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RESEARCH PAPER

Time-related mapping of quantitative trait loci controlling grain-filling in rice (*Oryza sativa* L.)

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Abstract

Grain-filling is a crucial process that determines final grain yield in rice (*Oryza sativa* L.). To understand the genetic basis of dynamics of grain-filling, quantitative trait locus (QTL) analysis was conducted using time-related phenotypic data on grain-filling collected from a population of 155 recombinant inbred lines (F_{12}), derived from a cross between Milyang 23 and Akihikari. Two QTLs detected on chromosomes 8 and 12 were strongly associated with increased filling percentage per panicle. These QTLs were not linked with those controlling spikelet numbers per panicle. This result confers the possibility of improving grain-filling together with an enlargement of sink size. The QTL for filling percentage per panicle on chromosome 8 exactly overlapped that for non-structural carbohydrate (NSC) content in the culm and leaf sheaths during grain-filling, and the Milyang 23 allele associated with increased grain-filling percentage per panicle was associated with decreased NSC content. Therefore, this QTL may be directly involved in NSC translocation from the culm and leaf sheaths to panicle. In addition, the Milyang 23 alleles of QTLs associated with greater spikelet number per panicle on chromosomes 1 and 6 were also related with a reduction in NSC content in the culm and leaf sheaths during grain-filling. These results indicate that NSC dynamics during grain-filling is partly dependent on sink size. NSC accumulation in the culm and leaf sheaths at the heading stage was mainly controlled by different genetic regulations from NSC dynamics during grain-filling. Nitrogen dynamics during grain-filling may also be involved in carbohydrate dynamics.

Key words: Grain-filling, non-structural carbohydrate (NSC) accumulation, quantitative trait locus (QTL), rice (*Oryza sativa* L.), translocation.

Introduction

In cereal crops, grain-filling is a critical and dynamic process that determines final grain yield. Numerous studies have elucidated that the final yield depends on carbohydrates derived from two different sources; leaf photosynthetic assimilates during grain-filling and accumulated non-structural carbohydrate (NSC) in culms and leaf sheaths prior to heading (Cock and Yoshida, 1972; Sumi *et al*., 1996; Nagata *et al*., 2001; Samonte *et al*., 2001). A recent study indicated that modern high yielding rice (*Oryza sativa* L.) cultivars with large sink size were short of available carbohydrates to fill their grains completely (Nagata *et al*., 2001). Therefore, it is obviously necessary to increase carbohydrate supply by leaf photosynthesis after heading and/or from the reserve NSC in culms and leaf sheaths before heading for breeders to develop higher yielding rice with successful grain-filling.

The contribution of reserved NSC in culms and leaf sheaths of rice plants is estimated at around 30% of the final yield depending on cultivar and environmental conditions (Yoshida, 1972; Song *et al*., 1990; Gebbing and Schnyder, 1999; Ntanos and Koutroubas, 2002). The stored NSC can serve as a buffer to support normal grain growth despite the fluctuations of weather (Yoshida, 1972). In addition, Weng *et al*. (1982) and Tsukaguchi *et al*. (1996) suggested that stored NSC played an important role in the formation of

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an active sink during the early grain-filling period through the determination of endosperm cell numbers. On the other hand, some studies have reported that a large amount of NSC, accumulated in culms and leaf sheaths before heading by rice cultivars such as the new plant type and F_1 hybrid, remained untranslocated to grains after heading, resulting in low yield with poor grain-filling (Tsukaguchi *et al*., 1999; Yang *et al*., 2002). Comprehensive understanding of the mechanism controlling plant carbohydrate dynamics during grain-filling has not yet been achieved.

Leaf nitrogen dynamics also seriously affects grain-filling. Leaf nitrogen is essential for maintaining photosynthetic capacity, while its remobilization to the panicle is also required for the formation of sound grains during grain-filling (Mae and Ohira, 1981). In addition, Yang *et al*. (2000*a*) indicated that translocation of stored NSC from vegetative tissues to the grains required the initiation of whole plant senescence, implying the necessity of nitrogen for carbohydrate dynamics. However, little was known about whether these phenotypic associations were due to a close genetic relationship or to independent gene expression.

