

Full Length Research Paper

Detection of elevated CO₂ responsive QTLs for yield and its components in rice

Guizhi Fan^{1,2}, Xiaocan Li², Qingsheng Cai^{1*} and Jianguo Zhu³

¹College of Life Sciences, Nanjing Agricultural University, Nanjing 210095, China.

²College of Life Sciences, Northeast Forestry University, Harbin 150040, China.

³Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China.

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A mapping population of IR24 (*indica*) chromosome segment substitution lines (CSSLs) in Asominori (*japonica*) background was used to detect quantitative trait loci (QTLs) for response to elevated CO₂ in yield and its components of rice in free air CO₂ enrichment (FACE, atmospheric CO₂ plus 200 μmol CO₂·mol⁻¹) and natural atmospheric CO₂ (Ambient) conditions. Transgressive segregation and continuous distribution in the CSSLs were observed for elevated CO₂ response values (the value in FACE minus that in Ambient), in panicle number per plant (PN), grain number per panicle (GN), 1000 grain weight (GW) and yield per plant (YD), suggesting that all the tested traits responding to elevated CO₂ were quantitatively inherited. Three (*qYD-1*, *qYD-10*, *qYD-12*), two (*qPN-5*, *qPN-10*) and one (*qGN-1*) QTLs were detected for CO₂ response to YD, PN and GN, respectively, but non-QTL for GW. Interestingly, both *qYD-1* and *qGN-1* were located at the same marker interval of C112-C2340, on chromosome 1, and had all positive response values from IR24. The results might be useful for understanding the genetic basis for responding to elevated CO₂ and breeding new rice varieties adapted to the higher atmospheric CO₂ environment in the future.

Key words: Free air CO₂ enrichment (FACE), quantitative trait loci (QTLs), rice (*Oryza Sativa* L.), yield and its component.

INTRODUCTION

Increasing atmospheric CO₂ will bring about seriously global warming, which is expected to affect crop production. Thus, determining the genetic basis for response to elevated CO₂ in rice is very meaningful work for breeding new rice varieties adaptable to higher CO₂ concentration environment in future. Free air CO₂ enrichment (FACE) facilities are presently considered to be the best establishment for manipulating atmospheric CO₂ concentration around plants growing under otherwise natural field conditions (Kobayashi, 2001; Holtum and Winte, 2003). Such system also has become an integral tool for studying the effects of increasing CO₂ concentration on the growth and development of plants. The enhancement of growth and yields by increasing CO₂ in the atmosphere has been reported in many species, including in rice

(Drake, and Gonzalez-Meler, 1997; Kimball et al., 2002; Kim et al., 2003; Yang et al., 2006). Though there were several reports on the use of molecular markers to map major genes or quantitative trait loci (QTLs) associated with yield and its components in rice under various natural conditions (Boonjung and Fukai, 1996; Moncada et al., 2001; Venuprasad et al., 2002; Hittalmani et al., 2003; Lanceras et al., 2004; Zhang et al., 2004), however, to the best of our knowledge, mapping of QTLs associated with elevated CO₂ response in rice has not been conducted yet. This is the first report on the identification of QTLs for elevated CO₂ responses with regard to yield and its components and their chromosomal localization on the molecular map of rice.

MATERIALS AND METHODS

Plant materials

The chromosome segment substitution lines (CSSLs), kindly pro-

*Corresponding author. E-mail: qscail@njau.edu.cn. Tel: +86-25-84395187. Fax: +86-25-84396542.

vided by Professor A. Yoshimura of Agricultural faculty of Kyushu University, Japan, was used in this study. A mapping population of 65 indica CSSLs in japonica background, derived from a cross between a japonica variety Asominori and an indica variety IR24 by the single seed descent, backcrossing and marked-assisted selection (Kubo et al., 2002), which have been used to detect QTL for resistance to ferrous iron toxicity and for grain dimension under different environments (Wan et al., 2003; Wan et al., 2005). The chromosome segments of IR24 in the CSSLs distributed among the whole Asominori genome.

