

# Genetic analysis of grain yield conditioned on its component traits in rice (*Oryza sativa* L.)

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**Abstract.** Grain yield (GY) of rice is a complex trait consisting of several yield components. It is of great importance to reveal the genetic relationships between GY and its yield components at the QTL (quantitative trait loci) level for multi-trait improvement in rice. In the present study, GY per plant in rice and its 3 yield component traits, panicle number per plant (PN), grain number per panicle (GN), and 1000-grain weight (GW), were investigated using a doubled-haploid population derived from a cross of an *indica* variety IR64 and a *japonica* variety Azucena. The phenotypic values collected from 2 cropping seasons were analysed by QTLNetwork 2.0 for mapping QTLs with additive (*a*) and/or additive × environment interaction (*ae*) effects. Furthermore, conditional QTL analysis was conducted to detect QTLs for GY independent of yield components. The results showed that the general genetic variation in GY was largely influenced by GN with the contribution ratio of 29.2%, and PN and GN contributed 10.5% and 74.6% of the genotype × environment interaction variation in GY, respectively. Four QTLs were detected with additive and/or additive × environment interaction effects for GY by the unconditional mapping method. However, for GY conditioned on PN, GN, and GW, 6 additional loci were identified by the conditional mapping method. All of the detected QTLs affecting GY were associated with at least one of the 3 yield components. The results revealed that QTL expressions of GY were contributed differently by 3 yield component traits, and provide valuable information for effectively improving GY in rice.

**Additional keywords:** yield component traits, QTL, conditional mapping.

## Introduction

Grain yield (GY) in cereals is one of the most important and complex traits in plant breeding experiments. Continued improvement of GY remains the top priority in most of the breeding programs (Yan *et al.* 2002). In rice, GY depends on various growth and component traits, and is the final outcome of a combination of different yield components, such as the panicle number per plant, the filled grain number per panicle, and the weight per grain (Yoshida 1983). Therefore, it is of significance to reveal the genetic contribution of yield component traits to GY. Since Donald (1968), many breeders have paid much attention to the concept of plant ideotypes and proposed several models for high-yielding rice, such as the 'heavy-panicle' and the 'multi-panicle' types. It was suggested that an increase in GY could be effectively achieved through yield component improvement since yield components have higher heritability than GY (Xiong 1992). The correlation and path analyses have revealed the relationships between GY and its yield components at both the phenotypic and genetic levels (Xiong 1992). However, the implications in those studies to breeding practice were limited due to complicated correlations between GY and its yield components, which were disturbed to varying degrees by numerous factors such as environmental effects and experimental error etc. (Risch 2000; Darvasi and

Pisanté-Shalom 2002). Selection of yield components was not highly effective in increasing GY, because of their negative correlations to each other (Li *et al.* 1998). Until now, our understanding of the genetic basis of correlation among quantitative traits has remained unresolved.

Recent advances in molecular marker techniques and statistical methods facilitated the analysis of quantitative trait loci (QTLs) (Lander and Botstein 1989; Wang *et al.* 1999). Many QTL mapping experiments for GY and its yield components have been conducted based on the separate phenotypic values (Lin *et al.* 1996; Lehmsiek *et al.* 2006; Musial *et al.* 2006). Some QTLs associated with GY, coinciding with those for yield components, were usually regarded as pleiotropic QTLs or closely linked loci (Lin *et al.* 1996). It was revealed that correlated traits often have QTL(s) at the same chromosomal locations (Albert *et al.* 1991; Paterson *et al.* 1991; Julier *et al.* 2007). Yan *et al.* (1999) reported that pairs of traits with higher genetic correlations would share more common QTL regions than those with smaller genetic correlations. Zhuang *et al.* (1997) suggested that pleiotropism rather than the close linkage of different QTLs might be the major reason for the correlation among related traits. A common problem of these analyses is that QTL mapping for related traits was conducted by considering the phenotypic values rather than conditioning one trait on the other

related traits. However, to condition one trait on the other(s) can provide statistical estimates of the effects and positions of QTLs, and detect their genetic relationships at the QTL level (Zhu 1999; Zhao *et al.* 2006). The genetic basis and relationship of GY with its yield components at the QTL level in rice are still poorly understood and needs to be determined.

Zhu (1995) proposed conditional analysis methods, which can be used to exclude the contribution of a causal trait to the variation of the resultant trait. The remaining variation of the resultant trait is defined as conditional variation, or net variation, which indicates the extra effects of genes that are independent of the causal trait (Atchley and Zhu 1997). This method has been used to study the dynamic behaviour of developmental traits in cotton (Zhu 1995) and mice (Atchley and Zhu 1997). Recently, this method was extended and applied to the analysis of conditional variation of the resultant trait on multiple related traits in cotton (Wu *et al.* 2004; Wen and Zhu 2005). To investigate the genetic relationship between 2 traits at the QTL level, a statistical procedure for analysing conditional genetic effects combined with the QTL mapping method, namely the conditional QTL mapping method, was proposed (Zhu 1999). It could distinguish whether the QTL of the target trait is associated with its component trait or not. The conditional QTL mapping method has been used to study the net QTL effects at different developmental stages of plant height and tiller numbers in rice (Yan *et al.* 1998a, 1998b; Cao *et al.* 2001), and to explore the QTLs contributed to the conditional variation of a resultant trait on its related traits (Guo *et al.* 2005).

