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# Analyzing quantitative trait loci for yield using a vegetatively replicated $F_2$ population from a cross between the parents of an elite rice hybrid

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**Abstract** Although $F_2$s are the most informative populations for genetic analysis, it has been difficult to use $F_2$ populations directly for QTL analysis because it is usually difficult to assess the reliability of the data, due to an inability to estimate the experimental errors. In this study, we performed a QTL analysis for yield and yield-component traits of an $F_2$ population based on data from replicated field trials over 2 years using vegetative shoots of ratooned plants, making use of the ratooning habit of rice. The objective of this study was to explore the possibility of conducting QTL analyses directly based on an $F_2$ population by means of ratooning plants. The experimental population was from a cross between 'Zhenshan 97' and 'Minghui 63', the parents of 'Shanyou 63', an elite rice hybrid widely grown in China. A genetic linkage map containing 151 molecular markers was constructed for QTL mapping. A total of 20 distinct QTLs were detected; eight of these were detected in both years and remaining 12 in only 1 year. Compared with the results of our previous analysis of the $F_{2:3}$ families from the same cross, it was shown that most of the QTLs detected in the ratooned $F_2$ population were also detected in the $F_{2:3}$ population. However, the estimates of both additive and dominant types of genetic effects for many of the QTLs based on $F_2$ ratoons were substantially larger than those based on $F_{2:3}$ families. The results indicate that vegetatively ratooned $F_2$ populations may have considerable utility in the mapping of QTLs, especially if dominant types of gene actions are of concern, although there were certain technical limitations in making use of such populations in the experiments.

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## Introduction

The development of high-density molecular-marker linkage maps in a number of crop species have generated considerable interest in mapping and analyzing genes controlling quantitative traits, commonly referred to as quantitative trait loci or QTLs. Several analytical methods, along with the availability of computer software (e.g. Lander and Botstein 1989; Zeng 1994); have made it possible to resolve the genetic basis of quantitative traits into individual Mendelian units, based on single-locus models.

Parallel to the analytical methods, there have also been rapid advances in population developments. Theoretically, $F_2$ is the most informative type of population for gene mapping and genetic analysi s, and there have been attempts to use $F_2$ populations directly for QTL analysis. However, it is usually difficult to assess the reliability of the data due to the inability to carry out replicated field tests with $F_2$ populations for data collection. To partly resolve this problem, several studies used $F_3$ families in place of the $F_2$ individuals frequently referred to as $F_{2:3}$ populations (Edwards et al. 1987; Yu et al. 1997) in making field measurements of the quantitative traits. However, the $F_3$ families are genetically heterogeneous; thus, it is impossible to have exact replications in field trials. Additionally, one generation of selfing theoretically reduces the level of heterozygosity by a half, and an additional cycle of meiosis would result in gene combinations that are different from those in the $F_2$ generation. Thus, the genotypes of $F_{2:3}$ families do not correspond exactly with those of the $F_2$ individuals, which may affect the estimation of genetic effects in QTL analysis.

Emphasis has also been given to constructing permanent populations, such as recombinant inbred (RI) lines (Burr et al. 1988) and doubled-haploid (DH) lines (Zhu et al. 1994), especially in self-fertilizing species. Plants

in such populations are homozygous at all loci throughout the genome, and individuals within lines are highly homogeneous and thus can be used for repeated trials both over times and locations. QTL studies using RI and DH populations have been reported in a number of cases, especially in rice (Wang et al. 1994; Lu et al. 1996; Yadav et al. 1997). However, the weakness associated with both DH and RI populations is that data from such populations do not provide any estimates regarding dominant types of genetic effects.

In the study reported in this paper, we performed a QTL analysis of an $F_2$ population of rice for yield and yield-component traits, based on field data from replicated trials over 2 years using vegetative shoots of ratooned plants, making use of the ratooning habit of rice. The objective of this study was to explore the possibility of conducting QTL analyses directly based on $F_2$ populations by means of ratooning plants.

