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ORIGINAL PAPER

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Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust

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Abstract The limited population sizes used in many quantitative trait locus (QTL) detection experiments can lead to underestimation of QTL number, overestimation of QTL effects, and failure to quantify QTL interactions. We used the barley/barley stripe rust pathosystem to evaluate the effect of population size on the estimation of QTL parameters. We generated a large ($n = 409$) population of doubled haploid lines derived from the cross of two inbred lines, BCD47 and Baronesse. This population was evaluated for barley stripe rust severity in the Toluca Valley, Mexico, and in Washington State, USA, under field conditions. BCD47 was the principal donor of resistance QTL alleles, but the susceptible parent also contributed some resistance alleles. The major QTL, located on the long arm of chromosome 4H, close to the *Mlo* gene, accounted for up to 34% of the phenotypic variance. Subpopulations of different sizes were generated using three methods—resampling, selective genotyping, and selective phenotyping—to evaluate the effect of population size on the estimation of QTL parameters. In all cases, the number of QTL detected increased with population size. QTL with large effects were detected even in small populations, but QTL with small effects were detected only by increasing population size. Selective genotyping and/or selective phenotyping approaches could be effective strategies for reducing the costs associated with conducting QTL analysis in large populations. The method of choice will depend on the relative costs of genotyping versus phenotyping.

Keywords *Hordeum vulgare* · *Puccinia striiformis* f. sp. *hordei* · Quantitative trait loci · Selective genotyping · Selective phenotyping · Random sampling

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Introduction

The limited population sizes used in many QTL detection experiments may have led to underestimation of QTL number, overestimation of QTL effects, and failure to quantify QTL interactions (Beavis 1998; Melchinger et al. 1998; Utz et al. 2000; Allison et al. 2002; Goring et al. 2001; Schön et al. 2004). The number of lines used in many QTL experiments has been about 100. Beavis (1998) suggested that even 200 individuals may be too few for reliable QTL detection, and addressed issues related to the choice of population size in QTL mapping experiments. He recommended the use of resampling techniques to obtain asymptotically unbiased estimates of QTL effects.

Random sampling (RS) has been used in experimental data and bootstrapping by Bennewitz et al. (2002) and cross-validation by Utz et al. (2000). In those studies, evaluation of the efficiency of the resampling techniques was limited by relatively small size of the source populations. Schön et al. (2004) used a large experimental population, 976 F_5 maize testcross progenies, and found that the effect of sample size on power and QTL detection as well as on accuracy and precision of QTL estimates was large.

In most QTL experiments, population size is limited by the cost of marker genotyping and/or the cost of trait phenotyping. If marker genotyping is costly, it may be worthwhile to exploit a large population by using selective genotyping (SG) (Lander and Botstein 1989), in which the entire population is phenotyped for a trait of interest and lines from the extreme tails of the resulting phenotypic distribution are selected for marker genotyping. This method should increase the power of QTL mapping because individuals from the extremes of the phenotypic distribution provide more linkage information and their phenotypes may be more accurately described based on the higher proportion of the + or − alleles at the QTL affecting the trait. SG has been used for efficiently mapping QTL that influence a single trait (e.g. Nandi et al. 1997; Foolad et al. 2001; Blum et al. 2003; Shen et al. 2003). Genotyping 10–20% of the population has been shown to be sufficient, in most cases, to detect the same QTL regions detected by interval mapping (Ayoub and Mather 2002). The SG method is not recommended for simultaneous analysis of multiple traits, but it may be cost/time effective for sequential analysis of more than one trait. SG may be performed with or without information on the map locations of molecular markers. The main limitation of SG is the risk of detecting spurious associations between markers and traits. An initial screening of all markers on the extreme tails of the population, followed by complete genotyping of significant markers, can eliminate spurious associations (Ayoub and Mather 2002).

If trait phenotyping is costly, it may be worthwhile to exploit a large population by using selective phenotyping (SP) in which the entire population is genotyped for markers and lines that are highly informative from a linkage mapping perspective are selected for trait phenotyping of these selected lines. Selection of subsets of individuals bearing complementary recombinational or radiation-induced breakpoints has been used in a procedure called "selective mapping" to map additional markers after an initial frame-work map was generated using the whole population (Vision et al. 2000), but the usefulness of selective phenotyping to extract QTL information from a large population has not been explored.

We used barley (*Hordeum vulgare* spp. *vulgare*) and barley stripe rust (BSR) to evaluate the effect of population size on the estimation of number, location, and interactions of QTL affecting BSR. BSR, caused by *Puccinia striiformis* f. sp. *hordei*, is a major disease of barley that can cause severe yield-losses and reduction of quality of the grain in Europe, the Asian continent, and the Americas (Dubin and Stubbs 1985; Chen and Line 2001). Sources of both qualitative and quantitative resistance to BSR have been reported and mapped (von Wettstein-Knowles 1992; Chen et al. 1994; Thomas et al. 1995; Hayes et al. 1996; Toojinda et al. 1998, 2000; Castro et al. 2002a, b, 2003a) and marker assisted selection for resistance has been successfully implemented (Castro et al. 2003a, b). For future resistance-breeding efforts, it is important to know what population sizes and sampling options will allow for the identification of the most important resistance factors. Accordingly we developed, phenotyped, and genotyped a large population to test if additional QTL —not previously identified—can be found, to validate existing QTL, and to empirically determine the effect of population size on the estimation of BSR QTL parameters using random sampling (RS), selective genotyping (SG), selective phenotyping (SP) approaches.

