Theor Appl Genet (2003) 106:649–658
DOI 10.1007/s00122-002-1113-z

K.H. Cui · S.B. Peng · Y.Z. Xing · S.B. Yu
C.G. Xu · Q. Zhang

Molecular dissection of the genetic relationships of source, sink and transport tissue with yield traits in rice

Received: 25 February 2002 / Accepted: 12 July 2002 / Published online: 24 October 2002

Abstract Source, sink, and translocation capacity of assimilates play important roles during the formation of grain yield. The present study was conducted to characterize the genetic bases of traits representing source, sink and transport tissue, and their relationships with yield traits in rice, by analyzing QTLs for these traits and various ratios among them. The genetic materials were a recombinant inbred population derived from a cross between two indica cultivars Zhenshan 97 and Minghui 63, the parents of the most-widely grown hybrid rice in China. Using a linkage map that covers a total of 1,796 cM based on 221 molecular marker loci, a total of 81 QTLs were identified for the 15 traits studied (three leaf areas as the source, total spikelets per panicle as the sink, the number of large vascular bundles in the stem as transport tissue, three source to sink ratios, three transport tissue to source ratios, one transport tissue to sink ratio and three yield traits). The amount of variation explained by individual QTLs ranged from 1.12% to 24.14%. Five QTLs were identified to show interaction effects with the environment, which explained from 3.19% to 9.15% of the variation. The results showed that close linkage or pleiotropy is the genetic basis for the correlations of grain yield traits with source, sink, transport tissue and the various ratios among them. Of the 25 QTLs identified for source-sink-transport tissue trait, and 43 for various ratios, 8 and 22 QTLs, respectively, were mapped to the similar genomic blocks harboring QTLs for yield traits, especially for grain weight. Co-location of QTLs for yield traits with those for ratios among source, sink and transport tissue may provide a genetic explanation for the physiological expression of yield traits, and also suggest that improvement in ratios among source, sink and transport tissue may result in improvement in yield potential.

Keywords *Oryza sativa* L. · Recombinant inbred lines · Molecular marker · QTLs · Source-sink relation · Yield traits

Introduction

In conventional breeding programs, breeders have been selecting for grain yield and its components to increase crop yield potential. However, grain yield and its components are extremely complex traits. The expressed yield is the result of various biochemical and physiological processes (Hageman et al. 1967), and genetic control of grain yield and its components is indirect and through the control of complex biochemical and physiological processes (Ashraf et al. 1994). Interaction between genotype and environment has great effects on plant growth and development (Kang 1998), and consequently on grain yield and its components such as grain-filling percentage and grain weight (Yoshida and Parao 1976; Venkateswarlu et al. 1987). Morphological characters affect grain yield through their influences on yield components (Hsu and Walton 1971). Understanding the genetic relationship between morpho-physiological traits and yield traits could contribute to breeding for improving crop yield potential.

Genetic studies for quantitative traits have been greatly facilitated by the development of various molecular markers. The use of quantitative trait locus (QTL) mapping has contributed to a better understanding of the genetic basis of many agronomically important traits such as grain yield. In rice, many researches have identified QTLs for grain yield and its components (Xiao et al. 1996; Li et al. 1997; Yu et al. 1997; Li et al. 2000; Xing et al. 2002). However, attention was only paid to yield

---

Communicated by C. Möllers

K.H. Cui · Y.Z. Xing · S.B. Yu · C.G. Xu · Q. Zhang (✉)
National Key Laboratory of Crop Genetic Improvement,
Huazhong Agricultural University, Wuhan 430070, China
e-mail: qifazh@mail.hzau.edu.cn
Tel.: +86-27-87282429, Fax: +86-27-87287092

S.B. Peng
Crop, Soil and Water Sciences Division,
International Rice Research Institute (IRRI),
DAPO Box 7777, Metro Manila, The Philippines

traits per se in those studies. Lack of physiological understanding limits the interpretation of those QTLs, and further limits genetic manipulation in crop improvement.

The importance of source-sink relationship in the formation of yield and its components has been studied by Herzog (1982), Lafitte and Travis (1984) and Ashraf et al. (1994). In rice, the upper leaves are the main source of assimilates for grain filling (Yoshida 1981). Large sink size is a prerequisite for high yield and high harvest index (Ashraf et al. 1994). Efficient transport of assimilates from leaves and stems to developing spikelets is required for better grain filling and high yield. The capacity to transport assimilates from source to sink could limit grain filling (Ashraf et al. 1994). Relations between the number of large vascular bundles and yield traits such as grain yield, number of filled grain and grain weight have been reported in wheat, oat and rice (Peterson et al. 1982; Ashraf et al. 1994; Li et al. 1999). In this paper, the areas of the topmost three leaves, the total spikelet number per panicle and the number of large vascular bundles were treated as source, sink and transport tissue, respectively, and used to quantify the ratios among source, sink and transport tissue.

Since permanent segregating populations, including the recombinant inbred lines (RIL) population, provide researchers with the characteristics of genetic homozygosity and further advantages for QTL mapping, those populations have been widely employed to identify genes for quantitative traits of importance. In this study, we identified QTLs controlling source, sink, transport tissue and yield traits, and various ratios among them, using a RIL population derived from a cross between the parents of an elite hybrid. Emphasis was given to the relations between the various ratios and yield traits. The objective of the study was to reveal the underlying genetic relationship between source-sink-transport characters and yield traits.

Materials and methods

Plant materials

A population consisting of 241 RI lines (F_{10} and F_{11}) constructed by a single-seed descendant from a cross between two indica cultivars Zhenshan 97 and Minghui 63 (the parents of Shanyou 63, the most-widely grown hybrid in China) was used in the study. Field experiments were conducted on the farm of the International Rice Research Institute in the Philippines in the dry season (January to May) of 1999 and 2000. Two parents, the F_1 and 241 RILs, were arranged in a randomized complete block design with three replications. Plot size was 1 × 2.4 m in 1999 and 1 × 3 m in 2000. Fourteen-day old seedlings were transplanted on 6 January in 1999 and 12 January in 2000. Other crop-management practices were identical in the 2 years. Transplanting spacing was 20 × 20 cm with three seedlings per hill. A total of 220 kg ha^{-1} of fertilizer N in the form of urea was applied in four splits [60 kg ha^{-1} at 1 day before transplanting, 60 kg ha^{-1} at about 20 days after transplanting (DAT), 60 kg ha^{-1} at about 40 DAT and 40 kg ha^{-1} at about 70 DAT] to ensure N sufficiency. Phosphorus (30 kg of P ha^{-1} as a single superphosphate), potassium (40 kg of K ha^{-1} as KCl), and zinc (5 kg of Zn ha^{-1} as zinc sulfate heptahydrate) were applied and incorporated in all plots 1 day before transplanting. Pests and diseases were intensively controlled using chemicals to avoid yield loss.