## Materials and methods

### Experimental materials and field data collection

The experimental population of 250 plants was maintained and propagated by ratooning 250 $F_2$ individuals derived from a cross between 'Zhenshan 97' and 'Minghui 63', the parents of 'Shanyou 63', an elite rice hybrid most widely grown in China.

The 250 $F_2$ individuals along with the parents and the $F_1$ were planted in Hainan Island (South China Sea) in the winter of 1993 and each of the individuals was separated into four plantlets by peeling off the tillers during the early tillering stage. At the time of harvest (spring of 1994), the stubs of all four vegetative clones of each plant were brought to Wuhan, and transplanted to the field (early May). One of the ratoons of each plant was grown for harvesting tissues for DNA extraction and the other three ratoons were for generating shoots to be used for testing agronomic traits. At approximately 30 days after transplanting, all vegetative shoots from the latter three ratoons of each plant were peeled off and collected as the seedlings for the field experiment. At the end of the season, the stub for one of the plants was saved and brought to Hainan Island. And essentially the same cycle of ratooning and propagation was repeated to provide the shoots for the field experiment of 1995.

In conducting the field experiment each year, the vegetative shoots were transplanted to a bird-net-equipped field in the experimental farm of Huazhong Agricultural University. The field planting followed a randomized complete block design with three replications. Seven shoots from each $F_2$ ratoon were laid out in a single-row plot with a distance of 26.5 cm between rows and 16.5 cm between plants within a row. The field management was similar to that under normal rice production conditions. Each plant was harvested individually at its maturity to prevent loss from over-ripening. Only five plants in the middle of each row were used for scoring the traits including: grain yield per plant measured as the weight of all filled grains of the plant, tillers per plant measured as the number of reproductive tillers of the plant, grain weight as the weight (g) of 1000 grains, grains per panicle scored as the number of filled grains per plant divided by the number of reproductive tillers.

The field experiment also included seed-born seedlings and ratoons of the parents and $F_1$ hybrids in each block for comparison, with the same lay-out as the ratooned plants.

### Molecular marker assay

Leaf tissues of the plants were harvested and ground to fine powder under liquid nitrogen. DNA extraction followed the procedure described by Murray and Thompson (1981).

Two classes of markers, restriction fragment length polymorphisms (RFLPs) and simple sequence repeats (SSRs), were used to survey DNA ploymorphisms in this population. RFLP analyses, including restriction digestion, Southern blotting and hybridization, were essentially as described by Liu et al. (1997). RFLP probes used to survey parental polymorphisms were selected at regular intervals from the high-density maps of Causse et al. (1994) and Kurata et al. (1994) in combination with six restriction enzymes. In case the selected markers for a genomic region failed to detect polymorphism between the parents, additional probes from the same region along with up to 14 restriction enzymes were added to the survey. In this way, 537 RFLP probes were used in screening parental polymorphisms. In addition, 54 primer pairs from published data (Wu and Tanksley 1993; Panaud et al. 1996; Xiong et al. 1998) were used to survey simple sequence repeat polymorphisms between the parents. The analysis, including PCR reactions and detection, followed essentially the methods of Wu and Tanksley (1993). The DNA markers that detected polymorphisms between the parents were used to assay the entire population of 250 $F_2$ individuals.

### Data analysis

A molecular linkage map was constructed using Mapmarker 3.0 (Lincoln et al.1992a) with LOD threshold 3.0. The entire genome was searched for QTLs for each trait using Mapmaker/QTL 1.1 (Lincoln et al. 1992b) with a LOD threshold of 2.4.

## Results

### Measurements of the yield traits

The measurements of yield and yield-component traits for the parents and the $F_1$ hybrid based on seed-born

