

## Analysis of QTLs for Flag Leaf Shape and Its Response to Elevated CO<sub>2</sub> in Rice (*Oryza sativa*)

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**Abstract:** To understand the responses of flag leaf shape in rice to elevated CO<sub>2</sub> environment and their genetic characteristics, quantitative trait loci (QTLs) for flag leaf shape in rice were mapped onto the molecular marker linkage map of chromosome segment substitution lines (CSSLs) derived from a cross between a japonica variety Asominori and an indica variety IR24 under free air carbon dioxide enrichment (FACE, 200 μmol/mol above current levels) and current CO<sub>2</sub> concentration (Ambient, about 370 μmol/mol). Three flag-leaf traits, flag-leaf length (LL), width (LW) and the ratio of LL to LW (RLW), were estimated for each CSSL and their parental varieties. The differences in LL, LW and RLW between parents and in LL and LW within IR24 between FACE and Ambient were significant at 1% level. The continuous distributions and transgressive segregations of LL, LW and RLW were also observed in CSSL population, showing that the three traits were quantitatively inherited under both FACE and Ambient. A total of 16 QTLs for the three traits were detected on chromosomes 1, 2, 3, 4, 6, 8 and 11 with LOD (Log<sub>10</sub>-likelihood ratio) scores ranging from 3.0 to 6.7. Among them, four QTLs (*qLL-6\**, *qLL-8\**, *qLW-4\**, and *qRLW-6\**) were commonly detected under both FACE and Ambient. Therefore, based on the different responses to elevated CO<sub>2</sub> in comparison with current CO<sub>2</sub> level, it can be suggested that the expressions of several QTLs associated with flag-leaf shape in rice could be induced by the high CO<sub>2</sub> level.

**Key words:** rice (*Oryza sativa*); flag leaf; leaf shape character; quantitative trait locus; chromosome segment substitution lines; carbon dioxide enrichment

The increase of atmospheric CO<sub>2</sub> concentration is one of the major factors in global warming and could affect crop production. In general, about 90% of rice grain yield depends on photosynthetic rate after flowering, and the flag leaf contributes a key role in grain filling<sup>[1-3]</sup>. The flag-leaf morphology significantly affects yield, grain quality, maturity, pest preference, absorption of plant growth regulators by canopy and other important production parameters in crops including rice<sup>[4-5]</sup>. Thus, understanding the genetic basis of elevated CO<sub>2</sub> responses of flag-leaf shape in rice has a significant implication on leaf morphological improvement under high CO<sub>2</sub> concentration in future. Recently, the free air carbon dioxide enrichment (FACE, 200 μmol/mol above current levels) facilities are considered the best apparatus for

manipulating atmospheric CO<sub>2</sub> concentration for plants growing under natural field conditions<sup>[6-7]</sup>. Such system has become an integral tool to observe the effects of increasing CO<sub>2</sub> concentration on the growth and development of plants. The previous studies under FACE verified the enhancement of growth, photosynthetic rate and yields of certain crops including rice<sup>[8-10]</sup>. Similar results were noted in our experiment with a japonica variety Asominori and an indica variety IR24. In the meantime, we also found the leaf area index was significantly increased under FACE<sup>[11]</sup>. Therefore, it might be suggested to investigate the genetic basis of flag leaf shape under elevated CO<sub>2</sub>. The purpose of this study was to identify the QTLs for rice flag leaf shape under two different free air CO<sub>2</sub> levels in a mapping population of chromosome segment substitution lines (CSSLs) derived from Asominori/IR24 in order to breed new varieties with the better leaf morphology under high

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CO<sub>2</sub> level.

## MATERIALS AND METHODS

### Experimental population and phenotypic measurements

A mapping population of sixty-five CSSLs with japonica background was derived from a cross between Asominori and IR24 using the single seed descent, backcrossing and marked-assisted selection [12]. The Asominori/IR24 CSSLs have been used to detect QTLs for seed dormancy, nitrogen content and resistance to ferrous iron toxicity in different environments [13-15].

