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ORIGINAL PAPER

Family-based mapping of quantitative trait loci in plant breeding populations with resistance to Fusarium head blight in wheat as an illustration

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Abstract Traditional quantitative trait loci (QTL) mapping approaches are typically based on early or advanced generation analysis of bi-parental populations. A limitation associated with this methodology is the fact that mapping populations rarely give rise to new cultivars. Additionally, markers linked to the QTL of interest are often not immediately available for use in breeding and they may not be useful within diverse genetic backgrounds. Use of breeding populations for simultaneous QTL mapping, marker validation, marker assisted selection (MAS), and cultivar release has recently caught the attention of plant breeders to circumvent the weaknesses of conventional QTL mapping. The first objective of this study was to test the feasibility of using family-pedigree based QTL mapping techniques generally used with humans and animals within plant breeding populations (PBPs). The second objective was to evaluate two methods (linkage and association) to detect marker-QTL associations. The techniques described in this study were applied to map the well characterized QTL, *Fhb1* for Fusarium head blight resistance in wheat (*Triticum aestivum* L.). The experimental populations consisted of 82 families and 793 individuals. The QTL was mapped using both linkage (variance component and pedigree-wide regression) and association (using quantitative transmission disequilibrium test, QTDT) approaches developed for extended family-pedigrees. Each approach successfully identified the known QTL location with a high probability value. Markers linked to the QTL explained 40–50% of the phenotypic variation. These results show the usefulness of a human genetics approach to detect QTL in PBPs and subsequent use in MAS.

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Introduction

An important product of quantitative trait loci (QTL) mapping studies is the identification of molecular markers that may be useful for plant breeding applications, such as marker assisted selection (MAS). Traditionally, QTL are mapped using populations specifically developed for that purpose and then QTL effects are validated within additional genetic backgrounds prior to widespread introgression via MAS.

There are some important limitations associated with traditional mapping methods. Some of which include limited polymorphism rates, and no indication of marker effectiveness in multiple genetic backgrounds. Often, by the time a QTL mapping population is developed and mapped, breeders have introgressed the new QTL using traditional breeding and selection methods. This can reduce the usefulness of MAS within breeding programs at the time when MAS could be most useful (i.e., shortly after new QTL are identified) (Jannink et al. 2001).

Efforts have been made to develop QTL mapping methods using multi-parental populations (Christiansen et al. 2006; Goffinet and Gerber 2000; Jannink and Jansen 2001; Jansen et al. 2003; Verhoeven et al. 2006; Xu 1998). A more suitable solution could include the use of plant breeding populations (PBPs) for QTL mapping (Beavis 1998). An approach could be the application of family-based methods

(Bink et al. 2002; Crepieux et al. 2005; Jannink et al. 2001) that are generally used within human and animal populations. Theoretically, family-based methods have the advantage of exploiting QTL variation associated with diverse genetic backgrounds to locate suitable polymorphic markers (Jannink et al. 2001). Few theoretical or practical examples exist where PBPs were used as mapping populations, though some examples include identical-by-decent (IBD) based variance component (VC) analysis (Crepieux et al. 2004b, 2005; Xie et al. 1998), mixed models (Arbelbide and Bernardo 2006a), and Bayesian methods for pedigree data (Bink et al. 2002). Crepieux et al. (2004b) demonstrated the VC-based IBD method for mapping a population composed of several subpopulations created by consecutive selfing or backcrossing. Furthermore, the method was validated to map wheat kernel hardness and dough strength among 374 F_6 lines derived from 80 unique parents (Crepieux et al. 2005). Arbelbide and Bernardo (2006a) successfully used a mixed-model on the same population and traits to validate the QTLs map by Crepieux et al. (2005). With respect to the time requirements for introgressing previously unmapped QTL into breeding programs these methods have limitations similar to the development of recombinant inbred lines (RIL) or near isogenic lines (NIL) families.

To speed the QTL mapping process, one prospect may include use of early generation materials. Through simulation experiments Xie et al. (1998) demonstrated the possible use of IBD-based VC methods in plant families (F_2, backcross, and full-sib) derived from multiple line crosses (from more than 50 parents). Bink et al. (2002) discussed a pedigree analysis approach for outbred plant species and applied Bayesian methods for mapping late blight resistance in single cross derived potato families. Similarly for outbred populations, Jansen et al. (2003) discussed a method based on haplotype sharing, and through simulation, demonstrated its use in early generation ($F_{2:3}$) maize populations derived from two-way crosses from multiple inbred parents.

Association mapping (also known as linkage disequilibrium mapping) has also received considerable attention for QTL mapping among lines from within breeding programs for multiple crops (Breseghello and Sorrells 2006a). The possibility of association mapping exists in self-pollinated plants as well for fine-mapping (Bink and Meuwissen 2004; Maccaferri et al. 2005). The most commonly used populations for association mapping are germplasm collections or natural populations (Breseghello and Sorrells 2006b; Gebhardt et al. 2004; Skøt et al. 2005; Yu and Buckler 2006). In contrast, the use of family-based association mapping techniques is limited. Malosetti et al. (2007) used pedigree-data for association mapping to map resistance loci to *Phytophthora infestans* in potato. Such association mapping techniques might be useful in marker development for MAS, in addition to being companion procedure for cross-validating results from linkage analysis (Breseghello and Sorrells 2006a; Flint-Garcia et al. 2003).

Studies are limited to early detection of QTLs (before gene of interest is introgressed into population) in typical PBPs derived from multi-parent crosses; additionally, software for such purpose is also limited. Typical PBPs are derived from small families of multi-way crosses. An effective way to integrate QTL mapping into breeding programs could be to employ robust and flexible family-based QTL mapping methods developed for use among humans and animals to PBPs.

Among linkage-based mapping procedures commonly used in humans, VC analysis is a powerful procedure to map unselected and normally distributed quantitative traits (Pugh et al. 1997). Sham et al. (2002) developed a pedigree-wide regression (PWR) procedure, more powerful than the conventional regression method developed by Haseman and Elston (1972). Both procedures, VC analysis, and PWR, can accommodate complex extended family-pedigrees with larger sibships. Several computer software packages have been developed to aid with the calculation requirements for both linkage-based mapping and association test for family-pedigrees in animals and humans. One example of is 'MERLIN' (Abecasis et al. 2002). Large number of markers can be analyzed with this software and it has the capacity to work with, tolerate genotyping errors, and missing values (Abecasis et al. 2002; Sham et al. 2002).

In addition to linkage-based mapping procedures, robust, and flexible family-based association tests are available. Association tests for quantitative traits in nuclear (Abecasis et al. 2000a) and extended families (Abecasis et al. 2000b) have been developed. The application of the transmission disequilibrium test in inbred population has been suggested in a situation where there is at least one heterozygote parent (Jannink et al. 2001). Suitable software packages are also available for this purpose. For example, 'QTDT' is software written to perform a quantitative transmission disequilibrium test (QTDT) for nuclear as well as extended pedigrees (Abecasis et al. 2000a, b).