**Table 1** Comparison of means of yield and yield-component traits based on the seed-born seedlings and ratoons planted in 1994 and 1995

| Trait | | 1994 | | | 1995 | | | LSD |
|---|---|---|---|---|---|---|---|---|
| | | Minghui 63 | Zhenshan 97 | $F_1$ | Minghui 63 | Zhenshan 97 | $F_1$ | |
| Yield (t/ha) | Seed-born | 8.0 | 6.1 | 11.2 | 8.1 | 6.3 | 10.9 | 1.36[a] |
| | ratooning | 7.3 | 6.0 | 10.7 | 4.7 | 3.4 | 8.6 | 1.85[b] |
| Tillers/plant | Seed-born | 14.7 | 14.0 | 17.7 | 14.2 | 15.3 | 16.7 | 1.92[a] |
| | ratooning | 18.0 | 17.0 | 19.7 | 10.3 | 6.3 | 16.6 | 2.62[b] |
| Grains/panicle | Seed-born | 95.2 | 86.1 | 104.0 | 102.2 | 78.5 | 107.0 | 8.44[a] |
| | ratooning | 74.8 | 72.1 | 97.9 | 77.3 | 66.7 | 93.4 | 11.47[b] |
| 1000-grain weight (g) | Seed-born | 25.6 | 22.4 | 27.2 | 27.7 | 22.0 | 27.3 | 0.83[a] |
| | ratooning | 24.2 | 23.1 | 25.2 | 24.5 | 21.7 | 24.7 | 1.12[b] |

