

## IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR LEAF TRAITS IN RICE

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Jia B., X. Zhao, Y. Qin, M. Irfan, T.-H. Kim, B. Wang, S. Wang, J. K. Sohn (2016). *Identification of quantitative trait loci for leaf traits in rice*. - Genetika, Vol 48, No. 2, 643-652.

A recombinant inbred lines (RILs) population of 90 lines were developed from a subspecies cross between an *indica* type cultivar, 'Cheongcheong', and a *japonica* rice cultivar, 'Nagdong' was evaluated for leaf traits in 2009. A genetic linkage map consisting of 154 simple sequence repeat (SSR) markers was constructed, covering 1973.6 cM of 12 chromosomes with an average map distance of 13.9 cM between markers. By composite interval mapping method a total of 19 QTLs were identified for the leaf traits on 5 chromosomes (Chr.1, Chr.3, Chr.6, Chr.8 and Chr.11). The percentage of phenotypic variance explained by each QTL varied from 8.1% to 29.4%. Five pleiotropic effects loci were identified on chromosomes 1, 6.

*Key words:* Leaf trait, QTL, SSR, Rice

### INTRODUCTION

Rice (*Oryza sativa* L.) is being one of principal food crops and utilized by one third of world population. It provides some 700 calories per person, mostly residing in developing countries. Rice production can be increased either by increasing its yield per unit area or bringing more area under its cultivation. It is held that any significant increase in rice growing area is not possible and to produce an extra 200 million tones from same area, rice production per unit area has to be increased (DAVOOD *et al.*, 2009). So the development of biologically superior and physiologically efficient genotypes with high yield potential is essentially required (YOSHIDA, 1981). The grain yield in rice is the product of spikelet yield (or sink) and ripening ability (or source) (YAMAGISHI, 2002). Leaves and panicles are two important traits of plant type, the former serves as the main source of photosynthetic products and the latter as the primary sink.

The top three leaves on a stem, particularly the flag leaf, are the primary source of the

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carbohydrates production (SICHER, 1993; FOYER, 1987; GLADUN *et al.*, 1993). The flag leaf, second leaf and third leaf characters are important to create an ideal type of plant (YUAN *et al.*, 1997). There are more than 80% gain yield due to the photosynthesis production of flag leaf and second leaf. The size and shape of the topmost three functional leaves (flag, second and third leaves on a stem) are considered to be the most important characters and key determinants of grain yield (WANG *et al.*, 2010). Large size and reasonable spatial distribution of the three functional leaves is required for elevated photosynthesis and accumulation of assimilates (HORTON, 2000).

It was reported that the source leaves, particularly the flag leaves, were associated with improved grain filling, 1000-grain weight and panicle weight as well as other yield-related traits in cereal crops (LI *et al.*, 1998; CUI *et al.*, 2003; MEI *et al.*, 2003; MA *et al.*, 2006), and the flag-leaf morphology significantly affects yield, grain quality, maturity, pest preference, and other important production parameters in rice (GLADUN *et al.*, 1993; JANG *et al.*, 2000; TAKAI *et al.*, 2006). Grain yield was positively and strongly correlated with flag leaf length and flag leaf width, and the correlations between flag leaf characteristics and yield components revealed that large leaf length, leaf width, and leaf area contributed to increased spikelet number per panicle (LI *et al.*, 1998). In contrast to a better understanding of physiology, our knowledge of the genetic basis underlying the relationship between plant type and yield traits remains limited.

## MATERIALS AND METHODS

### *Plant materials and field planting*

RILs population consisting of 90 F<sub>8</sub> lines was developed from a cross between cultivar 'Cheongcheong' (a Tongil variety) and 'Nagdong' (a Japnica variety). The RILs and their parents, Cheongcheong and Nagdong, were cultivated in 3 rows with 20 plants per row in the experiment field of Kyungpook National University. A randomized block design with two replications for each line was used in the experiment. The seeds of the RILs and their parents were grown at the seedling bed in greenhouse on the 1<sup>st</sup> May, 2010, 34-day-old seedlings were transplanted on 5<sup>th</sup> June, 2010. Fertilizer was applied at the rate of 90 kg N ha<sup>-1</sup>, 45 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 57 kg K<sub>2</sub>O ha<sup>-1</sup>. The field management, including irrigation and pest control, essentially followed normal agricultural practice.

