

Fine-mapping of *qRfg2*, a QTL for resistance to *Gibberella* stalk rot in maize

Dongfeng Zhang · Yongjie Liu · Yanling Guo ·
Qin Yang · Jianrong Ye · Shaojiang Chen ·
Mingliang Xu

Received: 30 August 2011 / Accepted: 14 October 2011 / Published online: 3 November 2011
© Springer-Verlag 2011

Abstract Stalk rot is one of the most devastating diseases in maize worldwide. In our previous study, two QTLs, a major *qRfg1* and a minor *qRfg2*, were identified in the resistant inbred line ‘1145’ to confer resistance to *Gibberella* stalk rot. In the present study, we report on fine-mapping of the minor *qRfg2* that is located on chromosome 1 and account for ~8.9% of the total phenotypic variation. A total of 22 markers were developed in the *qRfg2* region to resolve recombinants. The progeny-test mapping strategy was developed to accurately determine the phenotypes of all recombinants for fine-mapping of the *qRfg2* locus. This fine-mapping process was performed from BC₄F₁ to BC₈F₁ generations to narrow down the *qRfg2* locus into ~300 kb, flanked by the markers SSRZ319 and CAPSZ459. A predicted gene in the mapped region, coding for an auxin-regulated protein, is believed to be a candidate for *qRfg2*. The *qRfg2* locus could steadily increase the resistance percentage by ~12% across different backcross generations, suggesting its usefulness in enhancing maize resistance against *Gibberella* stalk rot.

Introduction

Most of the important agronomic traits in crops belong to quantitatively inherited traits, such as plant morphological features, yield-related components, abiotic stress tolerance,

most disease resistance, etc., and are normally conditioned by multiple loci and influenced by various environmental factors, thus resulting in continuous variations in segregating population. Accordingly, each quantitative trait locus (QTL) exerts its slight but distinct genetic effect on the phenotypic variation for a given trait. Although there is no definite criterion to describe a QTL as ‘major’ or ‘minor’, the proportion of the phenotypic variation explained by a QTL has been generally used to describe an individual QTL: a major QTL could account for a relatively large amount (e.g., >10%) and a minor QTL for <10% (Collard et al. 2005; Kou and Wang 2010). To date, map-based cloning of QTL has concentrated on those major ones, such as *tg1* and *tb1* in maize (Wang et al. 2005; Clark et al. 2006). However, no report has been found in plant species regarding map-based cloning of minor QTL, due to its tiny genetic effect and extreme difficulty in phenotypic evaluation.

A large number of QTLs for disease resistance have been identified in crops and only a few of them have been cloned so far. In wheat, a major QTL, *Yr36*, encoding a kinase with a putative START lipid-binding domain (WKS1), could confer resistance to diverse stripe rust races at relatively high temperatures (25–35°C) (Fu et al. 2009). Another QTL, *Lr34* of wheat, encoding the protein belonging to the subfamily of ABC transporter, supports broad-spectrum and durable resistance against stripe rust, leaf rust and powdery mildew (Krattinger et al. 2009). In maize, a QTL, *Rgc1*, belonging to the nucleotide-binding site, leucine-rich repeat (NBS-LRR) resistance gene family, showed resistance to anthracnose stalk rot caused by *Colletotrichum graminicola* (Ces.) (Abad et al. 2006).

Stalk rot poses a serious threat to maize production in the maize-growing regions of many countries, including China, India, Nepal, Bangladesh, Philippines, Thailand,

Communicated by T. Luebberstedt.

D. Zhang · Y. Liu · Y. Guo · Q. Yang · J. Ye · S. Chen ·
M. Xu (✉)
National Maize Improvement Center of China,
China Agricultural University, 2 West Yuanmingyuan Road,
Beijing 100193, People’s Republic of China
e-mail: mxu@cau.edu.cn

Zimbabwe, Israel, Greece, South Africa (Saxena 1982) and USA (Shim et al. 2006). The stalk rot symptom is observed during post-flowering and pre-harvest stage (Lal and Singh 1984). The rotting develops from the infected roots into the stalk and causes premature drying, ear dropping and stalk breakage, thus significantly reducing maize yield (Andrew 1954; Christensen and Wilcoxson 1966; Colbert et al. 1987; Sharma et al. 1993). Both fungal and bacterial pathogens have been found to cause stalk rot in maize (White 1999). *Fusarium graminearum* Schwabe, one of the major stalk rot pathogens, infests especially in northern China (Wu et al. 2007) and produces a wide variety of mycotoxins for pathogen invasion. Moreover, *F. graminearum* also causes ear rot and reduces grain yield; kernels contaminated with mycotoxins cannot be used as animal feed (Chen 2000).

In an early genetic study on stalk rot resistance disease, additive genetic effects were believed to have a major effect on *F. moniliforme* stalk rot (Russel 1961), and incomplete dominance of the F₁ hybrid was observed in resistance to *F. moniliforme* (Widakas et al. 1980). Moreover, using 20 reciprocal translocations derived from the highly susceptible line ‘57/75’ and the highly resistant ‘Syn. 61C’, three resistance genes were proposed to control the disease reaction (Younis et al. 1969). More recently, QTL analysis was adopted to reveal genetic factors underlying the resistance to stalk rot. Within the 112 F₃ families derived from the cross of the resistant line ‘B89’ and susceptible line ‘33-16’, resistance to *Gibberella* stalk rot showed quantitative variations, conditioned by five QTLs located on chromosomes 1, 3, 4, 5 and 10, respectively (Pè et al. 1993). A single dominant gene in the resistant line ‘1145’ was found to confer resistance to *Gibberella* stalk rot and this gene was mapped within the confidence interval of 5 cM (Chen and Song 1999; Yang et al. 2004). A total of 18 genotypes, including 6 single-cross hybrids and their 12 parental lines, were inoculated with the *F. graminearum* race FG36, and this further confirmed that genotype was the major factor that determines resistance to stalk rot (Szöke et al. 2007). Using the backcross population derived from the cross of the resistant line ‘1145’ and the susceptible line ‘Y331’ in our previous study, two QTLs, a major *qRfg1* and a minor *qRfg2*, which confer resistance to *Gibberella* stalk rot were detected in the resistant line ‘1145’. The major one could explain 36.3% of total phenotypic variation and was restricted to ~500 kb, whereas the minor *qRfg2* could only account for 8.9% of the total phenotypic variation and was mapped on chromosome bins 1.09/10 (Yang et al. 2010). Apart from genetic factors, a number of environmental elements, such as soil moisture, climate change and temperature among others, could also have enormous impact on the occurrence and prevalence of stalk rot disease. In Switzerland, stalk rot

significantly diversifies across different years and locations for the same set of maize inbred lines and hybrids under natural *Fusarium* infection (Dorn et al. 2009). Ahmad et al. (1996) found that solarization could significantly decrease maize infections to the fungal pathogens *Fusarium moniliforme* and *Macrophomina phaseolina*. Although application of chemical compound can curtail an incidence of stalk rot disease, the most effective method of disease control is to deploy resistant maize hybrids.

In the present study, we focus on fine-mapping of the *qRfg2* locus in a bid to clone the underlying gene. We also carefully checked the genetic effect of the *qRfg2* locus to assess its value in the improvement of maize resistance to *Gibberella* stalk rot.