In the present study, panicle number per plant (PN), filled grain number per panicle (GN), and 1000-grain weight (GW) in a rice doubled-haploid (DH) population were investigated and their genetic contribution to GY was calculated (Zhu 1995). Then, QTLs for observed and conditional phenotypic values of GY were obtained by the HAB method of QTL mapping (Yang *et al.* 2007). Our objective was to evaluate the genetic influence of variation in yield components on GY, and further to study the genetic relationships between GY and its 3 yield components at the level of an individual QTL by comparing the QTLs obtained by the unconditional mapping method for GY and conditional mapping methods for GY when conditioned on each of 3 yield components.

## Materials and methods

### Plant materials

A population of 129 DH lines developed from the cross between the *indica* variety IR64 and the *japonica* variety Azucena was used in the experiment. IR64 is a semi-dwarf variety carrying the *sd1* gene, Azucena is a traditional upland variety from the Philippines (Causse *et al.* 1994; Huang *et al.* 1994), and their DH lines were derived from F<sub>1</sub> anther culture following Guiderdoni *et al.* (1992). The seeds of all materials were provided by the International Rice Research Institution (IRRI), the Philippines. In previous studies, 6 restriction enzymes (*DraI*, *EcoRV*, *HindIII*, *ScaI*, *XbaI*, and *EcoRI*) were used for a parental polymorphism survey, and 175 markers comprising 146 RFLP, 3 isozymes, 14 RAPD, and 12 cloned genes were selected (Causse *et al.* 1994; Huang *et al.* 1994). The linkage map established by Huang *et al.* (1994), covering all 12 rice chromosomes of

2005 cM with an average spacing of 11.5 cM, was used for QTL analyses.

### Field experiments and trait evaluations

The field experiments were conducted at the experimental farm of the South China Agricultural University, Guangzhou, China (approx. 113°E, 23°N). The experiments were performed in 2 growing seasons, spring (March–August, denoted E1) and fall (August–December, denoted E2), in the year 2002. These 2 growing seasons are different in climate, especially day length. The same set of DH lines and the 2 parents were evaluated in each of the 2 growing seasons by randomised complete block design with 2 replications. The germinated seeds were sown in a seedling bed, and then seedlings were transplanted to a paddy field 30 days later, with a single plant per hill spaced at 0.3 m by 0.2 m. Each plot consisted of four 3-m-long rows with 16 plants. Ten plants in the middle of the inner 2 rows of each plot were investigated to score the following traits for each plant: panicle number (PN, the number of panicles at maturity), grain number (GN, the number of filled grains per main panicle at maturity), and 1000-grain weight (GW, weight in g of 1000 grains from bulk homogenised seeds at 12–14% moisture). Then the grain yield per plant (GY, weight in g of total filled grains of each plant) was calculated by  $GY = (PN \times GN \times GW)/1000$ . The average measurements over 10 individuals within a plot for each of the selected traits were used in the data analysis.

### Genetic model and conditional analysis

The genetic model for agronomic traits with genotype  $\times$  environment (GE) interaction effects (Zhu 1994) was used to study the inheritance of GY and its 3 yield components. Unconditional genetic analysis was conducted based on the phenotypic values ( $y$ ) for each trait, which can be partitioned by the following mixed linear model:

$$\begin{aligned} y &= \mathbf{1}\mu + \mathbf{U}_E \mathbf{e}_E + \mathbf{U}_G \mathbf{e}_G + \mathbf{U}_{GE} \mathbf{e}_{GE} + \mathbf{U}_B \mathbf{e}_B + \mathbf{e}_e \\ &= \mathbf{1}\mu + \sum_{u=1}^5 \mathbf{U}_u \mathbf{e}_u \end{aligned} \quad (1)$$

where  $\mu$  is the fixed population mean;  $\mathbf{1}$  is a vector with all elements equal to 1;  $\mathbf{e}_u$  is the  $u$ th vector of random effects,  $\mathbf{e}_u \sim N(\mathbf{0}, \sigma_u^2 \mathbf{I})$  ( $\mathbf{I}$  is an identity matrix);  $\mathbf{U}_u$  is the  $u$ th known design matrix for  $\mathbf{e}_u$ ; and the capital letter subscript, i.e.  $E$ ,  $G$ ,  $GE$ , and  $B$ , represents the environmental, general genotypic, GE interaction, and the block effects, respectively. Conditional genetic analysis was conducted based on the phenotypic values of GY conditioned on each yield component trait, which were obtained by the method described by Zhu (1995). The conditional phenotypic values of GY were partitioned by the following mixed linear model (Zhu 1994):