Materials and methods

Plant material

BCD47 and Baronesse are the parents of the mapping population. BCD47 is a two-rowed spring doubled haploid (DH) line, developed via marker-assisted selection for BSR resistance alleles at QTL on chromosomes 4H and 5H (Castro et al. 2003a). Baronesse is a two-rowed spring variety bred in Europe and grown extensively in the Pacific Northwest of the United States. Baronesse is susceptible to BSR under the intense epidemic conditions of the Toluca Valley, Mexico, (TVM) but observations under greenhouse and field conditions in Washington State, USA (WUSA) have indicated that this variety has a moderate level of high-temperature adult-plant resistance (unpublished data). The mapping population, consisting of 409 DH lines, was developed from the F_1 of BCD47/Baronesse using the *H. bulbosum* method, as described by Chen and Hayes (1989).

DNA isolation, molecular marker analysis, and linkage map construction

The population and the parental lines were grown in the greenhouse for DNA extraction. DNA was extracted from 30 to 50 mg of young leaf tissue using a Qiagen/Retsch MM300 mixer Mill and the Qiagen DNeasy 96 Plant Kit (Qiagen Inc, Valencia, CA). Simple Sequence Repeat (SSR) (Liu et al. 1996; Ramsay et al. 2000) and Expressed Sequence Tag (EST) markers (Struss and Plieske 1998; Thiel et al. 2003) were amplified by polymerase chain reaction (PCR) using a fluorescently tagged reverse primer and a non-labeled forward primer. One -to- three non-overlapping PCR products were analyzed simultaneously with an internal size standard using ABI PRISM DNA sequencers equipped with

Genescan and Genotyper software (PE Biosystems, Foster City, CA).

JoinMap 3 (Van Ooijen and Voorrips 2001) was used for linkage map construction, following the data management and map construction steps described by Hayes et al. (1997) and Toojinda et al. (2000), using the Haldane mapping function. Linkage groups and locus order were compared with published barley linkage maps (Ramsay et al. 2000; Cooper et al. 2004).

Phenotypic analysis

The population and the parental lines were grown in replicated alpha-lattice designs at the ICARDA/CIMMYT facilities in the Toluca Valley, Mexico (TVM), in 2001, and in 2002 with two planting dates, and in Mt. Vernon and Pullman, WA, USA (WUSA), in 2002. Each replicated assay is referred from now on as an 'experiment'. In TVM, the plant material was evaluated in two-row 1-m plots. Susceptible varieties infected with spores from the local winter nurseries were transplanted in hill plots every 1–3 plots. In addition, spreader rows consisting of a mixture of 8–10 susceptible lines were planted at 16-m intervals. The race composition (Chen and Line 2001) of the inoculum was not determined. In the WUSA experiments, the plant material was evaluated in single row 1-m plots. The spreader rows were planted with Steptoe barley. The field plots were not inoculated. The BSR population in the Mt. Vernon area contained races PSH-17, PSH-54, PSH-56, PSH-59, PSH-60, PSH-62, PSH-63, PSH-64, PSH-66, and PSH-67; PSH-54 and PSH-56 were the most predominant. In the Pullman area the predominant race was PSH-56, but races PSH-61 and PSH-63 were also found. Adult-plant BSR severity (%, on a whole canopy plot basis) assessments were performed between Feekes stages 9 and 10.5 at both locations.

Analyses of variance

Adjusted entry means (weighted least square means) and effective error mean squares derived from the analyses of variance of each experiment were used to calculate the combined analyses of variance for 409 DHs. The analyses were performed using the software PLABSTAT (Utz 2001). Random effects linear models were assumed. For each megaenvironment (TVM or WUSA) heritabilities were calculated on an entry mean basis as the quotient of genotypic and phenotypic variance $\left(\hat{h}^2 = \hat{\sigma}_g^2/(\hat{\sigma}_g^2 + \hat{\sigma}_g^2/E + \hat{\sigma}^2/\mathrm{RE})\right)$. Where $\hat{\sigma}_g^2$ represents the genotypic variance, $\hat{\sigma}_{ge}^2$ the genotype-by-experiment variance, $\hat{\sigma}^2$ the error variance, R number of replications and E number of experiments. Phenotypic correlation coefficients between pairs of experiments were calculated using the PROC CORR procedure of SAS (SAS Institute 2001).

Generation of subpopulations

Random sampling (RS)

From the experimental reference population of 409 lines, an array of subpopulations of size N ($N = 409$, 300, 200, 150, 100, 50) was sampled without replacement within each of the two megaenvironments: TVM and WUSA. Means across experiments within megaenvironment were used. After a new randomization step, this procedure was repeated resulting in a total of 40 different data sets (DS) for each size of N, except for $N = 409$ for which only one DS exists. Within each subpopulation, QTL analyses were performed for each DS individually. Fivefold crossvalidation (fivefold CV; Hjorth 1994; Schön et al. 2004) was used. Each DS was randomly subdivided into five samples without replacement. Means across all experiments within megaenvironments of four samples were used as an estimation data set (ES) for localization of QTL and estimation of their effects, and means across environments of the fifth independent sample were used as the test set (TS). The TS was used to validate QTL detected in the ES and to obtain asymptotically unbiased estimates of QTL effects and the variance explained by QTL. For each DS five different ES are possible, each with a corresponding TS. The randomization step of assigning lines to the five subsamples was repeated ten times resulting in 50 different ES and corresponding TS per DS.

