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ORIGINAL PAPER

# Detection of a quantitative trait locus controlling carbon isotope discrimination and its contribution to stomatal conductance in *japonica* rice

**Toshiyuki Takai · Akihiro Ohsumi · Yumiko San-oh · Ma. Rebecca C. Laza · Motohiko Kondo · Toshio Yamamoto · Masahiro Yano**

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**Abstract** Increasing leaf photosynthesis offers a possible way to improve yield potential in rice (*Oryza sativa* L.). Carbon isotope discrimination ($\Delta^{13}C$) has potential as an indirect selection criterion. In this study, we searched for quantitative trait loci (QTLs) controlling $\Delta^{13}C$, and assessed their association with leaf photosynthesis. Substitution mapping by using chromosome segment substitution lines (CSSLs), that carry segments from the *indica* cultivar Kasalath in the genetic background of the *japonica* cultivar Koshihikari, identified genomic regions affecting $\Delta^{13}C$ on chromosomes (Chr.) 2, 3, 6, 7, and 12. One of the CSSLs, SL208, in which most regions on Chr. 3 were substituted with Kasalath segments, showed higher leaf stomatal conductance for $CO_2$ ($g_s$) and $\Delta^{13}C$ than Koshihikari during the vegetative stage although leaf photosynthetic rate did not differ between them. These results suggest an association between $\Delta^{13}C$ and $g_s$. To test this association, we performed a QTL analysis for $\Delta^{13}C$ at vegetative and heading stages in an $F_2$ population derived from a cross between SL208 and Koshihikari. The results confirmed a QTL controlling $\Delta^{13}C$ on the long arm of Chr. 3. By using a near-isogenic line specific to *Hd6*, we ruled out the possibility that variation in $\Delta^{13}C$ was generated through the pleiotropic effect of heading date.

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T. Takai · T. Yamamoto · M. Yano (✉)
National Institute of Agrobiological Sciences,
2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
e-mail: myano@affrc.go.jp

A. Ohsumi · Y. San-oh · Ma. R. C. Laza · M. Kondo
National Institute of Crop Science,
2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

## Introduction

Photosynthesis, the process of use of light energy to manufacture carbohydrates via $CO_2$ assimilation reactions, is the basis of crop growth, biomass production and yield formation. The contribution to yield improvement made by raising leaf photosynthesis has proved controversial in a number of crop species (Evans 1993), but recent studies have shown that leaf photosynthetic rate (Pn) and stomatal conductance for $CO_2$ ($g_s$) were closely correlated with final grain yield in rice (Horie et al. 2006) and wheat (Fischer et al. 1998). These results suggest that improvement of leaf photosynthesis may offer a possible way to increase yield potential in cereals (Takai et al. 2006).

The advent of portable open gas-exchange monitors enables researchers to take precise measurements of Pn, $g_s$ and photosynthesis-related parameters in real time in the field (Long and Bernacchi 2003). So far, a number of studies of leaf photosynthesis in rice have used this method (Peng et al. 1998; Murchie et al. 2002; Ohsumi et al. 2007; Kanemura et al. 2007). Despite the benefits of portable monitors, real-time measurements remain inappropriate for genetic analysis and breeding programs because they are time-consuming and laborious and give only performance over a short period.

Stable carbon isotope discrimination ($\Delta^{13}C$) reflects the fact that plants discriminate against $^{13}C$ in ambient $CO_2$ in the process of photosynthesis (Farquhar et al. 1982). The major advantages of $\Delta^{13}C$ expressed on a dry matter basis are that it gives integrated data over a growing period and

is suitable for high-throughput screening, because samples can be stored for later automated measurement (Condon et al. 1987). $\Delta^{13}C$ has often been proposed as a criterion for selection of plants for leaf transpiration efficiency (TE), a major component of plant water-use efficiency, under water-limited conditions in $C_3$ crop species because both $\Delta^{13}C$ and TE are associated with the ratio of intercellular to ambient $CO_2$ concentration (Ci/Ca) (Farquhar et al. 1989). Ci/Ca value is determined by a balance between $g_s$ and carboxylation efficiency related to leaf N content and leaf thickness (Cook and Evans 1983; Condon et al. 2002; Ohsumi et al. 2007), and increase of each trait can contribute to improve Pn (Condon et al. 2002). Since $\Delta^{13}C$ represents the result of the balance, genetic factors controlling $\Delta^{13}C$ may reflect those controlling $g_s$ and/or carboxylation efficiency (Takai et al. 2006).

The advent of DNA markers has facilitated the genetic mapping of quantitative trait loci (QTLs) for photosynthetic traits. In rice, putative QTLs were detected for $\Delta^{13}C$ (Ishimaru et al. 2001; Price et al. 2002; Laza et al. 2006; Takai et al. 2006) in primary mapping populations such as recombinant inbred lines (RILs). RILs have several advantages in genetic analysis: they allow replicated trials and high recombination frequency leading to wide trait variations and most of the genome is homozygous for one parent or the other (Keurentjes et al. 2007). However, RILs often show a wide range of phenotypic variation in non-target traits. This variation often generates genetic noise making it technically difficult to detect target QTLs (Yin et al. 1999). For example, variation in flowering time (heading date) may have pleiotropic effects on many traits simultaneously (Yin et al. 1999; Ando et al. 2008). This problem can be solved by the detection and characterization of QTLs in the same genetic background (Yamamoto and Yano 2008) and the investigation of target traits at the same time. No such study of $\Delta^{13}C$ in rice has yet been undertaken, nor has a direct association of QTLs for $\Delta^{13}C$ with $g_s$ and/or carboxylation efficiency been studied.