### *Trait measurement*

Flag leaf length (FLL), flag leaf width (FLW), flag leaf area (FLA), second leaf length (SLL), second leaf width (SLW) and second leaf area (SLA) were evaluated on the 15<sup>th</sup> day after heading. Twelve representative plants in the middle of plot were chosen, and the leaves on the main stem were chosen for trait measurement and further analysis.

### *Genomic DNA extraction and PCR amplification*

Genomic DNA was extracted from fresh leaves by CTAB method (ROGERS and BENDCH, 1998). The PCR reactions were performed as described by WILLIAM (1990). The reaction mixture (total volume 12μl), contained 20ng template DNA, 1μl 5-10 pM primer, 0.375μl dNTPs, 0.5 U *Taq* polymerase, 1.2μl of 10×buffers with 1.8 mM MgCl<sub>2</sub>, 10mM Tris-HCL, and 7.325μl of distilled water. Reaction cycles were programmed as follows: one cycle (96°C, 5 min), 35 cycles (94°C, 15 s; 55°C, 15 s; 72°C, 1 min), and a final extension step (72°C, 7 min). PCR products were separated on 8% natural polyacrylamide gel using electrophoresis (Nihon Eido Co. NA-1114) with 350 volt for 1.5 hours. The gel was visualized by silver staining.

**Map construction and QTL analysis**

Five hundred and eighty simple sequence repeats (SSRs) markers from published genetic maps were used to detect polymorphism between Cheongcheong and Nagdong. One hundred and fifty-four polymorphic markers were distributed evenly on rice chromosomes were chosen to genotype the 90 lines. Mapmaker/EXP Version 3.0 was used to create the linkage map for the population (LINCOLN *et al.*, 1992).

**Data analysis**

Composite interval mapping (CIM) was implemented in Windows QTL Cartographer version 2.5 (WANG *et al.*, 2006). For CIM, a 2-cM widow size was used for genome scans. The threshold LOD scores to declare significance at  $P < 0.05$  was estimated empirically with 1000 permutations.

**RESULTS****Trait variations and correlations**

The phenotypic values of two parents and the RILs population for FLL, FLW, FLA, SLL, SLW and SLA are presented in table1. The data reveals that the parental differences in FFL, FLW, SLL, SLW and SLA were significant at 1% level or 5% level. The continuous distribution and transgressive segregation of the leaf traits were observed in the RILs population (Fig.1) and kurtosis were less than 1.0 suggesting that the leaf traits were quantitatively inherited traits and the population was suitable for QTL analysis.

*Table 1. Descriptive statistics of leaf traits of the parental and RILs population.*

Traits	Parents			RILs			
	Cheongcheong	Nagdong	<i>t</i> value	Mean	Range	Skew	Kurtosis
Flag leaf length	26.34	31.07	3.66**	27.08	18.38-38.23	0.569	-0.449
Flag leaf width	1.55	1.16	14.04**	1.46	1.11-1.79	-0.084	-0.311
Flag leaf area	31.30	27.35	2.22	30.27	18.86-48.46	0.840	0.328
Second leaf length	32.60	40.98	4.41**	36.55	23.80-49.73	0.336	-0.362
Second leaf width	1.35	0.99	7.80**	1.21	0.95-1.45	0.140	-0.794
Second leaf area	33.55	30.59	2.53*	33.40	22.02-53.63	0.846	0.286