Linkage-based methods offer high power to detect QTL's in genome-wide scans and association mapping has the benefit of increasing resolution (Remington et al. 2001). When used in succession, these two techniques will help in cross-validating results and increasing statistical power and identification of suitable markers for MAS (Wilson et al. 2004).

Fusarium head blight (FHB) caused by *Giberella zeae* Schw. (Petch) (*Fusarium graminearum* Schwabe) is one of the most important wheat diseases causing yield and quality losses worldwide (Goswami and Kistler 2004). The disease can affect wheat spikelets by causing what appears

to be premature ripening when healthy, green tissue would generally be expected. Diseased kernels become grayish-brown, lightweight, and may contain high mycotoxin concentrations. Severe epidemics can prevent normal development of seeds. The most commonly used source of FHB resistance within US wheat breeding programs is the *Fhb1* QTL derived from ‘Sumai 3’ and its derivatives (Bai and Shaner 1994; Liu and Anderson 2003a, b; Pumphrey et al. 2007). This QTL has been shown to explain 25–60% of phenotypic variation for Type II resistance (resistance to spread) (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002). The *Fhb1* QTL is located within the marker interval *Xbarc133–Xgwm493* on the distal end of the short arm of chromosome 3B (Cuthbert et al. 2006; Liu and Anderson 2003a, b). Genetic studies have shown quantitative inheritance with a moderate to high level of heritability (from 0.25 to 0.86) for *Fhb1* (Anderson et al. 1998, 2001; Buerstmayr et al. 2000, 2003; del Blanco et al. 1993; Miedaner 1997; Shen et al. 2003; Singh et al. 1995; Stack and Frohberg 1991).

The first objective of this study was to test, using the *Fhb1* locus in wheat as model, the feasibility of using family-pedigree based QTL mapping techniques generally used with humans and animals within PBPs. The second objective was to evaluate two methods (linkage and association) to detect marker-QTL associations. In each of these methods two statistical procedures were also evaluated.

We decided to use the *Fhb1* locus, which confers Type II resistance to FHB in wheat, as a model in which to test these methodologies because it has a well known and validated location, heritability, and effect on the phenotype. It is well established that Fhb1 consistently reduces symptoms across screenings (Pumphrey et al. 2007). As a model system *Fhb1* provides a positive control to test the efficacy of the analysis techniques utilized in this study.

Materials and methods

Plant material

Mapping populations were derived from three- and four-way crosses among a number of genotypes. Small three- or four-way F_1 families with backcross or testcross-like structures were derived. Founder plants were those that gave rise to other plants. Crossing schemes for each family are provided in Table 1. The term “family-pedigree” is defined here as a description of the ancestors of an individual going back to the parents used to generate the families in the mapping population. This does not include past breeding history of mapping population parents, most commonly known as the breeding pedigree.

There were 82 families consisting of 793 individual plants (256 founder and 483 non-founder plants). Average family size was 9 but ranged from 4 to 17. The common family structure was derived from three- or four-way crosses, which are similar to a three generation (grandparent, parents, and progenies) or four generation (great-grand parent, grand parent, parents, and progenies) human pedigree. To generate informative families for resistance, at least one parent in each family was known to carry *Fhb1*. Founders for the mapping population included 49 wheat genotypes used within the South Dakota State University (SDSU) spring wheat breeding program. Among founders, three were known to carry, *Fhb1*; SD3851 (ND 2897/SD3219//SD3414), SD3776 (1340_325-2-2-4///SDX17150//SD3005/GUARD/4/SD3420/5/SD3310), and Freyr (N94-0157//Sumai 3/Dalen). Other genotypes used for family construction were susceptible to FHB (based on previous greenhouse and field screening observations made by the SDSU spring wheat breeding program). It is important to notice that this material was developed for this study. The resistant parents were selected knowing that all three have the same resistance locus, Fhb1.

Phenotypic evaluation

Type II resistance (resistance to spread) was evaluated on inoculated individuals within families in a greenhouse testing environment during spring 2006. A single floret in the central portion of two spikes for each test entry was inoculated at anthesis with a highly aggressive *G. zeae* isolate (FG_4) using 10 μl of inoculum at a concentration of 100,000 conidia ml^{-1}. Following standard practices, disease severity was collected 14 and 21 days after inoculation (DAI) on every inoculated spike. Along with families, check genotypes, Sonalika and Wheaton were grown at regular spatial intervals within the greenhouse test.

Genotyping

DNA was extracted using a CTAB–chloroform extraction protocol (Doyle and Doyle 1990). Genotyping included simple sequence repeats (SSR) markers previously mapped to chromosome 3B included X*gwm389*, X*gwm533*, X*gwm493*, X*gwm114*, X*wmc754*, X*wmc623*, X*wmc777*, X*wmc787*, X*barc133*, X*barc084*, and *Xgwm340* (Korzun et al. 1997; Röder et al. 1998; Somers et al. 2004). Similarly, X*gwm136* on chromosome 1AS (Röder et al. 1998) and the STS marker linked to the leaf rust (*Puccinia recondita*) resistance gene, *Lr34*, on chromosome 7D (Lagudah et al. 2006) were used as non-linked check markers. The PCR amplification was performed as per Liu and Anderson (2003a). Primers used for PCR amplification were fluorescently labeled with 6-FAM, NED, VIC or PET,

