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Comparisons of quantitative trait locus mapping properties between two methods of recombinant inbred line development

Jixiang Wu[1,3], Johnie N. Jenkins[2], Jun Zhu[1], Jack C. McCarty Jr.[2] & Clarence E. Watson[3]
[1]*College of Agriculture & Biotechnology, Zhejiang University, Hangzhou, Zhejiang 310029;* [2]*Crop Science Research Laboratory, USDA-ARS, Mississippi State, MS 39762;* [3]*Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762*

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Summary

Theoretical comparisons for quantitative trait loci (QTL) mapping properties were conducted among simulated recombinant inbred (RI) populations developed by single-hill (SH), complete bulk, and single seed descent (SSD) procedures by Monte Carlo simulations based on various population sizes, heritabilities, and QTL effects. Our simulations included estimation of QTL effects, QTL positions, and statistical testing power in the RI populations by comparing the estimates with preset values. The simulation results showed that the single hill (SH) bulk and single seed descent RI populations were generally not significantly different with respect to quality of estimated QTL effects and positions. Furthermore, when each RI population had 150 lines, each could provide desirable properties for QTL mapping. The results implied that a SH RI population consisting of 75 or more F_2-derived families with two lines per family (corresponding population size of 150 or above) was appropriate for QTL mapping and was not significantly different than a SSD RI population of 150. Thus, the SH method could be used to develop large numbers of RI lines for achieving better results in QTL mapping. Simulations also showed that there was no significant difference between means using SH methods with 10 and 100 fruits per family. However, RI populations developed by the complete bulk method where F_2 identities are lost were not suitable for QTL mapping.

Introduction

Four types of experimental populations are commonly used for QTL mapping in crops: F_2 populations (Shappley et al., 1998; Lubberstedt et al., 1997), back cross (BC) populations (Park et al., 1999; Simko et al., 1999), doubled haploid (DH) populations (Quarrie et al., 1994; Yan et al., 1998), and recombinant inbred (RI) populations(Miklas et al., 2000; Messmer et al., 2000; Ittu et al., 2000). Among them, F_2 and BC populations are considered as temporary populations. To overcome the temporary nature of the F_2, progenies from F_2 populations were used in research by Shappley et al., 1998. The hidden assumption for direct uses of the progenies of F_2 or BC populations is that a quantitative trait is primarily controlled by QTL additive effects. If QTL dominance effects or epistatic effects exist, the progeny approach may not be appropriate since individuals in F_2 and BC populations are segregating. Unlike F_2 or BC populations, an RI line is produced by inbreeding the progeny of an F_2 derived from two well-established inbred lines (Burr et al., 1988). By the single seed descent (SSD) approach, almost all of the segregating loci come to homozygosity (Bailey, 1981; Burr et al., 1988; Burr & Burr, 1991). The primary advantage of RI populations is that they can be used indefinitely for mapping, hence, RI populations can be evaluated in many different environments, by different researchers, and at different times. Since a genotype in RI populations is represented by an inbred line, rather than by a heterozygous individual as in BC or F_2 populations, a

more precise assessment of QTL mapping properties can be achieved, especially for low heritability quantitative traits. Another property that distinguishes RI lines from F_2 or BC is that they undergo multiple rounds of meiosis before homozygosity is reached (Bailey, 1981; Burr et al., 1988). As a result, closely linked genes have a greater probability of recombination. This property was recognized by Haldane and Waddington (1931) in their study of inbreeding populations. In recent years, some researchers have used SSD methods for developing RI populations for QTL mapping (Liste and Dean, 1993; Burr et al., 1988; Li et al., 1995; Reiter et al., 1992; Haley et al., 1994; Schar et al., 1997). Many reports have used small RI populations developed by SSD and sometimes these may not adequately meet the requirement for effective QTL mapping (Miklas et al., 2000; Swarup et al., 1999; Yin et al., 1999; Burr et al., 1988). The problem of small RI population size can sometimes be overcome by combining data from several populations (Taylor et al., 1975; Burr et al., 1988), however, if the parents used in each RI population are different or if marker loci in the several populations have multiple alleles, this approach poses difficulties for linkage and QTL mapping. Using the modified SSD method in breeding programs can be traced back to the beginning of the 1970s (Empig & Fehr, 1971). Fehr (1987) also described the use of single-hill or the multiple-seed procedure for maintaining the founding population size. Macchiavelli & Beaver (2001), using Monte Carlo simulation reported that an increase in the number of seeds per pod from two to six increased the mean genetic similarity of plants represented in the F_6 generation. However, it still remains unknown if RI populations developed by modified SSD methods provide similar information and genetic variation for QTL mapping as the same sized SSD RI population.