Selective genotyping (SG): genotyping of subpopulations selected based on BSR severity

Subpopulations of 300, 200, 150, 100, and 50 DH lines from the full population were obtained based on the phenotypic distribution of BSR severity. Half of the lines for each subpopulation were obtained from the BSR-resistant tail of the phenotypic distribution, and the other half from the BSR-susceptible tail of the phenotypic distribution. Only genotypic data from the selected lines was used for further analysis. Since the selection of lines depended on the BSR severity scores, the composition of the subpopulations differed between the TVM and WUSA experiments.

Selective phenotyping (SP): phenotyping of subpopulations selected based on linkage mapping information

Subpopulations of 300, 200, 150, 100, and 50 DH lines from the full population with high recombination frequency and unique recombination patterns were obtained using MapPop (Vision et al. 2000). Phenotypic data from only the selected lines were used for further analysis. Since the selection of lines depended on genotypic information, the composition of the selected subpopulations was the same in the TVM and in the WUSA experiments.

QTL analysis

Adjusted entry means for BSR severity from each experiment, and means across experiments within megaenvironment, were used for QTL analysis. The QTL analyses of random sampled subpopulations (RS approach) were performed as described in Schön et al. (2004) using the software PLABQTL (Utz and Melchinger 1996). The LOD threshold used was 2.4. The parameters estimated included the number of QTL and the proportion of the genotypic variance explained by QTL as well as their arithmetic mean over all DS, ES, and TS for a given N (Schön et al. 2004). The QTL analyses of selected subpopulations (SG and SP approaches) were performed using the composite interval mapping (CIM) procedure (Zeng 1994) implemented in Windows QTL Cartographer 2.0 (Wang et al. 2001–2003). A forward-selection backward-elimination stepwise regression procedure was used to identify co-factors for CIM. A 10-cM scan window was used for all analyses. Experiment-wise significance ($P < 0.05$) likelihood ratio test statistics (LR) thresholds for QTL identification were determined with