Table 1 Description of the families used in this study

SN	Parent 1	Parent 2	Parent 3	Parent 4	Family size	Crossing Scheme
1	**SD3776**	SD3746	SD4032	–	8	SD3746/SD3776//SD4032
2		**FREYR**	SD3879	–	9	SD3776/FREYR//SD3879
3		GRANGER	TRAVERSE	–	9	SD3776/GRANGER//TRAVERSE
4		SD3641	SD4032	–	9	SD3776/SD3641//SD4032
5		**SD3851**	SD3943	–	8	SD3776/SD3851//SD3943
6			SD4011	–	7	SD3776/SD3851//SD4011
7		SD3870	SD4070	–	9	SD3776/SD3870//SD4070
8		STEELE-ND	SD3927	–	12	SD3776/STEELE-ND//SD3927
9			TRAVERSE	–	9	SD3776/STEELE-ND//TRAVERSE
10		SD3868	SD4032	–	9	SD3868/SD3776//SD4032
11		SD3900	SD3879	–	9	SD3900/SD3776//SD3879
12			TRAVERSE	–	6	SD3900/SD3776//TRAVERSE
13		SD4018	KNUDSON	–	6	SD4018//KNUDSON/SD3776
14	**SD3851**	BZ 998-447 W	BRIGGS	**SD3851**	7	BZ 998-447 W/BRIGGS//SD3851/3/SD3851
15		FN1405-350	98S0113-20-23	–	7	FN1405-350/SD3851//98S0113-20-23
16		FN1504-124	GLENN	–	10	FN1504-124/SD3851//GLENN
17		FN1504-19	SD3879	–	11	FN1504-19/SD3851//SD3879
18		FN1505-13	**FREYR**	–	8	FN1505-13/SD3851//FREYR
19			SD3900	–	7	FN1505-13/SD3851//SD3900
20		FN1505-40	SD4070	–	13	FN1505-40/SD3851//SD4070
21		FN1705-146	TRAVERSE	–	12	FN1705-146/SD3851//TRAVERSE
22		FN1905-53	SD3879	–	13	FN1905-53/SD3851//SD3879
23			SD4011	–	12	FN1905-53/SD3851//SD4011
24			SD4070	–	13	FN1905-53/SD3851//SD4070
25		**FREYR**	MN01057-3-1	–	6	FREYR//MN01057-3-1/SD3851
26		INIA 66	SD3879	–	16	Inia 66/SD3851//SD3879
27		MN01057-3-1	SD4011	–	9	MN01057-3-1/SD3851//SD4011
28			SD4023	–	10	MN01057-3-1/SD3851//SD4023
29			SD4032	–	12	MN01057-3-1/SD3851//SD4032
30		MN01164	BZ 998-447 W	SD4002	13	MN01164/BZ 998-447 W//SD3851/3/SD4002
31		MN01197	PI74494	SD4032	7	MN01197/PI74494//SD3851/3/SD4032
32			SD4070	–	6	MN01197/SD3851//SD4070
33		MN02306-2	SD3868	–	10	MN02306-2/SD3851//SD3868
34			SD3879	–	9	MN02306-2/SD3851//SD3879
35			TRAVERSE	–	5	MN02306-2/SD3851//TRAVERSE
36		SD3641	SD3943	–	10	SD3641/SD3851//SD3943
37			SD4037	–	16	SD3641/SD3851//SD4037
38			SD4070	–	12	SD3641/SD3851//SD4070
39		FN1705-146	**SD3851**	–	8	SD3851//FN1705-146/SD3851
40		FN1404-204	SD3943	–	5	SD3851/FN1404-204//SD3943
41			SD4070	–	5	SD3851/FN1404-204//SD4070
42		FN1704-298	SD3900	–	11	SD3851/FN1704-298//SD3900
43		GRANGER	SD4018	–	8	SD3851/GRANGER//SD4018
44			SD4032	–	9	SD3851/GRANGER//SD4032

Table 1 continued

SN	Parent 1	Parent 2	Parent 3	Parent 4	Family size	Crossing Scheme
45	**SD3851**	KNUDSON	SD3879	–	9	SD3851/KNUDSON//SD3879
46			SD4023	–	12	SD3851/KNUDSON//SD4023
47			TRAVERSE	–	11	SD3851/KNUDSON//TRAVERSE
48		SD3746	SD3868	–	6	SD3851/SD3746//SD3868
49			SD4002	–	10	SD3851/SD3746//SD4002
50			TRAVERSE	–	10	SD3851/SD3746//TRAVERSE
51			SD4018	–	7	SD3851/SD3776//SD4018
52		SD3900	**SD3851**	–	12	SD3851/SD3900//SD3851
53			SD3868	–	7	SD3851/SD3900//SD3868
54			TRAVERSE	–	12	SD3851/SD3900//TRAVERSE
55			ATILLA COMPLEX	–	12	SD3900//ATILLA COMPLEX/SD3851
56			FN1705-146	–	14	SD3900//FN1705-146/SD3851
57			FN1905-53	–	11	SD3900//FN1905–53/SD3851
58		SD3901	SD4018	–	14	SD3851/SD3901//SD4018
59			TRAVERSE	–	9	SD3851/SD3901//TRAVERSE
60		SD3927	GRANGER	–	12	SD3927//SD3851/GRANGER
61			KNUDSON	–	9	SD3927//SD3851/KNUDSON
62			SDSWX27158	–	16	SD3927//SDSWX27158/SD3851
63			SDSWX27150	–	6	SD3927//SDSWX27150/SD3851
64			SDSWX27151	–	15	SD3927//SDSWX27151/SD3851
65		SD3942	SD3642	–	6	SD3942//SD3642/SD3851
66			SD3641	–	11	SD3942//SD3641/SD3851
67			GRANGER	–	7	SD3942//SD3851/GRANGER
68		SD3943	BZ 998-447 W	BRIGGS	7	SD3943/3/BZ 998-447 W/BRIGGS//SD3851
69		SD4002	SD3900	–	6	SD4002//SD3851/SD3900
70			FN1705-146	–	6	SD4002//FN1705-146/SD3851
71		SD4011	MN01197	PI74494	13	SD4011/3/MN01197/PI74494//SD3851
72		SD4018	GRANGER	–	12	SD4018//SD3851/GRANGER
73		SD4032	FN1505-13	–	9	SD4032//FN1505-13/SD3851
74			INIA66	–	11	SD4032//Inia 66/SD3851
75			**SD3776**	–	6	SD4032//SD3776/SD3851
76			WEAVER COMPLEX	–	6	SD4032//WEAVER COMPLEX/SD3851
77		SDSWX27158	**SD3851**	–	7	SDSWX27158/SD3851//SD3851
78			SD4073	–	6	SDSWX27158/SD3851//SD4073
79		STEELE-ND	SD4073	–	14	STEELE-ND/SD3851//SD4073
80		TRAVERSE	MN01057-3-1	–	7	TRAVERSE//MN01057-3-1/SD3851
81		WEAVER COMPLEX	GLENN	–	12	WEAVER COMPLEX/SD3851//GLENN
82		WEEBILL 1	**SD3776**	SD4002	17	WEEBILL 1/SD3776//SD3851/3/SD4002

Founders carrying *Fhb1* are presented in bold

and fragment size analysis was performed using an ABI 3130XL (Applied Biosystems, Inc.) genetic analyzer with a LIZ fluorescently labeled internal size standard of 600 base pairs bin size. The resulting output was then scored using GeneMapper v3.7 software (Applied Biosystems, Inc.). Only founder parents and third generation offspring from all 82 family-pedigree were genotyped. The genotype of the second generation was predicted based on genotypes of their homozygous parents.

Statistical analysis

The software package PEDSTATS (Wigginton and Abecasis 2005) was used to test for Mendelian genotyping error

within pedigrees. Sex of progenies was arbitrarily assigned. No traits were defined as covariates. The family-based approach consisted of three methods. Two of these methods (VC and PWR) make used of linkage information, while the third one (QTDT) is an association-based method.

Linkage analysis was used to test for co-segregation of a chromosomal region with a trait locus of interest. Linkage-based QTL mapping in human genetic studies include different parametric (regression-based approach and VC approach) and non-parametric approaches (for review Majumder and Ghosh 2005). VC-based linkage analysis has been used from long time in QTL analysis in humans with wide range of modifications (Cherny et al. 2004). In contrast to pair-based (e.g., sib pair) methods the pedigree-based variance-component linkage methods are based on extension of the strategy of Amos (1994) to estimate the genetic variance attributable to the region around a specific genetic locus.

VC methods have been implemented in several software packages which include MERLIN (Abecasis et al. 2002), Genehunter (Pratt et al. 2000) and SOLAR (Almasy and Blangero 1998). A basically common feature of them to analyse general family-pedigrees is to partition the observed variation in quantitative trait into causal components including QTL genotype contribution from the chromosome segment, contribution from rest of genome, and residual (environmental) factors. Also IBD proportion is estimated using multiple marker information in the genome. MERLIN is a computer program that uses sparse inheritance trees for pedigree analysis and is based on Lander–Green's algorithm (Lander and Green 1987). This algorithm uses the Markov chain to calculate likelihoods for all gene flow patterns at arbitrary chromosomal locations. MERLIN estimates haplotypes by finding the most likely path of gene flow or by sampling paths of gene flow at all markers jointly.