$$y_{(GY|YC)} = \mathbf{1}\mu_{(GY|YC)} + \sum_{u=1}^5 \mathbf{U}_u \mathbf{e}_{u(GY|YC)} \quad (2)$$

where  $(GY|YC)$  denotes GY conditioned on one yield component; and  $y_{(GY|YC)}$  indicates the vector of conditional values of GY without the influence of one yield component trait. The definition of the remaining parameters and variables in Eqn 2 is same as those in Eqn 1. Both the unconditional

and conditional variance components were estimated by a minimum norm quadratic unbiased estimation (MINQUE) method in which all prior values were set to 1.0 (Zhu 1994). Then the total heritability ( $h_{G+GE}^2 = (V_G + V_{GE})/V_P$ ) was partitioned into general genetic heritability ( $h_G^2 = V_G/V_P$ ) and the interaction heritability ( $h_{GE}^2 = V_{GE}/V_P$ ) (Zhu 1994). In addition, the contribution ratio (CR) of a casual trait (C) to a target trait (T) was calculated by:  $CR_{C \rightarrow T} = 1.0 - V_{(T|C)}/V_{(T)}$  (where  $V_{(T|C)}$ ,  $V_{(T)}$  are the conditional and the unconditional variance, respectively). The Jack-knife re-sampling technique (Miller 1974) was applied to calculate the *t*-statistic for testing the significance of each of the parameters involved in the model. All of the aforementioned analyses were performed by the software QGASStation (Chen and Zhu 2003).

### QTL mapping

A newly developed QTL mapping method (Yang *et al.* 2007) was used to detect QTLs with additive and/or additive  $\times$  environment interaction effects. One-dimensional genome scan procedure was conducted to generate *F*-statistic profiles. The critical *F*-value to declare putative QTLs and to control the genome-wide type-I error was accommodated by 1000 permutation tests. When the *F*-values for a region exceed a pre-defined critical threshold value for type I error rates of 0.05, a QTL is indicated at that position with the regional maximum *F*-value. Both the observed phenotypic values ( $y_{(GY)}$ ) and the conditional phenotypic values ( $y_{(GY|YC)}$ ) were used for QTL mapping analyses. The QTLs identified from the observed phenotypic data were named as unconditional QTLs, and the QTLs detected for the conditional phenotypic values were defined as conditional QTLs attributed to the net genetic variation of GY independent of yield components. The analyses for both the unconditional and conditional QTL mapping were performed by the software QTLNetwork 2.0 (Yang *et al.* 2007).

## Results

### Trait performance

The average performance and other descriptive statistics of the 2 parents and the DH lines in 2 growing seasons for GY and its 3 yield components are summarised in Table 1. The results revealed that IR64 showed greater PN and GY but smaller GN

and GW than Azucena. The phenotypic performance of these traits coincided with the ideotypes of the 2 parents, namely IR64 is the ‘multi-panicle’ type while Azucena is the ‘large-panicle’ type (Guiderdoni *et al.* 1992). These results were also in agreement with those of Courtois *et al.* (1995) and Bagali (1997). No matter how large or small the trait difference between 2 parental lines, the variation of each trait in the DH population was large and continuous. The standard deviation (s.d.) ranged from 2.7 (in E2 for PN) to 37.5 (in E2 for GN). Significant transgressive segregations were observed for all traits examined, which might be attributed to the association of all the alleles of similar effects, either positive or negative, at the multiple QTLs. Environmental effects were also apparent for all 4 traits studied. The values of PN were larger but the other traits were smaller in E1 than in E2, with the exception that Azucena had larger GY in E1 than in E2.

### Components of variances and heritabilities

Table 2 compares the estimates of genetic variance and heritability components of GY and its 3 component traits. The PN and GW explained relatively large general heritabilities, general genetic variances accounting for ~43.3% and 40.6% of their phenotypic variances, respectively. Grain yield had the smallest general genetic heritability among the 4 traits investigated. The heritability of GY for GE interaction was larger compared with the 3 component traits. Among the 3 component traits of PN, GN, and GW, the GE interaction variances explained 0.8%, 2.3%, and 0.3% of their phenotypic variations, respectively. It was observed that GY and its yield components were mainly controlled by general genetic effects, but not much influenced by GE interaction effects. The result revealed that the inheritance of these traits was stable across environments.