The aim of this paper was to compare the QTL mapping properties between SH RI populations and the SSD RI population using Monte Carlo simulations. We also briefly examined a complete bulk method of developing RI lines. The emphasis was on comparing QTL mapping properties of the RI populations rather than statistical mapping methods or genetic models. The results of this study should extend our ideas on how to develop adequately sized RI populations for QTL mapping using methods other than SSD.

Methods

Procedures to develop different RI populations

Simulated SSD RI lines were created by the SSD method starting from F_2 individuals for seven generations (F_8). Simulated SH RI lines were developed as follows (Fehr, 1987),

Season 1: F_2 plants of a population are grown, and five fruits each with 20 seeds are self pollinated for each plant. The fruit are harvested and the 100 seeds for each plant are mixed.

Season 2: A 100 plant progeny row is grown for each family. Ten fruits each with twenty seeds are randomly self pollinated among the 100 plants in each family. These self pollinated fruits for each family are harvested and seeds are mixed. The 100 seeds are planted in a progeny row in the next season.

This procedure is repeated until the F_7. At the F_7, two individual plants in each progeny row are randomly selected and self pollinated seed are harvested. A variation of this method was simulated where 100 fruits in each progeny row were harvested and seed mixed and 100 plants were planted in the next generation and 100 fruits harvested. This continued until the F_7. In the F_7 two individual plants per progeny row were randomly selected and self pollinated seeds were harvested. To further simulate bulk based RI lines we simulated a completely bulked population starting in the F2 and bulking each generation until the F_7. The data are not shown for the complete bulk RI development; however results are discussed.

Presetting QTL effects and positions

For simplification, the genetic model used assumed that there were no dominance or additive additive (AA) epistatic QTL effects and no QTL environment interaction effects. In all simulations, four chromosomes and 64 unevenly distributed molecular markers (16 per chromosome with an average of 10 cM between adjacent markers), were employed. Four QTL each with different effects on the trait of interest were preset on four chromosomes. One QTL with 0.50 effect was set at position 14 cM, one with –1.34 effect was set at position 64 cM, one QTL with 1.27 effect was set at position 132 cM, and one QTL with –0.35 effect was set at position 106 cM on chromosomes 1, 2, 3, and 4 respectively.

Generating the data of markers and phenotypes

For simulation purposes, two levels of heritability (h^2) for the simulated quantitative trait were set as 0.20 and 0.50; and three population sizes were 100, 150, and 200. For the SSD RI lines these represented the final RI population sizes. For the SH RI populations the founding population sizes were 50, 75, and 100 $F_{2.3}$ populations with two RI lines chosen from each founding family in the F_7. Simulated data sets were generated for all combinations of above factors.

For generating each of the data sets, the theoretical genetic variance V_G was calculated first: $V_G = \sum_i \sum_j \rho_{ij} A_i A_j$, where $\rho_{ij} = 1 - 2r_{ij}$ under Haldane mapping function (Haldane, 1919), r_{ij} is the recombination fraction between QTLs i and j. With the V_G, the random error variance was then calculated as $V_e = \frac{1-h^2}{h^2} V_G$, where h^2 is the trait heritability magnitude. The genetic value G_k of individual k was obtained using additive model $G_k = \mu + \sum_f \alpha_{fk} A_f$, where μ is the mid-parent value, A_f is the additive effect of QTL_f (f = 1,2,3,4), coefficient α_{fk} is 1 for the QTL genotype $Q_f Q_f$ and –1 for $q_f q_f$ (Mather & Jinks, 1982). By generating a normal residual error $\varepsilon_k \sim N(0, V_e)$, the phenotypic value y_k of the kth RI line was calculated by the linear model $y_k = G_k + \varepsilon_k$. Mean simulated value from four replications for each line was used for QTL mapping.

Mapping methods

The composite interval mapping approach (CIM) (Zeng, 1994) was employed in all cases in this study. The stepwise linear regression procedure was used to select markers that showed significant contributions to the phenotypic variation (significant at 0.01). Then these selected markers were used as background genetic variation control when QTL mapping was conducted (Zeng, 1994; Wang, 1998; Wang et al., 1999). Simulations were repeated 200 times for each of the cases. Power is defined as number of significant simulations divided by the number of simulations. A significant test is defined as one in which the simulated QTL effect was significantly different from zero at probability level of 0.001.