Experimental design and field conditions

The experiments were conducted in China FACE Station (31°37' N, 120°28' E), Jiangsu province, China. The seeds of all 65 CSSLs together with their parents, Asominori and IR24, were sown in nursery seedling bed on May 18, 2004. The seedlings (3 plants per hill) at 30d after sowing, were transplanted into five control experiment plots with natural CO₂ level (Ambient, about 370 $\mu\text{mol}\cdot\text{mol}^{-1}$), and three FACE rings maintaining about 570 $\mu\text{mol}\cdot\text{mol}^{-1}$ CO₂ level, respectively. For each ambient plot or each FACE ring, a three-row-hill for each CSSL line and six-row-hill for each parent were transplanted at density of 16.7 × 25 cm. Each FACE ring is 12.5 m in diameter with pure CO₂ automatic injection for 24 h every day during rice-cropping season. Wind direction, wind speed and CO₂ concentration were monitored at the center of each ring. These data were used in a computer-controlled system, which maintained CO₂ concentration in FACE ring at about 200 $\mu\text{mol}\cdot\text{mol}^{-1}$ above Ambient. In addition, a safeguard plot at 1 m apart of inner ring was set up in each FACE ring. The other field managements in FACE were similar to ambient experiments according to the conventional practice for rice production.

Nine plants from each plot were harvested at 45d after heading in three or five replicates. After natural drying, grain yield per plant (YD) and its components, including panicle number per plant (PN), 1000 grain weight (GW), and grain number per panicle (GN), was surveyed according to the slightly modified method described by Xiao et al. (1998). Elevated CO₂ response values for studied traits were calculated as the value in FACE minus the value in Ambient. Those average values of each CSSL for the tested traits were used for QTL analyses.

Genetic mapping and QTL detection

The molecular linkage map covering 1275.4 cM with an average marker interval of 14.7 cM was constructed with 85 RFLP markers from the same CSSLs (Wan et al., 2002). QTL analysis was conducted with Windows QTL Cartographer 1.13a. A locus with a LOD threshold value of more than 2.0 was to be declared a putative QTL. In addition, the QTL effects were evaluated by a t-test to test the presence of significant differences between the phenotypic values of Asominori and those of CSSLs harboring QTL alleles derived from IR24. The QTLs were named according to the suggestions of McCouch et al. (1997).

RESULTS

Frequency distribution of elevated CO₂ response values for yield and its components

The elevated CO₂ response values of both parents, (Asominori, IR24), and the frequency distributions of for YD, GN, GW, and PN are listed in Figure 1. It was noted

that the two parents showed positive response values for all the tested traits. Among them, Asominori displayed much stronger response to GN than IR24. On the other hand, IR24 had a little higher response to PN than Asominori. All the positive responses verified that elevated CO₂ level are favorable for increasing grain yield and its components for both Asominori and IR24. In addition, continuous variation of elevated CO₂ response values for the four traits and transgressive segregation in both parental directions were observed, indicating that the studied traits; YD, GN, GW, and PN, responding to elevate CO₂, were quantitatively inherited in the CSSL population.

QTLs for elevated CO₂ response values of yield and yield components

Only one elevated CO₂ responsive QTL for GN was detected on chromosome 1, and tentatively designated as *qGN-1* (Table 1 and Figure 2), which located at interval markers between C2340 and C112, and explained 11.67% of the total phenotype variation. The IR24 allele in *qGN-1* increased 7.84 grains per panicle.

Two elevated CO₂ responsive QTLs for PN on chromosome 5 and 10 were identified, and tentatively named for *qPN-5* and *qPN-10*, respectively. The *qPN-5* (LOD = 2.0), flanked by C263-R2288, explained 9.38% of the total phenotype variation. The *qPN10* (LOD = 2.4), flanked by R1877-C405, accounted for 12.51% of the total phenotypic variation. Further, positive additive effect in *qPN-5* came from IR24, whereas *qPN-10* from Asominori.

Three elevated CO₂ responsive QTLs (*qYD-1*, *qYD-10*, *qYD-12*) for YD were detected on chromosome 1, 10 and 12, respectively. The *qYD-1*, located at the same interval markers between C2340 and C112 as *qGN-1*, showed the largest effect on the trait with a LOD value of 3.9 and explained 21.63% of the total phenotype variation. The *qYD-10* (LOD = 2.1), flanked by C1166-C809, accounted for 10.94% of the total phenotype variation. The *qYD-12*, flanked by C901- XNbp148 with a LOD value of 2.3, explained 15.87% of the total phenotype variation. In addition, additive effect in *qYD-10* and *qYD-12* came from Asominori, whereas *qYD-12* locus from IR24.