In this study, to detect chromosome regions underlying $\Delta^{13}C$, we used chromosome segment substitution lines (CSSLs), in which a particular chromosome segment of an *indica* cultivar Kasalath was substituted in the genetic background of a *japonica* cultivar Koshihikari. (Ebitani et al. 2005). A promising QTL for $\Delta^{13}C$ was detected on chromosome Chr. 3. We then conducted real-time measurements of leaf photosynthesis for a target CSSL, SL208, in which most regions on Chr. 3 were substituted with Kasalath segments, to clarify the QTL effects on leaf photosynthesis. Using backcross progeny of the CSSL, we mapped the QTL on the distal end of the long arm of Chr. 3. We also ruled out the pleiotropic effect of a gene associated with days-to-heading on $\Delta^{13}C$.

## Materials and methods

### Evaluation of CSSLs for $\Delta^{13}C$ and photosynthesis

Thirty-nine CSSLs derived from a cross between a *japonica* cultivar, Koshihikari, and an *indica* cultivar, Kasalath (Ebitani et al. 2005; Rice Genome Resource Center, http://www.rgrc.dna.affrc.go.jp/index.html), were grown in a paddy field in a randomized complete block design with three replications at the National Institute of Crop Science in Yawara (36°0′N, 140°1′E), Ibaraki, Japan, in 2005. Month-old seedlings were transplanted on 31 May at one seedling per hill at a spacing of 15 cm between hills and 30 cm between rows. Each plot consisted of three rows with 17 hills per row. In the present study, the amount of fertilizers depended on the soil fertility of the paddy field used in each experiment. Nitrogen (N) in the form of ammonium sulfate was applied as basal fertilizer at 63 kg $ha^{-1}$, and top-dressed 53 days after transplanting (DAT) at 42 kg $ha^{-1}$. Phosphorus (P) and potassium (K) were applied as basal fertilizers at 50 and 100 kg $ha^{-1}$, respectively. At vegetative stage (41 DAT) and at heading, four topmost fully expanded leaves or flag leaves per plot were collected, oven-dried, and ground to a fine powder for the analysis of carbon isotope composition ($\delta_p$). The $\delta_p$ of leaf samples was analyzed by an isotope ratio mass spectrometer connected to an elemental analyzer (Delta Plus XP, ThermoFinnigan Co., Bremen, Germany). The $\Delta^{13}C$ value was calculated as follows, on the assumption that the carbon isotope composition of the air ($\delta_a$) was −8‰ (Farquhar et al. 1989).

$$\Delta^{13}C = \frac{\delta_a - \delta_p}{1 + \frac{\delta_p}{1000}} \quad (1)$$

The significance of difference of $\Delta^{13}C$ between Koshihikari and the CSSLs was determined by Dunnett's test using commercial software (JMP 6.0.3, SAS Institute, Cary, NC, USA).

In 2006, Koshihikari and SL208, in which most regions on Chr. 3 were substituted with Kasalath segments in the Koshihikari genetic background, were sown in the seedling nursery on 28 April and transplanted on 30 May using the same cultivation techniques as in 2005. Basal fertilizers were applied at 40 kg N $ha^{-1}$, 160 kg P $ha^{-1}$, and 120 kg K $ha^{-1}$. Leaf photosynthesis was measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA). One leaf from each of three different plants in each plot was measured between 0900 and 1300 hours under a constant saturated light level of 2,000 μmol $m^{-2}$ $s^{-1}$ provided by red/blue light-emitting diodes (LEDs) on clear days. The leaf chamber temperature was maintained at 30°C, the reference $CO_2$

concentration was 380 μmol $mol^{-1}$, and relative humidity was 75 ± 5%. Gas-exchange parameters were recorded once the topmost expanded leaf was enclosed in the chamber and the system software indicated that $CO_2$, $H_2O$ and flow in the chamber were stabilized. The measurements were conducted four times between tillering and heading. After measurements, leaves were harvested and used for investigation of $\delta_p$ as described above. Leaf N was also determined by the elemental analyzer.

### Verification of allele effect of detected QTL

As SL208 showed highest $\Delta^{13}C$ at vegetative stage in 2005, we performed an additional QTL analysis of an $F_2$ population derived from a cross between SL208 and Koshihikari to confirm the existence and effect of a QTL for $\Delta^{13}C$. $F_2$ plants were grown in the paddy field at Yawara in 2006. Sowing, transplanting, and fertilizer application were performed using the same procedures as described for the photosynthesis experiment. The population plot consisted of 5 rows with 22 hills per row, and 93 plants (omitting border plants) were used for the QTL analysis. At vegetative stage (57 DAT), the topmost fully expanded leaf on the main stem or the primary tiller was taken from each plant for analysis of $\delta_p$. At heading stage, the flag leaf of each plant was sampled for the analysis of $\delta_p$.

Total DNA of each plant was extracted from leaves by the CTAB method (Murray and Thomson 1980). The genotypes on Chr. 3 were determined by 15 simple sequence repeat (SSR) markers developed by McCouch et al. (2002) and one cleaved amplified polymorphic sequence (CAPS) marker created by single nucleotide polymorphism (SNP) in *Hd6*, a QTL controlling flowering time identified on Chr. 3 (Takahashi et al., 2001). Linkage maps were constructed using MAPMAKER/EXP 3.0 (Lander et al. 1987). The chromosomal positions and effects of putative QTLs were determined by composite interval mapping (CIM) using QTL Cartographer 2.0 (Basten et al. 2002). The threshold of QTL detection was based on 1000 permutation tests at the 5% level of significance (Churchill and Doerge 1994; Doerge and Churchill 1996). The additive and dominant effects and phenotypic variance explained by each QTL were estimated at the peak of LOD score.