[a] $LSD_{0.05}$, [b] $LSD_{0.01}$ for the trait

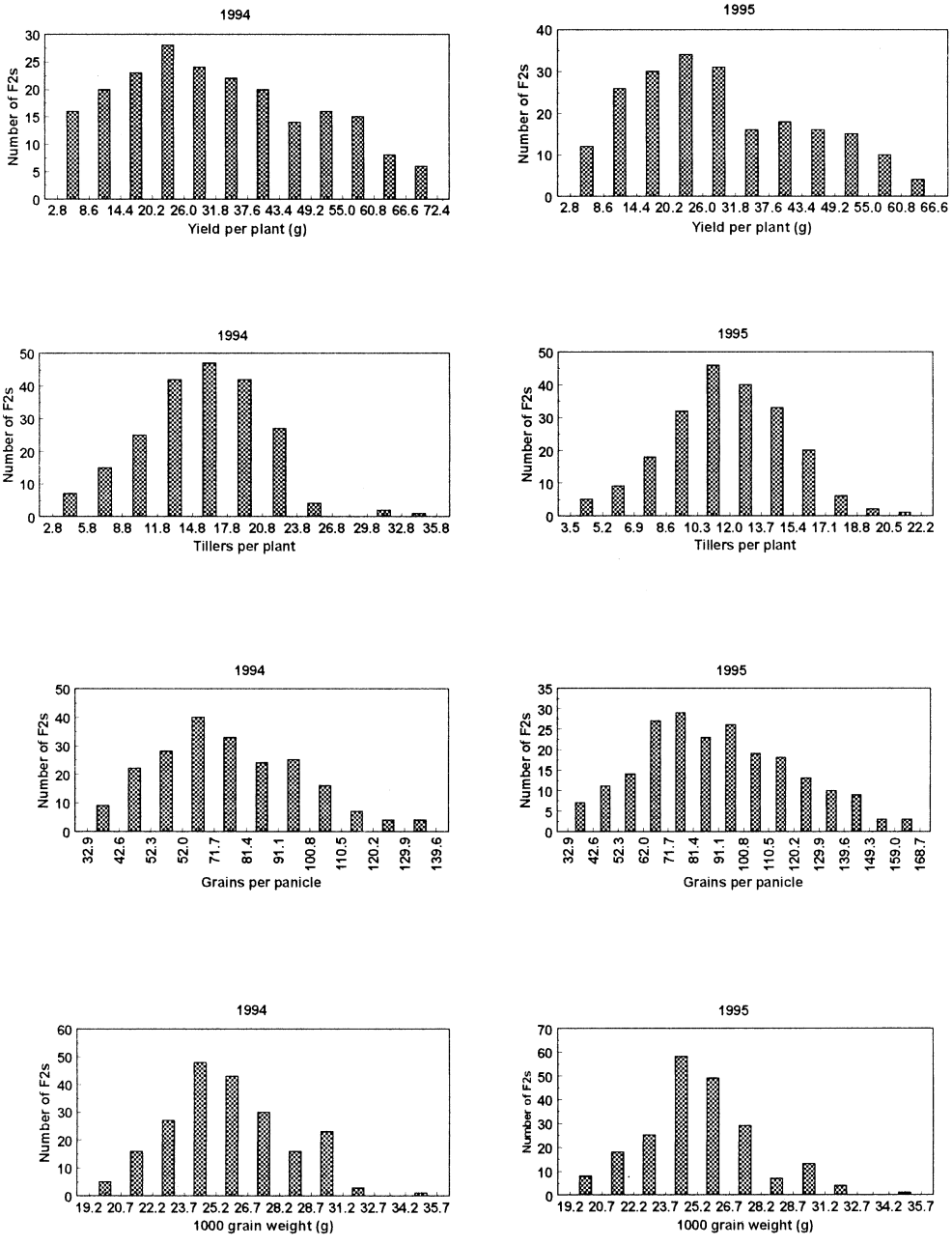


**Fig. 1** Distribution of measurements of yield and yield-component traits in the ratooned $F_2$ population grown in 1994 and 1995

seedlings and ratooned shoots, respectively, are given in Table 1. The measurements based on seed-born seedlings were very similar in the 2 years. In contrast, there were large differences between the measurements of the 2 years based on the ratooned-shoots in yield and its three component traits. The trait measurements of the parents and the hybrid of the ratooned plants grown in 1995 were consistently lower than those planted in 1994. It is also clear from Table 1 that the trait measurements of plants from ratooned shoots were generally lower than seed-born seedlings. This clearly indicated a reduction in productivity as a result of ratooning, although the extent of reduction differed from one genotype to another.

## Distribution of the trait measurements in the ratooned $F_2$ population

The distributions of the measurements of the four traits in the $F_2$ population from the ratooned plants were very similar in the 2 years (Fig. 1); the overall performance of the population was slightly lower in 1995 than in 1994. The distributions of the trait measurements were similar in the 2 years. The correlation coefficients between the measurements of the same traits in the 2 years were high in general, although they varied from 0.53 for tillers per plant, to 0.63 for yield per plant, to 0.68 for grains per panicle, and to 0.85 for grain weight.

**Table 2** Putative QTLs for yield and yield-component traits detected in 1994 and 1995

| Trait | QTL | Flanking markers | LOD | Var %[a] | Additive effect[b] | Dominance effect[c] |
|---|---|---|---|---|---|---|
| 1994 | | | | | | |
| Yield | yd7a | C1023–RG128 | 7.5 | 15.1 | – 9.1 | 6.2 |
| | yd8 | C483–C347 | 2.4 | 5.2 | 3.2 | 6.2 |
| Tillers/plant | tp1 | G359–RG532 | 3.3 | 7.4 | 1.9 | 1.1 |
| | tp7a | R1789–RM18 | 2.9 | 7.0 | – 1.7 | 1.2 |
| | tp7b | C1023–RG128 | 3.0 | 6.3 | – 1.7 | 1.1 |
| Grains/panicle | gp3 | C269–C1087 | 3.2 | 6.8 | 7.7 | 6.1 |
| | gp5a | RG360–C734 | 2.4 | 15.2 | – 2.5 | 17.3 |
| | gp7a | C1023–RG128 | 7.2 | 14.6 | –11.1 | 8.5 |
| Grain weight | gw3 | C1087–R1966x | 10.2 | 21.4 | – 1.9 | – 0.7 |
| | gw5a | RG360–R1674 | 9.4 | 20.4 | 1.7 | – 0.3 |
| | gw5b | C246a–RG528 | 2.7 | 7.1 | – 1.3 | 0.5 |
| | gw7 | C1023–RG128 | 5.4 | 16.4 | – 1.6 | 0.3 |
| | gw11 | RM4–RG98 | 2.9 | 8.3 | – 1.0 | 0.5 |
| 1995 | | | | | | |
| Yield | yd6 | R2147–RG424 | 2.5 | 18.5 | 7.3 | – 9.1 |
| | yd7a | R1440–C1023 | 9.3 | 19.8 | – 9.5 | 1.9 |
| | yd7b | R1789–RM18 | 4.4 | 9.2 | – 4.6 | 6.0 |
| Tillers/plant | tp7a | R1789–RM18 | 2.9 | 6.2 | – 0.9 | 0.9 |
| | tp7b | C1023–RG128 | 4.5 | 9.3 | – 1.4 | 0.0 |
| Grains/panicle | gp1 | RG532–RG173 | 2.7 | 6.8 | –10.8 | – 2.0 |
| | gp5b[d] | C624–C246a | 2.4 | 31.7 | – 3.9 | 33.0 |
| | gp6 | G200–R1014 | 3.3 | 7.8 | 11.6 | – 1.8 |
| | gp7a | R1440–C1023 | 9.1 | 20.4 | –18.6 | 6.7 |
| | gp7b | R1789–RM18 | 4.0 | 8.3 | – 8.8 | 10.3 |
| Grain weight | gw1 | R753–RM1 | 2.8 | 6.9 | 0.8 | 1.0 |
| | gw3 | C1087–R1966 | 9.0 | 19.3 | – 1.7 | – 0.9 |
| | gw5a | RG360–R1674 | 8.9 | 19.1 | 1.6 | – 0.1 |
| | gw7 | C1023–RG128 | 6.8 | 21.8 | – 1.8 | – 0.0 |
| | gw11 | RM4–RG98 | 2.3 | 6.1 | – 0.8 | 0.4 |