Table 1 Traits and derived traits

Trait abbreviation	Trait description
FL	Area of flag leaf (cm^2)
SL	Area of –2nd leaf (cm^2)
TL	Area of –3rd leaf (cm^2)
TS	Total spikelets per panicle
LVB	Number of large vascular bundles
FL/TS	Area of flag leaf per spikelet (cm^2 per spikelet)
SL/TS	Area of –2nd leaf per spikelet (cm^2 per spikelet)
TL/TS	Area of –3rd leaf per spikelet (cm^2 per spikelet)
LVB/FL	Ratio of LVB to area of flag leaf
LVB/SL	Ratio of LVB to area of –2nd leaf
LVB/TL	Ratio of LVB to area of –3rd leaf
LVB/TS	Ratio of LVB to total spikelets per panicle
GFP	Grain filling percentage (%)
GW	1,000-grain weight (g)
GY	Grain yield (t/ha)

Measurement of traits

The traits measured and abbreviations are listed in Table 1. At about 10 days after heading, ten uniform tillers from ten hills were sampled from each plot. The following traits were measured on each tiller: area of the flag leaf (FL, cm^2), area of the –2nd leaf (SL, cm^2), and area of the –3rd leaf (TL, cm^2) using an area meter (LI-COR Model 3100, LI-COR Incorporated Lincoln, Nebraska, USA).

Ten panicles were cut from the same ten tillers for measuring sink-related traits such as total spikelets per panicle. The number of large vascular bundles (LVB) was measured on the peduncle in each tiller. The transverse hand section about 1-mm thick was made at about 1-cm below the necknode of the panicle with a razor blade, and mounted on a slide. After staining with Safranin solution, the section was examined under a microscope and the LVB located in the inner parenchyma was counted.

On the basis of the data of the primary source-sink-transport tissue characters, the following derived traits were obtained: ratio of leaf areas to total spikelets per panicle (FL/TS, SL/TS, TL/TS), ratio of LVB to total spikelets (LVB/TS), ratios of LVB to the area of the flag leaf, and the –2nd and –3rd leaf (LVB/FL, LVB/SL, LVB/TL).

Twelve hills in the middle of each plot were harvested at maturity to determine grain yield expressed at the oven dry weight basis, the grain-filling percentage, and the 1,000-grain weight.

Data analysis

The trait measurements averaged over the 2 years were used for correlation analysis. Broad-sense heritability based on RI lines was estimated according to the following equation: $H_b^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_e/re + \sigma^2_{ge}/e)$, where r is the number of replications per year, e is the number of environments (years), σ^2_g is the genetic variance, σ^2_{ge} is the variance of genotype × environment interaction and σ^2_e is the residual variance.

Genetic-map construction and QTL detection

The molecular markers and the linkage map were described by Xing et al. (2002), which consisted of 221 marker loci and covered a total of 1,796 cM. Data obtained in 1999 and 2000 were used for combined analyses to determine putative QTLs and QTL

× environment interactions in a mixed linear model using QTL-Mapper (version 1.0) based on a threshold of LOD > 2.4 (Wang et al. 1999). Background genetic variation control was set as controlling the main and interaction effects of important markers. The computer software can map QTLs with main effects, epistatic effects and QTL × environment interaction. Separate QTL analyses for each year were also conducted and comparisons were made with results from combined analyses. Only results from the combined analysis of QTLs for the main effects were presented.

Results

Phenotypic variation

The phenotypic values of the 15 traits studied are given in Table 2. In comparison with Zhenshan 97, Minghui 63 had larger areas of the topmost three leaves, fewer spikelets per panicle and fewer large vascular bundles. Ratios of leaf areas to spikelets per panicle in Minghui 63 were greater than in Zhenshan 97. Zhenshan 97 had higher ratios of LVB to leaf areas than Minghui 63, while there was not much difference between the two parents in the ratio of LVB to spikelets per panicle. Minghui 63 had a higher grain weight, a lower grain-filling percentage and a lower grain yield than Zhenshan 97.

All traits of the RIL population segregated continuously; both skewness and kurtosis for all traits were less than 1.0 (Table 2), suggesting that segregation of all the traits in the RI population fits a normal distribution. Transgressive segregations were observed for all traits. Each trait had relatively high broad-sense heritability, which was consistent with the relatively high correlation between the 2 years for each trait (Table 3).

Simple correlation analyses

The simple correlation coefficients between the traits are given in Table 3. Areas of the topmost three leaves were not correlated with 1,000-grain weight, and were negatively correlated with grain-filling percentage and grain yield, respectively. Total spikelets per panicle and the number of large vascular bundles were also negatively correlated with grain weight, grain-filling percentage and grain yield.

The ratios of areas of the topmost three leaves to spikelets per panicle were positively correlated with 1,000-grain weight, whereas their correlations with

Table 3 Correlations between sources, sink, transport tissue and ratios among them, and yield traits in the RIL population derived from Zhenshan 97 × Minghui 63

Traits[a]	Correlation between 2 years	1,000-grain weight[b]	Grain filling percentage	Grain yield
FL	0.66**	0.02	–0.36**	–0.30**
SL	0.58**	0.04	–0.56**	–0.51**
TL	0.69**	0.07	–0.55**	–0.50**
TS	0.74**	–0.32**	–0.52**	–0.42**
LVB	0.77**	–0.02	–0.39**	–0.36**
FL/TS	0.64**	0.33**	0.14*	0.09
SL/TS	0.61**	0.41**	–0.05	–0.12
TL/TS	0.67**	0.37**	–0.15*	–0.20**
LVB/FL	0.54**	–0.08	0.09	0.04
LVB/SL	0.57**	–0.08	0.36**	0.33**
LVB/TL	0.66**	–0.13	0.38**	0.34**
LVB/TS	0.71**	0.35**	0.35**	0.22**

*,** Significant at 0.05 and 0.01 probability levels, respectively

[a] See Table 1 for abbreviations

[b] Correlation coefficient between the 2 years for grain yield is 0.72, 0.85 for grain-filling percentage, and 0.95 for 1,000-grain weight

Table 2 Variation of source, sink, transport tissue and ratios among them, and yield traits in the RIL population derived from Zhenshan 97 × Minghui 63