### Comparison of photosynthetically related traits among Koshihikari, SL208, and Kanto IL5

Koshihikari, SL208 and a near-isogenic line (NIL) of Koshihikari (Kanto IL5) were grown in the paddy field at the National Institute of Agrobiological Sciences in Tsukuba (36°1′N, 140°6′E), Japan, in 2007. Kanto IL5, carrying the Kasalath chromosome segment containing the *Hd6* allele, heads about 10 days later than Koshihikari under natural day length conditions in Japan (Takeuchi et al. 2006). Thirty-day-old seedlings of each line were transplanted, one per hill, with two replications on 16 May. Each plot consisted of two rows with 12 hills per row. Basal fertilizer was applied: 56 kg N $ha^{-1}$, 56 kg P $ha^{-1}$, and 56 kg K $ha^{-1}$. N fertilizer was top-dressed at 30 kg N $ha^{-1}$ 2 weeks after transplanting. Leaf photosynthesis, $\delta_p$ and leaf N of one leaf from each of different plants were measured in each plot as before. The significance of difference of each trait between Koshihikari and the other lines was determined by Tukey test.

## Results

### Comparison of $\Delta^{13}C$ between CSSLs and Koshihikari

The $\Delta^{13}C$ was significantly higher for Kasalath than for Koshihikari at both vegetative stage (41 DAT) and heading (Fig. 1). The $\Delta^{13}C$ values of the 39 CSSLs ranged from 21.1 to 22.7‰ at 41 DAT, and from 18.1 to 19.9‰ at

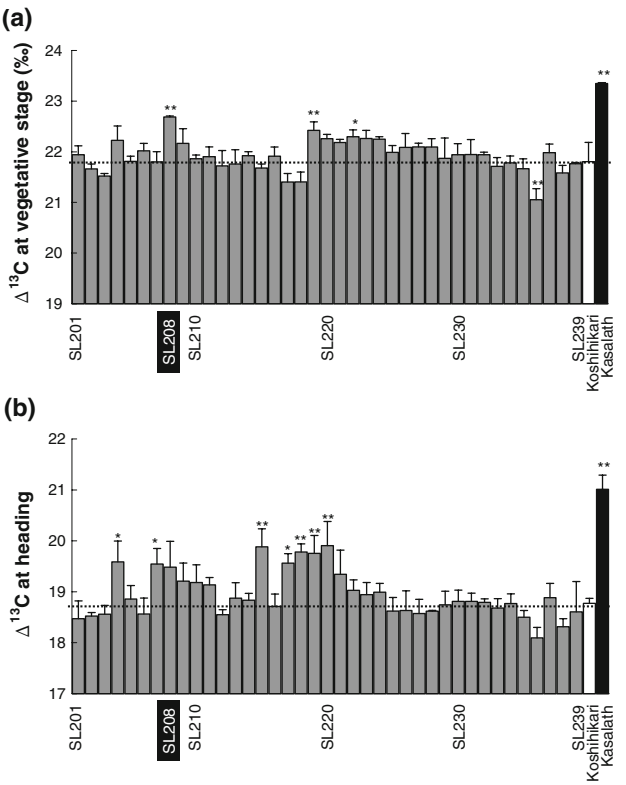


**Fig. 1** Carbon isotope discrimination ($\Delta^{13}C$) at vegetative stage (41 days after transplanting (DAT)) and at heading in 39 CSSLs and their parents, Koshihikari and Kasalath, in 2005. *Bars* indicate mean ± standard deviation. *Dotted lines* show the values of Koshihikari. **, * Significant difference between Koshihikari and CSSLs at 1 and 5% levels by Dunnett's test

