82 % at the seedling stage and from 9 to 33 % at the adult stage. The number of QTLs per population linked to plant height ranged from one in the F12 × S11 and Be × T populations to 11 in the A × B population; for earliness, the number ranged from two in the F12 × S11 population to ten in the F3 × R population.

STB QTL meta-analysis

The consensus map realized in this study pooled 8,856 markers together on 3,345 cM. The 115 STB QTLs were successfully projected onto the consensus map (Table 2). Meta-analysis resulted in 27 MQTLs comprising 105 initial STB QTLs and ten remaining individual QTLs (Table 3; Fig. 1). Of these 27 MQTLs, 14 co-localized with QTLs of height and/or

Table 2 Number of Septoria tritici blotch, earliness and height quantitative trait loci successfully projected on the consensus map[a]

Chromosome	Populations														Total	
	A × B		R × S		F3 × R		No × Bi		F12 × S11		Be × T		C × Nu			
1A	–	*1(1)*	–	*2(2)*	–	*2(2)*	–	–	–	–	–	–	–	–	–	*5(4)*
1B	–	*3(3)*	–	–	1(1)	*1(1)*	–	–	–	–	–	–	–	–	1(1)	*4(3)*
1D	1(1)	–	–	–	–	–	–	–	–	–	–	–	–	–	1(1)	–
2A	–	*1(1)*	1(1)	–	–	*2(2)*	1(1)	–	–	–	2(2)	*2(1)*	–	*1(1)*	4(4)	*6(5)*
2B	–	*3(3)*	–	–	1(1)	*3(3)*	–	–	2(2)	–	–	–	–	*1(1)*	3(3)	*7(7)*
2D	6(5)	*7(7)*	3(2)	–	–	*2(2)*	2(2)	–	–	–	–	*2(1)*	–	–	11(9)	*11(10)*
3A	–	*5(4)*	–	*1(1)*	–	*7(5)*	–	*2(2)*	–	–	–	–	–	–	–	*15(12)*
3B	1(1)	*1(1)*	–	–	–	–	–	*1(1)*	–	–	2(1)	*1(1)*	–	*1(1)*	3(2)	*4(4)*
3D	–	*2(2)*	–	–	–	–	–	*1(1)*	–	–	1(1)	*2(1)*	–	–	1(1)	*5(4)*
4A	–	–	–	–	–	*2(2)*	1(1)	–	–	–	–	–	3(2)	–	4(3)	*2(2)*
4B	1(1)	*1(1)*	1(1)	–	4(3)	*2(2)*	–	–	–	–	1(1)	–	2(2)	–	9(8)	*3(3)*
4D	2(2)	–	–	–	7(5)	*7(6)*	–	–	–	–	–	–	1(1)	–	10(8)	*7(6)*
5A	–	*1(1)*	1(1)	*2(1)*	–	*2(1)*	–	–	–	–	–	–	–	–	1(1)	*5(4)*
5B	1(1)	–	–	–	–	*1(1)*	–	–	–	–	–	–	–	–	1(1)	*1(1)*
5D	1(1)	–	–	–	–	–	–	–	–	–	–	–	–	–	1(1)	–
6A	–	–	–	–	–	*4(3)*	–	*1(1)*	–	–	–	–	–	–	–	*5(4)*
6B	–	–	–	*3(2)*	1(1)	*4(4)*	–	–	–	–	–	–	–	*1(1)*	1(1)	*8(7)*
6D	2(2)	*8(6)*	–	–	–	–	–	–	–	–	–	–	–	–	2(2)	*8(6)*
7A	1(1)	*1(1)*	–	–	2(2)	*2(2)*	–	–	1(1)	*4(1)*	1(1)	–	–	–	5(5)	*7(4)*
7B	3(3)	–	–	–	3(3)	–	–	–	–	–	–	–	–	–	6(6)	–
7D	–	*9(7)*	–	*1(1)*	–	–	2(2)	*2(1)*	–	–	–	–	–	–	2(2)	*12(9)*
Total	19	*43*	6	*9*	19	*41*	6	*7*	3	*4*	7	*7*	6	*4*	66	*115*

[a] The number preceding the parentheses is the number of projected quantitative trait loci (QTLs); "– "indicates a chromosome lacking a QTL; italicized values columns represent Septoria tritici blotch (STB) QTLs; other columns are development QTLs linked to earliness and height; number within parenthesis is the number of initial experiments with significant QTLs

of earliness. The 95 % CI of MQTLs ranged from 0.2 to 11.6 cM. MQTLs with the most precise CIs associated with loci were located on chromosomes 4D (0.2 cM), 7A (1.1 cM) and 4B (1.3 cM). The number of clustered initial QTLs ranged from 2 to 16 (chromosome 2D). In this study, MQTLs could group QTLs together from one (chromosome 1B) to 11 environments (chromosome 4D) and from one (chromosome 7A) to five different populations (chromosome 4B).

Association mapping

Structure analysis revealed seven sub-groups representing the seven initial biparental populations.

LD measurements were performed in the entire collection (613 genotypes) (ESM Fig. S1). Of the 63,881 intrachromosomal marker pairs, 48,322 (75.6 %) showed a significant level of LD ($P < 0.01$). The correlation between the r^2 and genetic distance in centiMorgans was significant; 10.6 % of all significant pairwise comparisons had an r^2 value of >0.2.

Both models (GLM and MLM) were compared for the investigated traits because the linked markers and the significance criteria were not equal for the two model approaches. The number of significant MTAs was much higher with GLM than with MLM. A reduction of significant associations was observed when the kinship matrix was implemented because this method is more stringent. For the trait PS, 24 MTAs were found with GLM and eight with MLM. For the trait BN, 35 and 13 MTAs were found with GLM and MLM, respectively. Twenty-one identical MTAs are shared by the two models. All of the MTAs found by MLM were also fully significant by GLM. Excluding these almost-identical MTAs, 38 unique MTAs for the GLM remained.