In 2003, two parents (Asominori and IR24) and sixty-five CSSLs were grown in Wuxi, Jiangsu Province, China (31° 35' N, 120° 30' E). During the experiments five plots were used as control under ambient CO<sub>2</sub> and three FACE rings (with ambient plus 200 μmol/mol CO<sub>2</sub>), while each FACE ring was 12.5 m in diameter with pure CO<sub>2</sub> injection for 24 hours a day during rice growth seasons. Meanwhile, the wind direction, wind speed and CO<sub>2</sub> concentration were measured at the center of each ring. The data were used in a computer-controlled system, which maintained CO<sub>2</sub> concentration at about 200 μmol/mol above Ambient, while the growth conditions under FACE and Ambient were kept as close as possible. The field managements for irrigation, high nitrogen fertilization, pesticide application and weed control were carried out according to the common methods [16].

Fifteen flag leaves from each CSSL were sampled and investigated for flag-leaf length (LL), width (LW), ratio of length to width (RLW) under

FACE and Ambient. Evaluation was similar to that described by Li et al [12]. Average values for each CSSL were used for QTL analysis.

### Genetic mapping and QTL detection

A linkage map for QTL detection comprised 87 RFLP markers and covered 1275.4 cM of the rice genome with an average marker interval of 11.8 cM [12]. LOD value of 3.0 was used as the threshold to detect the significant association between marker loci and gene loci. The correlations among molecular markers and flag leaf length, width, ratio of length/width were analyzed by using regression model. The effect and contribution of QTLs to whole variation were also analyzed by using mixed genetic model, and its analysis was conducted with Windows QTL Cartographer 1.13a [14-15]. The QTL were named according to the suggestions of McCouch et al [17].

## RESULTS

### Variations of three flag leaf shape traits

The phenotypic values of two parents and the CSSLs population for LL, LW and RLW under both FACE and Ambient are presented in Table 1. The data revealed that the parental differences in LL, LW and RLW were all significant at 1% level under FACE and Ambient. Furthermore, the means of CSSLs for the three traits were almost equal to the mid-parent values under FACE and Ambient. However, the continuous distribution and transgressive segregation of the three traits were observed in the CSSLs population under FACE and Ambient, suggesting that the three flag leaf shape traits are quantitatively inherited traits.

**Table 1. Rice flag leaf traits in CSSLs population and their parents.**

Trait	Treatment	Mean±SD	Range	Parent		Difference between two parents within the same CO <sub>2</sub> level
				Asominori	IR24	
Flag leaf length (LL, cm)	FACE	28.68±5.02	18.99–39.68	28.85±0.85	38.66±0.68	**
	Ambient	26.77±4.84	18.75–38.66	28.34±0.29	35.24±0.81	**
	Difference between CO <sub>2</sub> levels within the same parent				NS	**
Flag leaf width (LW, cm)	FACE	1.12±0.90	0.90–1.69	1.01±0.02	1.65±0.03	**
	Ambient	1.05±0.73	0.88–1.52	0.99±0.02	1.50±0.01	**
	Difference between CO <sub>2</sub> levels within the same parent				NS	**
Ratio of LL/LW (RLW)	FACE	25.67±4.23	18.63–34.21	28.56±1.12	23.43±0.06	**
	Ambient	25.38±4.84	18.50–34.81	28.63±0.86	23.49±0.48	**
	Difference between CO <sub>2</sub> levels within the same parent				NS	NS

FACE, The CO<sub>2</sub> level is 570 μmol/mol; Ambient, The CO<sub>2</sub> level is 370 μmol/mol; NS and \*\* indicate no significant difference at 5% level and significant difference at 1% level, respectively, by Fisher's protected LSD values.