\*\* $t_{0.01}=3.355$ , \* $t_{0.05}=2.306$ ,  $t_{0.10}=1.860$

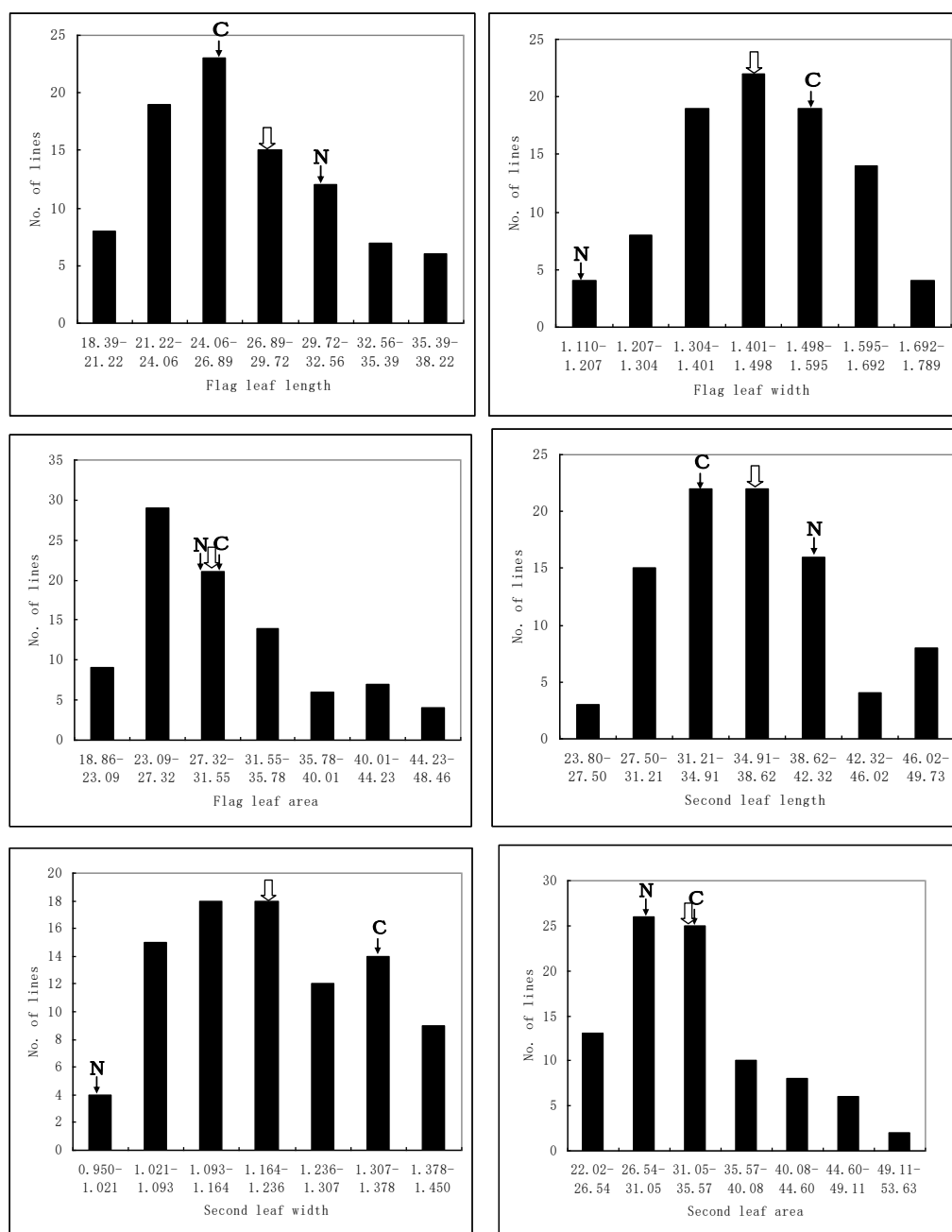


Fig 1. Distribution of top two leaves characters in the RILs population

**Construction of linkage map and QTLs identification**

A genetic linkage map consisting of 154 simple sequence repeat (SSR) markers was constructed, covering 1973.6 cM across 12 chromosomes with an average genetic distance of 13.9 cM between markers.