Regarding significant associations ($P < 0.001$), only the markers explaining >2 % of the phenotypic variations are presented in Table 4. Altogether 33 MTAs were identified for 26 of the overall markers. Of the total number of markers, seven were associated with PS and BN (*Xgpw332*, *XwPt-664251*, *XtPt-9405*, *XwPt-0724*, *XwPt-8330*, *XwPt-667584* and *XwPt-0298*); the others consisted of specific associations with a single trait. BN was involved in the highest number of MTAs (18) followed by PS (15). These 26 markers corresponded to 11 distinct genetic loci on the consensus map, which identified 11 A-QTLs associated with STB resistance.

Comparison meta-analysis/association mapping

By checking the corresponding locations of A-QTLs on our consensus map, we were able to compare the overlap between MQTLs and A-QTLs.

Eight A-QTLs and MQTLs fell in the same interval (A-QTL2/MQTL4; A-QTL3/MQTL9; A-QTL4/MQTL10; A-QTL5/MQTL11; A-QTL6/MQTL12; A-QTL8/MQTL20; A-QTL9/MQTL21; A-QTL10/MQTL22). For example, both markers detected by association mapping and clustered as A-QTL10, *Xgpw5176* and *Xgpw3087* were also closely linked to MQTL22. The closest marker linked to the resistance of MQTL20, *XtPt-4209*, was the only one detected by association mapping on chromosome 6A (A-QTL8). Two other A-QTLs were detected in the interval of independent QTLs (A-QTL1/QTL2; A-QTL7/QTL4). The CI of QTL4 ranged from 137.6 to 140.1 cM, and the unique marker detected by association mapping on chromosome 3B, *XwPt-0343* was located at 138.86 cM in the same interval.

Discussion

In this study, we analyzed seven biparental populations of *Triticum aestivum* using meta-analysis and association mapping approaches. MTA analysis revealed a total of 26 distinct markers corresponding to 11 A-QTLs associated with variation in *M. graminicola* resistance. The linkage analysis approach detected ten QTLs and 27 MQTLs linked to STB resistance. The meta-analysis approach detected a greater number of QTLs (27 MQTLs) than association mapping (11 A-QTLs). All of the A-QTLs were also found by the meta-analysis.

Relationship between STB resistance, plant height and earliness

Quantitative studies of STB resistance must take account the effect of plant architecture and development on the disease.

In this study, 18 height QTLs were located in the same interval as STB QTLs, and three MQTLs were located close to *Rht* dwarfing genes. On chromosome 4D, MQTL18 consisted of seven STB QTLs that explained individually up to 30 % of the STB phenotypic variation at the adult stage in population

Table 3 Meta-analysis results for STB resistance

Name	Chr.	CI (cM)	No. of pooled QTLs[a]	Type of initial QTL and populations				Markers linked to STB resistance	R^2 (STB) (%)	Postulated genes
				STB[b]		Height	Heading			
				Seedling	Adult					
MQTL1	1A	19.2–25.4	3	F3 × R	R × S F3 × R	–	–	wPt-7905, wPt-2527, gpw7072	12–18	
QTL1	*1A*	*48.5–66.3*	*1*	–	*R × S*	–	–	*wPt-669563*	*11*	
QTL2	*1A*	*107.3–112*	*1*	–	*A × B*	–	–	*wPt-732616, wPt-1011*	*10*	
MQTL2	1B	36.6–40.5	3	A × B	A × B	–	–	wPt-4325, gpw4069, tPt-8929	7–12	*Stb11*
MQTL3	1B	74.1–75.9	2	–	F3 × R	–	F3 × R	wPt-6975, wPt-4721,wPt-5281	11	
MQTL4	2A	81.7–88.5	7	–	F3 × R; B × T; C × Nu	R × S	B × T	wPt-740658, wmc177, wPt-9320, wPt-6711	11–17	*Ppd-1A*
MQTL5	2A	176–178.2	3	–	F3 × R; A × B	No × Bi	–	wPt-9277, wPt-6662,wPt-741584, wPt-7901	9–11	
QTL3	*2B*	*18.1–26.1*	*1*	–	*F3 × R*	–	–	*wmc764, wPt-0289, wPt-4453*	*20*	
MQTL6	2B	58.1–69.7	3	F3 × R; A × B	–	–	–	wPt-5672, gpw7438, wPt-6199, wPt-8583	11–22	*Stb9?*
MQTL7	2B	72.2–78.5	3	–	A × B	–	F12 × S11	wPt-0335, wPt-668084, gpw3032	17	
MQTL8	2B	85.9–87.5	3	–	C × Nu; F3 × R	F3 × R	–	wPt-1646, wPt-0189, wPt-8340	11–15	
MQTL9	2D	35.3–39.2	3	A × B	F3 × R	–	–	cfd36b, wPt-6003, wPt-6780	11–24	
MQTL10	2D	63.27–67.23	16	–	A × B	R × S; A × B	R × S; A × B	gwm332, gwm484, wPt-6419	10–26	*Ppd-D1, Rht8*
MQTL11	2D	86.73–89.7	4	–	B × T	–	No × Bi	wPt-665644, wPt-8330, wPt-0298	12	
MQTL12	3A	50.2–52.6	13	A × B; F3 × R	A × B; F3 × R; No × Bi	–	–	wPt-0714, wPt-5486, wPt-0951, wPt-0797	9–77	*Stb6*
MQTL13	3A	132.3–133.7	2	–	F3 × R; R × S	–	–	wPt-1036, wPt-1596, wPt-9761	15–18	
MQTL14	3B	75.6–83.9	4	A × B	No × Bi; C × Nu	–	B × T	wPt-0644, wPt-11278, wPt-3725, wPt-5358	6–15	