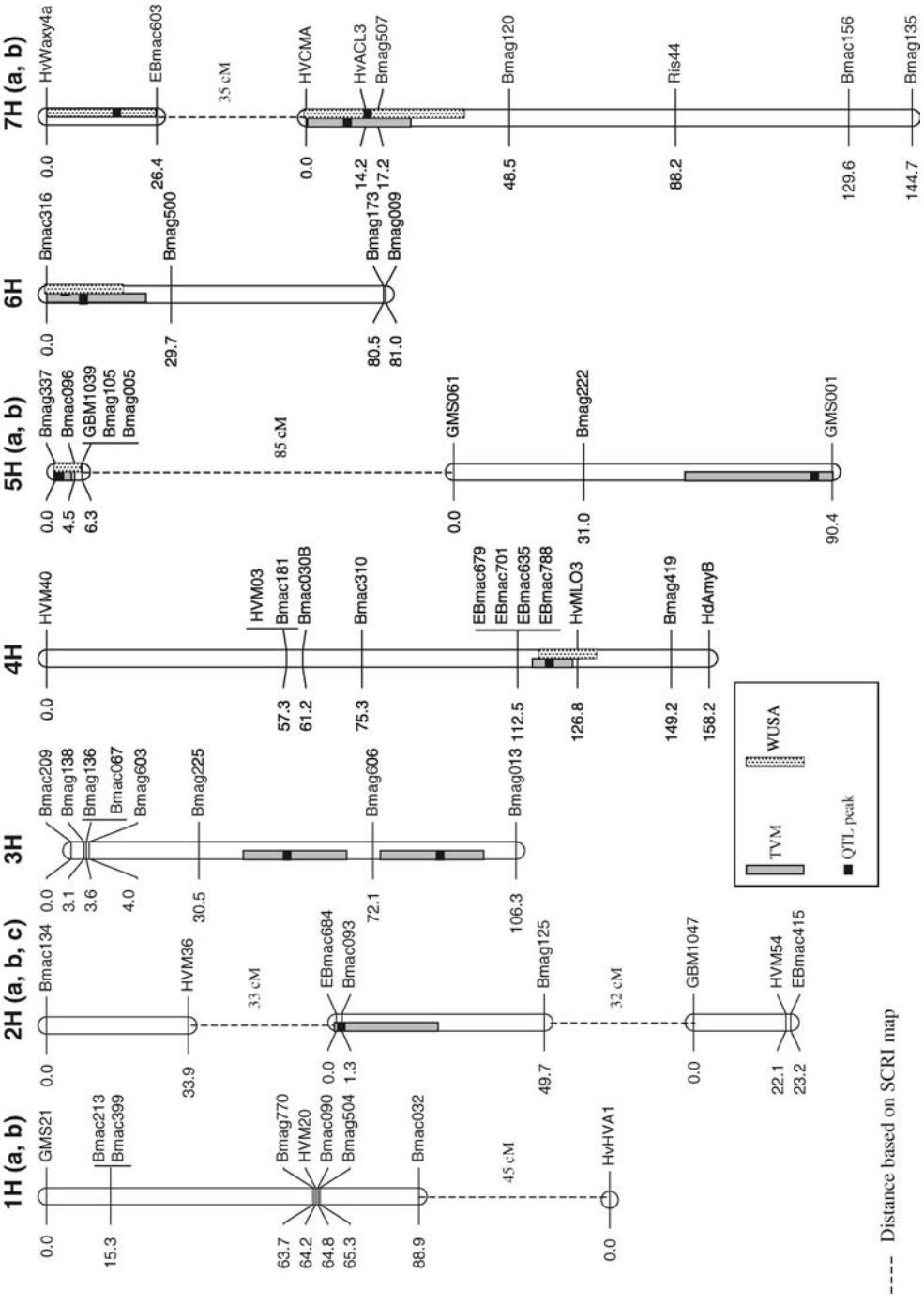

Fig. 1 Linkage map of the BCD47/Baronesse DH population constructed using all publicly available and polymorphic SSRs. The *dotted lines* indicate monomorphic regions with distances inferred from Ramsay et al. (2000)

1,000 permutations for the whole population and for each subpopulation and expressed as LOD (LOD = 0.217LR). The proportion of the total variance explained by the QTL was expressed as the average TR^2 (proportion of the total variance explained by the QTL conditioned on the cofactors) value obtained by QTL Cartographer. Tests for epistasis between QTL were evaluated using the MIM (Multiple Interval Mapping) method of QTL Cartographer. QTL x environment interactions were tested at an experiment-wide significance level of 0.05, using NQTL, a windows version of MQTL (Tinker and Mather 1995).

Results

Genotyping

Of the 128 DNA-based markers screened on the parents, eighty-six (67%) were polymorphic. Wherever two or more markers were expected to be completely linked, based on the Scottish Crop Research Institute (SCRI) linkage map (Ramsay et al. 2000), only one of them was assayed in the full population. Markers that were multicopy or difficult to amplify were also not assayed in the full population. Ultimately, 58 markers were assayed in the full mapping population. The linkage map spanned 809 cM, containing 57 loci forming eleven linkage groups and one unlinked locus, HvHVA1 (Fig. 1). The marker orders and distances were generally in agreement with published reports (Ramsay et al. 2000; Cooper et al. 2004). The number of linkage groups (11) was larger than the number of chromosomes (7); accordingly, the assignment of linkage groups to chromosomes was done based on prior reports. In order to indicate multiple groups per chromosome, each subgroup is indicated with a letter suffix, e.g., "a" after the chromosome number (Fig. 1). The average marker density was one marker per 23 cM. Segregation distortion ($P < 0.01$) in favor of BCD47 alleles was observed for four markers on chromosome 1H (Bmag770, HVM20, Bmac090 and Bmag504) and three markers on chromosome 2H (EBmac684, HVM54 and EBmac415). Two markers (GMS001 on 5H and HvHVA1) showed segregation distortion in favor of the Baronesse allele.

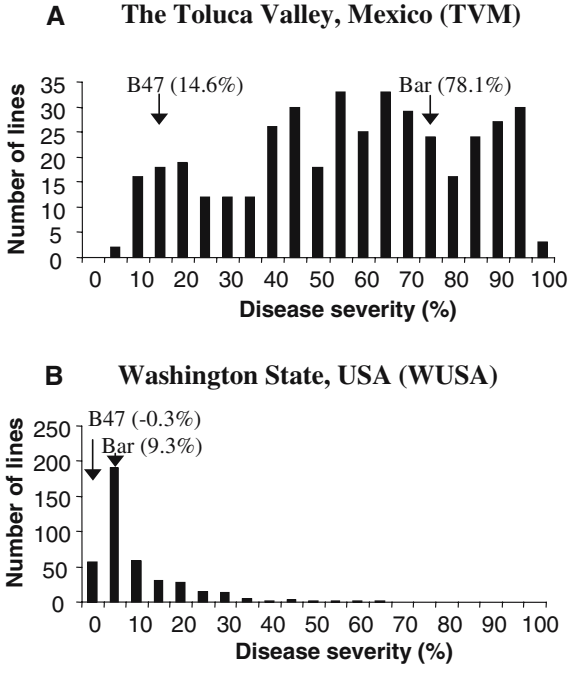

Fig. 2 Phenotypic distributions for barley stripe rust disease severity. **a** Adjusted entry means of three Toluca Valley, Mexico (TVM) experiments and **b** adjusted entry means of two Washington State (WUSA) experiments. *B47:* BCD47, *Bar:* Baronesse

Phenotyping

The population exhibited quantitative variation for BSR severity (Fig. 2a, b). In the WUSA experiments, the distribution of BSR severity values was skewed towards resistant values (Fig. 2b). Negative values resulted from adjusting the means based on the lattice design. The heritability of BSR, estimated on an entry-mean basis, was higher in the TVM experiments (95.7%) than in the WUSA experiments (65.7%) (Table 1). The correlation between the TVM and WUSA experiments was moderate ($r = 0.55$); therefore, these data sets were analyzed separately as two different megaenvironments (TVM and WUSA).