Simulation results

In this study, both QTL effects and locations were detected at the peak points (p_i) which had likelihood ratio (LR) values [testing additive effects of A_i] significant at α = 0.001. The results are summarized in Tables 1, 2, and 3.

Generally, estimates of QTL effects and QTL positions were closer to the preset value for larger population size than for small population size and for traits with high heritability compared to low heritability. More precise results were obtained for large QTL effects than for small QTL effects. Similar results were also found for statistical testing power.

Comparisons of estimated QTL effects and positions between two RI populations

Results (Tables 1, 2, and 3) indicated that for a population size of 100 and heritability of 0.20, and small QTL effects of 0.35 and 0.50 all three simulations gave poor estimates of QTL effects, positions, and showed low power; however for QTL with higher effects of 1.27 and 1.34 all three populaitons gave similar but good estimates. For example the deviations of estimated locations from preset locations for QTL_1 and QTL_4 were 11.6 cM and 0.2 cM for ten-fruit SH RI populations; 5.2 cM and 13.5 cM for the 100-fruit SH RI populations (Tables 2 and 3); the deviations for the same two QTLs were 2.2 cM and 7.4 cM for SSD RI populations, respectively (Table 1).

By increasing founding family size (increasing population size, correspondingly), generally, one will obtain a more unbiased estimate of QTL locations for traits with low heritability. When heritability of 0.20, founding family size was 75 with two lines per family, the deviations for QTL_1 was 0.9 cM and 2.7 cM for ten-fruit and 100-fruit SH RI populations at heritability of 0.20 (Tables 2 and 3). Very negligible biases for estimates of QTL locations were obtained for QTLs with large effects (1.27 and 1.34) using the various population sizes and the two different heritabilities (Tables 1, 2, and 3). Results also indicated that no significant differences for estimated QTL effects, positions, and statistical powers were detected (Tables 1, 2, and 3) for QTL with large effects. When founding population size increased to 75 and above, the QTL positions were closely estimated (most deviations were less than 5 cM) for various magnitudes of QTL effects and heritabilities. Therefore, these results suggest that SH RI populations containing 75 founding families with two lines per family are appropriate for mapping QTL positions for various situations.

Generally, estimated QTL positions and QTL effects in SH RI populations with larger population sizes

Table 1. Detection of QTL effects and positions for SSD RI populations

Population size	h^2	QTL	QTL effects		QTL positions (cM)		
			Preset value	Estimated value ± SD	Preset value	Estimated Value ± SD	Power ± SD
100	0.2	1	0.50	0.81±0.34	14.0	16.2±11.0	0.18±0.03
		2	–1.34	–1.28±0.22	64.0	63.8±2.7	0.98±0.01
		3	1.27	1.26±0.21	132.0	133.0±3.7	0.95±0.02
		4	–0.35	–0.67±0.53	106.0	98.6±23.9	0.12±0.02
	0.5	1	0.50	0.54±0.10	14.0	13.7±8.1	0.74±0.03
		2	–1.34	–1.23±0.17	64.0	64.3±2.6	0.98±0.01
		3	1.27	1.19±0.17	132.0	132.5±2.3	0.98±0.01
		4	–0.35	–0.50±0.09	106.0	104.6±13.9	0.38±0.03
150	0.2	1	0.50	0.66±0.27	14.0	19.8±21.9	0.42±0.03
		2	–1.34	–1.25±0.19	64.0	63.8±2.4	0.98±0.01
		3	1.27	1.21±0.25	132.0	132.3±3.5	0.97±0.01
		4	–0.35	–0.61±0.26	106.0	105.4±18.6	0.14±0.02
	0.5	1	0.50	0.51±0.11	14.0	14.7±7.4	0.93±0.02
		2	–1.34	–1.24±0.16	64.0	64.7±1.8	1.00±0.00
		3	1.27	1.17±0.15	132.0	132.1±1.9	1.00±0.00
		4	–0.35	–0.42±0.08	106.0	107.1±6.3	0.57±0.04
200	0.2	1	0.50	0.61±0.10	14.0	16.6±14.4	0.52±0.04
		2	–1.34	–1.25±0.17	64.0	64.1±2.4	0.99±0.01
		3	1.27	1.24±0.17	132.0	132.5±2.0	1.00±0.00
		4	–0.35	–0.56±0.29	106.0	101±18.3	0.21±0.03
	0.5	1	0.50	0.49±0.09	14.0	13.8±3.4	0.99±0.01
		2	–1.34	–1.24±0.14	64.0	64.8±1.6	1.00±0.00
		3	1.27	1.16±0.14	132.0	132.4±1.7	1.00±0.00
		4	–0.35	–0.38±0.07	106.0	105.2±6.9	0.75±0.03