Table 3 continued

Name	Chr.	CI (cM)	No. of pooled QTLs[a]	Type of initial QTL and populations				Markers linked to STB resistance	R^2 (STB) (%)	Postulated genes
				STB[b]		Height	Heading			
				Seedling	Adult					
QTL4	*3B*	*137.6–140.1*	*1*	–	*B × T*	–	–	*wPt-0343, wPt-6834, wPt-10758*	*15*	
QTL5	*3D*	*0–3.4*	*1*	–	*A × B*	–	–	*cfd141a, gwm161*	*8*	
MQTL15	3D	21.9–24	4	A × B	B × T; No × Bi	–	–	wPt-732185, barc125, wPt-731416	7–20	*Stb16*
QTL6	*4A*	*42.3–61.1*	*1*	–	*F3 × R*	–	–	*wPt-798213*	*17*	
QTL7	*4A*	*97.7–117.1*	*1*	–	*F3 × R*	–	–	*wPt-6997*	*11*	
MQTL17	4B	81.8–83.1	9	–	F3 × R	R × S; A × B F3 × R	C × Nu; B × T F3 × R	wPt-3991, wPt-1101, wPt-7365, wPt-6209	13	
MQTL16	4B	62.7–71.4	3	–	F3 × R; A × B	F3 × R	–	wmc617, wPt-3439, gwm149, wPt-8650	9–18	*Rht-B1*
MQTL18	4D	38.1–38.3	17	–	F3 × R	A × B; C × Nu F3 × R	–	wPt-5809, wPt-0431, wPt-3058, wPt-665313	10–30	*Rht-D1*
MQTL19	5A	24.7–34.2	4	F3 × R	R × S	–	R × S	wPt-2697, wPt-797382, tPt-6183	8–16	
QTL8	*5A*	*39.9–53.6*	*1*	–	*A × B*	–	–	*wPt-3620, wPt-0605, gwm129*	*17*	
QTL9	*5A*	*159.3–159.8*	*1*	*F3 × R*	–	–	–	*wPt-7061, wPt-1200, wPt-2873*	*16*	
QTL10	*5B*	*154.8–165.5*	*1*	*F3 × R*	–	–	–	*wPt-664788, wPt-3076*	*14*	
MQTL20	6A	18.4–20.3	5	F3 × R	F3 × R; No × Bi	–	–	wPt-0864, tPt-4209, wPt-1664, wPt-9132	10–15	
MQTL21	6B	40.7–42.5	9	F3 × R	F3 × R; R × S; C × Nu	–	F3 × R	wPt-729979, wPt-2587, wPt-2297, wPt-6437	6–20	
MQTL22	6D	26.86–30.1	8	A × B	A × B	–	–	gpw5176, gpw3087, wPt-665166	10–64	*Stb18*
MQTL23	7A	14.2–18.5	3	F3 × R	F3 × R	–	F3 × R	wPt-8171, wPt-3794, wPt-732309, wPt-1510	15–42	
MQTL24	7A	103.3–104.4	5	–	F12 × S11; A × B	–	–	wPt-5524, wPt-0639, wPt-0971, wPt-744897	11–32	

Table 3 continued

Name	Chr.	CI (cM)	No. of pooled QTLs[a]	Type of initial QTL and populations			Markers linked to STB resistance	R^2 (STB) (%)	Postulated genes
				STB[b]					
				Seedling	Adult	Height	Heading		
MQTL25	7D	87.7–91.7	7	A × B	–	–	–	wPt-744433, wPt-664469, gwm111	6–33	*Stb4*
MQTL26	7D	117.8–127.5	4	–	No × Bi	–	No × Bi	wPt-2258, wPt-7642	33	
MQTL27	7D	136.2–147.5	3	–	A × B; R × S	–	–	wPt-667894, wPt-7368, wPt-6553	8–15	

Chr chromosome, *CI*, 95 % confidence interval

QTLs given in italics represent unique QTLs for STB resistance; closest marker linked to the resistance are underlined

[a] Number of initial QTLs pooled into the MetaQTL

[b] R^2 is the percentage of variance explained by the initial QTLs

F3 × R, and eight height QTLs detected in three populations in seven different environments. The major gene, *Rht-D1*, was tightly linked (0.9 cM) to the MQTL18 on the consensus map. Parents of the A × B and F_3 × R populations were polymorphic for *Rht-D1* with the perfect marker, whereas parents of the C × N population were monomorphic with the perfect marker. However, the two gene alleles *Rht-D1b* (*Rht2*) and *Rht-D1d* (*Rht21*) cannot be differentiated by the perfect marker of *Rht-D1* (Pearce et al. 2011). We assumed that Cordiale and Nuage were polymorphic for these two alleles because a significant height polymorphism was observed for this population. All of these results prove that the MQTL18 corresponds to the *Rht-D1* gene. Furthermore, QTL for plant height, STB resistance and *Fusarium* head blight resistance have been found at the *Rht-D1* locus in the History × Rubens population, confirming the impact of this dwarfing allele on several traits, including STB (Miedaner et al. 2012).

On chromosome 2D, MQTL10, clustered many height QTLs detected in two populations. The major gene *Rht8*, located near *XwPt-4144* (Crossa et al. 2007), was located at a distance of 1.85 cM from MQTL10.

An association between STB resistance and the *Rht-B1 (Rht1)* gene previously described on chromosome 4B by Baltazar et al. (1990) was confirmed in this study. *Rht-B1* was found on the consensus map at 2.2 cM from the MQTL17 that grouped three height QTLs detected in three populations and one QTL of STB resistance. The *Rht-B1* perfect marker confirmed *Rht-B1* polymorphism in population F3xS, but not in the two other populations (R × S and A × B). According to the perfect marker, Robigus and Soissons were monomorphic for *Rht-B1* whereas Apache and Balance were monomorphic for *Rht-B1a* allele. We suspected that Soissons has *Rht-B1d* and Robigus has *Rht-B1b*. As the *Rht-B1* perfect marker is unable to distinguish these alleles, it cannot be used to reveal any existing polymorphism of *Rht-B1* in the population R × S (Pestsova et al. 2008). In the same way, we suspected that Apache and Balance have two different alleles (*Rht-B1a* and *Rht-B1c*) that cannot be distinguished by the *Rht-B1* perfect marker (Pestsova et al. 2008). Based on these results, we assume that MQTL17 corresponds to the *Rht-B1* gene.