QTL analysis using the full population

QTL analyses using the full population (409 DH lines) were performed on entry means for the TVM megaenvironment and for the WUSA data set. Using the TVM megaenvironment eight QTL were identified (Table 2). The resistance alleles came from BCD47, the resistant parent, at QTL on chromosomes 3H (two peaks), 4H, 5Ha, and 6H, and from Baronesse at QTL on chromosomes 2Hb, 5Hb, and 7Hb (Table 2). Within the TVM megaenvironment, the same QTL were identified in the three individual experiments (data not shown). Only the QTL on chromosome 4H showed significant QTL×experiment interaction and this was due to a change in magnitude of effect. This QTL had the largest effect, its BCD47 allele reduced disease severity by 14.75 percentage points and the average R^2 explained by this QTL was 34.0% (Table 2). The QTL with the second largest effect, on 3H, showed two poorly defined peaks spanning 24 and 21 cM, respectively (Table 2). The total amount of phenotypic variance explained by all of the BSR resistance QTL detected in the TVM data set was 60.4% (Table 2).

We identified BSR resistance QTL on 4H, 5Ha, 6H, 7Ha, and 7Hb in the WUSA megaenvironment (Table 2). The largest effect QTL was on chromosome 4H, followed

Table 1 First and second degree statistics for the parental lines BCD47 and Baronesse and DH lines derived from the cross BCD47/Baronesse for barley stripe rust resistance

Parameter	TVM	WUSA
Means	%	%
BCD-47	14.6	−0.3
Baronesse	78.1	9.3
DH lines	54.7	7.3
Range of DH lines	2.7–98.6	−2.7–64.3
LSD 5%	14.5	16.8
Variance components		
σ^2_g	602.2**	69.7**
σ^2_{ge}	54.6**	32.1**
σ^2 (Error)	54.2	81.6
Heritability (h^2)	95.7	65.7

TVM, The Toluca Valley, Mexico; WUSA, Pullman and Mt. Vernon, Washington State, USA
**: Significant at the 0.01 probability level

by the QTL on chromosome 6H and 7Hb. The BSR resistance QTL on 4H, 5Ha, 6H and 7Hb were in common between the TVM and WUSA megaenvironments. BSR resistance QTL on 2H and 3H (two peaks) detected in TVM were not detected in WUSA. The BSR resistance QTL on 7Ha (resistance allele from Baronesse) was detected only using the WUSA megaenvironment (Table 2). Within the WUSA megaenvironment there were no significant QTL×experiment interactions. The total amount of BSR phenotypic variance explained by QTL in the WUSA data set was 41.7% (Table 2).

Epistatic interactions between QTL were not detected in either the TVM or in the WUSA megaenvironments. Because of this, epistatic interactions were not evaluated in the subpopulations generated using the RS, SG, and SP approaches.

Effect of population size on the estimation of BSR QTL: random sampling (RS), selective genotyping (SG), selective phenotyping (SP)

Regardless of the sampling procedure used, more QTL were detected with the TVM than with the WUSA megaenvironment (Figs. 3a, 4a). In both megaenvironments, the number of BSR QTL detected increased as the population size increased, regardless of sampling procedure (Figs. 3a, 4a). With SG and $n = 50$, only the BSR resistance QTL on the long arm of chromosome 4H was identified and this QTL was significant for both TVM and WUSA (Figs. 3a, 4a). With SP and $n = 50$ only one BSR resistance QTL on chromosome 3H, was identified in the TVM data set, and two BSR resistance QTL were identified in the WUSA data set. The specific chromosome locations of QTL detected using the SG and SP approaches in the TVM and WUSA megaenvironments are included in the electronic supplementary tables. Based on the TVM and WUSA megaenvironments results, the SG and SP approaches do not reveal more QTL than RS when $n = 50$ or 100. For populations $n \geq 150$, there was a tendency to detect more BSR resistance QTL with SG and SP than with RS; however, in only some cases were the differences significant (Figs. 3a, 4a). Using SG or SP, $n = 300$ gave the same information regarding the number of significant QTL as the full population ($n = 409$) (Figs. 3a, 4a, Table 2).

In the case of RS there was not a significant difference between the percentage of phenotypic variance explained by the QTL detected for the different population sizes; however, the standard deviation increased dramatically as the population size decreased (Figs. 3b, 4b). The total amount of phenotypic variance explained by the QTL using SP was lower than when using SG

Table 2 Barley stripe rust resistance QTL detected in the BCD47/Baronesse DH population in the Toluca Valley, Mexico (TVM, average of three experiments) and in Washington State, USA (average of two experiments), using composite interval mapping

Linkage group	*TVM*				*WUSA*			
	QTL peak position and 2-LOD interval (cM)	LOD[a]	R^2 (%)[b]	Additive effect[c]	QTL peak position and 2-LOD interval (cM)	LOD[a]	R^2 (%)[b]	Additive effect[c]
2Hb	1.0 (0.0–25.3)	10.2	5.0	5.69				
3H	53.5 (42.5–66.5)	18.4	14.0	−9.44				
3H	87.1 (76.1–97.1)	18.2	12.8	−9.02				
4H	121.5 (117.5–124.5)	50.4	34.0	−14.75	126.8 (118.5–131.8)	18.5	13.7	−3.86
5Ha	0.0 (0.0–3.0)	5.5	2.6	−4.10	5.5 (0.0–6.3)	3.9	2.7	−1.69
5Hb	87.0 (57.0–90.4)	4.3	2.2	3.79				
6H	9.0 (0.0–23.0)	8.7	5.4	−5.90	4.0 (0.0–18.0)	9.4	7.8	−2.89
7Ha					19.0 (0.0–26.4)	8.1	6.5	2.65
7Hb	8.0 (0.0–26.2)	8.6	4.7	5.47	16.2 (0.0–39.2)	10.1	7.2	2.79
Total (%)[d]			60.4				41.7	