Variance components method

The VC-based linkage analysis was carried out using Multipoint Engine for Rapid Likelihood Inference (MERLIN) v.1.1-alpha 3 (Abecasis et al. 2002). This method is a parametric linkage analysis based on multipoint identity-by-descent (IBD) probabilities at each marker and equally spaced loci between each pair of markers. Multipoint linkage analysis increases the power to detect true linkages and decreases the false-positive rate. The observed variation from the quantitative trait is partitioned into the following components:

$$Y_i = \mu + Q_i + A_i + F_i + \varepsilon_i$$

where the phenotypic value (Y_i) is partitioned in to population average (μ), QTL genotype contribution from chromosome segment (Q_i), contribution from rest of genome (A_i), shared common family environment (F_i) and residual (experimental) error (ε_i). The following are variance associated with the components:

$$\sigma^2 y = \sigma^2 q + \sigma^2 a + \sigma^2 f + \sigma^2 e$$

where $\sigma^2 y$ is total phenotypic variation in trait of interest y, $\sigma^2 q$ is variation contributed from the chromosome segment, $\sigma^2 a$ is variation from rest of genome, $\sigma^2 f$ is variation due to shared common family environment, and $\sigma^2 e$ is residual experimental error variance.

Examples of applications of MERLIN-based parametric linkage analysis method, commonly termed as VC analysis in human genetics studies can be found in Aissani et al. (2006), Farbrother et al. (2004), and Malhotra et al. (2007).

The following assumptions were made for this analysis: (1) penetrance was assumed complete with a value of one, (2) the probability of an individual carrying the resistance gene was one-half, (3) founders were assumed unrelated to one another, and (4) consensus map distances from Somers et al. (2004) were used for distance measures between markers. Markers not available within the consensus map were placed on positions suggested by other studies (Lagudah et al. 2006; Korzun et al. 1997; Röder et al. 1998).

Pedigree-wide regression method

The second method was PWR (Sham et al. 2002). MERLIN-REGRESS, a procedure of MERLIN 1.1-alpha 3 software, was used for this purpose. This analysis is based on the regression of IBD sharing between relative pairs on the squared sums and squared differences of trait values. The method is considered to be less susceptible to violations of the normality assumption and is valid to diverse pedigree structures and even to pedigrees selected on the basis of trait value.

For a pedigree with n members, let the values of a quantitative trait X of the family members $X_1, X_2,\ldots, X_n$, respectively. The values of, $X_1, X_2,\ldots, X_n$, were standardized to mean 0 and variance 1 and joint multivariate normal distribution is assumed. For each pair of pedigree members, we define the squared sum $S_{ij} = (X_i + X_j)^2$ and the squared difference $D_{ij} = (X_i - X_j)^2$ for $i \neq j$. In addition, the proportion of alleles IBD for pedigree members i and j (denoted as π_{ij}) was estimated from the marker data and denoted as $\hat{\pi}_{ij}$. The calculation of these estimates was done using Lander–Green algorithm (Lander and Green 1987) implemented in Merlin (Abecasis et al. 2002). The arrays $[S_{ij}]$, $[D_{ij}]$, and $\left[\hat{\pi}_{ij}\right]$ of the entire pedigree were inserted into the vectors S, D, and $\hat{\prod}$, each having dimension of $n(n-1)/2$. The method is based on regressing IBD sharing on squared sums and squared differences (D). Thus $\hat{\prod}$ is regressed on S and D. The details on computational

procedure are given in Sham et al. (2002) and the whole process can be implemented with in Merlin software by Abecasis et al. (2002).

The two linkage-based procedures are usually used in human and animal genetic studies. The reason for considering both methods in this study was to explore the suitability and potential future application of each with PBPs, and also to cross-validate results. For both procedures (VC and PWR), linkage between markers and putative QTLs was deemed to be significant at a threshold LOD score of 3.0.

Using MERLIN v.1.1-alpha 3, 1,000 simulation scans of our empirical data (making 1,000 data sets with similar family structure, phenotype, marker spacing, allele frequencies and missing-data patterns) were performed to calculate the power of the test. The power of QTL mapping is defined as the ability of the test to detect a QTL if it actually exists. In other words, power is the probability of not committing a Type II error. A threshold LOD score of 3.0 was also used for this analysis. Thus, the power was calculated as the probability of observing two consecutive markers with a score greater than the threshold value within simulation iterations.

Association method

Results from the analysis described above were validated using the QTDT for extended pedigrees (Abecasis et al. 2000a, b). The software QTDT v 2.6.0 (Abecasis et al. 2000a, b) was used for association analysis. QTDT is a convenient method of testing family-based tests of LD which supports nuclear families, with or without parental genotypes, or extended pedigrees. IBD coefficients, calculated using MERLIN v.1.1-alpha 3 (Abecasis et al. 2002), were used as input for the QTDT software.

The QTDT can be used to test linkage, allelic transmission, and total association (joint linkage and association) depending upon model parameters specified (Lange et al. 2006; Mars et al. 2008; Deng 2003). Thus, using the QTDT program we can tests of population stratification, within-family association, and total association (including within- and between-family components). The tests implemented in the QTDT program were developed under a VC framework (Abecasis et al. 2000a, b). IBD coefficients, calculated using MERLIN v.1.1-alpha 3 (Abecasis et al. 2002), were used as input for the QTDT software. The orthogonal association model described by Abecasis et al. (2000b) for extended pedigrees was used to test the association of individual alleles of each locus with the trait in question. The following models were tested:

Null hypothesis: $\overline{X} = \mu + B$,
Alternative hypothesis: $\overline{X} = \mu + B + W$,

where B is between component of association and W is within component of association.

The orthogonal association model described by Abecasis et al. (2000b) for extended pedigrees was used to test the association of individual alleles of each locus with the trait in question. As default of the QTDT software the alleles that have less than 30 informative individuals were not tested. Informative individuals were defined as phenotyped offspring with at least one heterozygous parent for the allele in question.

Results

The frequency distribution of FHB severity scores is presented in Fig. 1; the *Q–Q* normal plots indicate approximate normal distributions for both disease observation dates. However, the Kolmogorov–Smirnov test of normality was significant ($P \leq 0.05$) at 14 DAI and not significant at 21 DAI. Data transformation was not applied to 14 DAI data as *Q–Q* normal plot shows approximation to normal distribution The percentage of spikelets infected in the susceptible checks, Sonalika and Wheaton was 78 and 75% at 14 DAI, respectively, and 100% for both at 21 DAI (Fig. 1). The percentage of spikelets infected in the resistant genotype SD3851 was 21 and 32% at 14 and 21 DAI, respectively (Fig. 1). All 13 markers tested were polymorphic and the number of alleles produced ranged from 2 to 7 (Table 3).