### Contribution ratios for each yield component to GY

Contribution ratios (CR) of 5 factors (phenotypic, environmental, general genetic, genotype  $\times$  environment interaction, and residual effect) for each yield component to GY in rice are presented in Table 3. Variations of all 5 factors in GY were associated with the phenotypic variation of yield components. In the phenotypic variation of GY, the

**Table 1. Phenotypic values of 2 parents and the DH population in 2 growing seasons for GY and its yield components in rice**

GY, PN, GN, and GW indicate grain yield per plant in rice and its 3 component traits (panicle number per plant, grain number per panicle, and 1000-grain weight), respectively. E1 and E2 represent the spring season and the fall season, respectively. s.d., Standard deviation

Trait	Environment	Parent		The DH population			
		IR64	Azucena	Mean	s.d.	Min.	Max.
PN	E1	15.7	6.2	11.8	3.0	5.5	21.4
	E2	13.1	5.2	10.0	2.7	1.8	18.7
GN	E1	83.3	153.6	71.1	36.1	0.3	160.5
	E2	102.1	160.5	107.4	37.5	27.0	225.2
GW	E1	23.3	27.4	23.5	3.2	13.0	32.7
	E2	25.3	29.9	25.1	3.2	17.8	34.9
GY	E1	30.4	26.1	19.8	10.2	0.2	48.7
	E2	33.8	24.9	27.0	8.3	5.4	54.4

**Table 2. Estimated variance and heritability components of grain yield and its components**

GY, PN, GN, and GW indicate grain yield per plant in rice and its 3 component traits (panicle number per plant, grain number per panicle, and 1000-grain weight), respectively. \* and \*\* indicate that the parameters are significantly different from zero at  $P = 0.05$  and  $0.01$ , respectively.  $V_G$  and  $V_{GE}$  are the general genetic variance and GE interaction genetic variance, respectively.  $h_G^2$  and  $h_{GE}^2$  are the general heritability and the GE interaction heritability, respectively

Parameters	PN	GN	GW	GY
$V_G$	3.936**	482.247**	4.425*	14.982**
$V_{GE}$	0.075	39.388	0.027	4.679*
$h_G^2$	0.433**	0.287**	0.406**	0.160**
$h_{GE}^2$	0.008	0.023	0.003	0.050

**Table 3. Estimated contribution ratios of each yield component to GY**  
 $CR_P$ ,  $CR_E$ ,  $CR_G$ ,  $CR_{GE}$ , and  $CR_e$  are the contribution ratios of a given yield component to phenotypic, environmental, general genetic, GE interaction, and error effects on GY, respectively. PN, GN, and GW indicate the given yield components (panicle number per plant, filled grain number per main panicle, and 1000-grain weight), respectively

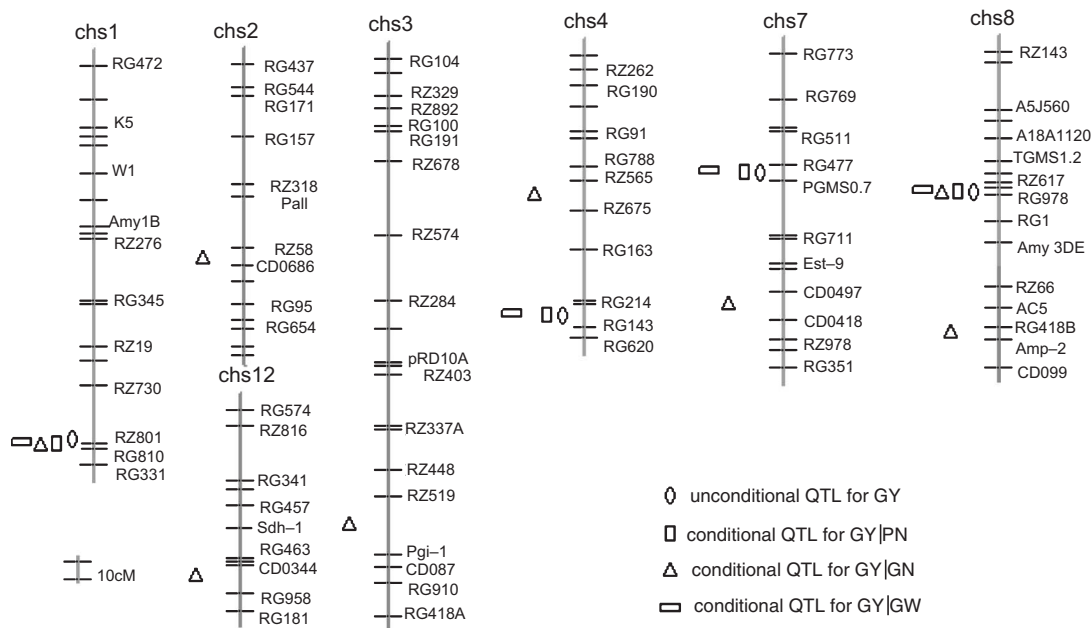
Yield components	$CR_P$	$CR_E$	$CR_G$	$CR_{GE}$	$CR_e$
PN	0.042	-0.498	0.048	0.105	0.104
GN	0.610	0.981	0.292	0.746	0.626
GW	0.011	0.164	-0.089	0.001	0.015