Materials and methods

Plant materials

A completely resistant inbred line ‘1145’ (the donor parent) was crossed with a highly susceptible line ‘Y331’ (the recurrent parent) to produce the F₁ hybrid, which was backcrossed to the recurrent parent ‘Y331’ to develop the backcross population (BC₁F₁). The resistant BC₁F₁ individuals were further backcrossed to ‘Y331’ to produce BC₂F₁, and the resistant BC₂F₁ individuals were again backcrossed to ‘Y331’ to produce the BC₃F₁ population. In 2007, using the markers anchored to both the *qRfg1* and *qRfg2* regions, we identified one BC₃F₁ individual that had the *qRfg2*, but not *qRfg1*, segments from the donor ‘1145’. This *qRfg2*-containing BC₃F₁ individual was used as the progenitor to produce all advanced backcross mapping populations. In the BC₄F₁ generation, recombinants in the *qRfg2* region were identified and then backcrossed to ‘Y331’ to produce the BC₅F₁ progeny, and such mapping process, including identification of new recombinants and backcrossing to ‘Y331’, was repeated in the following BC₅F₁ to BC₇F₁ backcross generations (Fig. 1). These advanced backcross populations shared almost the same ‘Y331’ genetic background with either heterozygous ‘Y331’/‘1145’ or homozygous ‘Y331’/‘Y331’ genotypes in the *qRfg2* region, hence insuring accurate assessment of the genetic effect of *qRfg2* on maize resistance to *Gibberella* stalk rot.

Development of PCR-based markers

In our initial QTL mapping effort, the minor QTL-*qRfg2* was detected in a confidence interval between SSR markers mmc0041 and phi307808, with a physical distance of ~39 Mb according to the B73 whole-genome physical map (<http://www.maizesequence.org/>). BAC sequences within the *qRfg2* confidence interval were downloaded

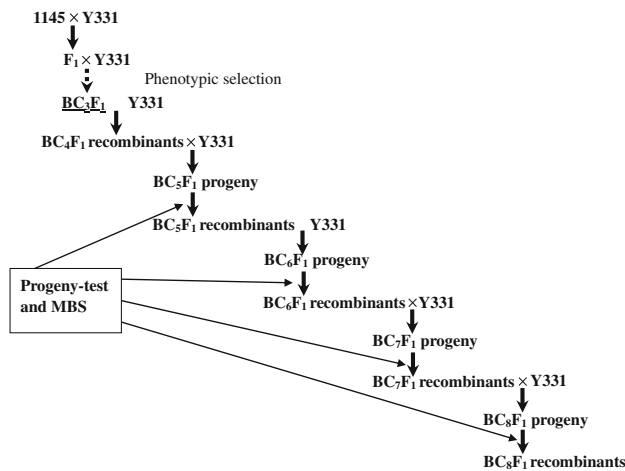


Fig. 1 Diagram of the progeny-testing and marker-based screening of recombinants in advanced backcross generations. One BC₃F₁ individual with the *qRfg2* region was selected as the progenitor to produce all advanced backcross mapping populations. The recombinants from each generation were identified with the *qRfg2*-tagged markers and backcrossed to the recurrent parent ‘Y331’, and this process was repeated from the advanced BC₅F₁ to BC₈F₁ backcross generations

from the Website. These sequences were used to develop new markers between the resistant ‘1145’ and susceptible ‘Y331’ parental lines. The sequences were first scanned by the software SSRHunter1.3 (Li and Wan 2005) to mine as many simple-sequence repeats (SSRs) as possible. Primers were designed by the software PRIMER 5.0 or PRIMER 3 (<http://frodo.wi.mit.edu/primer3/>) with the following criterion: ~20 nucleotides with 40–60% GC content, no consecutive tracts of a single nucleotide, and no secondary structure. Once the PCR products appeared polymorphic for two parental lines, SSR markers were deemed to be developed.

The retrieved sequences in the *qRfg2* region were further compared with the MAGI database (<http://magi.plantgenomics.iastate.edu/>) to mask those high-copy sequences. The resultant single-/low-copy sequences were used to develop either cleaved amplified polymorphic sequence (CAPS) or sequence-tagged site (STS) markers. Normally, primers were designed on those single-/low-copy sequences to amplify both parental lines, and the PCR products were cloned into the pGEM-T vector. At least three positive clones were selected for each PCR product and multiple sequence alignments were conducted with the DNAMAN software to guarantee that right sequences were obtained. Furthermore, the right sequence was compared to the B73 reference sequence to insure that the position of the amplified region was correct. Finally, pairwise alignment between two parents was performed for the correct sequences with the DNAMAN software to find out all polymorphisms. Consequently, sequences containing insertion/deletions (InDels) could be developed into STS markers, and single nucleotide

polymorphisms (SNPs) could be used to develop CAPS markers if the SNP sites were related to certain restriction sites.

Preparation of genomic DNA and genotyping

Leaf tissue was harvested in the field for DNA extraction according to the method described by Murray and Thompson (1980). Each DNA sample was genotyped at the markers as required, and PCR amplicons were electrophoretically analyzed on 1% agarose gel or 6% denaturing polyacrylamide gel and visualized by silver staining.

Artificial inoculation and scoring in the field

Fusarium graminearum Schwabe was kindly provided by Professor Xiaoming Wang, Chinese Academy of Agricultural Sciences. *F. graminearum* was cultured in darkness at 25°C on potato dextrose agar (PDA). After 1 week of culture, the reddish mycelia occupied the PDA medium and the compound secreted by the conidia sunk into the PDA. Preparation of maize kernel inoculums and field inoculation of plants were conducted as described by Yang et al. (2010).

Symptom scoring was conducted 1 month after inoculation and repeated twice at 1-week intervals. In the last scoring, the stem was longitudinally cut to observe mycelial development and degree of rotting inside the stem. Disease incidence was rated on six scales: 1, 2, 3, 4, 5 and 6, based on all scorings (Yang et al. 2010). Rating scales 1–3 were regarded as resistant and 4–6 were regarded as susceptible (Table 1).

Strategy for fine-mapping of the minor QTL-*qRfg2*

Fine-mapping is dependent on both high-density markers and sufficient recombinants in the target region. From BC₄F₁ to BC₇F₁ populations, we screened all possible recombinants using the existing as well as newly developed markers. The BC₄F₁ recombinants were backcrossed to ‘Y331’ to produce sufficient BC₅F₁ seeds. Since *qRfg2* is a minor QTL, the performance (resistant/susceptible) of a single individual cannot exactly reflect its genotype (presence/absence of *qRfg2*) due to the minor contribution of *qRfg2* and the large influence of environmental elements on the phenotype. To address this problem, we adopted a robust progeny-test strategy to obtain the precise genotype and phenotype of each recombinant for fine-mapping of *qRfg2*. Considering the weak contribution of *qRfg2* to stalk rot disease, we set up two to three replicates to obtain unbiased assessment of the phenotype for a given recombinant. All progeny were grown in two or three separate plots and each plot had a subset of >30 kernels. At seedling stage, leaf tissue was collected for all progeny for genotyping at markers on the *qRfg2* region.

Table 1 Symptom scoring and phenotypic determination

Rating scale	Symptom scoring			Phenotype	
	1st scoring	2nd scoring	3rd scoring		
1	No symptom	No symptom	No symptom	HR	Resistant individuals
2	No symptom	No symptom	Few symptoms	R	
3	No symptom	Few symptom	Few symptoms	MR	Susceptible individuals
4	No symptom	Few symptoms	Symptoms	MS	
5	No symptom	Symptoms	Symptoms	S	
6	Symptoms	Symptoms	Symptoms	HS	

HR highly resistant, R resistant, MR moderately resistant, HS highly susceptible, S susceptible, MS moderately susceptible

In each replicate, we divided the whole progeny into two subgroups, with (heterozygous ‘1145’/‘Y331’ genotype) and without (homozygous ‘Y331’/‘Y331’ genotype) the ‘1145’ donor segment, and calculated the resistance percentage for each subgroup (Fig. 2). Our assumption was that no significant difference ($P > 0.05$) between two subgroups in resistance to stalk rot disease indicated the absence of the QTL-*qRfg2* in the ‘1145’ donor segment (Fig. 2a). In contrast, significant difference ($P < 0.05$) between two subgroups indicated the presence of the QTL-*qRfg2* in the ‘1145’ donor segment (Fig. 2b). Statistical difference in resistance percentages between the two subgroups was tested by a pair-samples *t* test for all recombinants in the same genotype type. Comparison of the ‘1145’ donor regions to phenotypes for all genotype types allowed us to fine map the resistance QTL-*qRfg2*.