$h^2 = V_G/(V_G + V_e)$ = heritability; SD = standard deviation. Estimate was the average of the estimates at the peak points (p_i) with LR values (testing QTL additive effect) significant at 0.001, in the intervals each having at least one marker flanking a QTL. SD was obtained from these estimates. Power = No. of significant tests /No. of simulations.

were closer to the preset parameter values than in SSD RI populations with small population sizes for the same low heritability and small effects (Tables 1, 2 and 3). For example, the deviation for position of QTL_1 in SSD RI population with 100 lines was 2.2 cM (Table 1); the deviations for estimates of the same QTL in 10 fruit SH RI population with 150 lines and 200 lines were 0.9 cM and 0.5 cM, respectively (Tables 2 and 3).

Additionally, the statistical powers in SH RI populations with larger population sizes were higher than those in SSD RI populations developed from the same number of founding lines for small QTL effects (Tables 1, 2, and 3). For example, the statistical powers for QTL_1 and QTL_4 in ten-fruit SH RI populations consisting of 150 lines were 0.38 and 0.16, respectively; while the statistical powers for QTL_1 and QTL_4 in SSD RI populations consisting of 100 lines were 0.18 and 0.12, respectively (Tables 1and 2). Similar trends could also be found for estimates of QTL effects.

Comparisons of standard deviations of two RI Populations

Standard deviations (SD) for QTL positions were generally larger for small SH RI populations (100) than for the same sized SSD RI populations at low heritability of 0.20 (Tables 1, 2 and 3). For example, the SD's for QTL_1, QTL_2, and QTL_3 in ten-fruit SH RI populations were 30.3 cM, 3.4 cM, and 3.8 cM, respectively (Table 2), 20.5 cM, 7.3 cM, and 3.6 cM for 100-fruit SH RI populations (Table 3); while in SSD RI populations at the same conditions were 11.0 cM, 2.7 cM, and 3.7 cM, respectively (Table 1). The results implied that small SH RI populations might provide

Table 2. Detection of QTL effects and positions for SH RI populations developed by ten fruits per family and two RI lines per founding family

Population size	h^2	QTL	QTL effects		QTL positions (cM)		
			Preset value	Estimated value ± SD	Preset value	Estimated Value ± SD	Power ± SD
100	0.2	1	0.50	0.83±0.31	14.0	25.6±30.3	0.18±0.03
		2	–1.34	–1.29±0.22	64.0	64.1±3.4	0.97±0.01
		3	1.27	1.25±0.23	132.0	132.4±3.8	0.94±0.02
		4	–0.35	–0.71±0.39	106.0	105.8±24.0	0.10±0.02
	0.5	1	0.50	0.57±0.10	14.0	13.8±5.0	0.75±0.03
		2	–1.34	–1.26±0.16	64.0	63.9±2.7	0.99±0.01
		3	1.27	1.18±0.17	132.0	132.4±2.7	0.99±0.01
		4	–0.35	–0.49±0.14	106.0	105.7±16.8	0.39±0.03
150	0.2	1	0.50	0.70±0.21	14.0	14.9±16.6	0.38±0.03
		2	–1.34	–1.30±0.19	64.0	63.8±2.5	0.99±0.01
		3	1.27	1.22±0.19	132.0	132.2±2.9	0.98±0.01
		4	–0.35	–0.58±0.35	106.0	91.1±30.7	0.16±0.03
	0.5	1	0.50	0.51±0.12	14.0	15.0±11.6	0.89±0.02
		2	–1.34	–1.24±0.18	64.0	64.4±2.2	0.99±0.01
		3	1.27	1.15±0.17	132.0	132.3±2.2	1.00±0.00
		4	–0.35	–0.42±0.12	106.0	103.9±15.2	0.68±0.03
200	0.2	1	0.50	0.64±0.11	14.0	14.5±9.2	0.51±0.04
		2	–1.34	–1.25±0.17	64.0	64.2±2.8	0.97±0.01
		3	1.27	1.18±0.18	132.0	132.4±2.9	0.99±0.01
		4	–0.35	–0.62±0.11	106.0	105.0±18.4	0.23±0.03
	0.5	1	0.50	0.49±0.09	14.0	13.9±3.5	0.99±0.01
		2	–1.34	–1.21±0.18	64.0	64.5±2.5	1.00±0.00
		3	1.27	1.14±0.18	132.0	132.1±2.4	1.00±0.00
		4	–0.35	–0.39±0.07	106.0	104.4±11.5	0.78±0.03