Linkage method–variance component procedure

Results from VC-based linkage analyses produced similar results for both readings at 14 and 21 DAI (Fig. 2a). The QTL was found to most likely be located between the *Xbarc133* and *Xgwm493* marker loci. Markers that were located distantly on 3BS to the QTL of interest, and on completely different chromosomes, showed very low LOD scores in each of our analyses. *Xgwm136* on chromosome 1A (LOD = 0.005 for 14 DAI and 0.2 for 21 DAI) and *LR34* on 7D (LOD = 0.5 for both 14 DAI and 21 DAI) showed low LOD scores, which was indicative of no linkage to *Fhb1*.

Simulation results revealed that the probability of finding two consecutive markers with LOD scores >3.0 was 32% for 14 DAI and 34% for 21 DAI.

Linkage method–pedigree-wide regression procedure

Results from the PWR procedure produced similar results for severity at 14 and 21 DAI (Fig. 2b). The QTL was again found to most likely be located between the *Xbarc133* and *Xgwm493* marker loci. Non-linked markers

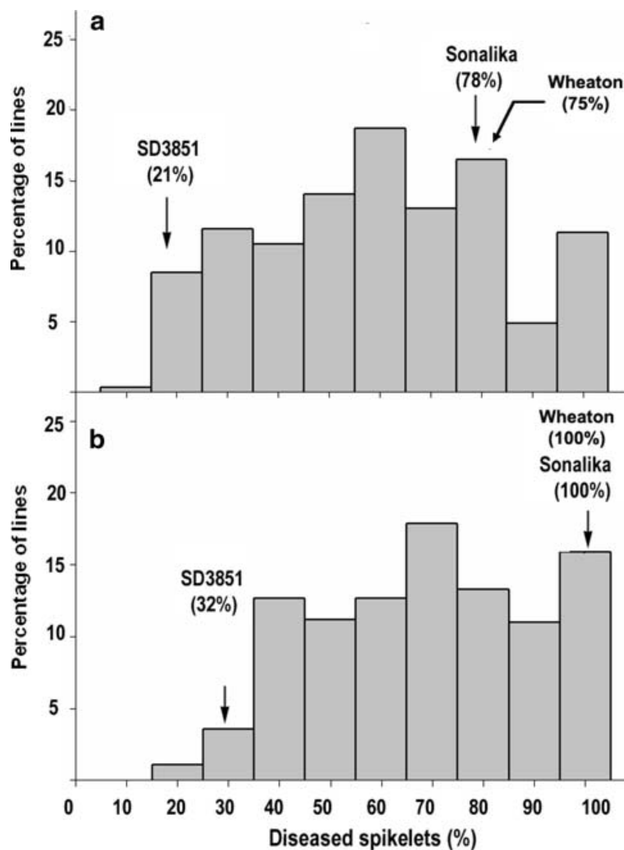

Fig. 1 Frequency distributions of FHB severity in population studied. **a** 14 days after inoculation, **b** 21 days after inoculation

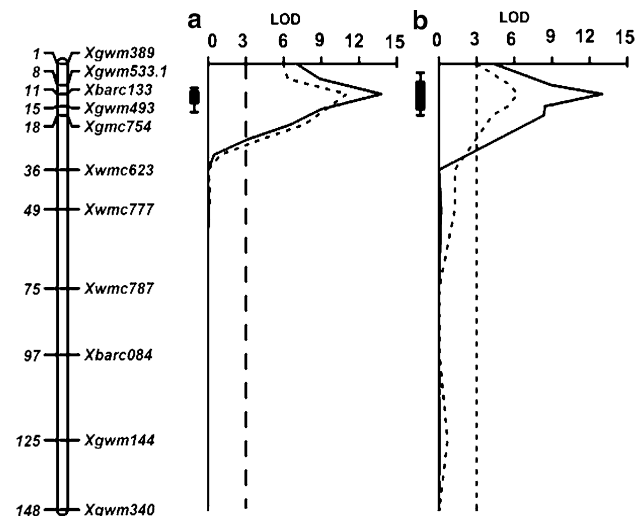

Fig. 2 Position of *Fhb1* based on **a** variance component-based linkage analysis using MERLIN and **b** pedigree-wide regression using MERLIN-REGRESS. *Dotted line* shows 14 days after inoculation and *solid line* shows 21 days after inoculation. QTL locations are indicated with 1 and 2 LOD confidence intervals

from both 3B and other chromosomes showed very low LOD scores. For example, *Xgwm136* on chromosome 1A (LOD = 0.09 for 14 DAI and 0.3 for 21 DAI) and *LR34* on 7D (LOD = 0.5 for both 14 DAI and 21 DAI). The most tightly linked marker locus, *Xbarc133* explained 46% of the total phenotypic variation for FHB severity.

Association method

Association mapping results using QTDT showed that the *Xgwm533.1*, *Xbarc133*, and *Xgwm493* were the only three loci significantly associated with FHB resistance ($P < 0.001$) (Table 2). In each of these three loci the allele derived from resistant parent showed the association to the QTL. The alleles showing the strongest association with the phenotypic data were *Xgwm533.1-158* ($\chi^2 = 15.5$, $P = 8 \times 10^{-5}$), *Xbarc133-118* ($\chi^2 = 11.2$, $P = 8 \times 10^{-4}$), and *Xgwm493-192* ($\chi^2 = 18.6, P = 2 \times 10^{-5}$) for 14 DAI (Table 2). These same alleles *Xgwm533.1-158* ($\chi^2 = 25.5$, $P = 4 \times 10^{-7}$), *Xbarc133–118* ($\chi^2 = 17.9, P = 2 \times 10^{-5}$), and *Xgwm493–192* ($\chi^2 = 25.4$, $P = 4 \times 10^{-7}$) showed the strongest association with the 21 DAI phenotypic data (Table 2). All of these alleles were transmitted from the resistant parents (SD3776, FREYER, and SD3851) (Table 3). The overall Bonferroni level of significance was $P < 0.0005$ and $P < 3 \times 10^{-5}$ for the 14 and 21 DAI, respectively.

Discussion

Evaluation of the breeding populations for FHB resistance suggested that disease severity had a distribution consistent with that of a quantitatively inherited trait (Fig. 1). The *Q–Q* normal plots show normal distribution for both disease observation dates, although the Kolmogorov–Smirnov test of normality was significant ($P < 0.05$) at 14 DAI ($P = 0.04$) and non significant for 21 days after inoculation ($P = 0.06$). Non-normality could have been due to a smaller than optimal population size (<1,000). Although the Kolmogorov–Smirnov normality test was significant, the *Q–Q* normal plot is often better suited than a *P* value to test for normality (Park 2006). All three procedures used in this study have some degree of tolerance to a minor deviation from normality as PWR is less sensitive to frequency distribution (Sham et al. 2002).