**Table 2 QTLs for leaf traits detected using a recombinant inbred lines (RILs) population**

Traits	QTL	Chrom.	Marker interval	Increase allele	LOD	R <sup>2</sup>	Addictive effect
FLL	<i>qfll1.1</i>	1	<b>RM1117-RM102</b>	Cheongcheong	4.55	15.6	-2.041
	<i>qfll1.2</i>	1	<b>RM315-RM104</b>	Nagdong	6.32	19.2	2.307
	<i>qfll6.1</i>	6	RM588-RM276	Nagdong	4.16	13.6	3.107
FLW	<i>qflw1.1</i>	1	RM302-RM102	Cheongcheong	3.24	19.5	-0.063
	<i>qflw6.1</i>	6	RM1161-RM162	Cheongcheong	5.86	19.3	-0.077
	<i>qflw6.2</i>	6	RM528-RM412	Cheongcheong	7.49	22.2	-0.082
	<i>qflw8.1</i>	8	RM477-RM72	Cheongcheong	3.27	14.7	-0.058
FLA	<i>qfla1.1</i>	1	<b>RM1117-RM102</b>	Cheongcheong	5.70	20.8	-3.612
	<i>qfla1.2</i>	1	<b>RM315-RM104</b>	Nagdong	4.45	14.5	2.942
SLL	<i>qsll1.1</i>	1	<b>RM1117-RM102</b>	Cheongcheong	5.55	14.6	-2.283
	<i>qsll1.2</i>	1	<b>RM315-RM104</b>	Nagdong	10.60	29.4	3.238
SLW	<i>qslw1.1</i>	1	RM495-RM576	Cheongcheong	3.31	8.1	-0.036
	<i>qslw1.2</i>	1	RM302-RM102	Cheongcheong	3.21	10.0	-0.041
	<i>qslw6.1</i>	6	RM1161-RM162	Cheongcheong	3.89	18.2	-0.056
	<i>qslw11.1</i>	11	RM1233-RM1812	Cheongcheong	4.37	12.1	-0.048
SLA	<i>qsla1.1</i>	1	<b>RM1117-RM102</b>	Cheongcheong	6.89	22.1	-3.277
	<i>qsla1.3</i>	1	<b>RM315-RM104</b>	Nagdong	6.58	17.5	2.897
	<i>qsla3.1</i>	3	RM130-RM148	Nagdong	4.20	10.6	2.283
	<i>qsla6.1</i>	6	RM162-RM412	Cheongcheong	3.76	10.7	-2.649

Note: FLL flag leaf length, FLW flag leaf width, FLA flag leaf area, SLL second leaf length, SLW second leaf width, SLA second leaf area

By composite interval mapping method, the significant QTLs identified for the six leaf traits are summarized in Table 2. A total of 19 QTLs were identified for the leaf traits on chromosomes 1, 3, 6, 8 and 11 (Fig. 2) with individual QTL explained phenotypic variation of 6.0% to 29.4%. For FLL, QTLs were identified on chromosome 1 and 6, accounting for 19.2% phenotypic variation. The Nagdong alleles contributed to long flag leaf at the *qfll1.2* and *qfll6.1*. Four QTLs for FLW were identified on chromosome 1, 6 and 8, accounting for 22.2 % phenotypic variation and the Cheongcheong alleles were associated with increasing the width of flag leaf at these loci. QTLs for FLA, which explained 20.8% of phenotypic variance were detected on chromosome 1. For SLL,

two QTLs were identified on chromosome 1, and 29.4 % of phenotypic variation were explained by these QTLs. Four QTLs for SLW were identified on chromosome 1, 6 and 11, which explained 18.2% of phenotypic variation. Cheongcheong alleles increased width of second leaf at these QTLs. For SLA, five QTLs were identified on chromosome 1, 3 and having explained phenotypic variance of 22.1 %. Three pleiotropic effects loci were identified on chromosome 1 at the interval of RM1117-RM102 for *qfl1.1*, *qfla1.1*, *qsl1.1*, *qsla1.1*, the interval RM315-RM104 for *qfl1.2*, *qfla1.2*, *qsl1.2*, and the interval RM302-RM102 for *qflw1.1* and *qslw1.2*. Two pleiotropic effects loci were identified on chromosomes 6 at the interval RM1161-RM162 for *qflw6.1* and *qslw6.1*, and at the interval RM162-RM412 for *qslw6.2* and *qsla6.1* respectively. These QTLs affecting leaf traits would be beneficial to leaf morphological improvement and high-yield rice improvement.