The association between plant height and STB resistance (Tavella 1978; Eriksen et al. 2003) was

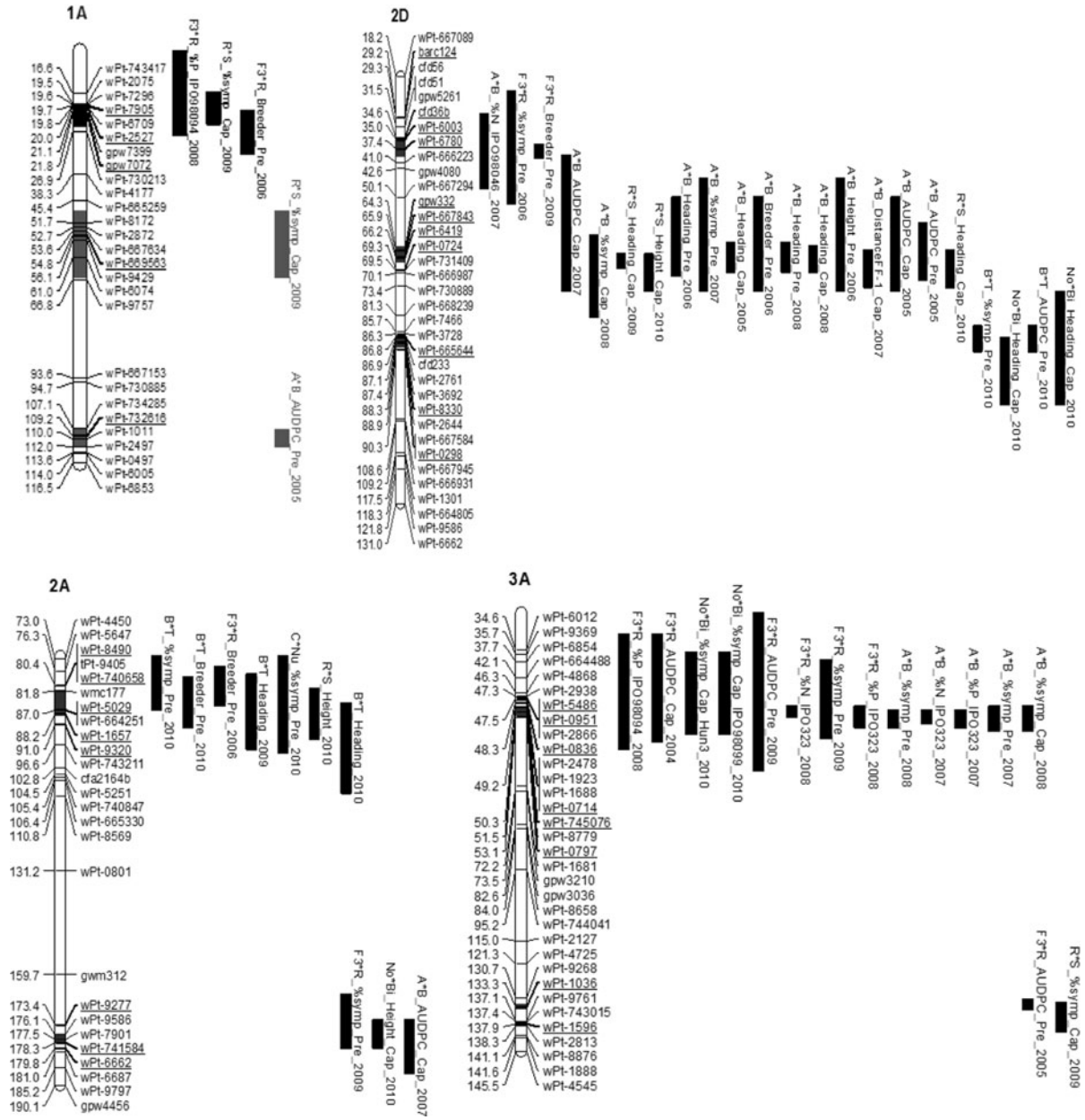

Fig. 1 Chromosomal genetic maps of quantitative trait loci (QTLs) and meta-QTLs (MQTLs). Genetic distance is given in centiMorgans (cM). *Highlighted chromosome bar segments* MQTLs, *rectangles* confidence intervals of QTL, *underlined markers* closest markers of the QTL in the initial biparental studies, *lighter QTLs* QTLs that remain as individual after the meta-analysis. The QTL name is the name of the population followed in succession by the type of scoring, the location and the year of the experiment

confirmed in our experiments. The main source of *M. graminicola* inoculum during the spring and summer are the pycnidiospores (Eriksen et al. 2001; Eriksen and Munk 2003), which are dispersed by splashing. These spores spread from the base and disperse upwards in the crop canopy, and the amount of inoculum transported decreases rapidly with height. In our study, artificial infection was applied, which decreased the impact of height on resistance. However, Rht genes *Rht-B1* and *Rht-D1* are associated with reduced cell elongation and, consequently, decreased cell size (Hoogendoorn et al. 1990) which could