$h^2 = V_G/(V_G + V_e)$ = heritability; SD = standard deviation. Estimate was the average of the estimates at the peak points (p_i) with LR values (testing QTL additive effect) significant at 0.001, in the intervals each having at least one marker flanking a QTL. SD was obtained from these estimates. Power = No. of significant tests /No. of simulations.

less precision for mapping QTL positions than the SSD RI populations with same number of lines. The possible reason is the genetic similarity in the two SH RI populations within each founding family result in less precision in estimation. Increasing the population sizes would increase the precision and decrease the differences between the two types of RI populations (Tables 1, 2, and 3).

In summary, generally the SH RI populations could provide similar mean estimates for QTL effects, QTL positions and statistical powers as the same size SSD RI populations, but SH RI populations would have less precise estimates. On the other hand, large RI populations gave better estimation for QTL effects and QTL positions than small SSD RI populations at same founding population size.

Discussion

RI lines have been recognized as a useful permanent mapping population for several reasons. However, with the difficulties involved in developing adequate SSD populations, small RI populations were used in some reported experiments (Miklas et al., 2000; Swarup et al., 1999; Burr et al., 1988). These populations may not have been appropriate for obtaining good mapping quality, especially for small QTL effects with low heritability. So it is important and necessary to consider using alternative methods to develop adequately sized RI population, while maintaining similar mapping properties as an adequately sized SSD RI population.

There are several other common methods such as multiple seed SSD and complete bulk for RI line de-

Table 3. Detection of QTL effects and positions for SH RI populations developed by 100 fruits per family and two RI lines per founding family

Population size	h^2	QTL	QTL effects		QTL positions (cM)		
			Preset value	Estimated value ± SD	Preset value	Estimated Value ± SD	Power ± SD
100	0.2	1	0.50	0.81±0.10	14.0	19.2±20.5	0.20±0.03
		2	–1.34	–1.30±0.23	64.0	64.1±7.3	0.99±0.01
		3	1.27	1.20±0.22	132.0	132.0±3.6	0.96±0.01
		4	–0.35	–0.58±0.62	106.0	92.5±33.9	0.10±0.02
	0.5	1	0.50	0.56±0.14	14.0	15.6±16.1	0.70±0.03
		2	–1.34	–1.25±0.20	64.0	64.9±4.7	0.99±0.01
		3	1.27	1.20±0.16	132.0	132.2±2.8	0.98±0.01
		4	–0.35	–0.50±0.09	106.0	103.0±13.1	0.31±0.03
150	0.2	1	0.50	0.69±0.22	14.0	16.7±10.6	0.33±0.03
		2	–1.34	–1.28±0.25	64.0	64.3±3.5	0.96±0.01
		3	1.27	1.20±0.18	132.0	132.2±3.3	0.99±0.01
		4	–0.35	–0.68±0.09	106.0	103.1±22.3	0.17±0.03
	0.5	1	0.50	0.50±0.09	14.0	14.4±9.4	0.90±0.02
		2	–1.34	–1.23±0.17	64.0	64.7±2.0	1.00±0.00
		3	1.27	1.16±0.17	132.0	132.3±2.0	0.99±0.01
		4	–0.35	–0.42±0.11	106.0	107.0±9.4	0.58±0.03
200	0.2	1	0.50	0.63±0.10	14.0	15.1±12.3	0.61±0.03
		2	–1.34	–1.27±0.19	64.0	64.1±2.1	0.99±0.01
		3	1.27	1.22±0.18	132.0	132.4±2.3	0.99±0.01
		4	–0.35	–0.59±0.21	106.0	104.9±18.7	0.21±0.03
	0.5	1	0.50	0.48±0.09	14.0	14.3±3.4	0.97±0.01
		2	–1.34	–1.22±0.17	64.0	64.6±2.0	1.00±0.00
		3	1.27	1.15±0.17	132.0	132.2±2.0	1.00±0.00
		4	–0.35	–0.39±0.07	106.0	105.7±8.3	0.81±0.03