Traits[a]	RIL population				Z.S. 97	M.H. 63	F_1	H^2_b
	Mean	Range	Skew	Kurt				
Source, sink, and transport tissue-related traits								
FL	35.34	17.54–59.12	0.44	0.63	26.51	31.08	39.54	79.7
SL	41.45	24.46–67.79	0.07	–0.00	31.24	39.95	41.82	73.6
TL	34.34	16.74–56.69	–0.06	–0.10	23.95	39.12	34.31	81.0
TS	107.7	63.02–176.40	0.63	0.54	107.74	87.93	106.60	85.0
LVB	15.80	11.63–24.03	0.39	1.13	17.01	14.27	14.81	86.9
Ratios among source, sink, and transport tissue								
FL/TS	0.34	0.19–0.47	0.00	–0.38	0.25	0.35	0.37	78.0
SL/TS	0.39	0.24–0.54	–0.02	0.04	0.29	0.46	0.40	75.6
TL/TS	0.33	0.19–0.53	0.29	0.04	0.22	0.45	0.33	79.9
LVB/FL	0.47	0.27–0.70	0.37	0.06	0.65	0.49	0.39	69.9
LVB/SL	0.39	0.27–0.58	0.52	–0.11	0.54	0.36	0.35	61.1
LVB/TL	0.48	0.29–0.80	0.84	0.51	0.72	0.36	0.43	76.1
LVB/TS	0.15	0.10–0.21	0.25	0.20	0.16	0.16	0.14	83.4
Grain yield and its components								
GW	25.79	19.13–31.85	–0.08	–0.54	25.47	27.10	27.11	95.1
GFP	47.08	13.20–76.70	–0.17	–1.02	53.80	18.71	54.10	89.1
GY	3.34	1.17–5.58	–0.20	–0.94	3.57	1.39	4.39	83.4

[a] See Table 1 for abbreviations

Table 4 Putative QTLs for leaf area, spikelets per panicle and vascular bundles

Trait[a]	QTL[b]	Chrom.	Interval[c]	LOD	Dist. (cM)[d]	Add.[e]	Var% (Ai)[f]	Var% (AEi)[g]
FL	qFL1-1	1	G359–RG352^{K}	14.51	0	2.47	11.19	
	qFL6-1	6	RZ667–RG424^{FY}	6.75	12	2.03	7.54	
	qFL6-1	6	Waxy-1496	4.05	2	1.33	3.24	
	qFL9-1	9	RM201–C472	3.29	8	–1.33	3.26	
	qFL10-1	10	RG561–R2625^{K}	2.65	2	1.04	1.99	
SL	qSL1-1	1	C904–R2632	5.46	2	1.83	6.17	9.15
	qSL3-1	3	RM227–R1925	4.60	4	1.29	3.09	
	qSL6-1	6	RZ667–RG424^{FY}	14.45	20	2.53	11.8	
	qSL11-1	11	R543a–RZ536	6.71	0	1.49	4.09	
	qSL12-1	12	G1314b–R643	5.60	20	1.60	4.70	
TL	qTL1-1	1	G1128b–C904	2.94	0	1.15	2.04	
	qTL6-1	6	RZ667–RG424^{FY}	20.05	18	3.55	19.47	
	qTL6-2	6	R2869–C474	3.45	2	–1.39	2.98	
	qTL10-1	10	RM258–RG561	5.92	0	1.74	4.66	
	qTL12-1	12	R887–G1314b	5.05	2	1.57	3.80	
TS	qTS2-1	2	R712–RZ324	3.93	4	–3.10	2.84	
	qTS3-1	3	RM227–R1925	2.48	4	2.57	1.96	
	qTS6-1	6	G200–Y4073L^{FY}	21.56	0	7.84	18.21	
LVB	qLVB1-1	1	C567–C2340^{K}	9.52	0	0.47	5.82	
	qLVB4-1	4	C2807–RM241^{Y}	2.40	2	–0.25	1.61	
	qLVB5-1	5	C624–C246	3.77	20	–0.34	3.04	
	qLVB6-1	6	R2147–RZ667^{FY}	9.73	0	0.43	4.88	
	qLVB8-1	8	G1149–R2272	3.81	8	–0.33	2.78	
	qLVB10-1	10	C677–RM258	15.59	2	–0.67	11.57	
	qLVB11-1	11	RG2–CDO534	5.88	0	–0.38	3.72	

[a] See Table 1 for abbreviations
[b] QTL nomenclature follows that of McCouch et al. (1997)
[c] The superscripts K,Y, F following the intervals indicate that a QTL for 1,000-grain weight, grain yield or grain-filling percentage was also located in or closely linked to the region
[d] Genetic distance (in cM) of the putative QTL from the right marker on the interval
[e] Negative value of the additive effect indicates that the allele from Zhenshan 97 increases the phenotypic score
[f] Var% (Ai) represents the relative contribution of the putative QTL to the trait
[g] Var% (AEi) represents the relative contribution of the additive × environment interaction effect for the trait

grain-filling percentage and grain yield were relatively weak and inconsistent. None of the correlations between the ratio of LVB to flag leaf area and the three yield traits was significant. However, the ratios of LVB to the areas of the –2nd and –3rd leaves were positively correlated with grain yield and grain-filling percentage, respectively. The ratio of LVB to spikelets per panicle was also positively correlated with the three yield traits.

QTLs for source-sink-transport tissue traits

Five QTLs each were identified for areas of the flag leaf, and the –2nd and –3rd leaf, respectively, explaining 27.88%, 29.83% and 32.20% of the total phenotypic variation of the respective traits (Table 4). The high-value parent Minghui 63 contributed the majority of alleles to increasing the topmost three leaf areas, while the low-value parent Zhenshan 97 also contributed two alleles to increasing phenotypic scores. It should be pointed out that one of the QTLs, located in the interval RZ667–RG424 on chromosome 6, appeared to have a large effect on the areas of all three leaves. In addition, two QTLs on chromosome 1 detected for areas of the –2nd leaf and the –3rd leaf, were located in nearby regions and it is likely that they were due to the effect of the same QTL. Similarly, the two QTLs on chromosome 12 detected for areas of the –2nd leaf and the –3rd leaf may also be due to the effect of the same QTL. Such common genetic control was consistent with the high correlation between areas of the two leaves (r = 0.91). Among 15 QTLs for leaf areas, only qSL1-1 for the area of the –2nd leaf was shown to have an environment interaction effect that explained 9.15% of total variation.