TVM, The Toluca Valley, Mexico; WUSA, Pullman and Mt. Vernon, Washington State, USA
[a]LOD is the log-likelihood at the QTL peak position. The LOD thresholds, based on 1,000 permutations and a type I error of 5% were 2.4 for both megaenvironments
[b]R^2 is the percentage of phenotypic variation explained by the QTL
[c]Negative and positive values indicate that BCD47 and Baronesse, respectively, contributed the resistance QTL allele
[d]Proportion of the total variance explained by the QTL conditioned on the cofactors

(Figs. 3b, 4b), but in both cases and for both megaenvironments the total amount of phenotypic variance explained by the QTL increased as the population size decreased (Figs. 3b, 4b). The amount of phenotypic variance explained by the QTL using SP was similar to that explained by random sampling in the TVM megaenvironment, and it was higher than random sampling in the WUSA megaenvironment (Figs. 3b, 4b).

Discussion

Relationships with published disease resistance QTL

Our results fit an oligogenic model with a few loci, with relatively large effects (reviewed in Tanksley 1993), high heritability, and a high probability of markers explaining a considerable proportion of the phenotypic variance. The number of BSR resistance QTL detected (eight in the TVM, and five in the WUSA megaenvironments) and the percentages of phenotypic variance explained (60.4% in the TVM and 41.7% in WUSA data sets) using the whole mapping population ($n = 409$) are within the ranges reported in the literature for quantitative disease resistance (reviews by Young 1996; Kover and Caicedo 2001). In these reviews, however, it was stated that estimates of QTL numbers were probably biased downward owing to small population sizes. Our data suggest that these estimates may be reasonable, given the population sizes feasible for most programs to assess. For the particular case of barley stripe rust, the evaluation of the whole population in the TVM megaenvironment detected the highest number of QTL detected in for barley stripe rust in a single population. BCD47, the resistant parent, contributed resistance alleles at QTL on chromosomes 3H, 4H, 5H and 6H whereas Baronesse, the susceptible parent, contributed resistance alleles at QTL on chromosomes 2H, 5H, and 7H. The fact that both parents contributed resistance alleles accounts for the positive and negative phenotypic transgressive segregants. This finding was validated by an analysis of the tails of the phenotypic frequency distribution. Lines with stripe rust severity scores lower than BCD47 had marker genotypes indicating the presence of BSR resistance alleles from both parents, while lines with stripe rust severity scores higher than Baronesse had marker genotypes indicating the presence of susceptible alleles from both parents (data not shown). The contribution of resistance alleles by susceptible parents has precedent (e.g., Toojinda et al. 1998, 2000; Castro et al. 2002b).

Some QTL affecting BSR resistance may remain undetected in the unmapped regions of the genome. Based on the 41 markers that were monomorphic between BCD47 and Baronesse and are of known map location, we can infer that these regions are also functionally monomorphic and not likely to harbor resistance gene polymorphisms. Considering the mapped and putatively monomorphic regions of the genome in this cross, we estimate that we have screened the full genome. The linkage map generated spanned 809 cM, which represents 67% of the genome (809 cM of 1,203 cM reported by Cooper et al. 2004). The remaining 33% is probably represented by monomorphic markers, which coincides with the level of monomorphism (33%) we found in our screening of the parental lines BCD47 and Baronesse.

Our study served to confirm the success of marker-assisted selection and also to validate BSR QTL previously found. We expected to see significant QTL effects on chromosomes 4H and 5H, since BCD47 was developed via marker-assisted selection for resistance alleles

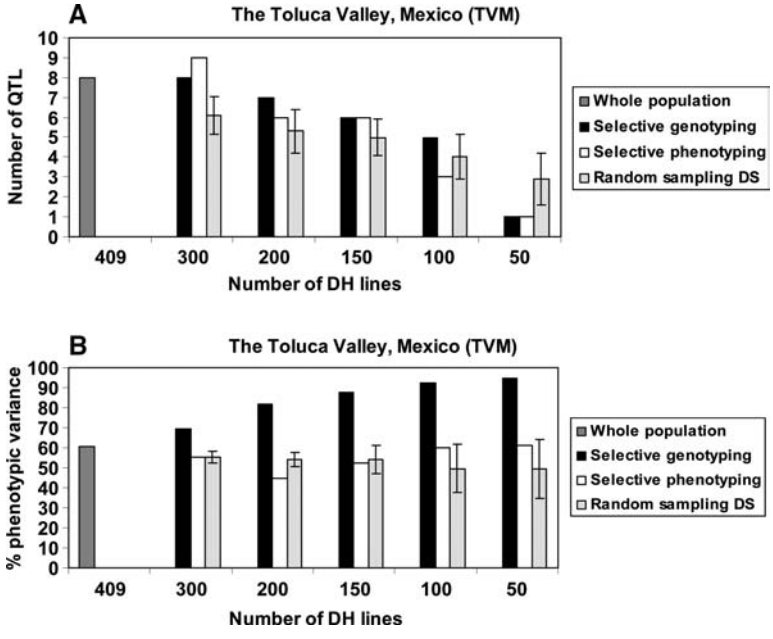

Fig. 3 Effect of population size on the estimation of number of significant QTL and percentage of phenotypic variance explained for barley stripe rust resistance in the Toluca Valley, Mexico (TVM) using selective genotyping, selective phenotyping, and random sampling (mean values and standard deviation shown for DS (data sets)