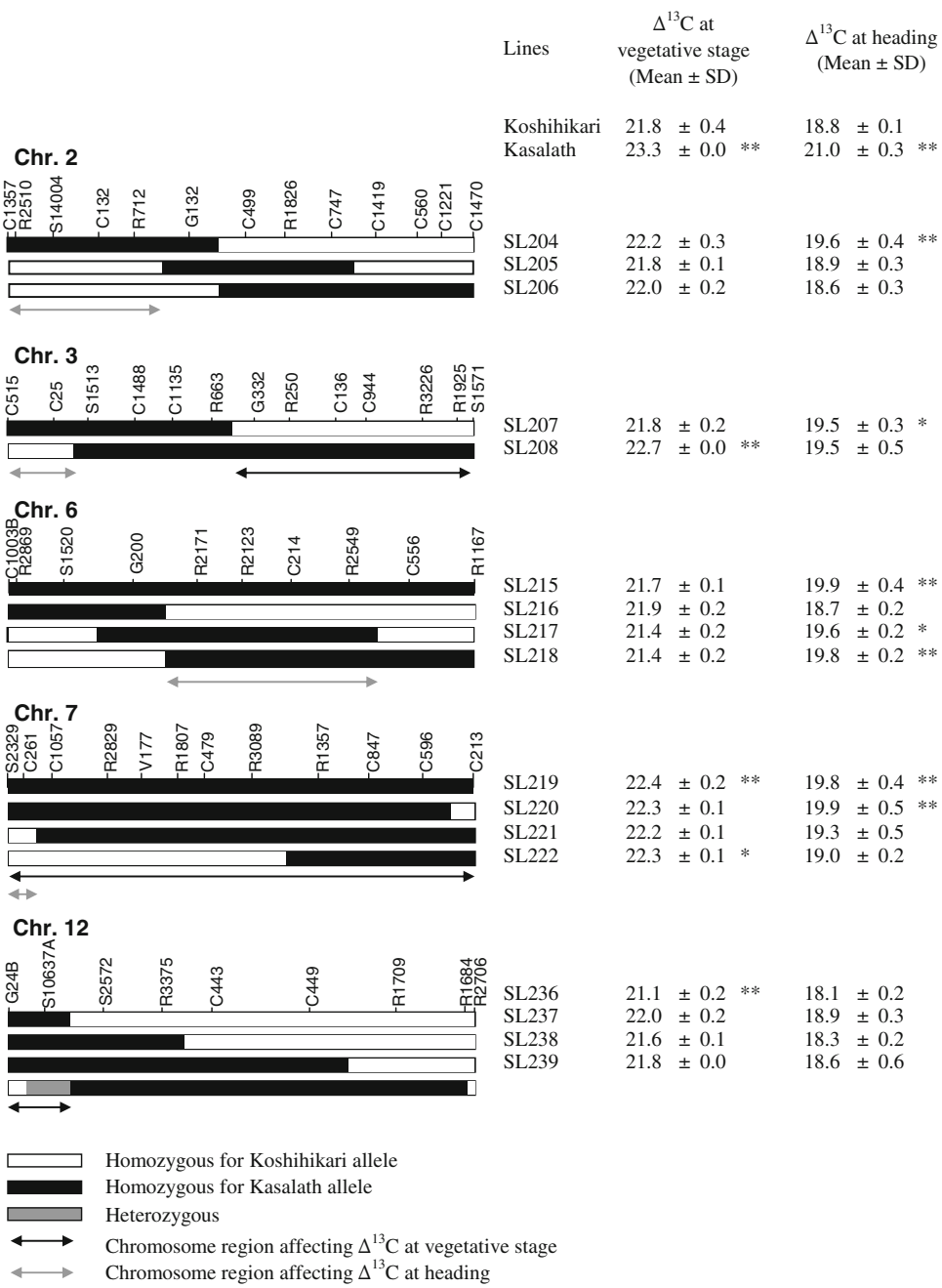


**Fig. 2** Substitution mapping of five chromosome regions affecting $\Delta^{13}C$ at vegetative stage (41 DAT) and at heading in the CSSLs. RFLP markers defined by Ebitani et al. (2005) are indicated at the top of each linkage map. Chromosome regions affecting $\Delta^{13}C$ (regions between *arrows*) were predicted from the difference in $\Delta^{13}C$ values between each CSSL and Koshihikari (shown at the *right*). **, * Significant difference between each CSSL and Koshihikari at 1% and 5% levels by Dunnett's test

heading. Significant differences including 1 and 5% level were detected between each of the four CSSLs (SL208, 219, 222, and 236) and Koshihikari at 41 DAT, and between each of the seven CSSLs (SL204, 207, 215, and 217–220) and Koshihikari at heading.

Using phenotype and genotype data of the CSSLs, we conducted substitution mapping (Ebitani et al., 2005) of chromosome regions affecting $\Delta^{13}C$. Three regions were detected as carrying putative QTLs for $\Delta^{13}C$ at 41 DAT: on Chr. 3, 7 and 12 (Fig. 2). The regions on Chr. 3 were successfully delineated by the markers G332 and S1571, while the regions on Chr. 7 and 12 could not be delineated. The candidate region between R1357 and C213 on Chr. 7, predicted by SL219 and SL222, was also shared in SL220 and SL221, and the region between G24B and S10637A on Chr. 12, predicted by SL236, was also shared in SL237–239. The Kasalath alleles on Chr. 3 and 7 increased the $\Delta^{13}C$. Four regions located on Chr. 2, 3, 6 and 7 were identified as carrying putative QTLs for $\Delta^{13}C$ at heading. These regions were delineated by C1357–R712 on Chr. 2, C515–C25 on Chr. 3, R2171–R2549 on Chr. 6, and S2329–C261 on Chr. 7. All Kasalath alleles increased the $\Delta^{13}C$.

Leaf photosynthesis ability in SL208

As SL208 showed the highest $\Delta^{13}C$ among lines at 41 DAT, we measured $\Delta^{13}C$, leaf photosynthesis and leaf N of SL208 and Koshihikari periodically until heading. The $\Delta^{13}C$ of SL208 was significantly higher than that of Koshihikari at vegetative stage (29 and 45 DAT) as shown above, but not at heading of Koshihikari (Fig. 3a). While Pn did not differ between SL208 and Koshihikari on any date (Fig. 3b), SL208 had a significantly higher $g_s$ (by 36%) than Koshihikari at 45 DAT, but not afterwards (Fig. 3c). The Ci/Ca of SL208 was also significantly higher than that of Koshihikari at 45 DAT (Fig. 3d). Leaf N did not differ at any date (Fig. 3e).