3 yield components of PN, GN, and GW contributed 4.2%, 61.0%, and 1.1%, respectively. The GY variation derived from environmental factors contributed by the phenotypic variation of GN and GW was 98.1% and 16.4%, respectively. The

environmental variation of GY was decreased by 49.8% due to the phenotypic variation of PN. It implied that environmental factors might cause the variation in PN, which then restrained the variation of GY. Correspondingly, 4.8% and 29.2% of the general genetic variation of GY was associated with the phenotypic variation of PN and GN. Since the contribution ratio of GW to GY was negative, the general genetic variation in GY would increase when excluding the influence of GW. It implied that the expression of genes on GY might be constrained by the phenotypic variation of GW. The phenotypic variations of the 3 yield components contributed partially to the GE interaction variation in GY, but the contributions of GN to GY were largest in magnitude among them. So it is suggested that improving GY with the ‘large-panicle’ type could be effective in special environments. In addition, the error in GY largely originated from the phenotypic variations of PN and GN, which accounted for 10.4% and 62.6% of the total error variation of GY, respectively. It was thus suggested that reducing the variations of PN and GN would enhance the accuracy of genetic analysis in GY.

*Unconditional and conditional QTLs in GY*

In total, 10 QTLs affecting GY were detected on chromosomes 1, 2, 3, 4, 7, 8, and 12 (Fig. 1). The estimated additive effects ( $a$ ) and predicted additive  $\times$  environment interaction effects ( $ae$ ) of the detected QTLs are listed in Table 4. The results indicated that chromosomes 4, 7, and 8 had 2 QTLs each. Each of the remaining chromosomes (1, 2, 3, and 12) had only one QTL (Fig. 1). Of these 10 QTLs, only 4 QTLs ( $I-15$ ,  $4-12$ ,  $7-5$ , and  $8-9$ ) were detected by the unconditional QTL mapping method, and the conditional QTL mapping method identified all



**Fig. 1.** Genome locations of unconditional and conditional QTLs detected for GY in rice. GY, GY|PN, GY|GN, and GY|GW indicate grain yield in rice and grain yield conditioned on one of 3 yield components (panicle number per plant, filled grain number per main panicle, and 1000-grain weight), respectively.

**Table 4. Unconditional and conditional QTLs with significant *a* effects and/or *ae* effects for GY in rice**  
 QTLs are nominated with the chromosomal number and the serial number of the marker interval on the chromosome. GY, GY|PN, GY|GN, and GY|GW indicate grain yield in rice and grain yield conditioned on one of 3 yield components (panicle number per plant, filled grain number per main panicle, and 1000-grain weight), respectively. *a* and *ae* are the additive and the additive × environment interaction effects. All estimated values were significant at the level of  $P < 0.05$ . The signs of QTL effects indicate the directions of IR64 alleles affecting GY. Numerals in parentheses are the contribution ratio of the corresponding QTL effect

QTL	GY		GY PN		GY GN		GY GW	
	<i>a</i>	<i>ae</i>	<i>a</i>	<i>ae</i>	<i>a</i>	<i>ae</i>	<i>a</i>	<i>ae</i>
1–15	2.852 (0.062)		1.757 (0.033)		1.202 (0.031)		3.292 (0.090)	
2–7					–1.604 (0.037)			
3–17					1.350 (0.034)			
4–8					1.164 (0.019)			
4–12	–2.050 (0.050)		–2.578 (0.058)		–		–2.131 (0.054)	
7–5	–0.769 (0.008)	–1.690 (0.030)	–0.820 (0.009)	–1.535 (0.027)	–	–	–0.809 (0.009)	–1.710 (0.031)
7–11					0.720 (0.011)	–0.753 (0.018)		
8–9	–2.429 (0.058)		–2.065 (0.051)		–1.375 (0.036)		–2.266 (0.056)	
8–15					1.348 (0.031)			
12–9					1.173 (0.023)			

QTLs (Fig. 1 and Table 4). The number of QTLs controlling GY conditioned on 3 component traits was different for each of the yield components. Four unconditional QTLs affecting GY were also detected when conditioned on PN or GW. Conditioned on GN, however, 2 unconditional QTLs (4–12 and 7–5) were not identified again, but 6 extra QTLs were detected for the first time. A total number of 8 QTLs, therefore, were defined conditioned on GN (Fig. 1 and Table 4). Comparing the effect difference of an unconditional QTL and a conditional QTL, one can find whether or not the QTLs for GY were associated with their yield components. When the effect of a conditional QTL is largely changed or unable to be detected again, the QTL is considered to be partially or completely contributed by the given/conditioned yield component trait. When a conditional QTL has a similar effect to its unconditional QTL, it demonstrates that the QTL for the unconditional trait (GY) is independent of its conditioned trait (yield component). In Table 4, the unconditional QTLs 4–12 and 7–5 controlling GY failed to be detected when GY was conditioned on GN, suggesting that the expression of these 2 QTLs is completely associated with the variation in GN. But these 2 QTLs were detected again with similar effects when given PN or GW; they thus were considered to be independent of PN and GW. Similarly, QTL 8–9 was independent of PN and GW, but was partially contributed by GN since the conditional and the unconditional effects of the QTL were largely different. QTL 1–15 for GY was detected with different effects conditioned on each of the 3 yield component traits compared with the unconditional QTL. It thus was considered to be partially associated with the variation of all 3 component traits.