Three QTLs were detected for spikelets per panicle, jointly explaining 23% of the total variation. The QTL located in the interval G200–Y4073L on chromosome 6 had the largest effect, with the allele from Minghui 63 in the direction of increasing the number of spikelets.

A total of seven QTLs were detected for the number of large vascular bundles, accounting for 33.43% of the total variation. Minghui 63 contributed alleles of increasing vascular bundles at two of the QTLs. At the other five QTLs, the alleles for increasing vascular bundles were contributed by Zhenshan 97.

QTLs for source to sink ratios

A total of nine QTLs for the ratio of flag leaf area to spikelets per panicle were detected, collectively explaining 43.28% of the total variation (Table 5). Variation explained by individual QTLs ranged from 1.12% to 13.71%. Minghui 63 alleles at six of the QTLs contributed to increasing the ratio, while alleles at the other three QTLs from the Zhenshan 97 alleles increased the ratio. Three QTLs were identified for the ratio of the –2nd leaf area to spikelets per panicle, explaining 32.56% of the total variation. Minghui 63 contributed all three alleles for increasing the ratio. Eight QTLs were detected for

Table 5 Putative QTLs for ratios among source, sink and transport tissue

Trait[a]	QTL[b]	Chrom.	Interval[c]	LOD	Dist.[d]	Add.[e]	Var% (Ai)[f]	Var% (AEi)[g]
FL/TS	qFLTS1-1	1	RM212–C567^{K}	17.5	2	0.021	13.71	
	qFLTS1-2	1	C904–R2632	5.03	0	0.010	3.11	
	qFLTS1-3	1	RG173–RM81A	4.26	12	0.009	2.52	
	qFLTS3-1	3	RM227–R1925	3.06	2	–0.009	1.99	
	qFLTS3-2	3	C746–C944^{K}	4.56	10	0.010	3.11	
	qFLTS5-1	5	C1447–RM31	6.37	10	0.013	5.25	
	qFLTS5-2	5	R830–R3166^{K}	2.56	6	–0.006	1.12	
	qFLTS6-1	6	RG424–R2549^{FY}	5.07	14	0.009	2.52	
	qFLTS9-1	9	RG667–RM215	6.76	4	–0.013	5.25	
SL/TS	qSLTS1-1	1	R2201–RM212^{K}	13.77	0	0.016	10.65	
	qSLTS1-2	1	C904–R2632	11.03	2	0.013	7.03	
	qSLTS4-1	4	RM241–G102^{Y}	17.11	0	0.018	13.48	
TL/TS	qTLTS1-1	1	R2201–RM212^{K}	12.22	0	0.015	5.88	6.33
	qTLTS1-2	1	G1128b–C904	6.24	0	0.011	3.16	
	qTLTS2-1	2	RM29–R1843	7.54	0	0.013	4.42	
	qTLTS4-1	4	G102–G235^{Y}	5.00	10	0.011	3.07	
	qTLTS6-1	6	RZ667–RG424$^{F\ Y}$	8.70	10	0.018	8.47	
	qTLTS8-1	8	RG978–R1394^{K}	2.68	0	–0.008	1.67	
	qTLTS10-1	10	C153A–RM222	4.04	6	–0.010	2.61	
	qTLTS12-1	12	R887–G1314b	6.63	2	0.012	3.76	
LVB/FL	qLVBFL1-1	1	RG532–RM259^{K}	7.87	6	–0.021	7.48	
	qLVBFL6-1	6	RZ667–RG424$^{F\ Y}$	2.77	4	–0.010	1.70	
	qLVBFL6-2	6	C764–RZ398	7.39	2	–0.023	8.97	
	qLVBFL9-1	9	R2638–RM257^{K}	14.05	0	0.024	9.77	
	qLVBFL11-1	11	RM20a–R3203	4.87	8	–0.015	3.82	
LVB/SL	qLVBSL1-1	1	RG173–RM81A	3.72	2	–0.012	2.62	
	qLVBSL1-2	1	C161–R753	2.68	8	0.010	1.82	
	qLVBSL4-1	4	G102–G235^{Y}	10.87	10	–0.021	8.02	
	qLVBSL6-1	6	RZ667–RG424$^{F\ Y}$	10.75	16	–0.022	8.80	
	qLVBSL9-1	9	R2638–RM257^{K}	5.66	0	0.014	3.56	
	qLVBSL11-1	11	R543a–RZ536	5.12	0	–0.013	3.07	
	qLVBSL12-1	12	G1314b–R643	5.75	6	–0.015	4.09	
LVB/TL	qLVBTL1-1	1	RG173–RM81A	3.91	6	–0.015	2.48	
	qLVBTL4-1	4	G235–R78^{Y}	5.16	0	–0.016	2.82	
	qLVBTL6-1	6	RZ667–RG424$^{F\ Y}$	16.61	14	–0.039	16.76	
	qLVBTL9-1	9	R2638–RM257^{K}	5.99	0	0.018	3.57	
	qLVBTL10-1	10	RG561–R2625^{K}	3.53	2	–0.015	2.48	
	qLVBTL12-1	12	G1314b–R643	7.73	20	–0.024	6.35	
LVB/TS	qLVBTS1-1	1	C2340–C86^{K}	28.27	4	0.009	18.58	
	qLVBTS6-1	6	RG653–G342	4.55	2	–0.003	2.06	
	qLVBTS6-2	6	P–G200$^{F\ Y}$	23.04	8	–0.008	14.68	
	qLVBTS9-1	9	R1164–RZ698	4.62	0	0.003	2.06	
	qLVBTS11-1	11	G4001–RM254	8.65	2	–0.004	3.67	4.13

[a] See Table 1 for abbreviations

[b] QTL nomenclature follows that of McCouch et al. (1997)

[c] The superscripts K,Y and F following the region indicate that a QTL for 1,000-grain weight, grain yield, or grain-filling percentage was also located in or closely linked to the region, respectively

[d] Genetic distance (in cM) of the putative QTL from the right marker on the interval

[e] Negative value of the additive effect indicates that the allele from Zhenshan 97 increases the phenotypic score

[f] Var%(Ai) represents the relative contribution of the putative QTL to the trait

[g] Var% (AEi) represents the relative contribution of additive × environment interaction to the trait

the ratio of the –3rd leaf area to spikelets per panicle, accounting for 30.68% of the total variation. Alleles from Zhenshan 97 contributed at two of the QTLs increased the ratio, while the remaining six QTLs alleles from Minghui 63 were in the direction of increasing the ratio. The QTL on chromosome 1 (qTLTS1-1) showed interaction with the environment, which explained 6.33% of the total variation.