QTL analysis in the advanced backcross generations

In progeny-test strategy, the difference in the resistance percentages between two subgroups is essentially attributed to the genetic effect of the ‘1145’ donor segments. Such difference in the resistance percentage was thus named as the ‘relative resistance percentage’.

All genotypic data at anchored markers in the *qRfg2* region from BC₄F₁ to BC₈F₁ generations were used to construct the linkage map by Mapmaker 3.0 (Lincoln et al. 1992), and the Kosambi function was used to convert recombination values to genetic distances (Kosambi 1944). QTL analysis was carried out by composite interval analysis (CIM) with the QTL cartographer (version 2.5 software package) (Basten et al. 1997). A QTL for resistance to *gibberella* stalk rot was declared significant at $P < 0.01$, with LOD score given by 1,000 permutations.

A model for declaration of a minor QTL and estimation of its genetic effect

The data structure in the present experiment could be described as: *y* for individual phenotype and *mm* (denoted as 0) and *Mm* (denoted to 1) for two QTL genotypes. An

individual with the genotype *Mm* tends to be more resistant than those with the genotype *mm*. To test whether this was the true situation for all backcross progeny, we formulated a simple regression model as:

$$y_i = \mu + z_i a + e_i$$

where y_i is the phenotypic value for the individual *i*, μ is the overall mean, z_i is the indicator variable that specifies the QTL genotype of individual *i* and is defined as

$$z_i = \begin{cases} 1 & \text{if QTL genotype is } Mm \\ 0 & \text{if QTL genotype is } mm \end{cases}$$

a is the additive effect of the QTL, and e_i is the random error, typically assumed to be normally distributed as $N(0, \sigma^2)$ (Doerge et al. 1997). In the regression model, individuals that carry the same genotype may not have exactly the same phenotypic value or resistance in our data. The hypotheses for the test can be formulated as:

$$H_0 : \mu_1 = \mu_0,$$

$$H_1 : \mu_1 \neq \mu_0.$$

where μ_0 is the mean value for the genotype *mm*; μ_1 is the mean value for the genotype *Mm*. A *t* test was used to check the difference in resistance percentages between the two mean values to declare the presence of a QTL, and a regression analysis was used to estimate the genetic effect of the QTL on maize resistance to stalk rot.

Results

Development of high-density markers in the *qRfg2* region

To resolve all possible recombinants for fine-mapping of the *qRfg2* locus, high-density markers are indispensable. Using the SSRHunter 1.3 software, a total of 400 SSR sequences were identified from 60 anchored BACs within the *qRfg2* region. However, only 17 of them were finally

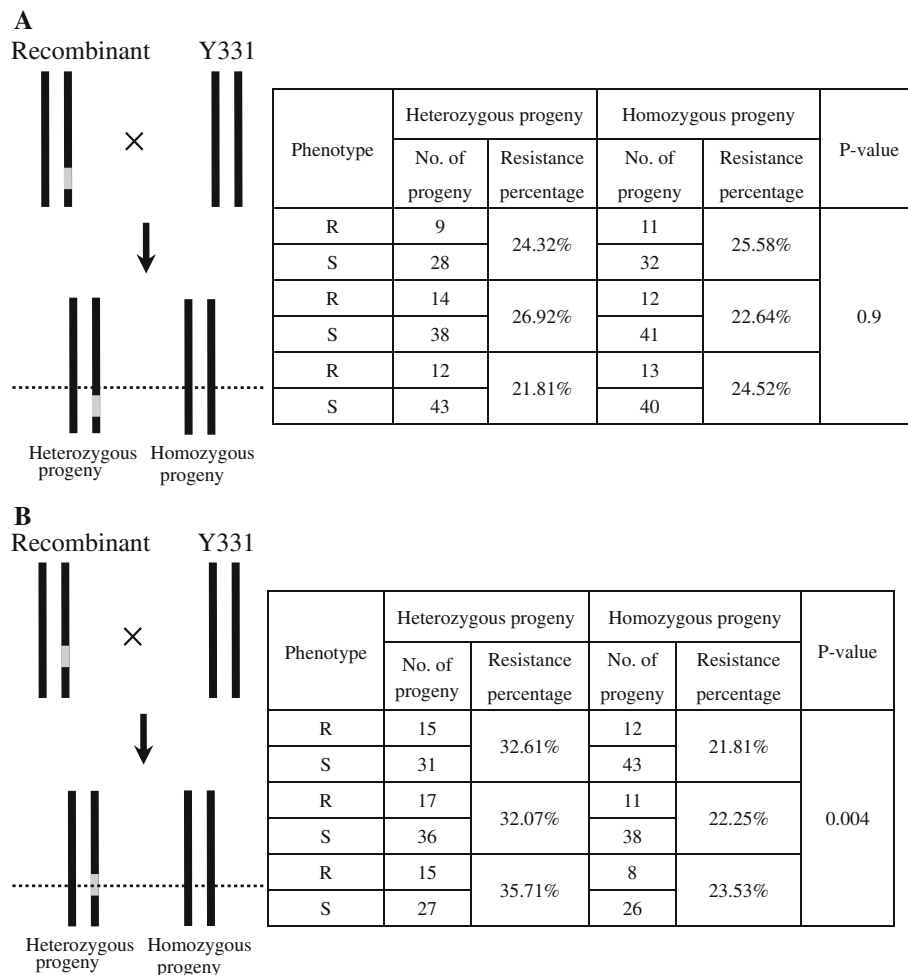


Fig. 2 Diagram of the progeny-test procedure. **a** A recombinant was backcrossed to ‘Y331’ to produce two genotypes at the ‘1145’ donor segment, heterozygous progeny (with the ‘1145’ donor segment) and homozygous progeny (without the ‘1145’ donor segment) at a theoretical ratio of 1 to 1. The progeny were planted in three replicate plots, and the resistance percentage was described as the resistant plants/total plants*100% for each genotype. Statistical difference in resistance percentages between the two genotypes was tested by a *t* test. In figure **a**, there was no difference in resistance percentages

between two kinds of genotypes ($P = 0.9$) in three replicates, indicating absence of the resistance QTL-*qRfg2* in the ‘1145’ donor segment. **b** Significant difference in resistance percentages between two kinds of genotypes ($P = 0.004$) was observed for three replicates, indicating the presence of the resistance QTL-*qRfg2* in the ‘1145’ donor segment. Comparison of the ‘1145’ donor regions to phenotypes for all recombinants allowed us to fine map the resistance QTL-*qRfg2*. Dotted line denotes the position of the resistance QTL-*qRfg2*

developed into new SSR markers polymorphic for the parental lines ‘1145’ and ‘Y331’. To accelerate the fine-mapping process, we tried hard to develop STS and CAPS markers within the mapped *qRfg2* region in each fine-mapping step to resolve the newly identified recombinants. Overall, we made use of 90 single-/low-copy sequences in the *qRfg2* region to develop two STS and three CAPS markers. Finally, 22 markers were developed to cover the QTL-*qRfg2* interval (Table 2).