The advent of molecular markers has made it possible to identify quantitative trait loci (QTLs) controlling complex traits and to analyse the genetic basis of association among traits (Tanksley, 1993). Using molecular markers, a large number of studies have been conducted to detect QTLs affecting grain yield and yield components in maize (Ajmone-Marsan *et al*., 1995; Austin and Lee, 1996), rice (Xiao *et al*., 1996; Li *et al*., 1997; Xing *et al*., 2002), wheat (Groos *et al*., 2003; Campbell *et al*., 2003), and soybean (Maughan *et al*., 1996; Concibido *et al*., 2003). However, attention was paid only to yield traits in those studies. Since final yield and yield components are the result of various biochemical and physiological processes, genetic analysis on final characters such as yield may not confer sufficient information on yield formation and/or yield-limiting processes (Cui *et al*., 2003). Horie *et al*. (2003) indicated that physiological traits as well as morphological traits limiting yield potential should be identified in the process of yield formation and should be incorporated into breeding programmes to break through the current limits to yield potential of rice. In this study, an attempt was made to clarify the genetic basis of the grain-filling mechanism in rice using time-related QTL mapping (Wu *et al*., 1999), because individual QTLs associated with yield formation must have different expression dynamics during the successive grain-filling period. Emphasis was given to QTL co-location and phenotypic association among traits.

Materials and methods

Plant materials and field experiment

A population consisting of 155 recombinant inbred lines (RILs) (F_{12} generation) constructed by a single seed descent method from a cross between Milyang 23 and Akihikari (Fukuta *et al*., 2004) was used in this study. Milyang 23 is a high-yielding Korean Indica-type cultivar with a heavy panicle. Akihikari is also regarded as a high-yielding variety among Japonica-type ones in Japan. Field experiment was conducted at the IRRI farm, Los Baños (14°11′ N, 121°15′ E, 21 m altitude), Laguna, Philippines, during the 2003 dry season from January to May. Two parents and 155 RILs were sown in the seedling nursery on 17 January and transplanted with one seedling per hill on 11 February. Each plot consisted of 7 rows with 12 hills per row and hill spacing was 0.2×0.2 m. Fertilizers of 40 kg N ha^{-1}, 40 kg P ha^{-1}, and 40 kg K ha^{-1} were applied as basal. Forty kg N ha^{-1} was topdressed at 2 weeks after transplanting. For pests, insects, and water, the practical field management was performed on the basis of IRRI experimental farm practices.

When 50% of hills had at least one stem which had started heading, heading stage was determined, and days-to-heading defined as the number of days from sowing to heading was counted in each RIL and the parents. Ten hills per plot were collected for the evaluation of traits associated with grain-filling at heading stage, and 14, 21, and 35 d after heading. Main stem or primary tiller was chosen from each plant and separated into green leaf blades, culm plus sheaths, and panicle. Dry weight of each organ was determined after oven-drying at 70 °C to constant weight. After weighing, leaf blades and culm plus sheath samples in each plot were bulked and ground to a fine powder, respectively. Leaf nitrogen content was determined by the Kjeldahl method (Bremner and Mulvaney, 1982). NSC content in culm and leaf sheaths was measured according to the method of Tsukaguchi *et al*. (1996) with some modifications: milled samples (0.5 g) were placed in 30 ml of water and heated at 100 °C for 30 s to extract NSC. After cooling, 1.5 mg α-amylase and 0.5 mg amyloglucosidase in 20 ml of 12.08 g l^{-1} KH_2PO_4 and 3.98 g l^{-1} $Na_2HPO_4.12H_2O$ buffer was added and incubated at 40 °C for 24 h to disbranch NSC into monosaccharides. After the incubated samples were filtered, the residues were dried at 80 °C for 3 h and weighed. NSC content was calculated from the weight difference between the initial sample and the residue. At 35 d after heading, panicles were hand-threshed after weighing, spikelet number per panicle was counted by multi auto counter (Kiya Seisakusho Ltd, Tokyo), and the spikelets were weighed. Filled spikelets were separated by submerging them in tap water. The number and weight of filled spikelets were determined after oven-drying at 70 °C to constant weight. Sink size and filling percentage per panicle at each sampling stage were calculated by the following equations, assuming that the weight of rachis and glume changes little during grain-filling (Cock and Yoshida, 1972).