The QTLs for ratios of the –2nd and –3rd leaf areas to spikelets per panicle shared three similar regions. Furthermore, qFLTS1-1, qSLTS1-1 and qTLTS1-1 were located in a similar region on chromosome 1 (R2201–RM212–C567 in 21.1 cM), and three QTLs (qFLTS1-2, qSLTS1-2 and qTLTS1-2) were also mapped in a similar region on chromosome 1 (G1128b–C904–R2632 in 4.9 cM). The region of RZ667–RG424–R2549 (42.5 cM) on chromosome 6 had effects on the ratio of flag leaf area to spikelets per panicle and the ratio of the –3rd leaf area to spikelets per panicle. The majority of alleles at those QTLs (15 out of 20 QTLs) increasing the source to sink ratios were contributed by the high-value parent Minghui 63.

QTLs for the ratios of transport tissue to source and to sink

Five QTLs were identified for the ratio of LVB to the flag leaf area, explaining 32.34% of the total variation (Table 5). Alleles for increasing the ratio were from

Table 6 Putative QTLs for yield and its components

Trait[a]	QTL[b]	Chrom.	Interval	LOD	Dist. (cM)[c]	Add.[d]	Var% (Ai)[e]	Var% (AEi)[f]
GW	qGW1-2	1	C2340–C86	13.28	2	0.53	4.82	
	qGW1-3	1	G359–RG532	19.79	0	–0.64	6.98	
	qGW3-1	3	C944–R321	15.12	0	0.56	5.40	
	qGW3-2	3	C1087–RZ403	38.08	0	0.95	15.41	
	qGW5-1	5	R3166–RG360	38.86	0	–0.91	14.20	
	qGW8-1	8	R1394–G2132	13.30	0	–0.49	4.15	
	qGW9-1	9	RM257–RM242	13.81	0	–0.55	5.15	
	qGW10-1	10	C371–C405a	4.52	0	–0.28	1.37	
	qGW11-1	11	G44–G257	7.38	2	0.45	3.43	
GFP	qGFP3-1	3	RG393–C1087	5.01	0	–1.90	1.55	
	qGFP6-1	6	G200–Y4073L	50.15	0	–7.50	24.14	4.54
GY	qGY4-1	4	G102–G235	6.89	12	–0.17	1.31	
	qGY6-1	6	RZ667–RG424	53.71	2	–0.51	12.18	3.19

[a] See Table 1 for abbreviations
[b] QTL nomenclature follows that of McCouch et al. (1997)
[c] Genetic distance (in cM) of the putative QTL from the right marker on the interval
[d] Negative value of the additive effect indicates that the allele from Zhenshan 97 increases the phenotypic score
[e] Var% (Ai) represent the relative contribution of the putative QTL to the trait
[f] Var% (AEi) represents the relative contribution of additive × environment interaction to the trait

Zhenshan 97 at all of the QTLs except qLVBFL9-1. A total of seven QTLs were detected for the ratio of LVB to the –2nd leaf area, together accounting for 31.74% of the total variation. Also, the high-value parent Zhenshan 97 contributed alleles for increasing the ratio at most of the QTLs. The six QTLs identified for the ratio of LVB to the –3rd leaf area explained 34.45% of the total variation. The QTL (qLVBTL6-1) on chromosome 6 had relatively large effects on the ratio and explained 16.76% of the total variation, at which the allele from Zhenshan 97 increased the ratio.

A comparison of the QTLs revealed that two regions (RZ667–RG424 on chromosome 6 and R2638–RM257 on chromosome 9) showed their effects on all three ratios of LVB to leaf areas. Additionally, three regions (RG173–RM81A on chromosome 1, G102–G235–R78 on chromosome 4, and G1314b–R643 on chromosome 12) exerted their effects on the ratio of LVB to the –2nd leaf area and the ratio of LVB to the –3rd leaf area. The Zhenshan 97 alleles at 14 out of 18 QTLs contributed to increasing the ratios of LVB to the leaf areas.

For the ratio of LVB to spikelets per panicle, five QTLs were identified. Two QTLs (qLVBTS1-1 and qLVBTS6-2) had relatively large effects on the ratio, explaining 18.58% and 14.68%, respectively. Interaction between the QTL (qLVBTS11-1) on chromosome 11 and the environment explained 4.13% of the total variation.

QTLs for yield traits

A total of nine QTLs were detected for 1,000-grain weight, jointly explaining 69.09% of the total variation (Table 6). Two QTLs (qGW3-2 and qGW5-1) had larger effects each explaining more than 14% of the total variation. Alleles for increasing grain weight at the nine QTLs were dispersed between the two parents. Two QTLs were mapped for grain-filling percentage. The QTL on chromosome 6 (qGFP6-1) explained as much as 24.14% of the total variation. This QTL interacted with the environment and the interaction explained 4.54% of the total variation. Two QTLs were detected for grain yield. The QTL on chromosome 6 (qGY6-1) explained 12.18% of the total variation. This QTL also interacted with the environment, which explained 3.19% of the total variation.

Alleles at QTLs increasing both grain-filling percentage and grain yield were provided by Zhenshan 97 at all of the QTLs. Two QTLs, qGW3-2 and qGFP3-1, were located in a similar genomic region on chromosome 3, but with opposite effects.

Co-location of QTLs for yield traits, source-sink-transport tissue characters, and ratios among source, sink and transport tissue

It is also noted that several QTLs for yield traits were located in similar regions with QTLs for other traits analyzed in this study. Nine of the 25 declared QTLs for source-sink-transport tissue traits shared five regions with the three yield traits (Tables 4 and 6). Of the 43 QTLs detected for the various ratios, 22 QTLs were mapped to similar genomic blocks harboring QTLs for the three yield traits, especially for 1,000-grain weight (Tables 5 and 6). More specifically, QTLs for the yield traits and ratios among source, sink and transport tissue shared regions RG532–RM243 and RM212–C86 on chromosome 1, C746–R321 on chromosome 3, G102–R78 on chromosome 4, R830–RG360 on chromosome 5,