**Table 2. Estimated genetic effects of QTL for flag leaf shape under FACE and Ambient.**

Trait	Treatment	QTL	Chromosome	Linked marker	LOD	R <sup>2</sup> (%) <sup>a</sup>	Additive effect	Donor of positive allele
Flag leaf length (LL)	FACE	<i>qLL-4<sup>F</sup></i>	4	XNbp331	3.0	10.48	-3.03	IR24
		<i>qLL-6*</i>	6	R2171	4.8	18.21	-3.99	IR24
		<i>qLL-8*</i>	8	R727	3.8	15.37	-3.31	IR24
		<i>qLL-11<sup>F</sup></i>	11	C1350	3.1	10.83	-3.90	IR24
	Ambient	<i>qLL-6*</i>	6	R2171	3.4	12.49	-3.21	IR24
		<i>qLL-8*</i>	8	R727	3.3	13.26	-2.99	IR24
Flag leaf width (LW)	FACE	<i>qLW-1<sup>F</sup></i>	1	C86	3.5	21.57	0.22	Asominori
		<i>qLW-2<sup>F</sup></i>	2	R3393	6.7	25.72	0.26	Asominori
		<i>qLW-3<sup>F</sup></i>	3	C1468	4.3	21.57	0.22	Asominori
		<i>qLW-4*</i>	4	XNbp331	3.4	15.96	-0.07	IR24
	Ambient	<i>qLW-4*</i>	4	XNbp331	3.2	13.55	-0.05	IR24
	Ratio of LL/LW (RLW)	FACE	<i>qRLW-6*</i>	6	R2171	4.1	19.46	-2.99
<i>qRLW-8<sup>F</sup></i>			8	R727	3.3	13.87	-2.62	IR24
<i>qRLW-11<sup>F</sup></i>			11	C1350	3.8	10.34	-3.10	IR24
Ambient		<i>qRLW-6*</i>	6	R2171	3.9	24.79	-3.14	IR24
		<i>qRLW-8<sup>A</sup></i>	8	XNbp41	3.2	19.64	-2.91	IR24

<sup>a</sup> R<sup>2</sup> indicates the percentage of variation explained; <sup>F</sup> and <sup>A</sup> indicate that the QTL was detected under FACE and Ambient, respectively; \* indicates the QTL was simultaneously detected under both FACE and Ambient.

In addition, LL and LW for two parents increased under FACE compared to those under Ambient, but there was a decrease in RLW. It was noted that responses to elevated CO<sub>2</sub> in LL and LW for IR24 were significant at 1% level, indicating that IR24 had greater response to elevated CO<sub>2</sub> than Asominori.

### Detection of QTL for flag leaf shape under FACE and Ambient

#### Flag leaf length (LL)

Four and two QTLs for LL were detected under FACE (*qLL-4<sup>F</sup>*, *qLL-6\**, *qLL-8\**, and *qLL-11<sup>F</sup>*) and Ambient (*qLL-6\** and *qLL-8\**), respectively (Table 2, Fig. 1). The *qLL-6\** and *qLL-8\** on chromosomes 6 and 8, were commonly detected under FACE and Ambient and located near the same markers R2171 and R727, respectively. While *qLL-4<sup>F</sup>* and *qLL-11<sup>F</sup>* were only detected under FACE. The *qLL-4<sup>F</sup>* with a LOD score 3.0 was located near XNbp331 on chromosome 4, with 10.48% of the total phenotypic variation explained, and *qLL-11<sup>F</sup>* (LOD=3.1) was detected near C1350 on chromosome 11, with 10.83% of the total phenotypic variation explained. Moreover, the IR24 alleles of all the four QTLs increased the trait with their additive values.

#### Flag leaf width (LW)

The result of the current experiment indicated

that four and one QTLs for LW were detected under FACE (*qLW-1<sup>F</sup>*, *qLW-2<sup>F</sup>*, *qLW-3<sup>F</sup>*, and *qLW-4\**) and Ambient (*qLW-4\**), respectively. Only *qLW-4\**, located near XNbp331 on chromosome 4, was commonly detected under both FACE and Ambient. The other three QTLs (*qLW-1<sup>F</sup>*, *qLW-2<sup>F</sup>* and *qLW-3<sup>F</sup>*) were only detected under FACE. The *qLW-1<sup>F</sup>* was located near C86 on chromosome 1 and explained 21.57% of the total phenotypic variation. The QTL, *qLW-2<sup>F</sup>* (LOD=6.7) was detected near R3393 on chromosome 2 and accounted for 25.72% of the total phenotypic variation. The other one *qLW-3<sup>F</sup>* (LOD=4.3) was detected near C1468, with 21.57% of the total phenotypic variation explained. The alleles from Asominori of *qLW-1<sup>F</sup>*, *qLW-2<sup>F</sup>*, *qLW-3<sup>F</sup>* and the allele from IR24 of *qLW-4\** increased the trait value.