Six additional QTLs (2–7, 3–17, 4–8, 7–11, 8–15, and 12–9) associated with GY, which failed to be detected in the unconditional mapping, were identified with significant effects when conditioned on GN. It was thus suggested that expressions of these QTLs for GY were completely suppressed by the trait GN, and their effects could only be revealed when the influence of the variation of GN was removed.

## Discussion

Grain yield in rice is a complex trait, which is the combination of different yield components (Yoshida 1983). The yield component traits, however, are less environmentally sensitive and have higher heritabilities than grain yield (Yano and Sasaki 1997). In the present study, the estimated general genetic heritabilities of PN, GN, and GW were 43.3%, 28.7%, and 40.6%, respectively; while only 16.0% in GY (Table 2). Therefore, yield components are often treated as indirect traits for improving grain yield. To dissect the complex relationship and to reveal the net contribution of one trait to another, the conditional analysis approach was proposed by Zhu (1995). This method can effectively analyse the dependencies among variables by estimating conditional variances after removing their causal influence. Using this method, we explored the net contribution of each yield component to the yield trait (GY). It was observed that the contribution of GN was the largest among the 3 yield components, and various types of variations of GY were mostly due to the phenotypic variation of GN. It suggests that GY can be effectively improved by enhancing the grain

number per panicle (GN) in rice plants. Meanwhile, reducing the variation in GN would enhance the accuracy of genetic analysis in GY.

In the classical Mendelian approach, it was very difficult to identify the individual genes controlling a quantitative trait (Comstock 1978). Recent advances in QTL mapping facilitated the analysis of the genetic basis of quantitative traits at the single-locus level (Lander and Botstein 1989; Zeng 1994, 2005; Zeng and Weir 1996; Yang *et al.* 2007). QTLs for rice yield and its components have been reported (Champoux *et al.* 1995; Courtois *et al.* 1995; Xing *et al.* 2002), and pleiotropy or close linkage of QTLs has been assumed as the basis of relationships among them (Paterson *et al.* 1991; Zhuang *et al.* 1997; Julier *et al.* 2007). However, a common problem associated with the QTL mapping analyses regarding yield and its components reported so far was based on the separate analysis of each trait. There is no obvious evidence that pleiotropy or close linkage of QTLs would result in genetic correlation among the traits of interest. It is nonetheless impossible to reveal the complex genetic basis of trait correlation by these methods (Zhu 1995; Guo *et al.* 2005). To effectively identify the influence of one trait on another trait at the QTL level, the conditional genetic analysis approach (Zhu 1995) combined with the QTL mapping method was proposed (Zhu 1999). Guo *et al.* (2005) applied this method to identify the QTLs for yield in rice with different component influences, but the QE effects were ignored due to the data being derived only from one environment in their study. The present investigation revealed several QTLs, i.e. the unconditional and conditional QTLs, independent, partial, and completely correlated QTLs associated with GY. Any QTLs for GY detected in this study were associated with at least one of the 3 component traits. It further demonstrates that application of unconditional and conditional mapping methods could reflect the net contribution of each of the causal traits to the resultant trait at the QTL level, which might be a useful way to improve the desirable (resultant) traits at the QTL level important for breeding.

Understanding expression patterns of QTLs is one of the major goals in quantitative genetics. According to the theory of developmental genetics, genes are expressed differently at different times and growth stages (Atchley and Zhu 1997). Many studies indicated that the expression of QTLs is affected by many factors, such as environments (Zhuang *et al.* 1997), genetic background (Li *et al.* 1998; Xing *et al.* 2002), developmental stages (Yan *et al.* 1998a, 1998b; Cao *et al.* 2001), and related traits (Guo *et al.* 2005; Zhao *et al.* 2006). According to expression patterns, QTLs can be classified into at least 5 types, namely non-specific, environment-specific, genotype-specific, stage-specific and trait-specific QTLs. Non-specific QTLs are the most stable since their expression is not influenced by inner and external factors, and they can be used under various conditions. The other 4 types of QTLs are unstable, and are suitable only under specific conditions. In this study, we found that 2 QTLs (7-5 and 7-11) were environment-specific since they had *ae* effects. One QTL (1-15) was found to be regulated by all 3 component traits, and the remaining 9 QTLs were regulated by GN. So these QTLs can be suggested as trait-specific, and might be suitable only in this population for marker-assistant selection and QTL cloning. Their expressions would be different if another population with different yield component

traits was used. Obviously, the genetic basis of quantitative traits is extraordinarily complex, and is the comprehensive effect of various types of QTLs on a trait. This perhaps is one explanation for why QTLs of a trait could be detected differently by using various environments, experimental materials, development stages, and mapping populations. In breeding practice, selections should be applied especially with non-specific QTLs due to their stable expression. It is also preferable to select the superior gene or trait combination rather than the single gene or trait due to the interactions between loci or traits.