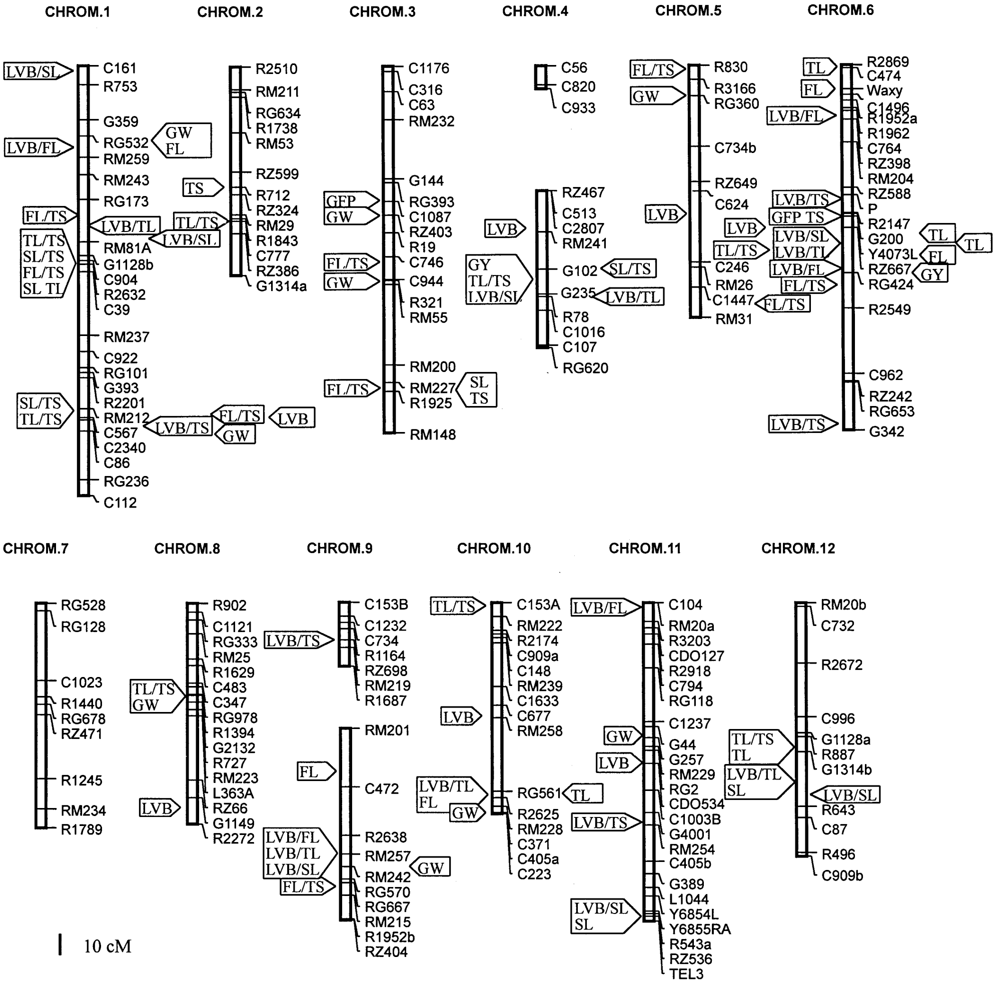

Fig. 1 Genetic linkage map showing locations of QTLs for source, sink, transport tissue and ratios among them, and yield traits in a recombinant inbred population derived from Zhenshan 97 × Minghui 63. The *arrows* indicate the position of the peak LOD. See Table 1 for abbreviations

P–R2549 on chromosome 6, R978–G2132 on chromosome 8, RM257–RM215 on chromosome 9, and RG561–C405a on chromosome 10 (Tables 4, 5 and 6, Fig. 1).

Discussion

In the present study, several morphological traits generally functioned as source, sink and transport tissue, and several derived traits as ratios among them were employed to understand the relationships of source, sink and transport tissue with yield traits in rice by QTL analysis. As pointed out by Ashraf et al. (1994) and in some literature, spikelet leaf ratio could reflect the relationships between sources and sinks in rice, and the number of LVB is generally connected with the capacity of the stem to transport assimilate. On the basis of facts, it is safe to infer that several ratios such as FL/TS, LVB/FL and LVB/TS may reflect relative relationship among source, sink and transport capacity, which are further associated with grain filling and development, although the physiology of those ratios are indefinite.

Detection of the QTLs

We compared the QTLs detected using the data of 1999 or 2000 separately, with the QTLs jointly detected using data of the 2 years (data not shown). Generally, the QTLs from the combined analysis of 2-year data were also detected in at least 1 year at the identical LOD and probability settings. The directions and magnitudes of additive effects of QTLs detected by the combined analysis were consistent with the same QTLs detected in 1 year. For example, three and eight QTLs were detected for the ratio of flag leaf area to spikelets per panicle in 1999 and 2000, respectively. Of them, two QTLs were detected in both years. Whereas, nine QTLs were detected when the combined analysis was done with the 2 year data. Therefore, the detection of QTLs by the combined analysis across environments should be more efficient. Paterson et al. (1991) believed that a single environment would underestimate the number of QTLs. Veldboom and Lee (1996a, b) indicated that the power to detect QTLs by using the mean of the two environments should be increased. Comparison made in the study also suggests that data from additional environments certainly add to the detection of QTLs.

Genotype by environment interaction (G × E) plays a very important role in trait performance. In the literature, detection of G × E was generally done by comparing QTLs in different environments (Paterson et al. 1991; Lu et al. 1996; Zhuang et al. 1997). In this study, a combined analysis using 2 year data revealed only five QTLs to have significant environmental interactions, suggesting that G × E had relative small effects on the tested traits. This is consistent with high correlation coefficients between 2 years in the tested traits.

Grain-filling percentage and grain yield are generally considered to be complex agronomic traits, involving many genes in the expression of the traits. However, it is noted that only two QTLs were identified for each of the two traits. This is consistent with the results of Lin et al. (1996), Lu et al. 1996, Xiao et al. (1996) and Li et al. (2000) in rice, and Veldboom and Lee (1996a, b) in maize. It is speculated that many genes had too small effects that did not reach the pre-set threshold for the detection. In addition, cancellations of the opposite effects of QTLs for the component traits may also account for the smaller number of QTLs for yield than its component traits.