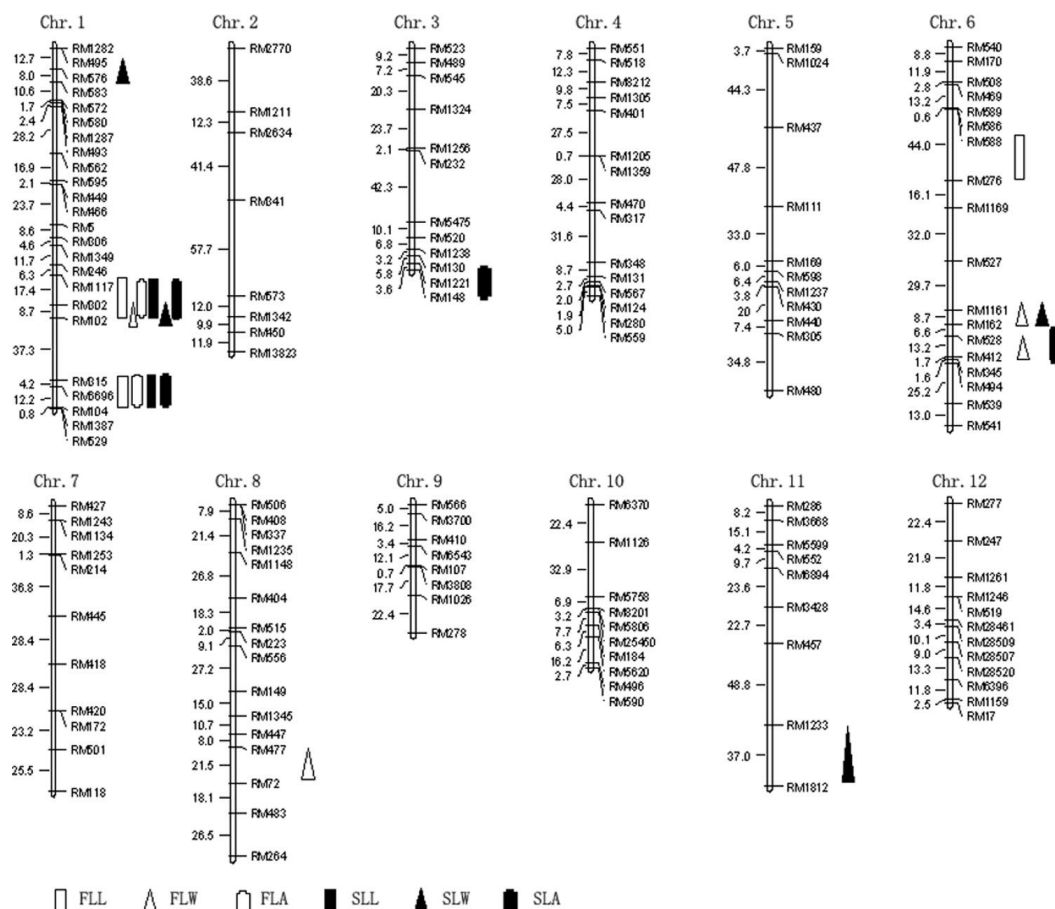


Fig. 2. Position of identified QTLs on rice chromosomes using RIL population. FLL flag leaf length, FLW flag leaf width, FLA flag leaf area, SLL second leaf length, SLW second leaf width, SLA second leaf area.