#### Ratio of leaf length/width (RLW)

Three and two QTLs for RLW were detected under FACE (*qRLW-4\**, *qRLW-6<sup>F</sup>*, *qRLW-11<sup>F</sup>*) and Ambient (*qRLW-4\** and *qRLW-8<sup>A</sup>*), respectively. One QTL, *qRLW-6\**, located near R2171 on chromosome 6, was simultaneously detected under both FACE and Ambient and the percentage of variation explained to the trait under FACE (19.46%) was lower than that under Ambient (23.79%), while the other three QTLs were detected only under one level of CO<sub>2</sub>. Meanwhile, *qRLW-8<sup>F</sup>* (LOD=3.3) and *qRLW-11<sup>F</sup>*

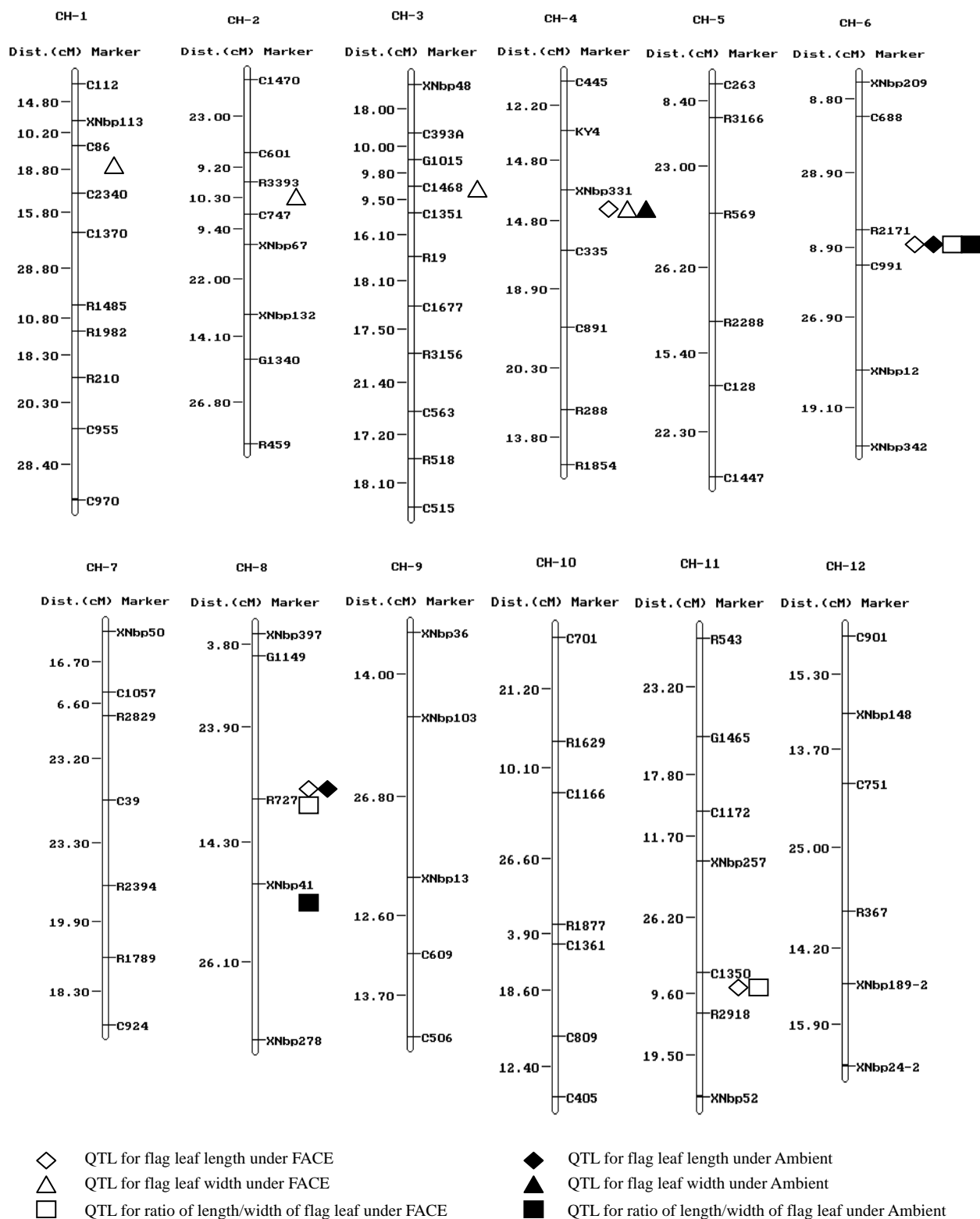


Fig. 1. Distribution of QTLs on the molecular linkage map.