[a] Variation explained by each QTL
[b] Positive values indicate that alleles from Zhenshan 97 are in the direction of increasing the trait score, and negative values indicate that alleles from Minghui 63 are in the direction of increasing the score
[c] Positive values indicate that heterozygotes have higher phenotypic values than the respective means of two homozygotes, and negative values indicate that heterozygotes have lower values than the means of the two homozygotes
[d] The large value s of the dominance effect and LOD may be partly due to the large gap in this region of the molecular linkage map

## Linkage map

The survey of the 591 molecular markers (537 RFLPs and 54 SSRs) identified a total of 151 markers that were polymorphic between the parents. These were essentially the same set of markers as that used by Yu et al. (1997) for analyzing the data of the $F_{2:3}$ population of the same cross. Mapmaker analysis at LOD 3.0 resolved the markers into 14 linkage groups, with chromosomes 1 and 9 each separated into two linkage groups (data not shown). The linkage map spanned 1841.9 cM in length with an average interval of 12.1 cM between adjacent markers. This map well-integrated the markers from the two high-density RFLP linkage maps of rice, with the exception that eight of the markers were placed in chromosomes different from where they appeared in the two original maps.

## QTLs detected in the ratooned $F_2$ population

QTLs detected using Mapmaker/QTL at LOD threshold 2.4 for yield and yield-component traits are listed in Table 2 and illustrated in Fig. 2. QTLs that were detected with LOD values ≥2.4 in 1 year and with LOD values of 2.0–2.4 are also listed in Table 2.

For yield, two and three QTLs were detected in 1994 and 1995, respectively. It is highly likely that the QTL located in the interval C1023-RG128 on chromosome 7 detected in 1994 is the same as the one located in the interval R1440–C1023 detected in 1995, since their LOD peaks lay very close to each other and the 1-LOD supporting intervals were completely overlapping. For two of the QTLs (yd7a and yd7b), alleles from Minghui 63 contributed to an increase of the trait values. For the other two QTL s (yd6 and yd8), allele s from Zhenshan 97 were in the direction of increasing the trait value.

Three and two QTLs were resolved for tillers per plant in 1994 and 1995, respectively. Two QTLs, both located on chromosome 7, were in common between the 2 years. In both cases alleles from Minghui 63 were in the direction of increasing the trait values. While at the third QTL on chromosome 1, detected only in 1994, the allele from Zhenshan 97 contributed to the increase of the trait score.