Fine-mapping of the *qRfg2* locus by the progeny-test strategy

The minor resistance QTL-*qRfg2* was mapped in a large chromosomal region (~39 cM) on bins 1.09/10, flanked

by the SSR markers, mmc0041 and phi308707, in our initial QTL analysis (Yang et al. 2010). The markers, mmc0041 and phi308707, together with eight newly developed SSR markers (SSRZ135, SSRZ171, SSRZ28, SSRZ56, SSRZ68, SSRZ81, SSRZ88 and SSRZ93) within the *qRfg2* region, were used to genotype 180 BC₄F₁ plants which were derived from one *qRfg2*-containing BC₃F₁ individual. The marker density was estimated to be 2–5 Mb per marker in the *qRfg2* region according to the B73 reference sequence. The resultant 15 BC₄F₁ recombinants within the *qRfg2* region were backcrossed to ‘Y331’ to produce the BC₅F₁ mapping populations (totally, 983 BC₅F₁ individuals). Based on their genotypes, the 15 BC₄F₁ recombinants could be classified into five types (Fig. 3a). Types I and II showed significant difference

Table 2 The list of 22 markers newly developed in the QTL-*qR/g2* region

Location (Mb)	BAC	Markers	Forward primer (5'-3')	Reverse primer (5'-3')	Annulling temperature (°C)	Type	Restriction enzymes
227.4 Mb	AC225222	SSRZ135	CCGATCCTCCTCCTTCAGTA	CTGACGCTAGTGTGGAGTG	60	SSR	
244.12 Mb	AC187338	SSRZ171	AGTAGTCGATGACACGATGG	GAAGGAACAACGTACCGAAG	57	SSR	
247.78 Mb	AC210272	SSRZ28	GATGACTGACTAGCGTGCCA	CCATCGGCTCTCCTTAATGA	60	SSR	
251.39 Mb	AC201886	SSRZ56	GCACTGAGATCGCAAGAATG	TGGTGAGCACACACGTAATG	60	SSR	
256.38 Mb	AC196645	SSRZ68	TGTCGTGTGTCGTCACAAGAG	GCCGAGATGATGATGTGAG	57	SSR	
258.3 Mb	AC210959	SSRZ293	TAGGACCTGGTCTTTGTTGG	TAGTGGCTTGTGATGCTCTCT	60	SSR	
259.53 Mb	AC202907	SSRZ307	CTTGGCATCCATCTCGTTCT	CTCTGAGTGGTCTCTGGTCTCT	60	SSR	
260.04 Mb	AC186007	CAPSZ406	GATACATGGCACAGAAAGCAG	GTCCATTGTACCCACTGAGC	60	CAPS	<i>NdeI</i>
260.69 Mb	AC195597	SSRZ312	CATCTGCGTTCTTCTTCTCT	AACTCCACGGCTAACTCTCGTT	60	SSR	
261.27 Mb	AC199953	SSRZ256	AAGCTGCTGAAGACGAGTTG	GGAGTGCAITTGACAGGTTGT	60	SSR	
261.46 Mb	AC197070	STSZ479	TAATTGATGAATCGCCACGA	GGAGATAGGAGGGCTGCAIT	60	STS	
261.65 Mb	AC190498	SSRZ319	CACCTTCTCTTGTGTAC	CTGCACCTGCTAGTCTCTGTC	56	SSR	
262.55 Mb	AC203019	STSZ514	ACGGGACAGAGGAGACACAC	GACCGTTTCATGCGTATGTG	60	STS	
261.94 Mb	AC211591	CAPSZ459	GCAATCGGAAATTTAGGGAAC	GGATAACTCGCCTGGCATAA	60	CAPS	<i>SdaI</i>
262.05 Mb	AC211591	CAPSZ454	GATGTGGGACACACGAAAGT	ACTAGTTTTCGGGGGGTTTC	60	CAPS	<i>TaqI</i>
262.29 Mb	AC210023	SSRZ263	CGAGTCCGGTAGTACGAAAGA	GAACGGACTGCTTCTCTAAT	56	SSR	
263.20 Mb	AC198412	SSRZ280	GGTTCGGCAGGTATCCTAAT	TTCTCGGCTAGATTCCACCAG	60	SSR	
264.79 Mb	AC194456	SSRZ81	CACACACACTACTCTGCGCC	ACCAITTCGATCACACACACG	60	SSR	
265.2 Mb	AC211638	SSRZ343	GAGTGCAGCGCAGTAGAAGT	AGCAGGAGGAGGAGGTGAGT	60	SSR	
266.25 Mb	AC194892	SSRZ88	GTGCGCCTTTCTCCATATT	CGGAGCAAGTACATGGTGTG	60	SSR	
266.97 Mb	AC196146	SSRZ93	AGTACGTTGTACGTGGCTGG	TGCTTGTGTGTTCCATCTT	60	SSR	
268.18 Mb	AC214515	SSRZ206	TTCTCGATCTCTGACCGGAC	CCATAATCTCTCTCTCTCG	60	SSR	

($P < 0.05$) between their heterozygous ('1145'/'Y331') and homozygous ('Y331'/'Y331') BC₅F₁ progeny in resistance to *Gibberella* stalk rot, indicating that the '1145' donor segments contained *qRfg2*. Accordingly, the corresponding parental recombinants were deduced to be resistant. On the contrary, the remaining three types (III, IV and V) exhibited no significant differences ($P > 0.05$) between their heterozygous ('1145'/'Y331') and homozygous ('Y331'/'Y331') BC₅F₁ progeny in resistance to stalk rot, indicating that the '1145' donor segments did not have any resistance QTL. Therefore, the corresponding parental recombinants were deduced to be susceptible. Type I recombinants contain the '1145' donor region covering the mmc0041/phi308707 interval where *qRfg2* had previously been mapped (Yang et al. 2010), while type II recombinants have the donor segment of SSRZ171/phi308707 located inside the mmc0041/phi308707 interval. Both types I and II show resistance to stalk rot, confirming that *qRfg2* was correctly mapped in our initial QTL analysis. The types III and IV had the closest crossing-over points upstream and downstream of the *qRfg2* locus, and both types were susceptible to stalk rot. These data allowed us to map the *qRfg2* locus between markers SSRZ68 and SSRZ93 (Fig. 3a).

In parallel, genotyping of all 983 BC₅F₁ individuals allowed us to identify more recombinants. Since the *qRfg2* locus has been mapped into the SSRZ68/SSRZ93 interval, we were only interested in those recombinants having recombination points located within or nearby the SSRZ68/SSRZ93 interval. As a consequence, 28 new BC₅F₁ recombinants were obtained. Within the SSRZ68/SSRZ93 interval, ten new markers (SSRZ293, SSRZ307, CAPSZ406, SSRZ312, SSRZ256, SSRZ263, SSRZ280, SSRZ81, SSRZ343 and SSRZ206) were used to resolve the recombination breakpoints for these 28 BC₅F₁ recombinants. The 28 BC₅F₁ recombinants could be divided into six types based on their genotypes. Meanwhile, these 28 BC₅F₁ recombinants were backcrossed to 'Y331' to produce 1,888 BC₆F₁ plants. The same progeny-test strategy was adopted to deduce the phenotypes of 28 BC₅F₁ recombinants. As a result, types I, II and III were deduced to be resistant ($P < 0.05$) and contained the resistance QTL-*qRfg2* in their '1145' donor segments, while types IV, V and VI were deduced to be susceptible ($P > 0.05$) and did not have the resistance QTL-*qRfg2* in their '1145' donor segments (Fig. 3b). Types I, II and III were resistant, confirming that *qRfg2* was present between markers SSRZ68 and umc1885. Types V and VI were susceptible and had the closest crossing-over points upstream and downstream of the *qRfg2* locus, thus further delimiting *qRfg2* into the SSRZ293/SSRZ263 interval with a physical distance of ~4 Mb based on the B73 reference sequence. Consequently, it is still necessary to further narrow down *qRfg2* until it is restricted to a few BAC clones.