(LOD=3.8) were only detected under FACE, with 13.87% and 10.34% of the total phenotypic variation explained, respectively. The *qRLW-8<sup>A</sup>* (LOD=3.2) was detected under Ambient only and accounted for

19.64% of the total phenotypic variation. In addition, alleles from IR24 in all QTLs detected for RLW increased the trait value.

## DISCUSSION

In rice crop, flag leaf strongly contributes to grain filling after heading, while the flag leaf shape is one of main factors determining its photosynthetic ability<sup>[1-3]</sup>. The previous results of QTL analysis with doubled haploid population in rice showed that leaf shape was a quantitatively inherited trait<sup>[2, 18]</sup>. Our study confirmed the results on the aspects of continuous distribution and transgressive segregation of LL, LW, and RLW in the CSSL population derived from Asominori/IR24 under both FACE and Ambient.

To date, QTL analysis for leaf shape are very few especially no report is available on elevated CO<sub>2</sub> responsive QTLs associated with flag leaf shape in rice. Therefore, this study was conducted to map QTLs controlling flag-leaf shape of rice under FACE and Ambient. Subsequently, a total of 16 QTLs for LL, LW and RLW were detected on chromosomes 1, 2, 3, 4, 6, 8 and 11, of which, four QTLs were commonly detected under both FACE and Ambient. In four common QTLs (*qLL-6\**, *qLL-8\**, *qLW-4\**, and *qRLW-6\**) except *qRLW-6\**, their percentages of variation explained to the respective traits were higher under FACE than under Ambient, which indicated that most QTLs detected under elevated CO<sub>2</sub> played more roles in determining the traits. Moreover, the other eight QTLs were only detected under FACE except *qRLW-8<sup>A</sup>*, indicating that the elevated CO<sub>2</sub> might induce the functional expressions of certain ‘dormancy genes/QTLs’ under current CO<sub>2</sub> level. The *qRLW-8<sup>A</sup>* was not effectively detected under FACE due to elevated CO<sub>2</sub>, which may inhibit the expression of *qRLW-8<sup>A</sup>* in IR24, or the genetic effect of *qRLW-8<sup>A</sup>* in Asominori might reach the same level under FACE.

In addition, with exception of three QTLs (*qLW-1<sup>F</sup>*, *qLW-2<sup>F</sup>* and *qLW-3<sup>F</sup>*, all determining the LW under FACE), the IR24 alleles of the other 13 QTLs enhance the respective traits, which supported that IR24 had greater positive response to elevated CO<sub>2</sub> than Asominori (Table 1).

Comparison of the genomic locations (Table 2 and Fig. 1) of QTLs for flag-leaf shape showed the two QTLs (*qLL-4<sup>F</sup>* and *qLW-4\**) for LL and LW, respectively, on the same chromosome are tightly

linked to the common marker XNpb331, two QTLs (*qLL-6\** and *qRLW-6\**) which were detected under FACE and Ambient, controlling LL and RLW, respectively, were tightly linked to the common marker R2171, two QTLs (*qLL-8\** and *qRLW-8<sup>F</sup>*) for LL and RLW, respectively, were linked to the common marker R727, two QTLs (*qLL-11<sup>F</sup>* and *qRLW-11<sup>F</sup>*) controlling LL and RLW, respectively, were linked to the common marker C1350. These results indicated the existence of either tight linkages between these QTLs or pleiotropic effects. Moreover, the closely linked molecular markers that flank these QTLs detected in our studies should be very useful for leaf morphological improvement of new rice varieties suitable for high CO<sub>2</sub> level through marker-assisted selection in future.

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