Three and five QTLs were detected for grains per panicle in 1994 and 1995. Again, the QTL located in the interval C1023–RG128 on chromosome 7 detected in 1994 is likely to be the same as the one located in the interval R1440–C1023 detected in 1995. The QTL, gp5b, detected in 1995 with a marginal LOD value (2.4) but showing a very large dominance effect and explaining a very large proportion of variance (31.7%) may partly be the result of a large gap (38.5 cM) in this region of the linkage map (Fig. 2). At the majority of the QTLs, alleles from Minghui 63 were in the direction of increasing grain numbers.

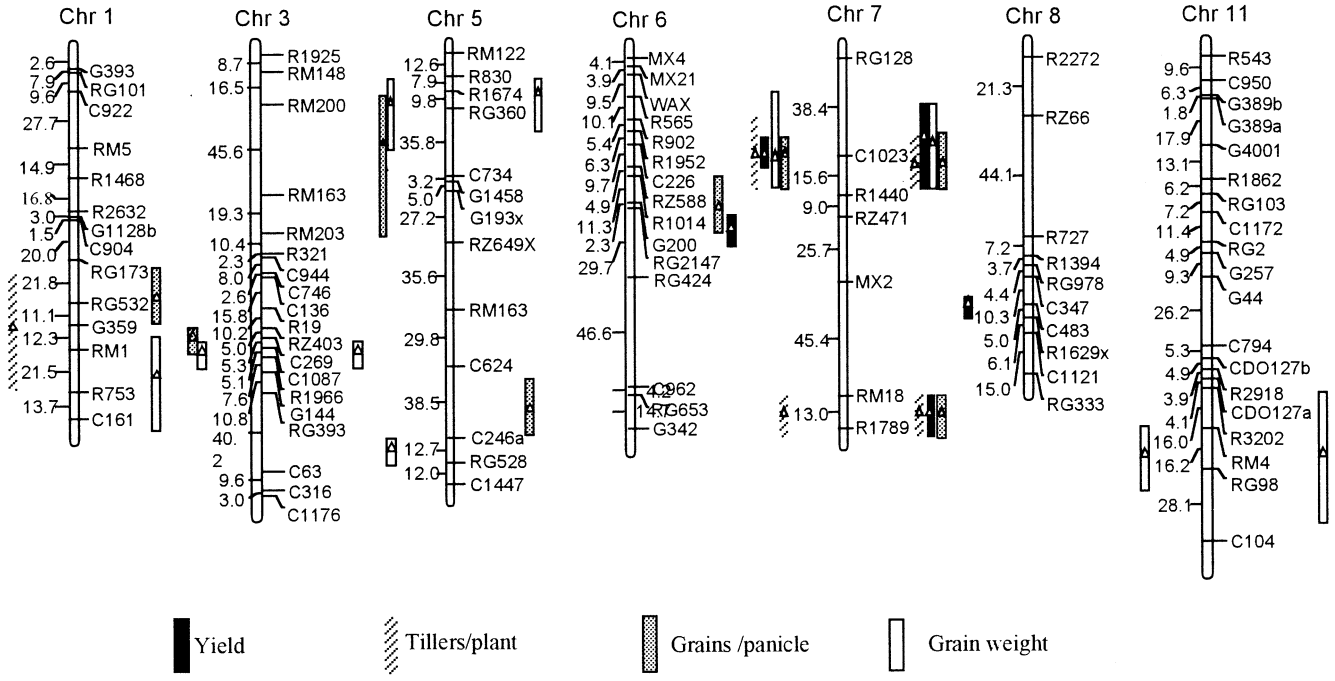


**Fig. 2** Distribution of QTLs in the molecular-marker linkage map based on the $F_2$ population from a cross between Zhenshan 97 and Minghui 63. *Numbers* on the left of each chromosome are map distances (cM) between adjacent markers. The *vertical bars* indicate the 1-LOD support intervals of the QTLs. *Bars* placed on the left of the chromosomes represen t QTLs detected in 1994 and those on the right are ones detected in 1995. A *triangle in a bar* indicates the maximum LOD position

Five QTLs were detected for grain weight in both 1994 and 1995. Four (gw3, gw5a, gw7 and gw11) of the five QTLs were in common between the 2 years. At one of the four QTLs (gw5a), the Zhenshan 97 allele was in the direction of increasing the grain weight, while Minghui 63 alleles at the other three QTLs contributed to an increase of grain weight. Two QTLs were detected in only 1 year: gw1 located on chromosome 1 was detected only in 1995 and gw3 located on chromosome 3 was detected only in 1994.