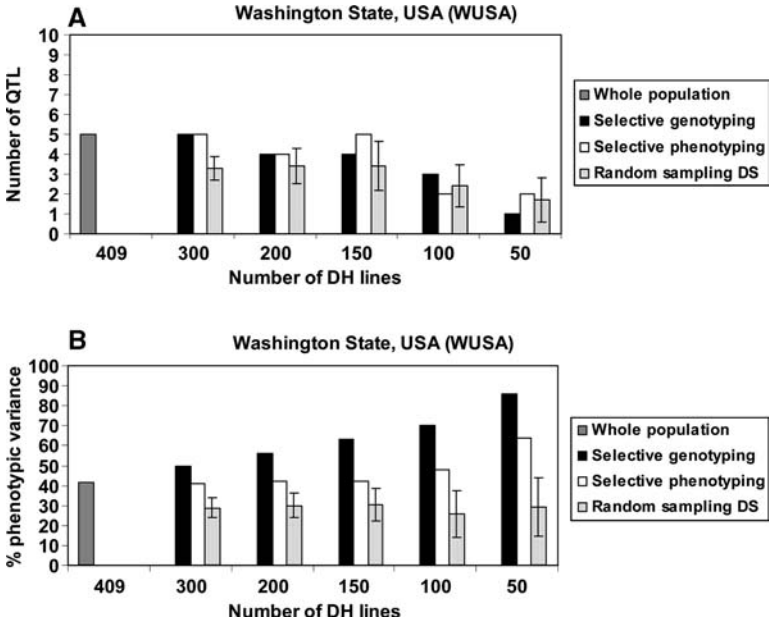

Fig. 4 Effect of population size on the estimation of number of significant QTL and percentage of phenotypic variance explained for barley stripe rust resistance in Washington State (WUSA) using selective genotyping, selective phenotyping, and random sampling (mean values and standard deviation shown for DS (data sets)

at these loci (Chen et al. 1994); and this expectation was fulfilled. The additional resistance QTL that were mapped using the full population coincided with the linkage map positions of previously reported resistance QTL and major genes. The QTL on chromosome 2H coincided with a BSR QTL mapped using the Shyri × Galena population (Toojinda et al. 2000). Two QTL were detected on chromosome 3H. The proximal QTL has not been reported previously; the more distal one was loacted in approximately the same region as in the Shyri × Galena population (Toojinda et al. 2000). The QTL on chromosome 4H was previously detected in several mapping populations: BSR41 × Steptoe (Toojinda et al. 1998), Calicuchima × Bowman (Chen et al. 1994; Castro et al. 2002b), the disease-resistance pyramid population (Castro et al. 2003a), and the AJ, BU and OP populations (Castro et al. 2003a, 2003b). As reported by Chen et al. (1994), the QTL on chromosome 4H spanned a 40 cM interval. By increasing marker data and including resistance phenotyping at the seedling and adult stages, Castro et al. (2002a) subdivided this 4H QTL into two separate QTL (called 4Ha and 4Hb), the first of which was significant at the adult-plant stage and the second of which was significant at the seedling stage. A QTL on chromosome 5H coincided with one reported in the Blenheim × E224/3 population (Thomas et al. 1995), the BSR41 × Steptoe population (Toojinda et al. 1998), and in the Calicuchima × Bowman population (Chen et al. 1994, Castro et al. 2002b). We detected an additional BSR QTL on chromosome 5H, close to Bmag222, using the TVM megaenvironment. The QTL on chromosome 6H was located approximately in the same region as the seedling and adult BSR QTL identified in the Shyri×Galena population (Toojinda et al. 2000), and in the Calicuchima-sib×Bowman population (Castro et al. 2002b). One of the QTL on linkage group 7Hb coincided with a BSR QTL detected in the Blenheim×E224/3 population (Thomas et al. 1995) and in the Calicuchima-sib×Bowman (Castro et al. 2002b). We detected an additional QTL on chromosome 7Ha, but only in the WUSA megaenvironment.

An interesting point is the change of magnitude of effects of the QTL on 4H and 5H in the BCD47/Baronesse population, in which the 4H effect was greater than the 5H effect, compared with the Cali-sib×Bowman population, in which the 5H effect was greater than the 4H effect (Chen et al. 1994; Castro et al. 2002b). This change in magnitude of effect may be due to more accurate estimates of QTL effect due to larger population size, and/or to the effects of genetic background. Castro et al. (2003a) also found the 4H QTL to be of larger effect than the 5H QTL, but Marquez-Cedillo et al. (2003) and Hayes (personal communication) found the opposite to be true in marker-assisted BSR introgression experiments in six-row barley. Additional research on QTL effects in different genetic backgrounds is warranted.