In 2010, five new markers within the SSRZ256/SSRZ263 interval, STSZ479, SSRZ319, STSZ514, CAPSZ459 and CAPSZ454, were used to resolve newly identified recombinants. A total of 36 BC₆F₁ recombinants within the SSRZ293/SSRZ263 interval were identified and backcrossed to 'Y331' to produce 2,817 BC₇F₁ plants. To obtain more recombinants, a large number of BC₇F₁ plants were grown in a 2009–2010 winter nursery to screen another 38 BC₇F₁ recombinants within the SSRZ293/SSRZ263 interval, which were backcrossed to 'Y331' to produce 3,022 BC₈F₁ plants. Both advanced BC₇F₁ and BC₈F₁ backcross generations were grown in the field to investigate their genotypes and resistance to stalk rot. For the 36 BC₆F₁ recombinants, the first three types (I, II and III) showed significant difference with very low P value ($P < 0.01$) between the heterozygous and homozygous BC₇F₁ progeny and were deduced to be resistant. The remaining four types showed no difference ($P > 0.05$) between the heterozygous and homozygous BC₇F₁ progeny and were hence deduced to be susceptible (Fig. 3c). For the 38 BC₇F₁ recombinants, six types (I, II, V, VI, VII and X) were deduced to be resistant and thus had *qRfg2* in their '1145' donor segments. On the contrary, the remaining four types (III, IV, VIII and IX) were deduced to be susceptible and thus had no *qRfg2* in their '1145' donor segments (Fig. 3d). Comparison of the '1145' donor sizes to phenotypes for 36 BC₆F₁ and 38 BC₇F₁ recombinants, we found that type VI BC₆F₁ and IX BC₇F₁ susceptible recombinants had the closest recombination breakpoints upstream of the *qRfg2* locus, and type IV BC₆F₁ and III BC₇F₁ susceptible recombinants had the closest recombination breakpoints downstream of the *qRfg2* locus, thus delimiting the *qRfg2* locus between the markers SSRZ319 and CAPSZ459 with a physical distance of ~300 kb (Fig. 3).

QTL analysis in advanced backcross generations

In 2008, a total of 11 markers (SSRZ135, mmc0041, SSRZ171, SSRZ28, SSRZ56, SSRZ68, SSRZ81, SSRZ88, SSRZ93, umc1885 and phi308707) were used to construct the genetic linkage map. The relative resistance percentages were estimated for the 15 BC₄F₁ recombinants. A QTL peak was detected in the confidence interval between the markers SSRZ68 and SSRZ93 by composite interval mapping (CIM). The QTL-*qRfg2* explained 40.24% of the total phenotypic variation with an LOD value of 4.21 and increased the resistance percentage by 12.85% (Fig. 4a). With the 28 BC₅F₁ recombinants, nine newly developed markers (SSRZ293, SSRZ307, CAPSZ406, SSRZ312, SSRZ256, SSRZ263, SSRZ280, SSRZ343 and SSRZ206) were added to construct a high-resolution genetic linkage map in the *qRfg2* region. The relative resistance percentages of the 28 BC₅F₁ recombinants were estimated and used for

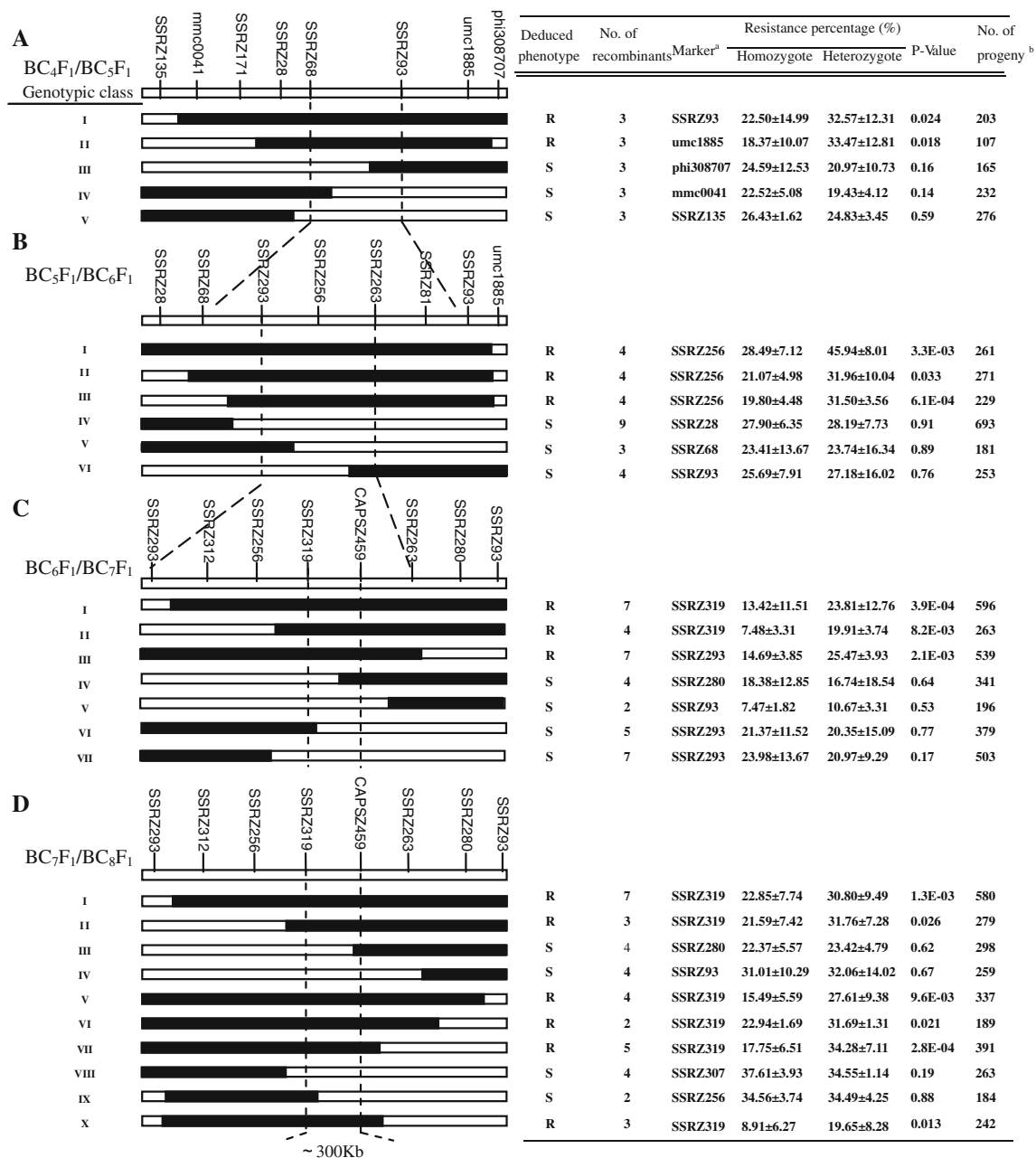


Fig. 3 Sequential fine-mapping of *qRfg2* with progeny-test strategy. Recombinants with the same '1145' donor regions were grouped as the same genetic class. The genetic structure for each genetic class is depicted as *open* and *filled rectangles*, corresponding to homozygous 'Y331'/'Y331' alleles and heterozygous '1145'/'Y331' alleles at the *qRfg2* region, respectively. The progeny for each genetic class were divided into two subgroups: homozygote and heterozygote, and the resistant percentages of both subgroups were calculated (on the right table). A *t* test is used to judge whether there is a significant difference between two subgroups. The significant difference ($p < 0.05$) between the two subgroups indicates the presence of

qRfg2 in the '1145' donor region, and thus denoted as resistant (R). In contrast, no significant difference ($p > 0.05$) between two subgroups means no *qRfg2* in the '1145' donor region, and denoted as susceptible (S). Analysis of both the donor region and the phenotype for all recombinants allowed the fine-mapping of *qRfg2*. **a** Progeny test with 15 BC_{4,5} families and their 983 BC₅F₁ progeny. **b** Progeny test with 28 BC_{5,6} families and their 1888 BC₆F₁ progeny. **c** Progeny test with 36 BC_{6,7} families and their 2817 BC₇F₁ progeny. **d** Progeny test with 38 BC_{7,8} families and their 3022 BC₈F₁ progeny. ^aMarker used to genotype progeny. ^bTotal number of progeny derived from the same recombinant type