$$Sink\ size = \frac{Filled\ spikelet\ weight}{Filled\ spikelet\ number} \times Spikelet\ number\ per\ panicle - Glume\ weight \quad (1)$$

$$Glume\ weight = Panicle\ weight\ at\ heading - Rachis\ weight\ at\ 35\ d\ after\ heading \quad (2)$$

$$Filling\ percentage\ per\ panicle\ at\ each\ state = \frac{Panicle\ weight\ at\ each\ stage - Panicle\ weight\ at\ heading}{Sink\ size} \quad (3)$$

Climate data were obtained from the Climate Unit in the Crop, Soil, and Water Sciences (CSWS) division of IRRI.

QTL analysis

Using 192 restriction fragment length polymorphism (RFLP) markers and 81 simple sequence repeat (SSR) markers, Fukuta *et al*. (2004)

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determined the F_{10} genotypes of marker loci and constructed a linkage map.

The chromosomal positions and effects of putative QTLs were determined by composite interval mapping (CIM) using QTL Cartographer 2.0 (Basten *et al*., 2002) since CIM is well known to have the advantages over simple interval mapping in more precisely detecting the position and effects of the QTLs (Zeng, 1994). QTL cartographer's Zmap QTL, Model 6 with a window size of 10 cM was used for CIM. The number of markers for the background control was set to 5. A likelihood ratio of 11.5, corresponding to a LOD score 2.5, was used as a threshold value to detect a putative QTL, since Yano and Sasaki (1997) pointed out that a higher threshold may underestimate putative QTLs and show a bias towards genes with larger phenotypic effects. To ensure the false positive (type I error) rate for QTL detection, 1000 permutation tests for each trait were conducted at the 5% level of significance (Churchill and Doerge, 1994; Doerge and Churchill, 1996). The peak of LOD score with one-LOD support interval was accepted as the location of putative QTL (van Ooijen, 1992), and the additive effect and phenotypic variance explained by each QTL were estimated at the peak of LOD score.

Results

Climate conditions

Solar radiation and temperature conditions during the growing season are shown in Fig. 1. Daily radiation was around 20 MJ m^{-2} throughout the season, although there was a drastic drop in radiation at the end of the season. During grain-filling, average daily radiation for the earliest and the latest matured RILs were 22.1±4.3 MJ m^{-2} (mean ±SD) and 22.8±4.5, respectively. Both maximum and minimum temperatures were fairly stable and showed a slight increase as the season progressed. Average maximum and minimum temperatures during grain-filling were 31.2±1.6 °C and 23.4±1.1 °C for the earliest matured RIL, and 33.3±1.4 °C and 25.1±0.7 °C for the latest one, respectively. These results indicate that grain-filling among 155 RILs had proceeded under favourable and similar climate conditions.

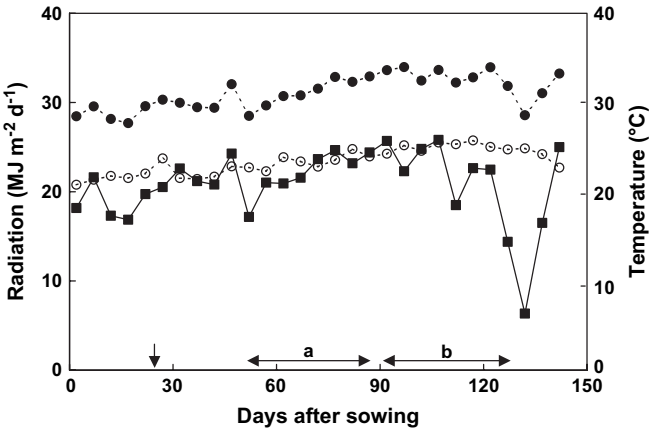

Fig. 1. Daily solar radiation (closed squares) and daily maximum (closed circles) and minimum (open circles) air temperatures during the entire growth period of rice grown at the International Rice Research Institute (IRRI), Los Baños, Laguna, the Philippines in the 2003 dry season. Each datum point represents the mean of five days. Vertical arrow stands for transplanting day. Horizontal arrows a and b show the grain-filling periods, defined here as 35 d since heading, for the earliest and the last matured recombinant inbred lines (RILs), respectively.