Variance component method

Results of the VC procedure were consistent with previously published conventional QTL mapping results (Anderson et al. 2001; Liu and Anderson 2003a, b) where tight linkage among *Fhb1* and the 3BS markers *Xgwm533.1*, *Xbarc133*, and *Xgwm493* were reported. Marker loci in the interval of *Xgwm389–Xgwm754* were significantly linked with the QTL, and had LOD scores

Table 2 Testing of association between marker loci (and their informative alleles) and Fusarium head blight severity in family-pedigrees studied using quantitative transmission disequilibrium test (QTDT)

Chr[a]	Marker	Allele Size (bp)	14 DAI[b]		21 DAI[c]	
			$\chi 2_{TDT}$	P	$\chi 2_{TDT}$	P
3B	*Xgwm389*	131	0.4	>0.05	0.04	>0.05
		135	0.34	>0.05	0.03	>0.05
3B	*Xgwm533.1*	114	6.4	0.01	8.25	0.004
		158	15.5	8×10^{-5}	25.5	4×10^{-7}
		155	8.5	0.003	15.1	0.0001
3B	*Xbarc133*	88	3.4	0.06	5.3	0.02
		120	7.3	0.006	11.1	0.0008
		118	11.2	0.0008	17.9	2×10^{-5}
3B	*Xgwm493*	137	8	0.004	8.7	0.003
		157	10.7	0.001	14.2	0.0002
		192	18.6	2×10^{-5}	25.4	4×10^{-7}
3B	*Xwmc754*	136	0.01	>0.05	0.7	>0.05
		158	0.02	>0.05	0.7	>0.05
3B	*Xwmc623*	94	0.67	>0.05	0.49	>0.05
		130	1.3	>0.05	1.14	>0.05
3B	*Xwmc777*	94	1.32	>0.05	1.04	>0.05
		114	0.55	>0.05	0.75	>0.05
3B	*Xwmc787*	118	0.97	>0.05	0.55	>0.05
		154	1.27	>0.05	1.01	>0.05
3B	*Xbarc084*	100	0.14	>0.05	0.17	>0.05
		122	0.47	>0.05	0.69	>0.05
3B	*Xgwm114*	100	0.43	>0.05	0.28	>0.05
		123	0.31	>0.05	0.38	>0.05
3B	*Xgwm340*	126	0.31	>0.05	1.35	>0.05
		146	0.08	>0.05	0.48	>0.05
1A	*Xgwm136*	118	0.008	>0.05	0.005	>0.05
		137	0.4	>0.05	0.32	>0.05
		310	0.019	>0.05	0.0102	>0.05
7D	*Lr34*	155	0.07	>0.05	0.48	>0.05
		242	0.35	>0.05	1.35	>0.05

[a] Chromosome number

[b] Maximum value for Fusarium head blight severity score at 14 days after inoculation

[c] Maximum value Fusarium head blight severity score at 21 days after inoculation

greater than 3.0 for both 14 and 21 DAI (Fig. 2). A LOD score of 12.0 was observed which is comparable with the 13.8 reported by Anderson et al. (2001).

The moderate statistical power of the VC procedure, 32 and 34% for 14 and 21 DAI, respectively, was comparable with results of previous studies for different population types (Arbelbide and Bernardo 2006b; Beavis 1998; van Ooijen 1992). Arbelbide et al. (2006b) applied a mixed model approach to map QTL in complex pedigrees of inbred lines and reported a power of <1–47%, with sample size variation of 600–1,200. Beavis (1998) reported that the power to detect QTLs varied between 9 and 57% depending upon sample size (100–500) for a QTL with an R^2 of 30%. Similarly, van Ooijen (1992) found a wide range power in the range of the tests depending upon the R^2 value in F_2 and backcross populations of size 100–200.

MERLIN was deliberately chosen over other genetic software packages because of its capacity to utilize a large number of markers and its tolerance to both genotyping error and missing values (Abecasis et al. 2002). MERLIN is also suitable for use with small to moderately large family-pedigrees. Our wheat pedigrees were composed of three generations which spanned from grand parents to grandchildren. MERLIN was found to have the capacity to analyze our data set. The most important advantage of plants, when contrasted with humans, is the use of informative parents selected for contrasting phenotypic value in designed crosses; in this example, resistant and susceptible genotypes to FHB.

Pedigree-wide regression method

PWR-based linkage analysis revealed that marker loci in the *Xgwm389–Xgwm754* interval were significantly linked with *Fhb1* and had LOD scores >3.0 for both 14 and 21 DAI. The PWR analysis showed that the marker locus *Xbarc133*, explained 46.0% of the total phenotypic variation. Previous conventional QTL mapping studies have shown a range of variations associated with *Xbarc133* that varied from 25 to 60%, depending on the population studied (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002).

Association method

The results from QTDT to detect association between marker loci and the phenotype were consistent with previously identified marker loci *Xgwm533.1*, *Xbarc133*, and *Xgwm493* (Anderson et al. 2001; Liu and Anderson 2003a, b). High probability values suggest that alleles of these markers were associated with *Fhb1*. In each marker locus the alleles originated in each parent with *Fhb1* were highly significantly associated with resistance. Of five markers found significantly linked to the QTL using VC and PWR, *Xgwm389* and *Xgwm754* were not significantly associated with *Fhb1* using QTDT (Table 2). These results show that QTDT test was more stringent than linkage-based methods. Also QTDT focus on transmission of particular alleles from different locus. This illustrated that QTDT can be a powerful tool to identify useful markers and their alleles for MAS. In this study the selection MAS should be focused on *Xgwm533.1-158*, *Xbarc133-118* and *Xgwm493-192*.

Table 3 Marker loci and their informative allele size for the pedigree's founders