Effect of population size

The evaluation of the effect of population size within each of the approaches used (RS, SG, and RS) is straightforward; however, comparison between the three sampling methods is confounded by at least two factors: (1) the fact that RS used repeated sampling while only one sample was available for SG and SP, and (2) the use of different programs for QTL analysis. The generation of multiple samples for each population size was not possible for SG and SP because of the nature of the selection process (selection of lines from the extremes of

the phenotypic distribution or the most informative ones based on marker genotypes, respectively). In relation to the software, QTL Cartographer was the first software of choice for QTL analysis because it is used by many researchers and because of its friendly interface. However, since it does not have the capacity to analyze multiple populations at the same time and provide statistics from the results, we used PlabQTL to analyze the populations generated for the RS approach. QTL analysis was performed on the whole population on both megaenvironments (TVM and WUSA) using both softwares. Using the TVM megaenvironment, QTL Cartographer detected eight significant QTL and explained 60.4% of phenotypic variance (Table 1), while PlabQTL detected ten significant QTL and explained 65% of the phenotypic variance (data not shown); in the case of the WUSA megaenvironment, QTL Cartographer detected five significant QTL and explained 41.7% of the phenotypic variance, while PlabQTL detected six QTL and 39.9% of phenotypic variance. The fact that the two programs use different strategies for the selection of cofactors and different techniques for detection and estimation of QTL (maximum likelihood in the case of QTL cartographer and multiple regression in the case of Plabqtl) could explain the differences observed in QTL detection. Based on QTL analyses performed on the whole population using both programs, we believe that the RS, SG, and RS methods used to evaluate the effect of population size can be compared in relation to the number of QTL detected and percentage of phenotypic variance explained, but the results should be interpreted carefully.

Assessment of large populations may not be feasible and accordingly subsampling or assessment of smaller populations may be warranted. The principal limitation of SG is that it can only be used for one trait at a time, and this is problematic for multiple trait mapping. In our analyses, when $n = 50$ or $n = 100$, SG and/or SP do not seem to offer an advantage over random sampling (Figs. 3a, 4a): the number of QTL detected using SG and SP is within the range of number of QTL obtained using random populations in the data sets (DS). Small populations can lead to the detection of spurious QTL which we have not observed (see electronic supplementary tables). We have detected additional QTL on chromosome 4H using SG of 100 and 150 lines in the TVM megaenvironment and we believe that in this case the selection method rather than population size could be the cause of detecting these potentially spurious QTL. With population sizes of $n \geq 150$, SG and/or SP are in general superior to random sampling. In theory, SP improves the accuracy of QTL detection, thanks to the use of individuals with a higher number of recombination events. Jannink (2005) reports that SP will improve the accuracy of QTL mapping for QTL of small effect and in cases of sparse marker coverage. The simulation assumes that there are no marker errors, that recombination can be unambiguously identified, and that a progeny can have only a single observable recombination event in any given marker interval. The effect of double recombination in an interval has not been explored and it is not clear how this would affect QTL accuracy. The method of choice to reduce population size for QTL analysis will depend mainly on the relative cost of genotyping and phenotyping. In the case of BSR, evaluations under field conditions are relatively easy, so SG of $n \geq 150$ lines would be the most efficient and cost/time-effective approach. However, the main concern is the overestimation of percentage of phenotypic variance explained using this approach. The percentage of phenotypic variance explained was inversely proportional to population size and number of QTL detected, this was probably due to the nonrandom selection of lines, and it was especially evident in the case of small samples sizes.

The number of BSR resistance QTL we identified increased with population size. This supports published experimental and simulated data (Beavis 1998; Melchinger et al. 1998; Utz et al. 2000; Schön et al. 2004). We used the methodology of Schön et al. (2004) to empirically assess the effect of population size on estimates of the number QTL and the proportion of variance explained. The results were very similar in both studies: the number of QTL increased as the population size increased. Furthermore, QTL with large effects were detected with small populations, but it was necessary to increase the population size to be able to detect QTL with small effects. We found that a population of 300 DH lines was as effective as the population of 409 DH lines. This finding is in accordance with some reports (Beavis 1998; Utz et al., 2000), although Schön et al. (2004) found that increasing population size from 488 to 976 had a relatively large effect on the amount of genotypic variance explained.

In summary, we found that using a large ($n = 409$) population was useful for revealing more stripe rust resistance QTL than any of our previous mapping efforts based on $n = 94$. Interestingly, most of the resistance QTL identified in this study were detected in other cross combinations we have analyzed over the past 16 years. Therefore, the same catalog of resistance genes could be generated using one set of experiments involving a large population or multiple sets of experiments involving smaller populations. Compared to various subsampling strategies designed to reduce population size, a large population was useful for detecting more QTL, although there was little improvement beyond 300. At $n = 100$, the population size closest to the $n = 94$ that is most commonly employed, selective genotyping, selective phenotyping and random sampling were equally effective. Between $n = 100$ and $n = 300$, however, the selective approaches were superior to random sampling. The choice of sampling procedure will be dictated by the relative costs of genotyping versus phenotyping. In terms of the latter, our results confirm that trait evaluations under optimum conditions are essential for optimizing estimates of QTL number, effect and interactions. Interestingly, there was no QTL × QTL interaction detected in this study and QTL × E interac-

tion within megaenvironments was all due to changes in magnitude of response. These barley stripe rust data show high heritability, and involve ≤ 10 QTL, being therefore a best-case scenario for QTL analysis and suitable candidates for marker-assisted selection (Schon et al. 2004).

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