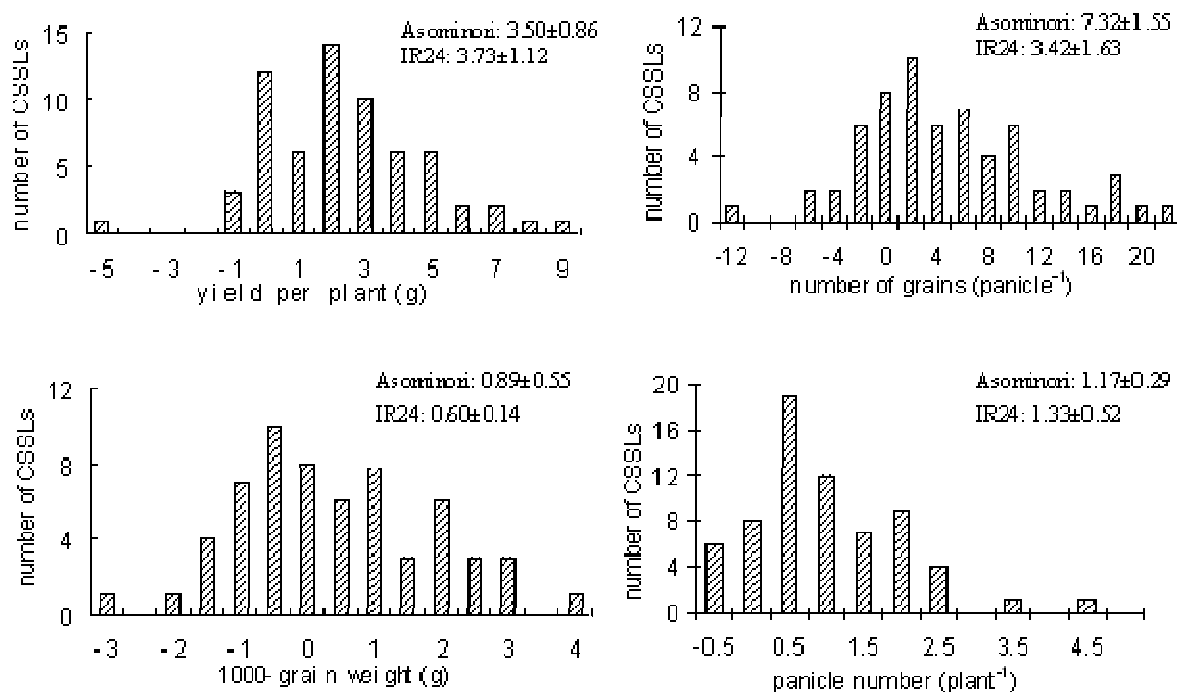
DISCUSSION

Usually, QTL × environment interaction effects (*QE*) were treated as random effects especially in different years and different places (Lu et al., 1996; Kamoshita et al., 2002; Kobayashi et al., 2003; Piepho et al., 2005). They imply the extents that QTLs may be affected by unknown environment. At a specific environment, the total effects of a QTL should include all the genetic main effects and *QE*. In considering the fact of QTLs having *QE*, the relative gene expression of these QTLs could be mainly induced by given environment. In our study, we firstly

Table 1. QTLs for elevated CO₂ response values of YD, GN and PN and estimated genetic effects.

CO ₂ response values	Chr	QTL Name	Marker interval	Peak LOD value	R ² (%)	Additive effect	Positive allele
GN*	1	<i>qGN-1</i>	C112- C2340	2.1	11.67	-7.84	IR24
PN	5	<i>qPN-5</i>	C263-R2288	2.0	9.38	-0.58	IR24
	10	<i>qPN-10</i>	R1877-C405	2.4	12.51	1.12	Asominori
YD	1	<i>qYD-1</i>	C112- C2340	3.9	21.63	-3.36	IR24
	10	<i>qYD-10</i>	C1166-C809	2.1	10.94	2.29	Asominori
	12	<i>qYD-12</i>	C901- XNbp148	2.3	15.87	2.47	Asominori

*GN, PN and YD indicated grain number per panicle, panicles per plant and grain yield per plant, respectively; Chr: chromosome; R²: the percentage of variation explained by QTL analysis.