Markers	Size (bp)	Parents
Xgwm389	131	SD3776, MN1197, FN1705-146, SD4017, SD4032, WEEBIL, SD4018, SD4011, SD3868, SD3641
	135	SD4070, SD3746, FREYER, STEEL-ND, WEAVER COMPLEX, TRAVERSE, SD4037, SD3851
Xgwm533.1	114	MN01164, FN1705-146, SD4017, SD3879, MN01057-3-1, GRANGER, SD3900, WEAVER COMPLEX, SD4023, SD4037, GLENN, SD3943, SD3868, INIA 66, SD3641
	158	**SD3776, FREYER, SD3851**
	155	SD4073, SD4070, MN02-306-2, SD4017, 98S550113-20-23, SDWX27158, SD3942
Xbarc133	88	MN01164, SD4070, MN1197, SD4017, SD3879, SD3746, SD3940, SD3927, MN01057-3-1, GRANGER, 98S550113-20-23, SD3870, SD4009, SD4017, INIA 66
	120	SD4032, WEEBIL, STEEL ND, SD3942, KNUDSON, SD4018, SD4023, GLENN, SD3641
	118	**SD3776, SD3851**
Xgwm493	137	SD3879, SD3927, SD3901, MN01164, SD3940, MN1197, 98S550113-20-23, SD3942, SD3868, MN02-306-2, SD3900, INIA 66, SD4037
	157	SD4009, SD4073, SD4017, GRANGER, SD3870, WEEBIL, MN01057-3-1, SD4004
	192	**SD3776, FREYER, SD3851**
Xwmc754	136	SD4073, SD3776, MN01164, MN1197, FN1705-146, SD4017, SD3879, SD3746, SD4032, STEEL-ND, MN02-306-2, SD3942, SD3940, SD3927, KNUDSON, SD4018, WEAVER COMPLEX, TRAVERS, 98S550113-20-23, SD3870, SD4023, SD4037, SD3901, SD3868, SD4009, SD4017, INIA 66, SD3641, SD3851
	158	SD4070, FREYER, WEEBIL, MN01057-3-1, GRANGER, SD3943
Xwmc623	130	SD4073, SD3776, MN01164, SD4070, MN1197, SD37746, FREYER, SD4032, WEEBIL, STEEL-ND, SD3827, KNUDSON, MN01057-3-1, GRANGER, SD3900, TRAVERSE, 98S550113-20-23, SD3870, SD4037, SD3901, SD3943, SD4009, SD3641, SD3851
	94	FN1705-146, SD3879, SDWX27158, SD3942, SD3940, SD4018, SD401, SD3999, SD3868, SD4017, INIA 66
Xwmc777	94	SD4073, MN01164, SD4070, MN1197, FN1705-146, SD4017, SD3879, SD3746, FREYER, SD4032, STEEL-ND, MN02-306-2, SDWX27158, SD3940, KNUDSON, MN01057-3-1, SD4018, TRAVERSE, 98S550113-20-23, SD3870, SD4011, SD4023, SD3999, SD3901, GLENN, SD3943, SD3868, SD4017, SD4004, INIA66, SD3641, SD3851
	114	SD3776, SD3942, GRANGER, SD4037
Xbarc084	100	SD4073, SD3776, SD4032, WEAVER COMPLEX, INIA 66, MN01164, WEEBIL, SD3900, SD4018, SD4023, SD4037, SD3943, SD3868, MN1197, SD4017, STEEL ND, MN02-306-2, SDWX27158, 98S550113-20-23, SD3870, SD3641
	122	SD3940, SD3851, GRANGER, FN1705-146, SD3999, SD4004, SD3942
Xgwm114	101	SD4004, 98S550113-20-23, SD3870, INIA 66, SD3776, STEEL ND, SDWX27158, SD4018, SD4037, SD4017
	123	SD4073, MN01164, MN1197, SD4017, SD4032, MN02-306-2, SD3940, KNUDSON, GRANGER, SD3900, WEAVER COMPLEX, SD3943, SD4009
Xgwm340	126	SD4017, MN02-306-2, GRANGER, SD3900, SD3927, WEAVER COMPLEX, SD4009, INIA 66
	146	MN1197, FN1705-146, SDWX27158, SD3942, SD3940, SD4018, TRAVERSE, 98S550113-20-23, SD3870, SD3943, SD3641, SD4073, SD4037, SD4070, SD3776, KNUDSON, SD3901, WEEBIL, SD4017
Xwmc787	118	SD4009, SD3776, MN1197, SD4023, KNUDSON, SD4017, WEEBIL, SD4004, SD4070, FN1705-146, STEEL ND, SD3868, INIA 66, SD3641, SD3943, SDWX27158, SD3851
	154	SD4073, SD3940, SD3901, SD3927, SD4032, MN01164, GRANGER, SD4018, SD3900, SD4017, 98S550113-20-23, MN02-306-2
Xgwm136	118	MN01164, SD4017, SD3746, FREYER, SDWX27158, GRANGER, SD3900, SD4018, SD4023, SD3901, GLENN, SD3868
	137	SD3940, WEAVER COMPLEX, SD3943, INIA 66, SD3641
	310	SD3879, SD4032, SD3942, SD3851
Lr34	155	SD3879, FREYER, SD3927, MN01057-3-1, SD4011, SD4023, SD3999, SD4037
	242	SD4073, SD3776, MN1197, FN1705-146, SD4017, SD3746, STEEL ND, MN02-306-2, SDWX27158, SD3942, SD3900, SD4018, 98S550113-20-23, SD3870, SD3901, INIA 66, SD3641, SD3851

Founders carrying *Fhb1* are presented in bold

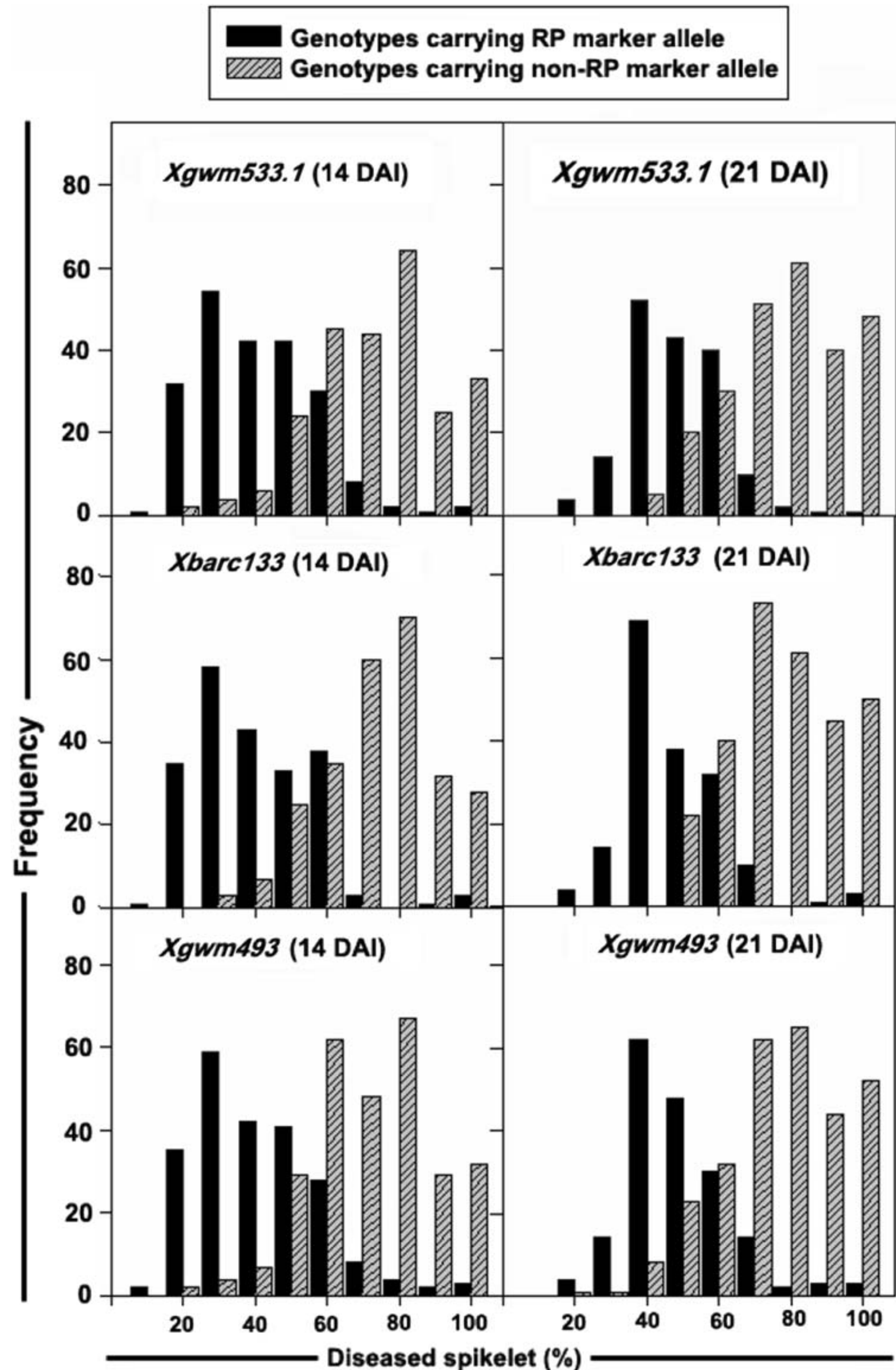

Fig. 3 Frequency distribution of genotypes carrying resistant parent type allele (RP) and susceptible parent type alleles (SP) for three most linked or associated markers (see Table 3 for allele size detail)