Relationship between source-sink characters and yield traits

It would be expected that grain filling depends largely on the source leaves. However, correlations between leaf areas and yield traits were not always strong (Rutger et al. 1971; Herzog 1982). Both positive and negative correlations between grain yield and leaf-area index (LAI) have been reported, depending on LAI value. A negative relationship between LAI at flowering and grain yield was often observed at a high LAI in rice (Dingkuhn et al. 1991; Chau and Bhargava 1993). In this study, areas of the topmost three leaves were negatively correlated with both grain-filling percentage and grain yield. Three QTLs for leaf areas (qFL6-1, qSL6-1 and qTL6-1), and a QTL for grain-filling percentage (qGFP6-1) and one for yield (qGY6-1) were mapped to a similar genomic region, but QTLs for leaf areas and ones for yield traits showed opposite directions of additive effect. This is consistent with the results from correlation analysis. However, it is inconsistent with the report of Li et al. (1998) that alleles increasing source leaf size were associated with increased grain yield. From the viewpoint of crop physiology, firstly, it is often noted in a rice canopy that the –2nd and –3rd leaves are mutually shaded during grain filling. In this case, their net photosynthetic capacity is low and may become net consumers of assimilates. Richards (1996) also suggested that reducing the size of the flag leaf may increase grain number and yield because of an increase in assimilates to the developing ear in wheat. Secondly, it is often observed that negative correlation occurs between leaf area and photosynthetic rate (Herzog 1982; Bhagsari and Brown 1986). Such observations suggest that larger leaf area does not always provide more assimilates to the grain during grain filling. Assimilate partitioning should be considered in designing the plant type for yield improvement.

Relationship between source to sink ratio and yield traits

Grain weight is determined by both source and sink (Reynolds et al. 1999). The ratio of leaf area to spikelet number represents the available source per spikelet and could be a critical physiological parameter influencing grain weight. Our results showed that areas of the topmost three leaves per spikelet were positively correlated with 1,000-grain weight. The ratio of source to sink traits influenced grain weight by creating different intrinsic growth potential in the grains (Herzog 1982) because seed growth rate is one of the components determining seed size (Egli 1998). In this study, six QTLs for source to sink ratio (qFLTS1-1, qFLTS3-2, qFLTS5-2, qSLTS1-1, qTLTS1-1 and qTLTS8-1) were closely linked to regions harboring QTLs for 1,000-grain weight, and the parental contributions of the alleles also appeared in the same direction. Moreover, the flag leaf area to spikelet ratio and 1,000-grain weight shared three similar regions. The flag leaf is often regarded as the most important source of the assimilate-supply to the ear, and was associated with spikelet sterility, grains with high-density, 1,000-grain weight and grain yield in wheat and rice (Hsu and Walton 1971; Herzog 1982; Ghosh et al. 1990).

Assimilate supply is closely associated with both final grain weight and grain-filling percentage, and consequently with grain yield (Egli 1998; Kobata et al. 2000). The topmost three leaves are considered to be sources for yield formation. Although significant correlations between areas of the topmost three leaves and dry matter production were observed (data not shown) respectively, results from correlation analysis and QTL mapping revealed that relationships between source to sink ratios and two yield traits (grain-filling percentage and grain yield) were weak and inconsistent. Flag leaf area per spikelet was positively correlated with yield traits. However, the –2nd and –3rd leaf area per spikelet was negatively correlated with grain yield and grain-filling percentage (Table 3). This is consistent with the fact that QTLs for source to sink ratio (qSLTS4-1, qTLTS4-1, qFLTS6-1 and qTLTS6-1) and QTLs for grain-filling percentage and grain yield (qGFP6-1, qGY4-1 and qGY6-1), which were co-located in the vicinity of the similar genomic blocks on chromosome 4 and 6, showed opposite genetic effects. This seems to indicate that grain filling and yield in the RI population are limited by source availability under the conditions of this study.

Roles of transport tissue in relation to yield traits

LVB in the rice stem play important roles in the translocation of assimilates and movement of water and nutrients. The capacity of the conducting tissue could limit the amount of assimilates accumulated in the grains during the filling period, especially under environmental conditions favorable for both photosynthesis and a large sink capacity (Nátrová and Nátr 1993). The relationship of vascular bundles to yield traits such as filled grain number, grain weight and grain yield per panicle was established in oat and wheat (Peterson et al. 1982; Li et al. 1999). In our study, a negative correlation between the number of LVB and yield traits was observed. Peterson et al. (1982) also observed a negative correlation between kernel weight and the number of bundles and the phloem area. However, our data indicate that the number of LVB relative to spikelets per panicle was positively correlated with yield traits. QTL analysis provides the genetic basis of this relationship. Two QTLs for the ratio of LVB to spikelets per panicle (qLVBTS1-1 and qLVBTS6-2) and three QTLs for yield traits (qGW1-1 and qGFP6-1 and qGY6-1) were mapped to similar genomic regions and showed the same direction of the genetic effect. A high number of LVB per spikelet may contribute to the rapid accumulation of assimilates in filling grains.

The results also showed that five regions (G359–RM259 on chromosome 1, G102–R78 on chromosome 4, G200–RZ667–RG424 on chromosome 6, R2638–RM242 on chromosome 9 and RG561–R2625–C405a on chromosome 10) were shared by QTLs for LVB to leaf area ratios and yield traits in the study. Alleles at two genomic regions (G102–G235–R78 on chromosome 4 with a length of 14.8 cM and G200–RZ667–RG424 on chromosome 6 with a length of 31.3 cM) from Zhenshan 97 increased LVB to the leaf area ratios, grain-filling percentage and grain yield. The positive correlation between LVB to leaf area ratios and yield traits suggests that transport tissue relative to the source may be a limitation for assimilate translocation in the tested materials.

It is noted that the region R2638–RM242, which had effects simultaneously on the ratios of LVB to the areas of the topmost three leaves, was linked to qGW9-1 for 1,000-grain weight. However, parental contributions of the genetic effects of the ratios of LVB to leaf areas and grain weight were in opposite directions. Two QTLs, qGW1-2 and qLVBFL1-1, which shared the similar genomic region on chromosome 1, had the same parental direction of the genetic effect. These results suggest a complex relationship between 1,000-grain weight and LVB to leaf area ratios.

The implications

This study analyzed QTLs for traits that we regarded as source, sink, transport tissues, various ratios among them and their relationships with yield traits. This analysis provided useful information for understanding the expressions and physiological basis of yield traits on a perspective of source-transport-sink. It should be noted that the two parents, Zhenshan 97 and Minghui 63, and their hybrid are adapted to the subtropical and temperate environments in central and southern China. Most of the RILs are probably not adapted to the tropical environment where the experiment population was planted in this study, as can be seen from the relatively low percentage of grain filling (Table 2). This may to a certain extent affect the interpretation of the results, especially with respect to yield traits.

Co-localization of the QTLs for various traits, as the results of either pleiotropic effects or close linkage, can provide an explanation for the genetic basis of correlations between the traits. However, it should also be pointed out that co-localization between some of the QTLs may have occurred just by chance, due to the large number of the QTLs detected in this study.