Phenotypic variation

Continuous variation was observed in the RILs for all measured traits, which were quantitatively inherited and transgressive segregation was demonstrated in both parents' directions (Fig. 2). Akihikari reached heading stage earlier than Milyang 23; days-to-heading for Akihikari was 62 d, whereas that of Milyang 23 was 84 d. Spikelet number per panicle of Akihikari (65.9) was less than that of Milyang 23 (127.6), confirming that Milyang 23 is a heavy panicle type. Akihikari ripened more rapidly than Milyang 23; Akihikari had already attained nearly 100% of grain-filling at 21 d after heading, while Milyang 23 ripened slowly and the final filling percentage per panicle at 35 d after heading was 81.2%. The NSC content in the culm and leaf sheaths at heading was higher in Milyang 23 than that in Akihikari. The accumulation of NSC was still occurring in Akihikari 14 d after heading although the reduction of NSC was already being observed in Milyang 23. The decrease in NSC content of Milyang 23 was greater at 21 d after heading than that of Akihikari, suggesting that a large amount of NSC reserve in the culm and leaf sheaths was translocated to the panicle in Milyang 23. Because the reduction of leaf nitrogen proceeded at a similar rate in both parents, Akihikari, with deeper green leaves at heading stage, maintained a higher leaf nitrogen content than Milyang 23 throughout the grain-filling period.

Phenotypic relationships among grain-filling traits

Days-to-heading was positively correlated with spikelet number per panicle (r=0.28; P <0.01) and NSC content in the culm and leaf sheaths at heading (r=0.35; P <0.01) in RILs, but negatively correlated with leaf nitrogen content at heading (r = −0.64; P <0.01) (Table 1). There was no significant correlation between days-to-heading and filling percentage per panicle.

Modern rice cultivars which produced a large number of spikelets often resulted in a poor grain-filling (Peng *et al*., 1999). As in the previous studies, spikelet number per panicle was negatively correlated with filling percentage per panicle during grain-filling although only weakly (r = −0.16 to −0.21). This result suggests that a large sink may delay and decrease grain-filling. Negative correlations were observed between spikelet number per panicle and NSC content in the culm and leaf sheaths during grain-filling except heading stage (r = −0.25 to −0.36; P <0.01). Filling percentage per panicle was also negatively correlated with NSC content at 14 d and 21 d after heading (r = −0.24 and −0.27; P <0.01). These results imply that a large panicle needs translocation of a considerable amount of