Verification of a QTL on chromosome 3 in advanced backcross progeny

To delimit the location of a QTL for $\Delta^{13}C$, we conducted a QTL analysis in an $F_2$ population derived from a cross between SL208 and Koshihikari. $\Delta^{13}C$ showed continuous variation among $F_2$ plants and transgressive segregation (Fig. 4), ranging from 21.2 to 22.8‰ (21.7‰ in Koshihikari and 22.3‰ in SL208) at vegetative stage (57 DAT), and from 18.2 to 21.3‰ (19.9‰ in both Koshihikari and SL208) at heading. In contrast, days-to-heading showed a bimodal distribution ranging from 101 to 128 days, corresponding to the parental values (103 days in Koshihikari and 127 days in SL208). Days-to-heading was significantly correlated with $\Delta^{13}C$ at both 57 DAT and heading: positively at 57 DAT and negatively at heading (Table 1). There was no correlation between $\Delta^{13}C$ at 57 DAT and $\Delta^{13}C$ at heading. A QTL for $\Delta^{13}C$ at 57 DAT was identified at marker RM1221. The phenotypic variance explained by the QTL ($R^2$) was 31.1% (Table 2; Fig. 5). The additive effect of the Kasalath allele was 0.3‰, indicating that the Kasalath allele contributed to discrimination of $^{13}C$. A QTL for $\Delta^{13}C$ at heading was detected near RM6970. The $R^2$ of the QTL was 79.0%. The additive effect of the Kasalath allele of the QTL was −1.0‰, indicating that the Kasalath allele reduced discrimination of $^{13}C$ (Table 2; Fig. 5). A major QTL for days-to-heading was detected also at RM6970. The $R^2$ of the QTL was 90.1%. The additive effect of the Kasalath allele of the QTL was 9.4 days.

Investigation of the effect of flowering time QTL *Hd6* on $\Delta^{13}C$, $g_s$ and Pn

To clarify whether $\Delta^{13}C$ and days-to-heading are controlled by different genetic factors or not, we compared photosynthetically associated traits among Koshihikari, SL208 (Fig. 6a) and Kanto IL5 (Fig. 6b). Kanto IL5 and SL208 headed 10 and 14 days, respectively, later than Koshihikari (Fig. 6c). At heading, Kanto IL5 had a

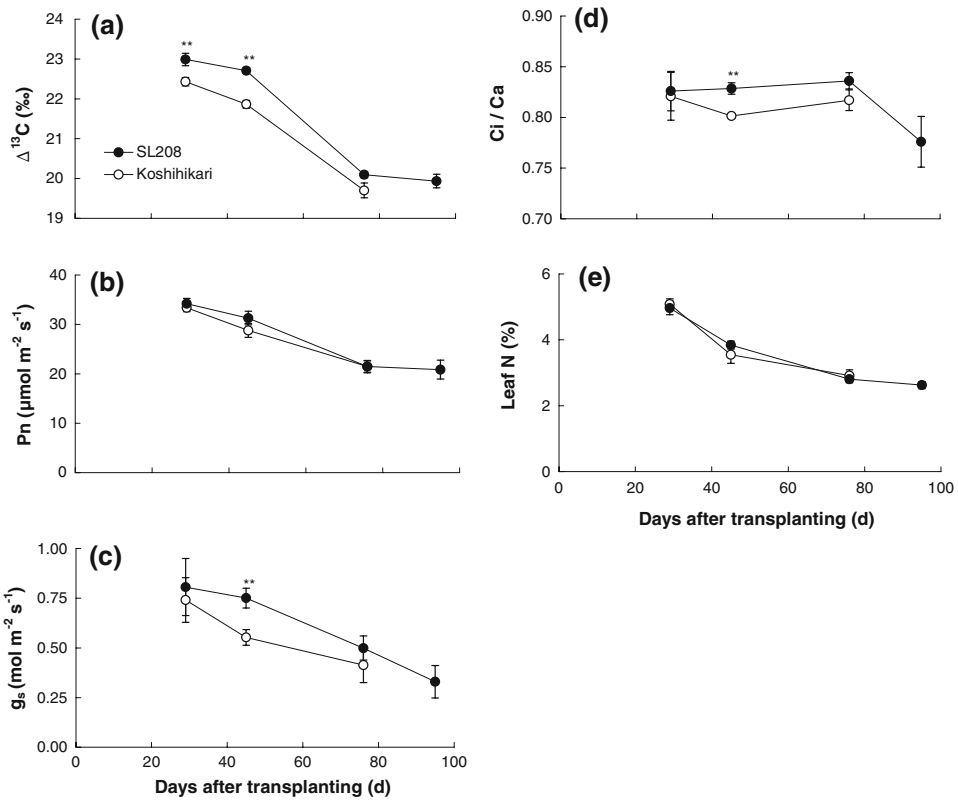


**Fig. 3** Change in leaf photosynthetically related traits over time until heading of Koshihikari and SL208 in 2006. **a** $\Delta^{13}C$, **b** leaf photosynthetic rate (*Pn*), **c** stomatal conductance for $CO_2$ ($g_s$), **d** the ratio of intercellular to ambient $CO_2$ concentration (Ci/Ca), **e** leaf *N*. Symbols show mean ± standard deviation. ** Significant difference on measuring date between Koshihikari and SL208 at 1% levels by *t*-test