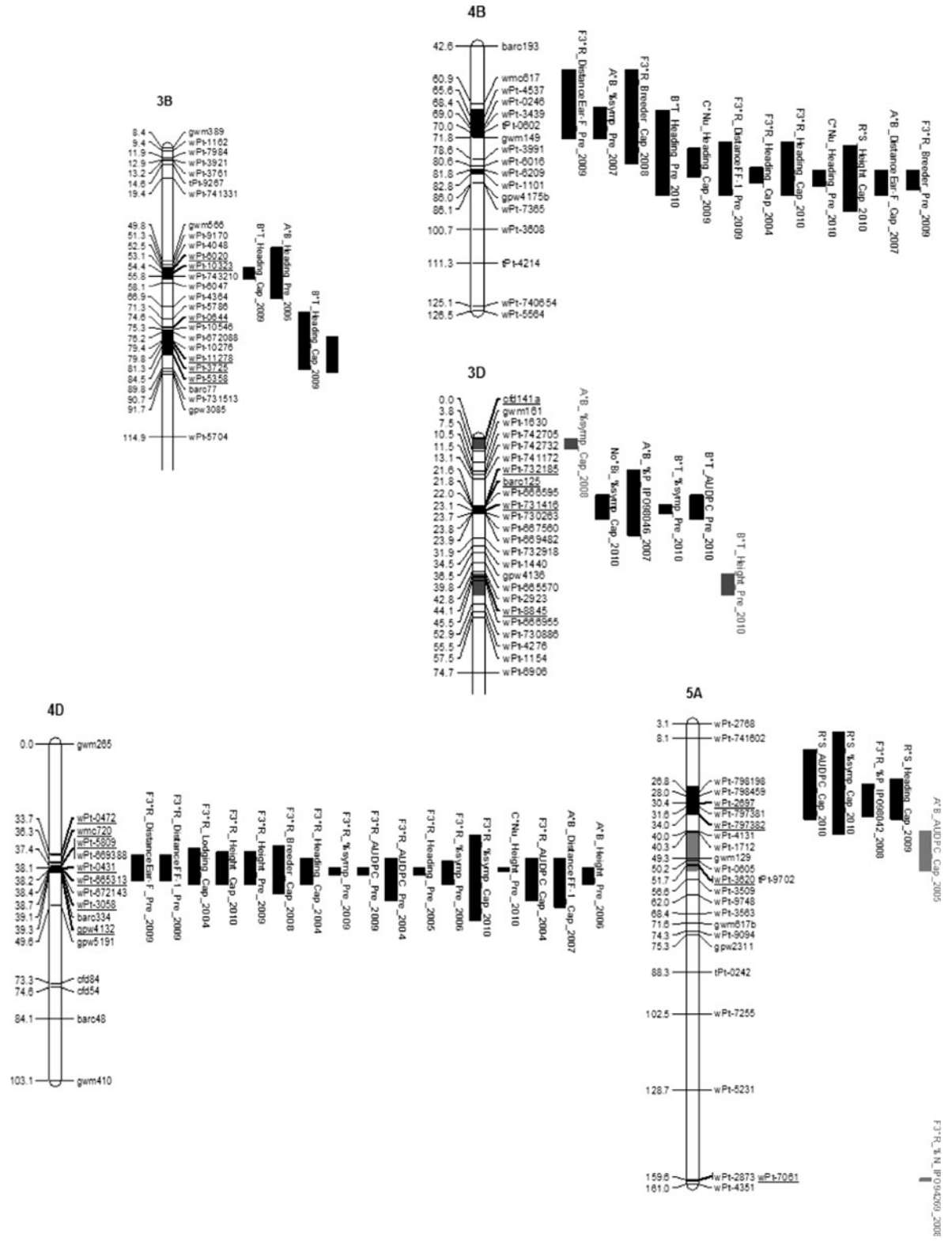

Fig. 1 continued

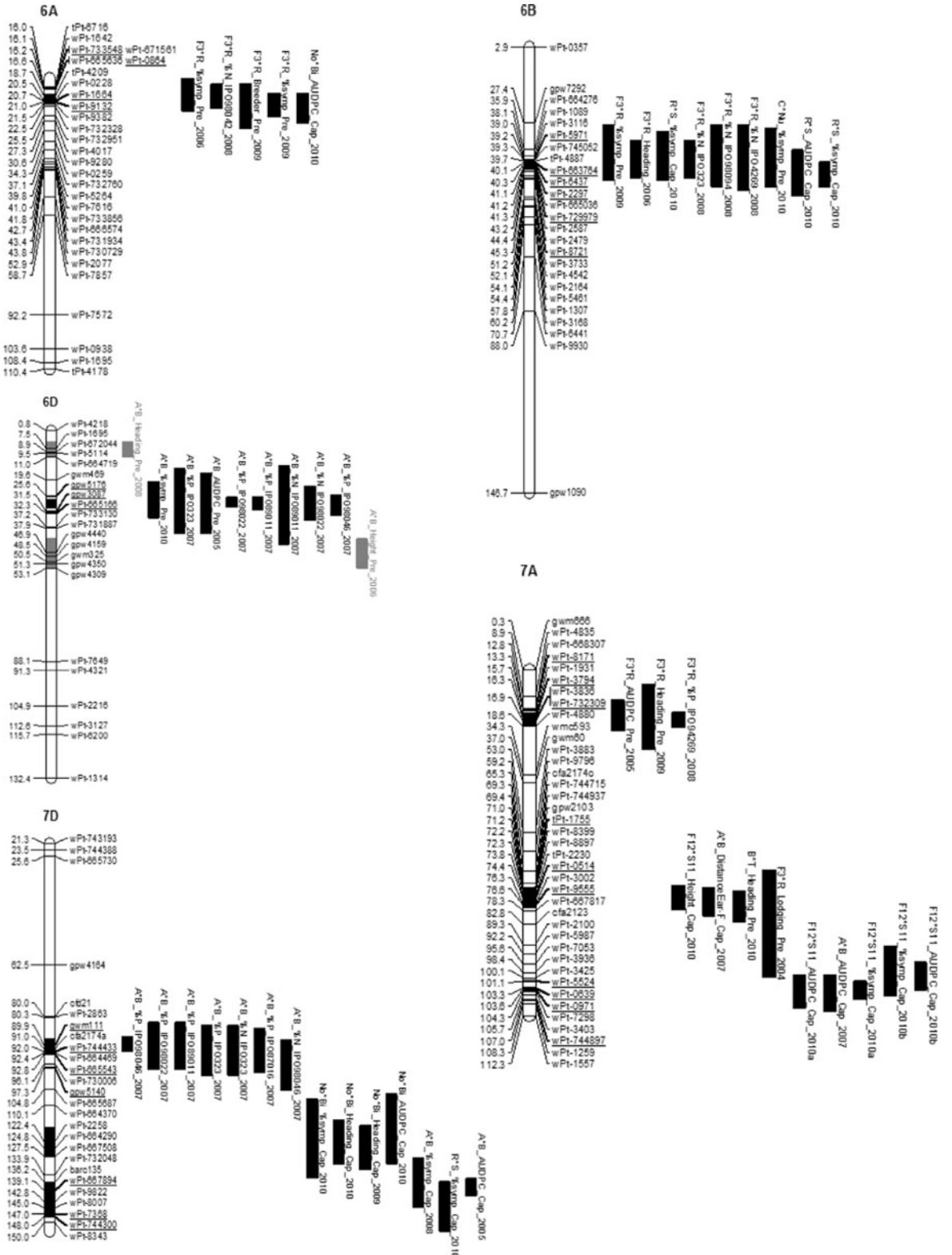

Fig. 1 continued

Table 4 Associations between markers and traits obtained with the generalized linear model (GLM) and mixed linear model (MLM)