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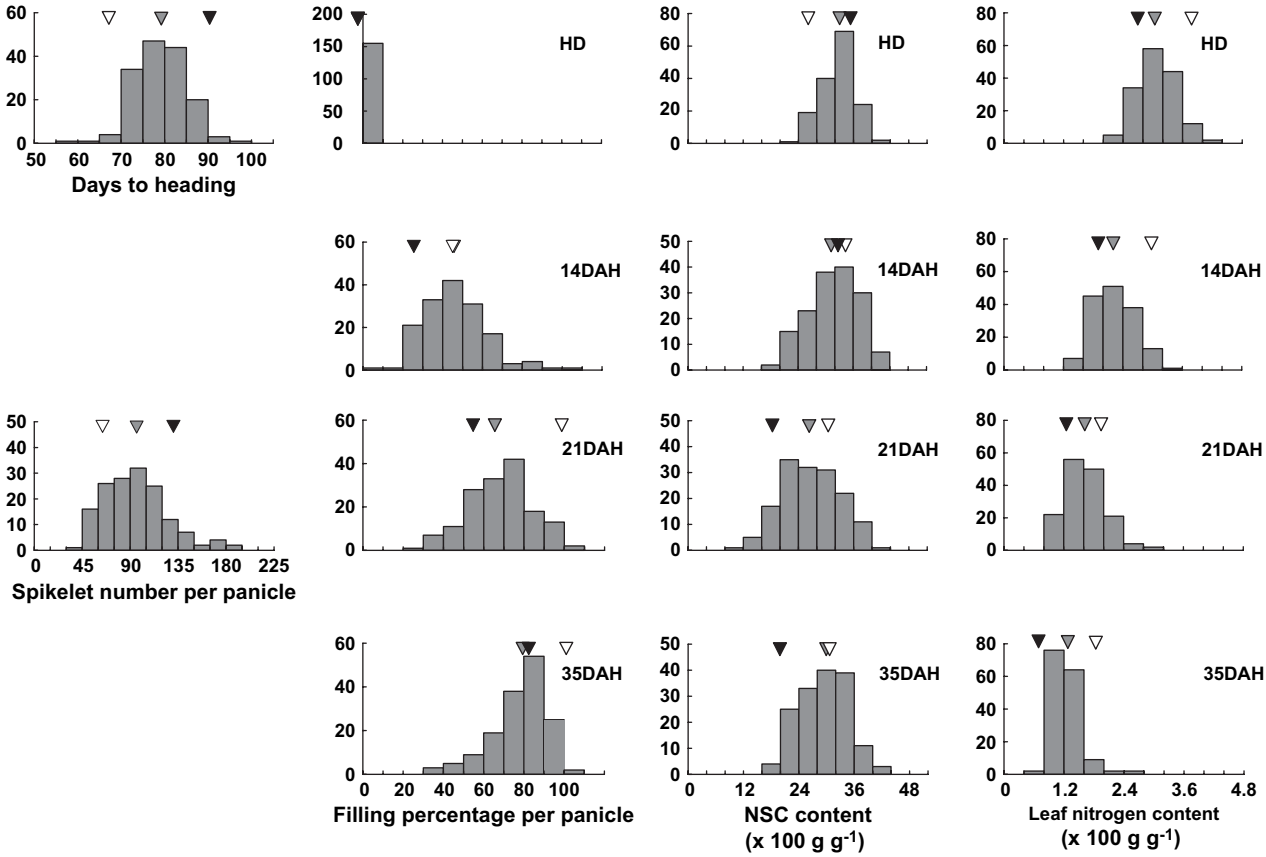

Fig. 2. Frequency distributions of five traits associated with grain-filling in RILs derived from Milyang 23 and Akihikari. Mean values were shown for the RILs (grey triangles) as well as Milyang 23 (closed triangles) and Akihikari (open triangles). HD: heading stage, DAH: days after heading.

NSC from the culm and leaf sheath for successful grain-filling. In addition, filling percentage per panicle was negatively correlated with leaf nitrogen content at 14 d and 21 d after heading ($r = -0.40$ and -0.34; $P < 0.01$), suggesting that nitrogen remobilization may be also related with grain-filling. A high negative correlation was observed between NSC content and leaf nitrogen content at heading ($r = -0.47$; $P<0.01$). This negative correlation at the heading stage agrees with previous studies that higher content of total carbohydrate in plants led to lower nitrogen content in leaves (Matsushima and Wada, 1958; Weng *et al*., 1986).

Quantitative trait loci for grain-filling traits

A total of three, six, five, ten, and nine genomic regions significantly affecting days-to-heading, spikelet number per panicle, filling percentage per panicle, NSC content, and leaf nitrogen content were detected on all chromosomes except for chromosome 7 during grain-filling (Table 2; Fig. 3). The phenotypic variance explained by each QTL (R^2) ranged between 5.6% and 16.6%.

For days-to-heading one and two QTLs were detected on chromosomes 2 and 10, respectively. All QTLs showed a small effect, ranging from 6.0–7.8% of total variance. The Milyang 23 allele of QTLs on chromosome 2 and on the distal end of chromosome 10 delayed time of heading, but hastened it for the QTL close to *XNpb133* on chromosome 10.

A large-effect QTL for spikelet number per panicle was identified in the vicinity of *XNpb90* on chromosome 1, explaining 16.5% of total variance. This QTL was located near the region where Yagi *et al*. (2001), Nagata *et al*. (2002*a*), and Kobayashi *et al*. (2004) detected QTLs controlling spikelet number per panicle, indicating that these were common QTL. R^2 of other five QTLs for spikelet number per panicle detected on chromosomes 4, 6, and 8 ranged between 5.6% and 11.6%. The Milyang 23 alleles of QTLs on chromosomes 1, 6, and 8 contributed to increased spikelet number per panicle, whereas the Akihikari alleles of three QTLs on chromosome 4 positively affected spikelet number per panicle.