$h^2 = V_G/(V_G + V_e)$ = heritability; SD = standard deviation. Estimate was the average of the estimates at the peak points (p_i) with LR values (testing QTL additive effect) significant at 0.001, in the intervals each having at least one marker flanking a QTL. SD was obtained from these estimates. Power = No. of significant tests /No. of simulations.

velopment (Fehr, 1987). Our simulations showed that RI population developed from complete bulk-based procedure resulted in very poor QTL mapping quality (results not provided) for various situations. It is probably that genetic information from the bulk population will be lost during the random selection. Therefore, complete bulk-based RI populations are not recommended for mapping QTLs. Multiple seed SSD procedure is typically similar to SH method. Initially, the single-hill procedure was used to maintain the founding population size in SSD RI line development (Fehr, 1987). In this research, we extended this idea for enlarging the RI population size through choosing two or more lines within each founding progeny. Simulations show that genetic variation appears to be more important than genetic similarity in SH bulk and SSD. If less than 100 lines are used a poor estimate is sometimes obtained for both methods, however, if more than 150 lines are used then both methods gave similar results. Generally, the following results can be expected through comparing simulated SH and SSD RI populations: (1) For the same RI population sizes, a larger founding size is better than a smaller founding size; (2) For the same size founding population, a larger RI population is better than a smaller RI population; (3) If RI population is small (100 or less) both methods gave poor mapping results; and (4) For a large number of RI lines (150 or more) both SH and SSD RI populations have similar mapping properties.

The number of bulk seeds harvested within each founding progeny in each season may influence the QTL mapping properties. Our simulations (Tables 2 and 3) showed that when 10 fruit and 100 fruit each with 20 seeds were randomly selected from 100 plants

within each family, QTL mapping properties for both SH RI populations were very similar with respect to the mean estimates but standard deviations tended to be smaller with 100 fruits per generation. This suggested that increasing number of fruits within each family should be appropriate during SH RI population development for obtaining better mapping quality. SH RI populations developed from smaller founding size with a larger number lines per family (i.e. four lines or eight lines per family) resulted in similar mean estimates but with less precision (larger SD, simulation results not shown). Other factors such as number of seeds per fruit, variation in seed number, and harvest time may also slightly affect the QTL mapping properties for single-hill RI populations. These issues can be examined in another study.

We have used the SH bulk method employed in this study to develop a RI population of upland cotton (*Gossypium hirsutum* L.). The SH bulk methods for developing RI lines can vary based on properties of different species. For example, for self-pollinated crops like rice and soybean, several plants can be randomly selected within each F_2-derived family at each generation at harvest season, then, a number of seeds are randomly selected from the mixed seeds within each family for the next generation. This procedure is repeated until homogeneity of each individual is reached. At this time, several lines can be randomly selected from each family to composite an RI population. For cross-pollinated crops, enforced self pollination would be needed; however the other procedures remain the same.

In this study, we only addressed the comparison of the QTL mapping properties between two RI populations rather than comparisons of mapping methods or different genetic models. The genetic effects of some QTLs can be more complicated than we assumed in this study. The additive QTL model in this study is extendable to more complicated genetic models such as additive additive epistatic effect models and QTL $\times$ environment interaction effect models. Since the 1980s, many mapping methods have been proposed (Weller, 1986; Lander & Botstein, 1989; Jansen, 1993; Zeng, 1994; Zhu, 1998; Zhu & Weir, 1998; Wang, 1998; Wang et al., 1999). Obviously, these statistical methods will have different testing powers for different genetic models.

Compared to the SSD method, SH technique could provide several advantages during the process of developing RI lines: (1) Large RI populations can be developed fairly easily through SH procedure which are suitable for QTL mapping even if less than 100 F_2-derived families are available; (2) This method may reduce the influences of factors that would result in reducing the family size using the SSD method; (3) The bulk based method also allows for developing several RI populations simultaneously from one F_2 population. This study provides important theoretical support for future studies on QTL mapping based on RI lines developed by SH method.

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