Comparing both the unconditional and conditional QTL mapping methods, some new QTLs controlling the target trait could be detected by conditional QTL mapping (Yan *et al.* 1998a, 1998b; Cao *et al.* 2001; Guo *et al.* 2005). In this study, conditional QTL mapping detected 6 new QTLs, which failed to be detected by unconditional QTL mapping, and the total number of QTLs detected for GY was larger than that detected only by the unconditional mapping method. Using the same mapping population as this study, we had a comprehensive work based on phenotypic evaluation and detailed GE analysis for 11 traits (Hittalmani *et al.* 2003). Although it was conducted at 9 different locations across 4 countries in Asia, only 3 GY QTLs were identified by the unconditional mapping method, and their expressions were suggested to be environment-specific. In the given study, we detected 10 GY QTLs by the method of unconditional mapping combined with conditional mapping based on data only in 2 different seasons at one location, and 2 of the QTLs were identified to be environment-specific. In addition, conditional QTL mapping may be helpful to distinguish the pleiotropic effect or gene linkage for QTLs that contribute to both traits in the same genomic intervals. For example, QTL 1-15 might be pleiotropic since it controls GY and 3 yield components simultaneously. Generally, 2 close-vicinity QTLs for 2 traits may be regarded as a pleiotropic QTL if their effects change due to conditional mapping. Conversely, they are more likely to be closely linked QTLs if the effects of unconditional and conditional QTLs coincide.

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## References

- Albert BSB, Edwards MD, Stuber CW (1991) Isoenzymatic identification of quantitative trait loci in crosses of elite maize inbreds. *Crop Science* **31**, 267–274.
- Atchley WR, Zhu J (1997) Developmental quantitative genetics, conditional epigenetic variability and growth in mice. *Genetics* **147**, 765–776.
- Bagali PG (1997) RFLP mapping of quantitative trait loci controlling yield related traits and resistance to leaf blast disease in rice (*Oryza sativa* L.). M.Sc.(Agric.) thesis, University of Agricultural Sciences, Bangalore, India.
- Cao GQ, Zhu J, He CX, Gao YM, Yan JQ, Wu P (2001) Impacts of epistasis and QTL  $\times$  environment interaction for developmental behavior of plant height in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **103**, 153–160. doi: 10.1007/s001220100536
- Causse MA, Fulton TM, Cho YG, Ahn SN, Chunwongse J, *et al.* (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* **138**, 1251–1274.

- Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. *Theoretical and Applied Genetics* **90**, 969–981. doi: 10.1007/BF00222910
- Chen GB, Zhu J (2003) Software for the classical quantitative genetics. Institute of Bioinformatics, Zhejiang University, Hangzhou, China. URL: <http://ibi.zju.edu.cn/software/qga/index.htm>
- Comstock RE (1978) Quantitative genetics in maize breeding. In 'Maize breeding and genetics'. pp. 191–206. (Wiley-Interscience: New York)
- Courtois B, Huang N, Guiderdoni E (1995) RFLP mapping of genes controlling yield components and plant height in an indica × japonica doubled haploid population. In 'Proceedings of the International Rice Research Conference on Fragile Lives in Fragile Ecosystems'. pp. 963–976. (International Rice Research Institute Publishing: Los Baños, The Philippines)
- Darvasi A, Pisanté-Shalom A (2002) Complexities in the genetic dissection of quantitative trait loci. *Trends in Genetics* **18**, 489–491. doi: 10.1016/S0168-9525(02)02767-1
- Donald CM (1968) The breeding of crop ideotypes. *Euphytica* **17**, 385–403. doi: 10.1007/BF00056241
- Guiderdoni E, Galinato E, Luistro J, Vergara G (1992) Anther culture of tropical japonica × indica hybrids of rice (*Oryza sativa* L.). *Euphytica* **62**, 219–224. doi: 10.1007/BF00041756
- Guo LB, Xing YZ, Mei HW, Xu CG, Shi CH, Wu P, Luo LJ (2005) Dissection of component QTL expression in yield formation in rice. *Plant Breeding* **124**, 127–132. doi: 10.1111/j.1439-0523.2005.01093.x
- Hittalmani S, Huang N, Courtois B, Venuprasad R, Shashidhar HE, et al. (2003) Identification of QTL for growth- and grain yield-related traits in rice across nine locations of Asia. *Theoretical and Applied Genetics* **107**, 679–690. doi: 10.1007/s00122-003-1269-1
- Huang N, McCouch SR, Mew T, Parco A, Guiderdoni E (1994) Development of an RFLP map from a doubled haploid population in rice. *Rice Genetics Newsletter* **11**, 134–137.
- Julier B, Huguet T, Chardon F, Ayadi R, Pierre JB, Prosperi JM, Barre P, Huyghe C (2007) Identification of quantitative trait loci influencing aerial morphogenesis in the model legume *Medicago truncatula*. *Theoretical and Applied Genetics* **114**, 1391–1406. doi: 10.1007/s00122-007-0525-1
- Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.
- Lehmensiek A, Eckermann PJ, Verbyla AP, Appels R, Sutherland MW, Martin D, Daggard GE (2006) Flour yield QTLs in three Australian doubled haploid wheat populations. *Australian Journal of Agricultural Research* **57**, 1115–1122. doi: 10.1071/AR05375
- Li ZK, Pinson SRM, Stansel JW, Paterson AH (1998) Genetic dissection of the source-sink relationship affecting fecundity and yield in rice. *Molecular Breeding* **4**, 419–426. doi: 10.1023/A:1009608128785
- Lin HX, Qian HR, Zhuang JY, Min SK, Xiong ZM, Huang N, Zheng KL (1996) RFLP mapping of QTLs for yield and related characters in rice. *Theoretical and Applied Genetics* **92**, 920–927. doi: 10.1007/BF00224031
- Miller RG (1974) The Jackknife: a review. *Biometrika* **61**, 1–15.
- Musial JM, Lowe KF, Mackie JM, Aitken KS, Irwin JAG (2006) DNA markers linked to yield, yield components, and morphological traits in autotetraploid lucerne (*Medicago sativa* L.). *Australian Journal of Agricultural Research* **57**, 801–810. doi: 10.1071/AR05390
- Paterson AH, Deverna JW, Lanini B, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. *Genetics* **127**, 181–197.
- Risch NJ (2000) Searching for genetic determinants in the new millennium. *Nature* **405**, 847–856. doi: 10.1038/35015718
- Wang DL, Zhu J, Li ZK, Paterson AH (1999) Mapping QTLs with epistatic effects and QTLs by environment interaction by mixed linear model approaches. *Theoretical and Applied Genetics* **99**, 1255–1264. doi: 10.1007/s001220051331
- Wen YX, Zhu J (2005) Multivariable conditional analysis for complex trait and its components. *Acta Genetica Sinica* **82**, 289–296.
- Wu JX, Jenkins JN, McCarty JC, Zhu J (2004) Genetic association of yield with its component traits in a recombinant inbred line population of cotton. *Euphytica* **140**, 171–179. doi: 10.1007/s10681-004-2897-5
- Xing Y, Tan YF, Hua JP, Sun XL, Xu CG, Zhang QF (2002) Characterization of the main effects, epistatic effects and their environmental interactions of QTLs on the genetic basis of yield traits in rice. *Theoretical and Applied Genetics* **105**, 248–257. doi: 10.1007/s00122-002-0952-y
- Xiong ZM (1992) Research outline on rice genetics in China. In 'Rice in China'. (Eds ZM Xiong, HF Cai) pp. 40–57. (Chinese Agricultural Science Press: Beijing)
- Yan JQ, Zhu J, He C, Benmoussa M, Wu P (1999) Molecular marker-assisted dissection of genotype × environment interaction for plant type traits in rice (*Oryza sativa* L.). *Crop Science* **39**, 538–544.
- Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1998a) Molecular dissection of developmental behavior of plant height in rice (*Oryza sativa* L.). *Genetics* **150**, 1257–1265.
- Yan JQ, Zhu J, He CX, Benmoussa M, Wu P (1998b) Quantitative trait loci analysis for the developmental behavior of tiller number in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics* **97**, 267–274. doi: 10.1007/s001220050895
- Yan W, Hunt LA, Johnson P, Stewart G, Lu X (2002) On-farm strip trials vs replicated performance trials for cultivar evaluation. *Crop Science* **42**, 385–392.
- Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. *Bioinformatics* **23**, 1527–1536. doi: 10.1093/bioinformatics/btm143
- Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. *Plant Molecular Biology* **35**, 145–153. doi: 10.1023/A:1005764209331
- Yoshida S (1983) Rice. In 'Potential productivity of field crops under different environments'. (Eds WH Smith, SJ Banta) pp. 103–127. (International Rice Research Institute Publishing: Los Baños, The Philippines)
- Zeng ZB (1994) Precision mapping of quantitative trait loci. *Genetics* **136**, 1457–1468.
- Zeng ZB (2005) QTL mapping and the genetic basis of adaptation: recent developments. *Genetica* **123**, 25–37. doi: 10.1007/s10709-004-2705-0
- Zeng ZB, Weir BS (1996) Statistical methods for mapping quantitative trait loci. *Acta Agronomica Sinica* **22**, 535–549.
- Zhao JY, Becker HC, Zhang DQ (2006) Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. *Theoretical and Applied Genetics* **113**, 33–38. doi: 10.1007/s00122-006-0267-5
- Zhu J (1994) General genetic models and new analysis methods for quantitative traits (Chinese). *Journal of Zhejiang Agricultural University* **20**, 551–559.
- Zhu J (1995) Analysis of conditional genetic effects and variance components in developmental genetics. *Genetics* **141**, 1633–1639.
- Zhu J (1999) Mixed model approaches of mapping genes for complex quantitative traits. *Journal of Zhejiang University (Natural Science)* **33**, 327–335.
- 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. *Theoretical and Applied Genetics* **95**, 799–808. doi: 10.1007/s001220050628

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