QTL analysis. In this case, the confidence interval was contracted into the SSRZ293/SSRZ263 interval (Fig. 4b). QTL-*qRfg2* could explain 47.01% of the total phenotypic

variation with an LOD value of 3.86. The genetic effect of *qRfg2* in the resistance to stalk rot was 13.35%, almost the same as that detected in BC₄F₁ recombinants. In 2010, an

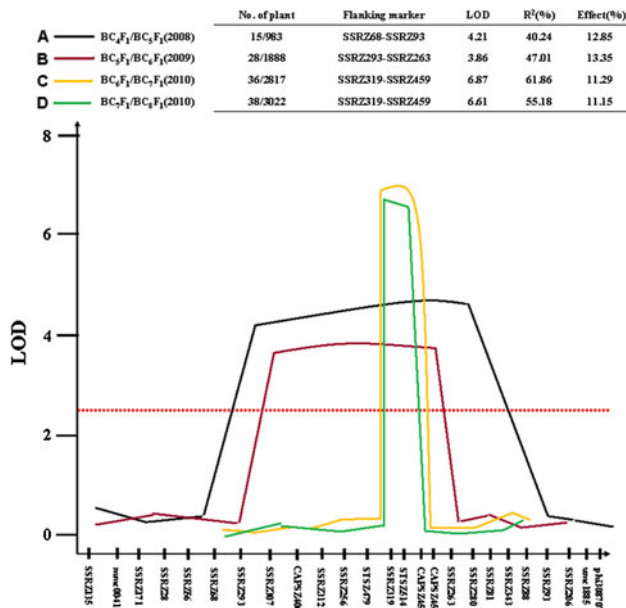


Fig. 4 Diagram of QTL plots for *qRfg2* in four advanced backcross generations. The logarithm of odds (LOD) profile, relative position of *qRfg2* and relevant markers are displayed by using QTL cartographer version 2.5. **a** QTL plot of 15 BC_{4:5} families consisting of 983 BC₅F₁ plants. **b** QTL plot of 28 BC_{5:6} families consisting of 1888 BC₆F₁ plants. **c** QTL plot of 36 BC_{6:7} families consisting of 2817 BC₇F₁ plants. **d** QTL plot of 38 BC_{7:8} families consisting of 3022 BC₈F₁ plants

additional five newly developed markers (STSZ479, SSRZ319, STSZ514, CAPSZ459 and CAPSZ454) were added to genotype 36 BC₆F₁ and 38 BC₇F₁ recombinants, thus constructing two high-resolution genetic maps. Consequently, two sharp QTL peaks appeared in the narrow confidence intervals between markers SSRZ319 and CAPSZ459 with high LOD values (Figs. 4c, d). The genetic effect of QTL-*qRfg2* in the resistance to stalk rot was almost the same across the four advanced backcross generations (Fig. 4).

Impact of the residual ‘1145’ segments on resistance of *qRfg2* to stalk rot

To know the possible impact of the residual ‘1145’ segments in genetic backgrounds on the resistance of *qRfg2* to stalk rot, a total of 50 SSR markers that were evenly distributed on maize genome were selected to scan the genetic backgrounds across different backcross generations. In BC₆F₁ mapping population, only 90% of the SSR markers were the same as those in the recurrent parent ‘Y331’, whereas 100% markers were identical to ‘Y331’ in BC₇F₁ and BC₈F₁ mapping populations. Since the genetic effect of *qRfg2* was quite stable in the resistance to stalk rot across different backcross generations (Fig. 4), it demonstrated that the residual ‘1145’ segments in the genetic

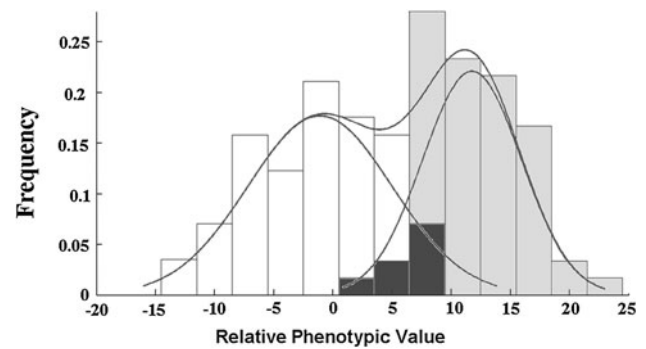


Fig. 5 A histogram of phenotypic data from 117 recombinant plants. The light gray columns correspond to recombinants with *qRfg2*, and the white column correspond to those recombinants without *qRfg2*; the dark bars represent those overlapping recombinants. All the data were tested by Shapiro–Wilk normality test to reveal the presence of *qRfg2*, which could steadily increase the resistance frequency by ~12% in the ‘Y331’ genetic background

backgrounds were less likely to have any impact on the resistance of *qRfg2* to stalk rot.

Analysis of data structure and estimation of the *qRfg2* contribution to resistance to stalk rot

The relative resistance percentages of 117 recombinants were summarized to study their distribution by Shapiro–Wilk normality test. It seemed unlikely that a normal distribution was present ($P = 0.07$), suggesting the presence of a QTL in these 117 recombinants that played a role in resistance to stalk rot (Fig. 5). The 117 recombinants were further divided into two subgroups based on their genotypes at the *qRfg2* locus, resulting in 60 recombinants with *qRfg2* versus 57 recombinants without *qRfg2*. In the subgroup with *qRfg2*, the relative resistance percentage was 11.77% on average and the phenotypic dataset corresponded to normal distribution ($P > 0.05$), while the subgroup without *qRfg2* showed a relative resistance percentage of -1.08% on average and also corresponded to normal distribution ($P > 0.05$) (Fig. 5). Again, this finding confirmed the presence of a resistance QTL-*qRfg2* ($P < 0.001$), which contributed to stalk rot resistance (Fig. 5). By combining data from 117 recombinants, it was found that the QTL-*qRfg2* could enhance the resistance percentage by ~12%.

Discussion

The genetic basis underlying quantitatively inherited traits often involve multiple QTLs, including QTLs with large and small effects (Flint and Mackay 2009; Holland 2007). With the advent of modern biotechnology and statistical methods, it has become more and more convenient to

obtain high-resolution genotype data and to use sophisticated methods in QTL analysis (Daetwyler et al. 2010). The most important but difficult issue in QTL mapping is, therefore, how to accurately evaluate the phenotype. Although the theory for QTL mapping had been established and applied to plants since the early twentieth century (Sax 1923; Wang et al. 2005), cloning of QTL has been achieved in less than a dozen years. Almost all QTLs cloned so far can explain large proportions of the phenotypic variations and are responsible for important traits, such as flowering time, disease resistance, yield components, etc., (Mackay et al. 2009). As indicated, the major QTL with large effect could segregate as a single Mendelian locus in an isogenic background and its genetic effect could be maintained consistently across different generations. In contrast, accurate phenotypes of minor QTLs are very difficult to evaluate due to their minor contributions (<10%) to the total phenotypic variation, and to make things even worse, their genetic contributions are often undetectable in advanced mapping populations. In addition, the confidence interval of a minor QTL is generally larger than that of the major QTL due to its minor genetic effect. No matter how small the genetic effect of a minor QTL, its role in the improvement of the target trait cannot be ignored; moreover, stacking of multiple minor QTLs related to a given agronomic trait via marker-assisted selection would result in a big improvement in the phenotypic performance. Therefore, it is of great importance to fine map and clone minor QTLs.