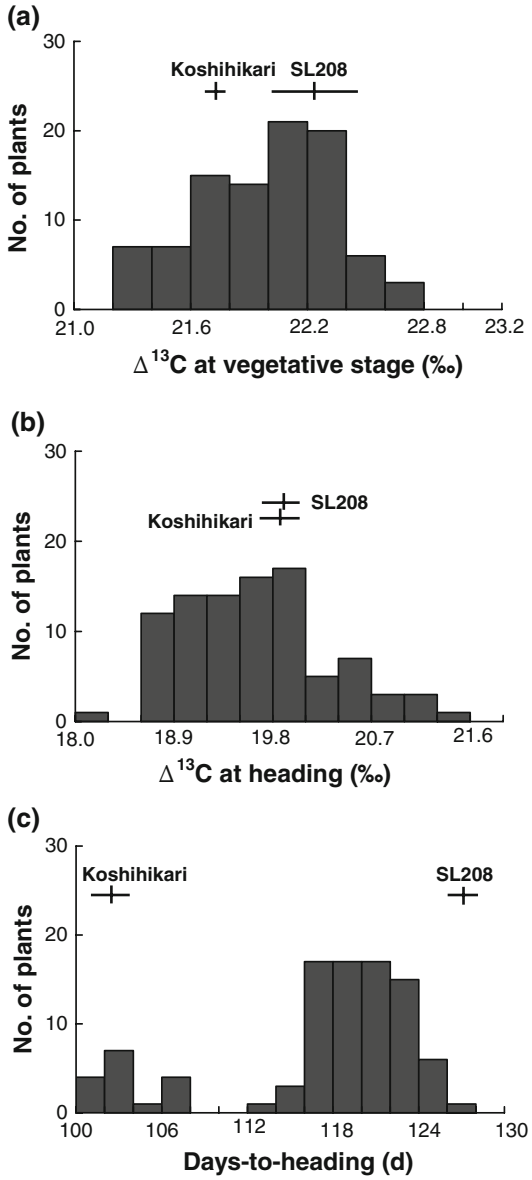


**Fig. 4** Frequency distribution of $\Delta^{13}C$ at vegetative stage (57 DAT) and at heading, and of days-to-heading in the $F_2$ population derived from Koshihikari × SL208. *Vertical bars*, mean; *horizontal bars*, standard deviation

**Table 1** Coefficients of correlation among $\Delta^{13}C$ at vegetative stage (57 days after transplanting (DAT)), $\Delta^{13}C$ at heading and days-to-heading in an $F_2$ population derived from Koshihikari × SL208

| | $\Delta^{13}C$ at vegetative stage | $\Delta^{13}C$ at heading |
|---|---|---|
| $\Delta^{13}C$ at heading | −0.12 | |
| Days-to-heading | 0.56 ** | −0.43 ** |

** 1% level of significance

significantly lower $\Delta^{13}C$ than Koshihikari (by 0.7‰, Fig. 6d). To avoid the effects of variation in days-to-heading, we compared traits at the same measuring time (same DAT) (Fig. 6e). Values of $\Delta^{13}C$ did not differ between Kanto IL5 and Koshihikari. On the other hand, SL208 had a substantially higher $\Delta^{13}C$ than Koshihikari and Kanto IL5 at any date. Although Pn and leaf N did not differ among three varieties, significantly higher $g_s$ (by 32%) and Ci/Ca in SL208 were confirmed than those in Koshihikari and Kanto IL5 at vegetative stage (43 DAT) (Table 3).

## Discussion

By substitution mapping of the CSSLs, we identified regions affecting $\Delta^{13}C$ on Chr. 2, 3, 6, 7 and 12 (Fig. 2). All regions except for those on Chr. 7 and 12 for $\Delta^{13}C$ at vegetative stage (41 DAT) were successfully delimited. In the regions on Chr. 7 and 12, there was an inconsistency between phenotype and genotype data in the target CSSLs (Fig. 2). One of the possible reasons for the inconsistency might have been two or more QTLs involved with opposed direction on the same chromosome, or genetic interaction between loci (epistasis). The power to detect epistasis by CSSLs may be lower than by RILs because CSSLs contain only a single substituted segment (Keurentjes et al. 2007). Besides, statistical soundness of substitution mapping by Dunnett's test between Koshihikari and each CSSL may not be greater than QTL analysis by RILs where all lines were used in the test of each genomic region. These results represent the limits of genetic analysis by substitution mapping of the CSSLs and show the necessity of additional analysis to validate the regions detected by the CSSLs. In this study, since the region on Chr. 3 was well defined, and the effect was the highest for $\Delta^{13}C$ among the CSSLs at 41 DAT (SL208 in Fig. 1), we focused on Chr. 3 for further study. Although QTL analysis in the $F_2$ population derived from a cross between SL208 and Koshihikari confirmed the QTLs for $\Delta^{13}C$ at vegetative stage (57 DAT) and heading on the long arm of Chr. 3, the QTLs were co-located with that for days-to-heading (Fig. 5). In this region, the flowering time QTL *Hd6* was mapped and cloned in studies using backcross progeny derived from a cross between *japonica* cultivar Nipponbare and *indica* cultivar Kasalath (Yamamoto et al. 2000; Takahashi et al. 2001). Since the sequence of the Koshihikari allele of *Hd6* is identical to that of the Nipponbare allele (unpublished data), we considered the detected QTL for days-to-heading to be *Hd6*.

It was of interest that the effect of the QTL for $\Delta^{13}C$ at heading was opposite to that at 57 DAT (Fig. 5; Table 2): the Kasalath allele increased $\Delta^{13}C$ at 57 DAT but decreased it at heading. It is possible that trait values are influenced by variation in days-to-heading when investigated at heading (Keurentjes et al. 2007; Ando et al. 2008). To clarify whether the QTLs for $\Delta^{13}C$ were different from

**Table 2** Putative QTLs controlling carbon isotope discrimination ($\Delta^{13}C$) and days-to-heading detected in an advanced backcross population derived from SL208 × Koshihikari

| Trait | Chr. | Flanking marker | LOD | LOD threshold | $A^a$ | $D^b$ | $R^{2\ c}$ |
|---|---|---|---|---|---|---|---|
| $\Delta^{13}C$ at vegetative stage (57DAT) | 3 | RM1221 | 8.5 | 2.7 | 0.3 | −0.1 | 31.1 |
| $\Delta^{13}C$ at heading | 3 | RM6970 | 18.8 | 2.6 | −1.0 | 1.1 | 79.0 |
| Days-to-heading | 3 | RM6970 | 54.2 | 2.8 | 9.4 | −5.2 | 90.1 |