A-QTL	Marker	Chromosome	Position	Trait[a]		Method[b]		R^2
				Percentage of symptoms	Breeder's notation	GLM	MLM	
A-QTL1	wPt-6005	1A	114.02		X		X	0.025
A-QTL2	tPt-9405	2A	80.43	X	X	X		0.042–0.044
	wPt-664251	2A	87	X	X	X		0.041–0.049
A-QTL3	barc124	2D	29.18	X		X		0.126
	cfd56	2D	29.27	X		X		0.115
	cfd51	2D	31.53	X		X		0.178
	gpw5261	2D	31.53	X		X		0.158
	cfd36b	2D	34.58	X		X		0.171
A-QTL4	wPt-667584	2D	63.21	X	X		X	0.023–0.025
	gpw332	2D	64.32	X	X	X		0.24
	wPt-0724	2D	69.27	X	X	X	X	0.02–0.032
	wPt-731409	2D	69,51		X	X	X	0.024–0.025
	wPt-666987	2D	70.07		X		X	0.023
A-QTL5	wPt-8330	2D	88.27	X	X	X	X	0.019–0.043
	wPt-0298	2D	90.33	X	X		X	0.021–0.023
A-QTL6	wPt-1923	3A	49.18	X		X	X	0.072–0.156
	wPt-0797	3A	53.07	X		X	X	0.044–0.057
A-QTL7	wPt-0343	3B	138.86		X	X	X	0.036–0.038
A-QTL8	tPt-4209	6A	18.74		X		X	0.03
A-QTL9	wPt-745052	6B	39.26	X			X	0.024
	wPt-729979	6B	41.26		X	X		0.04
A-QTL10	gpw5176	6D	25.6		X	X		0.154
	gpw3087	6D	31.46		X	X		0.199
A-QTL11	gpw4440	6D	46.93		X	X	X	0.059–0.068
	gpw4159	6D	48.45		X	X		0.058
	gpw4350	6D	51.31		X	X		0.115

A-QTL association-analysis-based QTL

[a] X in the column "Trait" indicates the presence of an association between a marker and a specific trait

[b] X in the column "Method" indicates the method used to detect this association; only markers explaining more than 2 % of the phenotypic variation ($R^2 > 0.02$) are shown

explain an effect of height on *Septoria* resistance, i.e. a difference in infection rate between smaller and larger cells. A relationship between small plants and susceptibility to STB has been reported (Baltazar et al. 1990). Breeders could select tall individuals to obtain varieties resistant to *M. graminicola*, but these phenotypes are often discarded because they are susceptible to lodging. To meet both objectives, i.e. improve resistance to lodging and to STB, phenotypes with intermediate height should be selected for.

Twenty-four QTLs for earliness overlapped with STB QTLs, and two MQTLs were located close to major genes affecting earliness. MQTL10 positioned on chromosome 2D was close to *XwPt-4144,* reported to be linked to the major gene *Ppd-D1* (*Ppd1)* (Crossa et al. 2007). This MQTL comprised six earliness QTLs detected in the populations R × S and A × B in six different environments. The MQTL10 region was linked to the major gene *Ppd-D1* but also to the dwarfing gene *Rht8*. MQTL4 clustered four STB QTLs and two QTLs of earliness detected in the B × T population. *Ppd-1A* (*Ppd3*) was located in the same region, linked to *Xwmc177* (Wilhelm et al. 2009). This marker was found at a distance of 0.1 cM

from MQTL4, within its CI. Therefore, we assume that the earliness *Ppd-1A* locus is tightly linked to MQTL4.

There was a significant negative correlation between earliness and STB infection. Levels of STB were substantially lower in late heading lines compared to early heading lines, a finding which agrees well with previous results (Tavella 1978; Chartrain et al. 2004). Arama et al. (1999) reported no influence of heading date when cultivars were evaluated at the same developmental stage and under similar weather conditions. As these authors mention, to compare the resistance of a range of cultivars, disease severity should be measured not at the same date, but at the same stage of development. In our experiments, disease development was measured on the same day at the adult stage for all cultivars. QTLs/MQTLs of resistance that overlapped with QTLs of earliness were due to differences in leaf age and as well as to differences in the duration of the period leaves were exposed to the disease. Therefore, these loci of resistance could not be considered as foliar resistance per se.

MQTL and other independent QTL/gene studies

Of the 27 MQTLs, six were assumed to be major genes of STB resistance (*Stb* loci). The others, which did not overlap with QTLs for height and/or earliness, could be considered as potentially new loci of STB resistance.

MQTL2 and the *Stb11* locus were located in the same marker interval. Chartrain et al. (2005) fine-mapped the *Stb11* locus on chromosome 1B in wheat line TE9111 near to the marker *Xbarc008*, which was on our consensus map located at 1.83 cM from MQTL2. MQTL2 consisted of three STB QTLs detected in the population A × B at both stages, explaining from 7 to 12 % of the phenotypic variation. In addition, similar to the Mexican isolate IPO90012 (Chartrain et al. 2005), isolates IPO323 and IPO87016 used in this study were avirulent on lines carrying the *Stb11* locus (Tabib Ghaffary et al. 2011a). Therefore, we conclude that MQTL2 in cultivar Apache represents *Stb11*. MQTL2 associated with *Stb11* in the A × B population was linked to DArT marker *XwPt-4325*. This marker could be used in addition to *Xbarc008* as a tool for marker-assisted selection of *Stb11*.

MQTL6 and the *Stb9* locus were located in the same marker interval on chromosome 2B, extrapolating from the results obtained by Chartrain et al. (2009), where *Stb9* was positioned between markers *Xfbb226* and *XksuF1b* in cultivar Courtot. On our consensus map, the marker *Xfbb226* and MQTL6 are 2.5 cM distant from each other (Table 3). *Stb9* was identified using isolate IPO89011, and MQTL6 was detected with isolates IPO98094, IPO98046 and also IPO89011. As IPO98094 has virulence V9 (Tabib Ghaffary 2011), we cannot assume that MQTL6 and the *Stb9* locus are the same resistance locus identified in varieties Balance and Robigus. However, MQTL6 consisted of three STB QTLs detected only at the seedling stage, indicating that this resistance locus was not effective at the adult stage as is *Stb9*. MQTL6 was found to be closely linked to DArT marker *XwPt-6199*.