The QTL-*qRfg2* could just explain 8.9% of the phenotypic variation in the initial mapping and increased the resistance percentage by ~12% in the current study. Provided only F₂ or BC₁ mapping populations were used for fine-mapping of *qRfg2*, the minor contribution of *qRfg2* would be inundated in experimental errors resulting from different genetic backgrounds and environmental elements, making fine-mapping impossible. This may occur even if F_{2:3} or BC₁F₂ progeny were used to estimate the phenotype of the parental F₂ or BC₁ individual. Thus, how to evaluate the phenotypic performance is crucial toward the successful fine-mapping of a minor QTL. In the present study, the progeny-test strategy was used to evaluate the phenotypes for all recombinants, leading to successful fine-mapping of the minor QTL-*qRfg2*. Overall, the progeny-test strategy showed many advantages in reducing the experimental errors in the fine-mapping of *qRfg2*. Firstly, advanced backcross populations (from BC₅F₁ to BC₈F₁) were used for fine-mapping and their genetic backgrounds were almost recovered to the recurrent parent ‘Y331’, thus dramatically reducing the influences from variable genetic backgrounds on the resistance to stalk rot. Secondly, all progeny (either heterozygous or homozygous genotypes at the *qRfg2* region) derived from a single parental

recombinant were grown in the same plot to share exactly the same environmental conditions, such as soil moisture and nutrition, temperature and solarization, thus reducing perturbation of environmental elements in the resistance to stalk rot. Thirdly, the number of progeny was increased to reduce the statistical error in the phenotypic difference between two subgroups with/without the *qRfg2* region. In the present study, progeny were grown in two to three replicate plots and numbered >72 individuals. As shown, such progeny size was sufficient to reveal the accurate genetic effect of *qRfg2* on the resistance to stalk rot. The larger the progeny population, the more accurate was the phenotype obtained. Theoretically, it is possible to reveal any genetic effect of a minor QTL just by increasing progeny size. Fourthly, we normally selected as many recombinants as possible in the *qRfg2* region, especially in the final fine-mapping step, to reduce the sampling error. For those key recombinants with the closest recombination breakpoints to the *qRfg2* locus, phenotypic evaluation was repeated across different locations and years. The performance of all recombinants could be referred to one another to confirm the *qRfg2* locus. The more the recombinants selected in the *qRfg2* region, the more accurate is the position of the *qRfg2* locus. In principle, the progeny-test strategy has no difference with normal fine-mapping approach; both focus on exploring a relationship between genotype and phenotype. If the gene can determine the target trait, it is very simple to know the accurate genotype solely based on phenotype. In contrast, if the gene can only partially contribute the target trait, especially for the minor QTLs, the genetic effects would be accurately revealed only by diminishing all experimental errors. The progeny-test strategy is very powerful in controlling all experimental errors, so as to reveal the authentic genetic effect of a given QTL. Moreover, new recombinants within the mapped region can be obtained in each mapping generation by genotyping all progeny to guarantee the sequential narrowing down of the target gene.

There are many reports on fine-mapping of QTL in maize and rice (Ducrocq et al. 2009; Song et al. 2007). Generally, phenotypic evaluation of recombinants was based solely on homozygous progeny. Although the method looks simple and straightforward, it is sometimes difficult to obtain accurate phenotypic data and to narrow down the target locus for QTL, especially the minor ones. In our experience, phenotypic variations are frequently observed for the same set of progeny in different years and experimental plots; thus, it seems impossible to evaluate the minor genetic effects of some QTLs. In other words, it is impossible to minimize environmental errors by using homozygous progeny or near isogenic lines. Repeated evaluation may be helpful to obtain accurate phenotypic data; however, it is not an efficient and cost-effective

approach. Furthermore, limited numbers of homozygous progeny or near isogenic lines are often helpless to narrow down the target gene into a short interval, since fine-mapping depends on large numbers of key recombinants in the target region.

In each mapping population, we combined genotypes with relative resistance percentages for all recombinants by QTL cartographer to reveal QTL parameters for the *qRfg2* locus (Fig. 4). As the recovery rate increased from BC₄F₁ to BC₇F₁, the LOD values and coefficient of determination (R^2) increased. The most striking change was the confidence interval, from ~10 Mb in BC₄F₁ to ~300 kb in BC₇F₁, whereas the genetic contribution of the *qRfg2* locus in resistance to stalk rot remained almost the same. The result is in favor of the fine-mapping of a minor QTL using advanced backcross generations. In early backcross generations, determination of a minor QTL is so difficult that the confidence region would cover a big chromosomal region. This situation can be greatly improved in advanced backcross generations, in which the genetic background has been almost recovered to the recurrent parent, and the minor QTL thus plays a major role in phenotypic variation. On comparing QTL analysis with progeny-test strategy, we found that both methods were suitable to identify the *qRfg2* location. In view of the simple and straightforward nature of the progeny-test strategy, in which only pair-simples *t* test is required, we recommend it.

Within the ~300 kb interval of QTL-*qRfg2*, four B73 BACs were identified and the corresponding B73 sequence was retrieved. Several genes were predicted; one of them was annotated to code for an auxin-regulated protein and other open reading frames were related to retrotransposons and thus unlikely to be candidate for *qRfg2*. As known, auxin belongs to one of five kinds of phytohormones and is responsible for regulating the whole course of plant growth and development. Interestingly, recent results have shown that auxin also participates in resistance response in plants through positively or negatively changing the host auxin biosynthesis (Park et al. 2007; Wang et al. 2007). Several auxin response genes have been identified to have direct relationships with disease resistance, including GH3-5 coding for IAA-amino synthetase (Zhang et al. 2007), GH3.12 coding for an acyl adenylase in *Arabidopsis* (Nobuta et al. 2007), and GH3-8 coding for an indole-3-acetic acid-amino synthetase in rice (Ding et al. 2008). Also, auxin signaling in *Arabidopsis* is down-regulated by microRNA to induce immune response, thereby increasing resistance to pathogens in *Arabidopsis* (Navarro et al. 2006). The *Pseudomonas syringae* type III effector AvrRpt2 which promotes bacterial virulence in *Arabidopsis thaliana* plants lacking a functional *RPS2* gene increased the levels of free indole acetic acid (IAA) (Chen et al.

2007). These reports show that perturbation in the auxin signal transduction pathway results in imbalance of plant host hormone, which either suppresses or promotes host susceptibility to the bacterial pathogen and disease symptom development (Manulis et al. 1994; Chung et al. 2003; Maor et al. 2004; Vandeputte et al. 2005). All evidences convince us that the predicted gene coding for an auxin-regulated protein is a good candidate for *qRfg2*. To restrict the *qRfg2* locus into a single gene or further to reveal the sequence variations related to gene function, we continue creating and screening new recombinants within the *qRfg2* locus.

Acknowledgments The authors cordially thank Mr. Chao Wang and Ms. Nan Zhang for their technical assistance in both genotyping and field evaluation. This study was financially supported by the National ‘863’ High-Tech Program of China and the National ‘973’ Basic Research Program, Grant No: 2009CB118401.