Taken together, 20 distinct QTLs, distributed on 7 of the 12 chromosomes, were identified for these four traits, eight of the QTLs were observed in both years, and the remaining 12 were detected in only 1 year. It is also clear that these QTLs were highly concentrated in a few chromosomal regions, or QTL hot-spots. This is particularly the case for the region around the C1023 locus on chromosome 7 where QTLs for all four traits were detected in both years.

## Dominance

A locus is recognized as exhibiting overdominance if the ratio of the dominance effect to the absolute value of the additive effect is larger than unity. Thus, two (yd7b and yd8) of the four QTLs for yield showed overdominance, one (yd7a) showed partial dominance, and the other one (yd6) showed negative dominance (Table 2). For tillers per plant, the effects of the QTLs varied from no dominance to partial dominance to full dominance. There were also differences between the 2 years in the levels of dominance of the QTLs that were detected in both years. For grains per panicle, overdominance was observed in one of three QTLs detected in 1994 and two of the five QTLs in 1995. Negative dominance was also observed in two of the QTLs detected in 1995. For grain weight, overdominance was observed in only one QTL detected in 1995. Negative dominance was observed in two of the QTLs in both years.

## Comparison of QTLs detected in $F_2$ ratoons and $F_{2:3}$ populations

The analysis of the $F_{2:3}$ population of the same cross was reported previously by Yu et a l. (1997), and can be used for a comparison of the QTL detection. Table 3 presents the QTLs for yield and yield-component traits that were simultaneously detected at least once in both of the $F_2$ ratoons of this study and the $F_{2:3}$ population of the previous study (Yu et al. 1997).

For yield, two QTLs, located on chromosome 7 and 8, were detected in both studies. At both of the QTLs, both additive and dominance effects detected in the $F_2$ ratoons were much larger than in the $F_{2:3}$ population. Five QTLs were detected in both studies for grains per panicle. Again, almost all the QTLs detected in $F_2$ ratoons showed larger effects than in the $F_{2:3}$ population. For grain weight, five QTLs were detected in both studies. The magnitudes of both additive and dominance effects resolved for each of the QTLs were very similar in both studies.

**Table 3** Comparison of QTLs for yield and yield component traits detected in the $F_2$ ratoons and $F_{2:3}$ (Yu et al. 1997) families. See footnotes of Table 2 for the explanations of the columns

| Trait | Chrom. | Flanking markers | Population | LOD | Var % | Additive effect | Dominance effect |
|---|---|---|---|---|---|---|---|
| Yield | 7 | C1023–RG128 | $F_2$ | 7.5 | 15.1 | – 9.1 | 6.2 |
| | | | $F_{2:3}$ | 3.5 | 9.4 | – 1.8 | 2.3 |
| | 8 | C483–C347 | $F_2$ | 2.4 | 5.2 | 3.2 | 6.2 |
| | | C483–R1629 | $F_{2:3}$ | 3.2 | 6.6 | 0.8 | 2.4 |
| Grains/panicle | 1 | RG532–RG173 | $F_2$ | 2.7 | 6.8 | –10.8 | – 2.0 |
| | | | $F_{2:3}$ | 7.1 | 17.8 | – 9.1 | – 2.8 |
| | 3 | C269–C1087 | $F_2$ | 3.2 | 6.8 | 7.7 | 6.1 |
| | | R1966–G144 | $F_{2:3}$ | 8.3 | 16.9 | 10.0 | – 3.6 |
| | 5 | RG360–C734 | $F_2$ | 2.4 | 15.2 | – 2.5 | 17.3 |
| | | | $F_{2:3}$ | 2.4 | 8.9 | – 1.3 | 10.3 |
| | 6 | G200–R1014 | $F_2$ | 3.3 | 7.8 | 11.6 | – 1.8 |
| | | | $F_{2:3}$ | 2.5 | 5.5 | 5.0 | 0.9 |
| | 7 | R1440–C1023 | $F_2$ | 9.1 | 20.4 | –18.6 | 6.6 |
| | | | $F_{2:3}$ | 4.7 | 9.5 | – 5.2 | 6.5 |
| Grain weight | 1 | R753–RM1 | $F_2$ | 2.8 | 6.9 | 0.81 | 1.0 |
| | | R753–C161 | $F_{2:3}$ | 3.5 | 12.1 | 0.7 | 1.5 |
| | 3 | R1966–C1087 | $F_2$ | 10.2 | 21.1 | – 1.9 | – 0.7 |
| | | R1966–G144 | $F_{2:3}$ | 11.4 | 22.5 | – 1.7 | – 0.7 |
| | 5 | RG360–R1674 | $F_2$ | 9.4 | 20.4 | 1.7 | – 0.3 |
| | | | $F_{2:3}$ | 12.9 | 24.1 | 1.6 | – 0.3 |
| | 7 | C1023–RG128 | $F_2$ | 6.8 | 21.8 | – 1.8 | – 0.0 |
| | | | $F_{2:3}$ | 5.2 | 17.7 | – 1.4 | – 0.4 |
| | 11 | RM4–RG98 | $F_2$ | 2.9 | 8.3 | – 1.0 | 0.5 |
| | | | $F_{2:3}$ | 3.5 | 8.6 | – 0.9 | 0.6 |