On chromosome 3A, MQTL12 and *Stb6* were in close vicinity but not within the same marker interval. Brading et al. (2002) fine-mapped the *Stb6* locus between the markers *Xwmc11* and *Xgwm369* positioned at 47.3 cM on our consensus map, whereas MQTL12 was located between 50.2 and 52.6 cM. All of the CI of initial QTLs covered the marker interval of the MQTL12 locus, indicating that no significant difference in QTL positions can be found in the entry-data. Moreover, MQTL12 could explain from 9 to 77 % of the phenotypic variation, which is typical of a major gene reaction. According to these initial data, we assume that this MQTL and *Stb6* locus are the same resistance locus. The *Stb6* locus was identified in cultivars Balance, Nogal and Robigus and was closely linked in our study to marker *XwPt-0797* (1.5 cM).

MQTL15 was located next to *Stb16* locus. As described by Tabib Ghaffary et al. (2011b), the *Stb16* locus was positioned between *Xbarc125* and *Xbarc128* on chromosome 3D. The SSR marker *Xbarc125* was located on our consensus map at 0.1 cM from MQTL15. One of the initial QTLs of MQTL15 was detected in line Nogal, which is derived from a synthetic hexaploid wheat as line M3 used to identify the *Stb16* resistance locus (Tabib Ghaffary et al. 2011b). We assume that MQTL15 and the *Stb16* locus are the same resistance locus that originated from synthetic hexaploid wheat. This locus explained up to 20 % of the variation at the adult stage (No × Bi population) and could be placed near the DArT marker *XwPt-731416* (0.1 cM).

On chromosome 6D, MQTL22 and *Stb18* were located in the same marker interval. The *Stb18* locus was fine-mapped between markers *Xgpw5176* and

Xgpw3087 (Tabib Ghaffary et al. 2011a), and on our consensus map MQTL22 was located within this marker interval. This MQTL clustered STB QTLs detected at both the seedling and adult stages in population A × B, confirming the results of Tabib Ghaffary et al. (2011a). The *Stb18* resistance locus found in Balance in this study likely corresponds to the QTL *Qstb.lsa.fb-6D* identified in cultivar Florett by Risser et al. (2011). Indeed, *Qstb.lsa.fb-6D* was located at a distance of 1.55 cM from MTL22 on the consensus map. According to our consensus map, the closest marker for *Stb18* was the DArT marker *XwPt-665166* (3.3 cM).

MQTL25 was located near the *Stb4* locus on chromosome 7D. According to Adhikari et al. (2004), the *Stb4* locus is positioned at 0.7 cM from *Xgwm111*, and *Xgwm111* was located on our consensus map in the MQTL25 confidence interval. MQTL25 consisted of seven STB QTLs detected only at the seedling stage in population Apache × Balance, indicating that this resistance locus was not effective at the adult stage. This MQTL was detected with isolates IPO323, IPO87016, IPO89011, IPO98046 and IPO98022. We assume that MQTL25 and the *Stb4* locus are the same resistance locus identified in variety Apache.

Reliability of projection in the meta-analysis

Meta-analysis results underline the substantial role played by precise initial QTL detection and accurate QTL projection quality on the consensus map. The CI of MQTLs were sometimes slightly tighter than the most precise initial QTLs, such as the CI of MQTL5 (2.2 vs. 3.9 cM), and some markers that were linked to STB QTLs in individual studies were located outside the CI of the corresponding MQTL after meta-analysis. Similar observations were found by Löffler et al. (2009).

The results of meta-analysis (number, position and accuracy of MQTLs) are strongly dependent on the precision of the initial QTL positioning, CI and projection quality (Goffinet and Gerber 2000). First, particular attention needs to be paid to factors on which the reliability of the initial QTL characteristics depends (Veyrieras et al. 2007). In our study, initial QTL parameters (position, CI, R^2) were all reported, contrary to some studies reported in the literature (Löffler et al. 2009) where these characteristics are only partially described. Nevertheless, even reported, the accuracy and the homogeneity of their estimation may be variable. QTL position varies according to the characteristics of the genetic map (marker density, marker type, map coverage). For example, QTLs located at an extremity of their initial chromosome map will often have a poor projection quality (Hanocq et al. 2007). Initial CI estimated by a decrease of one unit in the LOD score value is also a source of heterogeneity because this CI can either be underestimated or overestimated and can therefore impact on reliability. Independently of the initial QTL mapping parameters, the projection from the original map onto the reference map has to be verified (Hanocq et al. 2007). Indeed, common markers may be rare and poorly distributed or inverted, both of which will impact effectiveness of map and QTL projections. The projection task is essential because an error of a few centiMorgans can be critical when computing the number and position of MQTLs. For example, on chromosome 2B, three different MQTLs (MQTL6–MQTL7–MQTL8) were detected, probably due to a problem of projection.

Association mapping

Power of detecting associations with GLM and MLM

The suitability of the MLM method for association mapping has been demonstrated in humans and in many crops (Yu et al. 2006; Zhao et al. 2007; Stich et al. 2008, 2009). In our study, all of the MTAs detected by MLM were also detected by GLM with a variable percentage of explained variance, but some of the known loci, such as major MQTLs or genes, could only be detected with the GLM approach. For example, marker wPt-729979, located in the CI of MQTL21, was only detected with GLM. This observation is in accordance with Yu et al. (2009) who stated that the GLM approach with the Q matrix was probably more sensitive than the MLM approach in some cases because the use of both matrices (Q and K) leads to a loss of degrees of freedom and reduces the power of detecting associations (Stich et al. 2008). Neumann et al. (2010) carried out a genome-wide association mapping study on a winter wheat collection of 96 accessions using both the GLM and MLM models to compare the results with already known loci. In their study as in ours, some of the known loci could only be detected with the GLM approach.