References

- Abad L, Wolters P, Stucker D, Davis P (2006) Advances in anthracnose stalk rot resistance. The fifth national IPM symposium, “delivering on a promise”. <http://www.ipmcenters.org/ipmsymposiumv/posters/037.pdf>
- Ahmad Y, Hameed A, Aslam M (1996) Effect of soil solarization on corn stalk rot. *Plant Soil* 179:17–24
- Andrew RH (1954) Breeding for stalk-rot resistance in maize. *Euphytica* 3:43–45
- Basten CJ, Weir BS, Zeng ZB (1997) QTL cartographer: a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh
- Chen J (2000) Status and perspective on research of ear rot and stalk rot in maize. *J Shenyang Agric Univ* 31:393–401
- Chen SJ, Song TM (1999) Disease resistance of maize stalk rot. Simple genetics controlled by a single gene. *Acta China Agric Univ* 4:56
- Chen Z, Agnew JL, Cohen JD, He P, Shan L, Sheen J, Kunkel BN (2007) *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. *Proc Natl Acad Sci USA* 104:20131–20136
- Christensen JJ, Wilcoxson RD (1966) Stalk rot of corn. *Am Phytopathol Soc Monograph* 3:1–59
- Chung KR, Shilts T, Erturk U, Timmer LW, Ueng PP (2003) Indole derivatives produced by the fungus *Colletotrichum acutatum* causing lime anthracnose and postbloom fruit drop of citrus. *FEMS Microbiol Lett* 226:23–30
- Clark RM, Wagler TN, Quijada P, Doebley J (2006) A distant upstream enhancer at the maize domestication gene *tb1* has pleiotropic effects on plant and inflorescent architecture. *Nat Genet* 38:594–597
- Colbert TR, Kang MS, Myers O, Zuber MS (1987) General and specific combining ability estimates for pith cell death in stalk internodes of maize. *Field Crops Res* 17:155–162
- Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica* 142:169–196
- Daetwyler HD, Pong-Wong R, Villanueva B, Woolliams JA (2010) The impact of genetic architecture on genome-wide evaluation methods. *Genetics* 185:1021–1031

- Ding XH, Cao YL, Huang LL, Zhao J, Xu CG, Li XH, Wang SP (2008) Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independent basal immunity in rice. *Plant Cell* 20:228–240
- Doerge RW, Zeng ZB, Weir BS (1997) Statistical issues in the search for genes affecting quantitative traits in experimental populations. *Stat Sci* 12:195–219
- Dorn B, Forrer HR, Schürch S, Vogelgsang S (2009) *Fusarium* species complex on maize in Switzerland: occurrence, prevalence, impact and mycotoxins in commercial hybrids under natural infection. *Eur J Plant Pathol* 125:51–61
- Ducrocq S, Giauffret C, Madur D, Combes V, Dumas F, Jouanne S, Coubriche D, Jamin P, Moreau L, Charcosset A (2009) Fine-mapping and haplotype structure analysis of a major flowering time quantitative trait locus on maize chromosome 10. *Genetics* 183:1555–1563
- Flint J, Mackay TFC (2009) Genetic architecture of quantitative traits in flies, mice and humans. *Genome Res* 19:723–733
- Fu DL, Uauy C, Distelfeld A, Blechl A, Epstein L, Chen XM, Sela H, Fahima T, Dubcovsky J (2009) A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science* 323:1357–1360
- Holland JB (2007) Genetic architecture of complex traits in plants. *Curr Opin Plant Biol* 10:156–161
- Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugen* 12:172–175
- Kou YJ, Wang SP (2010) Broad-spectrum and durability: understanding of quantitative disease resistance. *Curr Opin Plant Biol* 13:1–5
- Krattinger SG, Lagudah ES, Spielmeier W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 323:1360–1362
- Lal S, Singh IS (1984) Breeding for resistance to downy mildews and stalk rots in maize. *Theor Appl Genet* 69:111–119
- Li Q, Wan JM (2005) SSRHunter: development of a local searching software for SSR sites. *Hereditas (Beijing)* 27:808–810
- Lincoln S, Daly M, Lander E (1992) Mapping genes controlling quantitative traits with MAPMAKER/QTL 1.1. Whitehead Institute Technical Report, 2nd edn
- Mackay TFC, Stone EA, Ayroles JF (2009) The genetics of quantitative traits: challenges and prospects. *Nat Rev Genet* 10:565–577
- Manulis K, Shafir H, Epstein E, Lichter A, Barash I (1994) Biosynthesis of indole-3-acetic acid via the indole-3-acetamide pathway in *Streptomyces* spp. *Microbiology* 140:1045–1050
- Maor R, Haskin S, Levi-Kedmi H, Sharon A (2004) In planta production of indole-3-acetic acid by *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. *Appl Environ Microbiol* 70:1852–1854
- Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res* 8:4321–4326
- Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science* 312:436–439
- Nobuta K, Okrent RA, Stoutemyer M, Rodibaugh N, Kempema L, Wildermuth MC, Innes RW (2007) The GH3 acyl adenylase family member PBS3 regulates salicylic acid-dependent defense responses in *Arabidopsis*. *Plant Physiol* 144:1144–1156
- Park JE, Park JY, Kim YS, Staswick PE, Jeon J, Yun J, Kim SY, Kim J, Lee YH, Park CM (2007) GH3-mediated auxin homeostasis links growth regulation with stress adaptation response in *Arabidopsis*. *J Biol Chem* 282:10036–10046
- Pè ME, Gianfranceschi L, Taramino G, Tarchini R, Angelini P, Dani M, Binelli G (1993) Mapping quantitative trait loci (QTLs) for resistance to *Gibberella zeae* infection in maize. *Mol Gen Genet* 241:11–16
- Russel WA (1961) A comparison of five types of testers in evaluating the relationship of stalk rot resistance in com inbred lines and stalk strength of the lines in hybrid combinations. *Crop Sci* 1:393–397
- Sax K (1923) The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* 8:522–560
- Saxena SC (1982) Epidemiology and control of *Erwinia* stalk rot of maize. PhD Thesis, GB Pant Univ Agric Techn, Pantnagar, India
- Sharma RC, Leon CD, Payak MM (1993) Diseases of maize in South and South-East Asia: problems and progress. *Crop Prot* 12:414–422
- Shim WB, Sagaram US, Choi YE, So J, Wilkinson HH, Lee YW (2006) *FSR1* is essential for virulence and female fertility in *Fusarium verticillioides* and *F. graminearum*. *MPMI* 19:725–733
- Song XJ, Huang W, Shi M, Zhu MZ, Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nat Genet* 39:623–630
- Szöke C, Árendás T, Rácz F, Pintér J, Nagy E, Marton CL (2007) Correlation between maize genotypes and the stalk rot caused by maize *Fusarium*. *Acta Agron Hung* 55:447–452
- Vandeputte O, Oden S, Mol A, Vereecke D, Goethals K, El Jaziri M, Prinsen E (2005) Biosynthesis of auxin by the gram-positive phytopathogen *Rhodococcus fascians* is controlled by compounds specific to infected plant tissues. *Appl Environ Microbiol* 71:1169–1177
- Wang H, Nussbaum-Wagler T, Li BL, Zhao Q, Vigouroux Y, Faller M, Bombliès K, Lukens L, Doebley JF (2005) The origin of the naked grains of maize. *Nature* 436:714–719
- Wang D, Pajeroska-Mukhtar K, Culler AH, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Curr Biol* 17:1784–1790
- White DG (1999) Compendium of corn diseases, 3rd edn. APS Press, Saint Paul, p 78
- Widakas W, Sandaland PC, Liu H (1980) Inheritance of resistance to *Diplodia zeae*, *Gibberella zeae* and *Fusarium moniliforme* stalk rot in corn. *Maize Genet Coop Newsl* 34:97–98
- Wu HY, Sun SR, Fan ZW, Liu CG, Yang TY, Zhu JH (2007) Research Condition and Prevention *Countermeasures* of Maize Stalk Rot. *J Maize Sci* 15:129–132
- Yang DE, Zhang CL, Zhang DS, Jin DM, Weng ML, Chen SJ, Nguyen H, Wang B (2004) Genetic analysis and molecular mapping of maize (*Zea mays* L.) stalk rot resistant gene *Rfg1*. *Theor Appl Genet* 108:706–711
- Yang Q, Yin GM, Guo YL, Zhang DF, Chen SJ, Xu ML (2010) A major QTL for resistance to *Gibberella* stalk rot in maize. *Theor Appl Genet* 121:673–687
- Younis SEA, Abo-El Dahab MK, Mallah GS (1969) Genetic studies of the resistance to *Fusarium* stalk rot in maize. *Indian J Genet* 29:418–425
- Zhang Z, Li Q, Li Z, Staswick PE, Wang M, Zhu Y, He Z (2007) Dual regulation role of GH3.5 in salicylic acid and auxin signaling during *Arabidopsis*–*Pseudomonas syringae* interaction. *Plant Physiol* 145:450–464