The only peculiar case occurred in tillers per plant, for which no common QTL was detected in the two studies.

## Discussion

Making use of the vegetative clones by ratooning the plants over seasons and years, we were able to perform replicated field trials of an $F_2$ population over 2 years to collect yield data for QTL mapping. Theoretically, data collected from this sort of field experiment best serve the purpose of QTL analysis, because the $F_2$ is genetically the most informative population that enables a direct estimation of all genetic components, and replicated field trials can provide estimates and also reduce the experimental errors.

The analysis identified a total of 20 distinct QTLs for yield and yield-component traits, distributed on 7 of the 12 chromosomes; eight of the QTLs were observed in both years, and the remaining 12 were detected only in 1 year. A very interesting feature is the highly concentrated distribution of the QTLs in a few chromosomal regions, or the existence of QTL hot-spots. Such a concentrated distribution of QTLs was also observed in previous studies (Xiong et al. 1999; Zhang and Yu 1999). Particular attention should be given to such QTL hot-spots in future studies of gene cloning and functional genomics.

When the results of QTL mapping based on the ratooned shoots of the $F_2$ population were compared with those based on the $F_{2:3}$ population, it is clear that many of the QTLs were detected in both studies, which reinforced the results of both studies. However, for most of the QTLs that were detected in both studies, larger effects were evident in the ratooned $F_2$ population than in the $F_{2:3}$ population. This is expected because there is extensive genetic heterogeneity within each of the $F_{2:3}$ families that would lead to an underestimation of all types of genetic effects, as well as an overestimation of the experimental errors which would result in a further underestimation of the genetic effects. Also, one generation of self-fertilization reduces the level of heterozygosity by 50% on average, which would result in an underestimation specifically of the dominance type of genetic effects.

However, there are also a number of technical limitations associated with the use of a ratooned $F_2$ population for QTL analysis. An obvious limitation is the difficulty to maintain such a population of vegetative clones, as it requires a large amount of tedious work. The most serious limitation is the reduction of productivity as a result of continuous ratooning. Moreover, different genotypes appeared to have a different level of tolerance to ratooning, as indicated by the variable amount of reduction displayed by the various genotypes in the populations. Thus, the estimates of the genetic effects detected for the QTLs for the yield traits may have suffered from such confounded effects of a reduction in productivity caused by ratooning. This may explain why fewer QTLs were detected in the present study using the ratooned $F_2$ population than those resolved in the $F_{2:3}$ population.

In summary, vegetatively ratooned $F_2$ populations may have considerable utility in the mapping QTLs, especially if dominant types of gene actions are of con-

cern, although there are certain technical limitations in experiments making use of such populations.

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