However, in Neumann et al.'s study, very many of the associations detected by GLM could not be detected with MLM, suggesting that these may be false positives. Therefore, new loci should be identified definitively by the MLM approach, rather than by GLM, to avoid false positives that would not be removed by comparison with other reference studies. More comparisons of the different models of association mapping should be made to examine this phenomenon in depth.

Artificial population composed of seven biparental crosses

A specific aim of this study was to collect biparental populations to create an artificial association mapping panel. Association mapping studies have generally been performed on populations composed of unrelated individuals (Crossa et al. 2007; Neumann et al. 2010; Bordes et al. 2011). The MLM, including the kinship relations and an estimate of the population structure, has improved the control of both type I and type II error rates and has allowed the analysis of more or less related individuals.

However, in our study, relatedness (Yu et al. 2006) and artificial structure (Pritchard et al. 2000) in a panel composed of biparental populations might have led to a significant increase in the number of false positives. However, the high consistency between the results of the meta-analysis and those of association mapping suggest that no detectable error was present in our association study. The position of MQTLs and A-QTLs in close proximity confirm that biparental populations can be used in association mapping studies without creating spurious MTAs. This conclusion is particularly interesting for breeding companies which have at their disposal numerous biparental populations originating from many initial crosses.

Nevertheless, our meta-analysis detected a greater number of true loci than the association study (e.g. MQTL15 linked to the *Stb16* locus was not detected by genome-wide association mapping). Therefore, we conclude that meta-analysis remains the most appropriate method to detect QTLs from biparental populations, although in our specific situation association mapping was able to confirm the loci of interest for breeding.

Comparison of results from association mapping and meta-analysis

All markers detected in association mapping with both models were associated with MQTLs or QTLs of STB resistance. This conclusion is not in agreement with the results of other comparative studies in which only a proportion of A-QTLs could be covered by QTLs from a linkage mapping approach. In *Brassica napus,* more than half of the loci to which associated markers corresponded were located within the QTL intervals that had been identified during previous linkage mapping (Zou et al. 2010). In another oilseed rape population (Jestin et al. 2010), several marker alleles displayed a significant association with stem canker resistance, even though no resistance QTLs were detected in these regions by linkage analysis. While previous studies mainly compared the results from standard linkage mapping populations with those from association studies on germplasm from elite and/or wild genotypes (Wilson et al. 2004; Jestin et al. 2010; Zou et al. 2010), in our study we compared meta-analysis and association mapping approaches on the same initial dataset. This difference likely explains why all A-QTLs were confirmed by linkage analysis. In contrast, in some regions detected by linkage mapping, such as MQTL15 or MQTL25, no marker was identified by the association mapping approach to be linked to STB resistance. These differences between linkage analysis and association mapping have been reported previously (Jestin et al. 2010) and could be due to a number of reasons. First, some markers located in the MQTL CI could not be tested in association mapping due to their low frequency (<0.05) in the population. Second, although markers in the MQTL regions were tested, no association was detected either because the LD in the region was too low to detect an association at the marker density used or because of inadequate phenotypic data (Jestin et al. 2010). Moreover, in our study, marker alleles with a P of <0.001 were declared significantly associated with the trait; $P < 0.001$ is a stringent significance value (Bordes et al. 2011) and was specifically chosen to reduce the number of false positives. Many previous association studies in crops used a P value of <0.05 rather than 0.001 (Neumann et al. 2010). Demanding such high significance, however, may lead to the loss of true positives in some regions.

Linkage analysis and association studies are clearly complementary to each other in terms of providing prior knowledge, cross-validation and statistical power (Yu et al. 2006). The combination of the two methods provides the most powerful experimental approach because it exploits the strengths and weaknesses of each method and allows efficient QTL evaluation of the genome. The resolution of linkage mapping studies is often on the order of $\geq$ 10 cM, which corresponds to millions of bases, while association mapping can provide a high resolution alternative even compared with meta-analysis (Veyrieras et al. 2007) because association studies make use of the historical accumulation of recombination events that occurred in natural populations (Atwell et al. 2010). However, linkage mapping remains a powerful tool in combination with association mapping for two reasons (Wilson et al. 2004). First, controlling for population structure in association mapping is necessary for reducing the false positive rate, but this approach also introduces false negatives. QTL mapping, for its part, is not affected by population structure and can therefore facilitate in distinguishing true from spurious associations and may be an excellent alternative for detecting false negatives. Second, although the use of mapping populations in QTL studies tests a maximum of only two alleles, these populations have substantial statistical power to contrast these alleles, given the high frequency of each allele (Wilson et al. 2004). QTL studies have the potential to detect rare alleles that remain undetected by association mapping studies. On the other hand, there is a higher chance to detect the causal alleles in association mapping, as allelic diversity is higher in association studies than in linkage studies. The combination of linkage and association mapping clearly outperforms each method used in isolation (Brachi et al. 2010). The complementarity of association mapping and classical linkage mapping has been particularly well demonstrated in a mouse association mapping study (Manenti et al. 2009) and in *Arabidopsis thaliana* where the combination of association mapping with linkage mapping resulted in F_2 crosses that enabled the identification of both false positives and false negatives (Zhao et al. (2007). Brachi et al. (2010) observed that combining association mapping with coarse mapping using RILs and near-isogenic lines enhanced their ability to distinguish true from false associations.

In our study, association mapping did not detect new loci when compared with meta-analysis. Nevertheless, association mapping can be used as a validation method based on QTLs previously detected by classical methods, as suggested by Thornsberry et al. (2001) and Bordes et al. (2011). For example, two individual QTLs (QTL 2 and QTL 4) would not have been used in breeding without their confirmation by association mapping (A-QTL1 and A-QTL7). Indeed, after decades of standard linkage mapping studies, only a few of the QTLs so identified could be validated and used in breeding programs because for the majority their expected effects usually "disappeared" after introgression into another background (Zou et al. 2010). The main interest in combining approaches is to detect robust loci that will be functional in many genetic backgrounds rather than just in the specific background of the biparental population under study. This reason explains the growing enthusiasm for the simultaneous use of linkage and association mapping because these approaches offer the possibility using markers directly in marker-assisted-selection.

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