[a] Additive effect of the allele from Kasalath compared with that from Koshihikari

[b] Dominant effect of the allele from Kasalath compared with that from Koshihikari

[c] Percentage phenotypic variance explained by each QTL

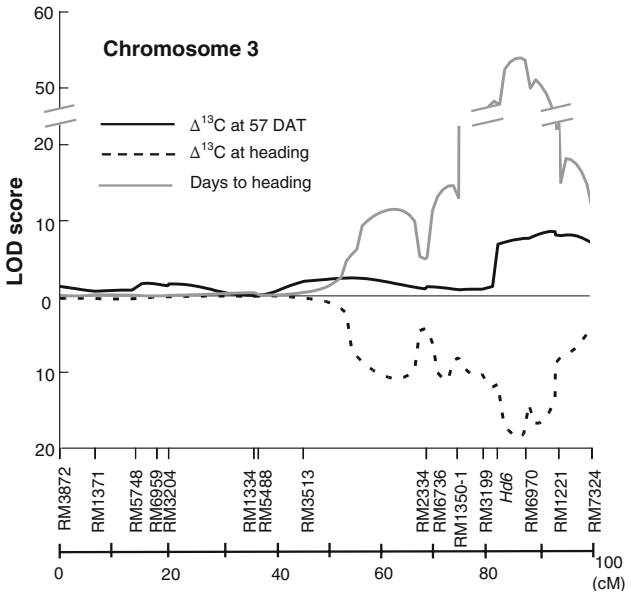


**Fig. 5** LOD scores of QTLs controlling $\Delta^{13}C$ at vegetative stage (57 DAT) and at heading and of QTLs controlling days-to-heading on Chr. 3, computed by composite interval mapping. *Upward* (*downward*) scores indicate positive additive effects of Kasalath (Koshihikari) alleles

*Hd6* or not, we compared the values of $\Delta^{13}C$ among Koshihikari, SL208 and Kanto IL5 (Koshihikari *Hd6* NIL) before heading. When $\Delta^{13}C$ was compared at the same sampling time, Koshihikari and Kanto IL5 showed no difference in $\Delta^{13}C$ before heading of Koshihikari, but $\Delta^{13}C$ in Kanto IL5 decreased after Koshihikari heading (Fig. 6e). Thus, when $\Delta^{13}C$ was compared at the heading date of each cultivar, $\Delta^{13}C$ of Kanto IL5 was significantly lower than that of Koshihikari (Fig. 6d). These results demonstrate that the difference in $\Delta^{13}C$ at heading between Koshihikari and Kanto IL5 resulted from the reduction of $\Delta^{13}C$ in Kanto IL5 by the delayed heading date. This is supported by previous reports that $\Delta^{13}C$ decreased continuously throughout plant development (Samejima 1985; Horie et al. 2003), and a negative correlation between days-to-flowering and $\Delta^{13}C$ was observed in a wide range of crop species (Ehdaie et al. 1991; Hall et al. 1994). On the other hand, $\Delta^{13}C$ of SL208 remained significantly higher than those of the other two varieties before heading of Koshihikari (Fig. 6e). These differences indicate a Kasalath QTL potentially increasing $\Delta^{13}C$ at least during the vegetative stage on the long arm of Chr. 3. Therefore, our results indicate no direct genetic association between the QTL for $\Delta^{13}C$ and *Hd6*. The apparent association may be due to the tight linkage of two different loci. This can be proved by the cloning of the QTL detected in this study. Because agronomical traits are dynamic and vary as plants grow up, evaluation at the same developmental stage is often proposed (Yin et al. 1999). However, our results indicate that genetic analysis to detect potential QTLs for $\Delta^{13}C$ or other target traits needs attention to ensure that indigenous variation in the traits is not confounded with variation in days-to-heading. Similar care for genetic analysis of $\Delta^{13}C$ is suggested in wheat (Rebetzke et al. 2008). Analysis at the same sampling time would avoid variation in heading date among different genetic lines although developmental stages may be different among the lines. In case that $\Delta^{13}C$ was to be investigated at heading stage, significant difference of 0.7‰ resulted from 10 days difference of heading date between Koshihikari and Kanto IL5 should be taken into account.

$\Delta^{13}C$ is determined by the balance between $g_s$ and carboxylation efficiency (Farquhar et al. 1982). The theory suggests that QTLs for $\Delta^{13}C$ may have functions controlling $g_s$ and/or carboxylation efficiency. SL208, carrying the QTL for $\Delta^{13}C$, had a significantly higher $g_s$ and Ci/Ca than Koshihikari during the vegetative stage, but leaf N as one of the traits related with carboxylation efficiency (Makino et al. 1984; Ohsumi et al. 2007) did not differ between SL208 and Koshihikari (Fig. 3; Table 3). These results imply that the increased $\Delta^{13}C$ may have resulted from an enhanced $g_s$. In *Arabidopsis*, $\Delta^{13}C$ was regulated by *ERECTA* gene which played a role in controlling both $g_s$ and carboxylation efficiency (Masle et al. 2005). The BLAST search for *ERECTA* showed that no homolog of *ERECTA* was located on the long arm of chromosome 3 in rice (data not shown). Therefore, the QTL detected for