**Figure 1.** Frequency distribution of elevated CO₂ response values for yield components in 65 CSSLs.

conducted QTL analysis for elevated CO₂ responsive values (the increased phenotypic value in yield and its components between FACE and Ambient). A total of six elevated CO₂ responsive QTLs were detected and distributed on 4 chromosomes, including three ones for YD, one for GN and PN. These results could explain the transgression and continuous distributions for elevated CO₂ responsive values in the CSSL population. In comparing with genomic positions of total twenty QTLs for yield and its components detected in the same CSSL population, only *qYD-12* was overlapped with the one QTL for grain yield detected under Ambient on the 12th chromosome (Fan et al., 2005), suggesting that the QTL not only can be expressed under Ambient condition, but also was elevated CO₂ sensitive QTL for grain yield in

rice. However, the remaining five QTLs (*qYD-1*, *qYD-10*, *qPN-5*, *qPN-10*, *qGN-1*) for elevated CO₂ response in rice only can be expressed under FACE, which suggested that high CO₂ concentration can stimulate/induce the expressions of “dormancy QTLs/genes” affecting yield and its components under natural condition. Undoubtedly, there is possibility that some QTLs/genes for affecting yield and its components under present CO₂ condition cannot be expressed or lost their function with the increase of CO₂ concentration in future. Therefore, screening and isolation of rice novel genes/QTLs effectively expressing under higher CO₂ concentration is challenge to dealing with global warming due to increase of CO₂ concentration.

In addition, judging from the present results that non

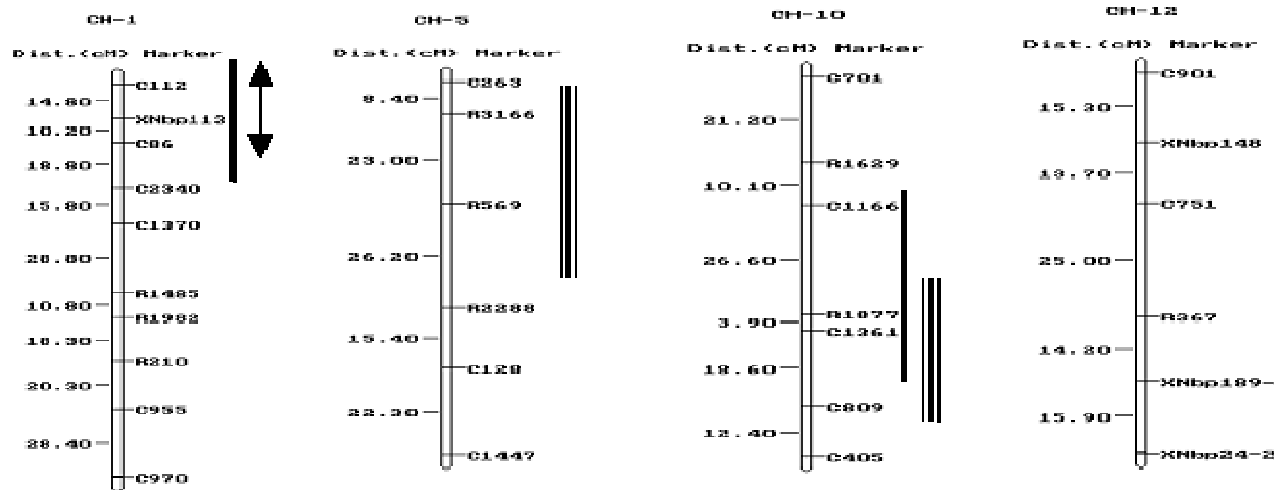


Figure 2. Distribution of QTL on the molecular linkage map for elevated CO₂ response values of yield (YD, |), grain number per panicle (GN, †) and panicle number per plant (PN, ||).

elevated CO₂ responsive QTLs for 1000 grain weight was detected in this study, it could be concluded that expression of these QTLs controlling grain weight is not easily affected by elevated CO₂ environments. Similar result was also found in other environment study, such as salt stress and non-stress (Gong et al., 2000). Interestingly, both *qYD-1* and *qGN-1* locus flanked by the same genomic region of C112-C2340 on chromosome 1, and furthermore their positive alleles all came from IR24, suggesting that they were tightly linked or had pleiotropic effects. Similar results were obtained by the experiments of Li et al. (2000) and Septiningsih et al. (2003).

The elevated CO₂ responsive values with regard to the yield and its components showed positive and negative phenotypic variation and transgressive segregation in CSSLs, and some lines even showed greater enhancement in FACE than their parents (Figure 1), which showed the possibility of breeding new rice varieties more adaptable for higher CO₂ level in rice. The molecular markers tightly linked to elevated CO₂ responsive QTLs lead to the isolation of loci and will help us to better understand elevated CO₂ response mechanisms.

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