Assimilate supply is closely related to grain yield and its components. As major sources, the importance of leaves is expected. However, as revealed by the results of this study, various ratios among source, sink and transport tissue gave somewhat different pictures on the relationship with yield traits from those individual source, sink or transport tissue per se with yield traits. Therefore, the optimizing source to sink ratio should be considered for improving yield potential in the breeding program. Lafitte and Travis (1984) reported that sink to source ratios can be genetically altered for increasing grain yield. Results from this study suggest that transport tissue to source and sink ratios should also be considered in the genetic manipulation of morpho-physiological traits for improving crop yield potential. This suggests that more attention should be paid to the integration of source, sink and transport tissue with whole-system viewpoints, rather than only to individual source, sink or transport tissue. Identification of QTLs controlling source, sink, transport tissues and yield traits will enhance the understanding of the complex interrelationships among them, and will also facilitate the genetic modifications of these characters in order to improve the yield.

Acknowledgements This study was supported in part by a grant from the National Natural Science Foundation of China and a fellowship granted to K.H. Cui by the International Rice Research Institute.

References

Ashraf M, Akbar M, Salim M (1994) Genetic improvement in physiological traits of rice yield. In: Slafer GA (ed) Genetic improvement of field crops. Marcel Dekker Incorporates New York, pp 413–455

Bhagsari AS, Brown RH (1986) Leaf photosynthesis and its correlation with leaf area. Crop Sci 26:127–132

Chau NM, Bhargava SC (1993) Physiological basis of higher productivity in rice. Indian J Plant Physiol 4:215–219

Dingkuhn M, Schnier HF, De Datta SK, Dorffing K, Javellana C (1991) Relationship between ripening-phase productivity and crop duration, canopy photosynthesis and senescence in transplanted and direct-seeded lowland rice. Field Crops Res 26:327–345

Egli DB (1998) Seed biology and the yield of grain crops. CAB International, UK

Ghosh S, Sahai VN, Saran S (1990) Role of flag leaf on grain yield and spikelet sterility in rice cultivars. Oryza 27:87–89

Hageman RH, Leng ER, Dudley JW (1967) A biochemical approach to corn breeding. Adv Agron 19:45–86

Herzog H (1982) Relation of source and sink during the grain-filling period in wheat and some aspects of its regulation. Physiol Plant 56:155–160

Hsu P, Walton PD (1971) Relationships between yield and its components and structures above the flag leaf node in spring wheat. Crop Sci 11:190–193

Kang MS (1998) Using genotype-environment interaction for crop cultivar development. Adv Agron 62:199–252

Kobata T, Sugawara M, Takatu S (2000) Shading during the early grain filling period does not affect potential grain dry matter increase in rice. Agron J 92:411–417

Lafitte HR, Travis RL (1984) Photosynthesis and assimilate partitioning in closely related lines of rice exhibiting different sink: source relationships. Crop Sci 24:447–452

Li J, Wei F, Ding X (1999) Relationship between the vascular bundle system of rachis and rachilla and ear productivity in wheat (in Chinese). Acta Agron Sinica 25:315–319

Li JX, Yu SB, Xu CG, Tan YF, Gao YJ, Li XH, Zhang Q (2000) Analyzing quantitative trait loci for yield using a vegetatively replicated F_2 population from a cross between the parents of an elite rice hybrid. Theor Appl Genet 101:248–254

Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.). Genetics 145:453–465

Li Z, Pinson SRM, Stansel JW, Paterson AH (1998) Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). Mol Breed 4:419–426

Lin HX, Qian HR, Zhuang JY, Lu J, Min SK, Xiong ZM, Huang N, Zheng KL (1996) RFLP mapping of QTLs for yield and related characters in rice (*Oryza sativa* L.). Theor Appl Genet 92:920–927

Lu C, Shen L, Tan Z, Xu Y, He P, Chen Y, Zhu L (1996) Comparative mapping of QTLs for agronomic traits of rice across environments using a doubled-haploid population. Theor Appl Genet 93:1211–1217

McCouch SR, Cho YG, Yano M, Paul E, Blinstruub M (1997) Report on QTL nomenclature. Rice Genet Newslett 14:11–13

Nátrová Z, Nátr L (1993) Limitation of kernel yield by the size of conducting tissue in winter wheat varieties. Field Crops Res 31:121–130

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE. Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127: 181–197

Peterson DM, Housley TL, Luk TM (1982) Oat stem vascular size in relation to kernel number and weight. II. Field environment. Crop Sci 22:274–278

Reynolds MP, Rajaram S, Sayre KD (1999) Physiological and genetic changes of irrigated wheat in the post-green revolution period and approaches for meeting projected global demand. Crop Sci 39:1611–1621

Richards RA (1996) Increasing the yield potential of wheat: manipulating sources and sinks. In: Reynolds MP, Rajaram S, McNab A (eds). Increasing yield potential in wheat: breaking the barriers. International Maize and Wheat Improvement Center, Mexico, pp 134–149

Rutger JN, Francis CA, Grogan CO (1971) Diallel analysis of ear leaf characteristics in maize (*Zea mays* L.). Crop Sci 11:194–195

Veldboom LR, Lee M (1996a) Genetic mapping of quantitative trait loci in maize in stress and nonstress environments. I. Grain yield and yield components. Crop Sci 36:1310–1319

Veldboom LR, Lee M (1996b) Genetic mapping of quantitative trait loci in maize in stress and nonstress environments. II. Plant height and flowering. Crop Sci 36:1320–1327

Venkateswarlu B, Vergara BS, Visperas RM (1987) Influence of photosynthetically active radiation on grain density of rice. Crop Sci 27:1210–1214

Wang DL, Zhu J, Li ZK, Paterson AH (1999) Mapping QTLs with epistatic effects and QTL × environment interactions by mixed linear model approaches. Theor Appl Genet 99:1255–1264

Xiao J, Li J, Yuan L, Tanksley SD (1996) Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. Theor Appl Genet 92:230–244

Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. Theor Appl Genet 105:248–257

Yoshida S (1981) Fundamentals of rice crop science. International Rice Research Institute, Manila, The Philippines

Yoshida S, Parao FT (1976) Climatic influence on yield and yield components of lowland rice in the tropics. In: Climate and rice. International Rice Research Institute, Manila, The Philippines, pp 471–494

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang QF, Saghai Maroof MA (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Zhuang JY, Lin HX, Lu J, Qian HR, Hittalmani S, Huang N, Zheng KL (1997) Analysis of QTL × environment interaction for yield components and plant height in rice. Theor Appl Genet 95:799–808