## DISCUSSION

In rice, there are more than 80% grain yield due to the photosynthesis, production of flag leaf and second leaf (GLADUN and KARPOV, 1993). By using the Cheongcheong/Nagdong RILs, 19 QTLs associated with flag and second leaf traits were identified in the present study. These QTLs distributed on 11 regions of chromosomes 1, 3, 6, 8 and 11. Among these nine regions, RM1117-RM102 increased FLL, FLA, SLL and SLA with the Cheongcheong allele, and *qfla1.1* and *qsla1.1* explained 20.8% and 22.1% of phenotypic variance, respectively. The marker interval RM315-RM104 increased FLL, FLA, SLL and SLA with the Nagdong allele, and *qsll1.2* explained phenotypic variance of 29.4%. In our study, three QTLs for flag leaf length was detected on chromosome 1 and 6 which is quite similar with results of CUI *et al.*, (2003) detected five QTLs for flag leaf area on chromosomes 1, 6, 9 and 10 with variation ranged from 1.99 to 11.19% . The marker intervals for the flag leaf length reported in our study showed similarity with *QFll1b* (MEI *et al.*, 2005) and *qFll1* (HAN-HUA *et al.*, 2007). The region RM588-RM276 increased FLL with the Nagdong allele, and *qfl6.1* explained 13.6% of the phenotypic variation. This QTL is similar with *qFLL-6* (WANG *et al.*, 2004) and *QFll6* (YUE *et al.*, 2006). The region RM1161-RM162 increased FLW and SLW with the Cheongcheong allele, and *qflw6.1* and *qslw6.1* explained phenotypic variation of 19.3% and 18.2% respectively. The region RM528-RM412 increased FLL with the Cheongcheong allele, and *qflw6.2* explained 22.2% of the phenotypic variation. There was a region on the chromosome 4 can increase both FLL and FLW, and its effects were very powerful and stable across the environments (KOBAYASHI *et al.*, 2003). Flag leaf length is considered to be a key factor in high yielding potential of rice (MA *et al.*, 2008).

Five main effect QTLs on chromosome 1, 4, 6, 8 and 12 and three digenic epistatic QTLs were reported for flag leaf width in RILs population of 254 rice individuals (MEI *et al.*, 2003). YUE *et al.*, (2006) reported five QTLs for flag leaf width in two years of data with highest variation of 35.81% and this variation is greater than our study. KEBRIYAE *et al.*, (2012) reported two QTLs for flag leaf width on chromosome 1 and 2 with explained phenotypic variance of 10.18 and 12.26 % using SSR and AFLP markers respectively. In this study two QTLs for flag area were detected on chromosome 1 having additive effects of -3.612, 2.942. The phenotypic variation of these QTLs is lower than previous reports (YUE *et al.*, 2006). Some studies suggested that leaf area is positively correlated to grain yield (LI *et al.*, 1998).

For second leaf area, four QTLs were detected on chromosome 1, 3 and 6 and among these *qsla1.1* could be declared as major QTL for having phenotypic variation of 22.1%. Similar findings were also reported by CUI *et al.*, (2003) declaring QTLs on chromosome 1, 3, 6, 11 and 12, but the phenotypic variance explained by these QTLs is lower than our study. In this study some QTLs for second leaf length and width are reported which are not reported yet according to best of our knowledge based on previously published literature. So, these are some novel findings of this study. Some QTLs reported in this study is different from previous reports and these differences might be due to the population used, markers, software and environments. These regions identified can be further study for improving flag and second leaf size.

Received December 05<sup>th</sup>, 2015

Accepted May 25<sup>th</sup>, 2016

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## IDENTIFIKACIJA LOKUSA KOJI KONTROLIŠU KVANTITATIVNE OSOBINE LISTA KOD PIRINČA

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### Izvod

Formirana je populacija od 90 recombinantnih (RILs) linija ukrštanjem subvrsta i *indica* tipa 'Cheongcheong' i *japonica* kultivara pirinča. Ocena osobina lista kultivara 2009'Nagdong' je vršena u toku 2009. Genetička mapa ukopčanosti (genetic linkage map) se sastoji od 154 jednostavno ponovljive sekvence (SSR) markera. Konstruisana je pokrivanjem 1973,6 cM 12 hromozoma sa prosečnom map distancom od 13,9 cM između markera. Kompozitnom metodom mapiranja intervala identifikovano je ukupno 19 QTL (Quantative Traits Lokus) lokusa koji kontrolišu osobine lista na 5 hromozoma (Chr.1, Chr.3, Chr.6, Chr.8 and Chr.11). Procenat fenotipske varijanse udaljenosti objašnjene za svaki QTL varira od 8,1 do 29,4 % . Pet lokusa sa plejotropskim efektom je identifikovano na hromozomima br.1 i 6.

Primljeno 05. XII 2015.

Odobreno 25. V. 2016.