The popular transmission disequilibrium test (TDT) of association in humans was originally devised for studying qualitatively inherited traits among independent trios (Spielman et al. 1993). QTDT, developed by Abecasis et al. (2000b), allows use of extended family-pedigrees and uses all the information in a pedigree to construct tests of association. The test is a nested type analysis applicable to quantitative traits that avoids the effects of stratification. In contrast to other types of association mapping tests family-based association tests do not produce misleading or ambiguous results in non-homogeneous population studies. Also, the capacity of QTDT to accommodate missing parental or progeny genotypes makes it very flexible and particularly suitable for breeding applications.

Phenotypic distribution of genotypes possessing resistant and susceptible alleles

Phenotypic distribution by genotypes possessing resistant or susceptible type marker alleles of QTL is presented in Fig. 3. The distributions were fitting as expected, i.e., marker genotype conditional distribution is a mixture of two normal distributions (Broman 2001). Indeed a few individuals with resistant marker alleles have extreme susceptible phenotypic score, suggesting the presence of recombinants between the marker locus and QTL.

Applicability of family-based QTL mapping approach in plants

Results from this study show that each analysis method identified the presence of *Fhb1* on the short arm of chromosome 3B in the interval flanked by SSR loci *Xbarc133* and *Xgwm493*. This marker interval is consistent with those identified in previous studies (Anderson et al. 2001; Bai et al. 1999; Buerstmayr et al. 2002; Cuthbert et al. 2006; Liu and Anderson 2003a, b; Shen et al. 2003) and implies that the application of these methods in the context of cultivar development programs is a definite possibility.

There is a general understanding among researchers that approaches traditionally used for QTL mapping in humans or animals can be useful in plants (Bink et al. 2002; Crepieux et al. 2005; Jannink et al. 2001; Xie et al. 1998). A general limitation in the use of human or animal genetics approaches is availability of software specifically written for plants. This study shows that the software packages used here, i.e., MERLIN and QTDT, certainly have the potential for applications in plant-based studies. A few assumptions were made to accommodate the use of this software. For association or linkage analysis, the relationship among founder genotypes was assumed to be 0 in order to simplify the analysis (Crepieux et al. 2004a). This is a practical mean of dealing with incomplete or confusing breeding pedigrees and simplifies the analysis. If breeding-pedigrees were added to the family-pedigree, the complexity and computational requirements would be increased, thereby limiting practicality of the approach. In addition, breeding-pedigrees are separated by cycles of crossing and selfing in inbred crops, and the genes or QTLs in parents may be lost during segregation. However, Bink et al. (2002) demonstrated the possibility of using breeding pedigrees to map QTLs. Although such an approach has the obvious advantage of using existing material, mapping results are obtained after plant breeders have ultimately introgressed the gene or QTL of interest. The advantage of the procedures detailed in this manuscript is that they can be incorporated while the breeding is being carried out, as demonstrated by their accuracy in correctly mapping *Fhb1* in only three generations.

We decided to use consensus map distances in the linkage analysis to better represent the recombination differences within various genetic backgrounds. Since the order of the different markers used in this experiment is well established, this should not significantly alter the power of this approach. There are alternatives for creating pedigree specific linkage maps by establishing a reference pedigree as in animals (Neff et al. 1999; Barendse et al. 1997; Vaiman et al. 1996; Crawford et al. 1995; Green et al. 1990). The application of such approaches needs testing in plants and will be subject to future experiments. This approach of use an established maker–marker linkage maps instead of developing a new one is frequently used in QTL mapping studies in humans (Aissani et al. 2006; Mertin et al. 2002; Elbein and Hasstedt 2002). The QTDT does not use linkage distance while evaluating association by scoring transmission of alleles from parents to progeny.

In this study, the genome scan was limited to a single chromosome to illustrate that this approach is useful in plants. However, for a de novo QTL search, whole genome scans will be required and possibly more than one QTL loci will be identified. To maximize the power of this approach, pedigrees should be designed using one ‘donor’ parent carrying the QTL to map and a wide range of ‘recurrent’ parents. In this study we were able to use the resistant parents due to the knowledge that the three of them were derived from the same original source and carried the same QTL allele. In such experiments, linkage analysis, using both VC and PWR should be followed by transmission disequilibrium analysis with QTDT. Our results agree with findings in other studies (Glazier et al. 2002; Mackay 2001) showing that linkage analysis is more useful for a genome-wide scan for QTL while association analysis gives more precise location of an individual QTL. An example of such sequential application of MERLIN and QTDT software in linkage and association analysis can be found in Li et al. (2003) where the mapping of human genes influencing obesity was demonstrated.

For an initial validation of a family-pedigree based QTL mapping approach in plants we deliberately choose a well-studied large effect QTL ($R^2 \sim 45\%$). Although family-based approaches have been used to map small effect QTLs in humans and animals (Lee and van der Werf 2005; de Koning and Haley 2005) the power of this approach to map small effect QTL in plants using the approach described here needs to be further studied.

The method discussed here is based on single plant phenotyping, analogous to single individuals in human or animal studies. As such, this method might not be applicable to all traits. Possible modifications include a progeny test that can help to determine phenotype more accurately

(Zhang and Xu 2004). Progeny test can help to increase replication and multi-environments evaluations. In addition, the putative QTLs can be further validated in the same populations in later generations. These modification need to be tested in future studies. In this case given that *Fhb1* consistently reduces symptoms of infections across screenings (Pumphrey et al. 2007) single plant phenotypes are considered to be sufficiently accurate to demonstrate the utility of this approach.

This experiment demonstrates how useful family-pedigree based approaches are in plants. Both types of analyses were helpful in cross-validating results as well as finding suitable associated markers alleles. The results show the potential application of this approach to detect QTLs in early generation PBPs and their further application in MAS. More than one potential allele at a locus of interest could be further validated in the same breeding program for their effectiveness in selection progress. The method can be completed as early as in 2 years in contrast to at least 4–6 years for mapping methods based on recombinant inbred lines. This approach can also be used to validate previously mapped QTLs for multiple traits in several genetic backgrounds of breeders' interest. At the same time the QTL of interest is introgressed in multiple genetic backgrounds for breeding purposes.

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