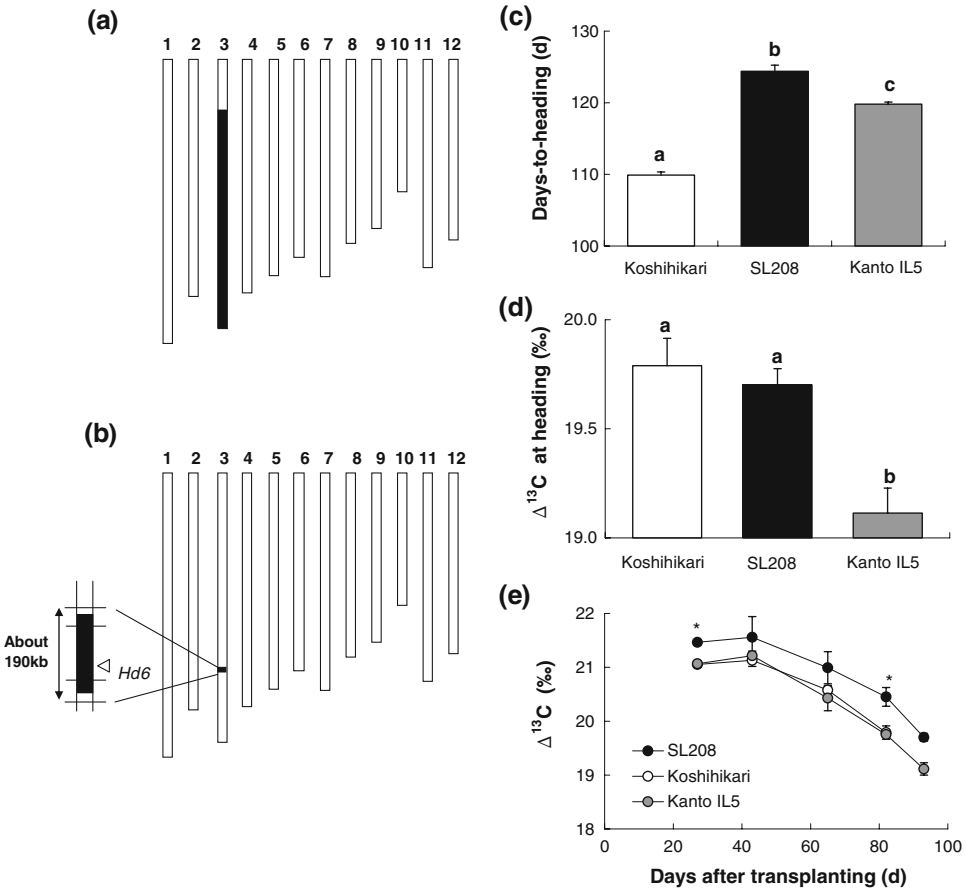


**Fig. 6** Comparisons of photosynthetically related traits among Koshihikari, SL208 and Kanto IL5 in 2007. **a** Graphical genotypes of SL208, **b** graphical genotypes of Kanto IL5. *White blocks*, chromosome regions derived from Koshihikari; *black blocks* from Kasalath, **c** days-to-heading, **d** $\Delta^{13}C$ at heading, **e** $\Delta^{13}C$ at each measuring time until heading. * Significant difference compared to other varieties at 5% levels by Tukey test

**Table 3** Leaf photosynthetic rate (*Pn*), stomatal conductance for $CO_2$ ($g_s$), the ratio of intercellular to ambient $CO_2$ concentration (Ci/Ca), and leaf *N* among Koshihikari, Kanto IL5, and SL208 at vegetative stage (43DAT) in 2007

| | *Pn* (μmol $m^{-2}$ $s^{-1}$) | $g_s$ (mol $m^{-2}$ $s^{-1}$) | Ci/Ca | Leaf *N* (%) |
|---|---|---|---|---|
| Koshihikari | 35.1 ± 1.8[a] a[b] | 0.62 ± 0.06 a | 0.78 ± 0.00 a | 3.4 ± 0.2 a |
| Kanto IL5 | 33.6 ± 0.3 a | 0.55 ± 0.02 a | 0.77 ± 0.01 a | 3.6 ± 0.1 a |
| SL208 | 38.4 ± 0.9 a | 0.82 ± 0.01 b | 0.81 ± 0.01 b | 3.8 ± 0.2 a |

[a] Values are shown as mean ± standard deviation

[b] Means followed by different letter are significantly different at 5% level among varieties with the Tukey test

$\Delta^{13}C$ in this study may be different from the homolog of *Arabidopsis ERECTA*. In addition, higher $g_s$ (by 32–36%) in SL208 than in Koshihikari did not lead to significantly improved Pn, although higher tendency of Pn (by 9%, but not significant) was observed in SL208 than in Koshihikari at vegetative stage (Fig. 3; Table 3). Ohsumi et al. (2007) indicated that the response of Pn to increased $g_s$ was smaller than that to increased leaf N and that improvement of $g_s$ or N alone wouldn't enhance Pn largely in rice. The combination of the QTL detected for $\Delta^{13}C$ with another QTL controlling $g_s$ and/or leaf N may be necessary to improve Pn. To our knowledge, however, this is the first study that investigated a relationship between leaf photosynthesis, $g_s$ and $\Delta^{13}C$ based on genetically well-characterized materials under field conditions in rice. To further test this relationship, fine mapping of the QTL for $\Delta^{13}C$ is now being conducted.

So far, numerous QTL analyses of traits with agronomic and economic importance have been conducted by using mapping populations such as RILs. However, a limited number of studies have subsequently confirmed the character and effects of the QTLs in crop species (Yamamoto and Yano 2008). Recently a QTL for $\Delta^{13}C$ in tomato was confirmed by fine mapping (Xu et al. 2008). In rice, by combining CSSLs, backcross progeny and a NIL, this study successfully revealed a QTL controlling $\Delta^{13}C$ at least during the vegetative stage on the long arm of Chr. 3 and suggested its association with $g_s$.

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