Among five genomic regions showing significant association with filling percentage per panicle, two QTLs on chromosomes 8 and 12 could be detected continuously between 21 d and 35 d after heading, and between 14 d and 35 d after heading, respectively. R^2 of the QTL on chromosome 8 increased from 6.7% to 15.2% during the period, while the QTL on chromosome 12 constantly accounted for around 15% of total variance (14–16.6%)

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Table 1. *Correlation coefficients among five traits associated with grain filling in RILs derived from Milyang 23 × Akihikari*

HD: heading stage, DAH: days after heading. * and ** The 5% and 1% levels of significance, respectively.

	Days to heading	Spikelet number per panicle	Filling percentage per panicle			NSC content				Leaf nitrogen content		
			14 DAH	21 DAH	35 DAH	HD	14 DAH	21 DAH	35 DAH	HD	14 DAH	21 DAH
Spikelet number per panicle	0.278**											
Filling percentage per panicle												
14 DAH	0.152	−0.158*										
21 DAH	0.009	−0.164*	0.626**									
35 DAH	−0.144	−0.209**	0.530**	0.577**								
NSC content												
HD	0.351**	0.002	0.206**	0.131	−0.024							
14 DAH	−0.029	−0.249**	−0.240**	−0.146	−0.284**	0.578**						
21 DAH	0.014	−0.358**	−0.142	−0.269**	−0.300**	0.464**	0.786**					
35 DAH	0.066	−0.283**	0.041	0.056	−0.129	0.457**	0.672**	0.747**				
Leaf nitrogen content												
HD	−0.636**	−0.291**	−0.139	−0.051	0.089	−0.470**	−0.104	−0.010	−0.075			
14 DAH	−0.557**	−0.150	−0.398**	−0.140	0.000	−0.538**	−0.128	−0.052	−0.108	0.758**		
21 DAH	−0.498**	−0.170*	−0.262**	−0.337**	0.016	−0.489**	−0.117	0.093	−0.033	0.687**	0.757**	
35 DAH	−0.488**	−0.223**	−0.126	−0.101	0.064	−0.462**	−0.152	0.012	0.079	0.624**	0.708**	0.773**

between 14 d and 35 d after heading. The other three QTLs on chromosomes 9, 10, and 11, explaining R^2 from 6.6–9.6%, were identified only at 14 d after heading. The parent Akihikari alleles of QTLs on chromosomes 10, 11, and 12 were associated with increased filling percentage per panicle, while the Milyang 23 alleles of QTLs on chromosomes 8 and 9 acted to enhance filling percentage per panicle. The QTL on chromosome 10 detected at 14 d after heading overlapped that for days-to-heading.

Six out of ten chromosome regions detected for NSC content were associated with NSC accumulation in the culm and leaf sheaths at heading stage. Four QTLs on chromosomes 2, 3, and 9 contributed to reservation of NSC in the culm and leaf sheaths with the Milyang 23 allele, while the Akihikari alleles of two QTLs on chromosomes 1 and 6 acted to increase NSC content. R^2 of those QTLs affecting NSC accumulation ranged from 6.3% to 14%. The QTL on chromosome 6 for NSC content was repeatedly detected at the same region at 21 d and 35 d after heading, in addition to the heading stage, suggesting that this QTL may have relevance to NSC dynamics during grain-filling as well as NSC accumulation at heading. This QTL was co-located with the one controlling spikelet number per panicle. Two QTLs close to *XNpb90* on chromosome 1 and *RM44* on chromosome 8 were continuously identified between 14 d and 35 d after heading, respectively. The Milyang 23 alleles of both QTLs were associated with decreased NSC content in the culm and leaf sheaths. Both loci on chromosomes 1 and 8 showed a peak expression at 21 d after heading, accounting for 13.9% and 14.9% of total variance, respectively. This result corresponds well with the large reduction in NSC content of Milyang 23 at 21 d after heading (Fig. 2). It is of interest that the region of the QTL on chromosome 1 coincided with that for spikelet number per panicle, whereas the QTL on chromosome 8 was closely linked to that for filling percentage per panicle. Besides, a QTL was repeatedly detected on chromosome 5 at 21 d and 35 d to heading explaining 6.4% and 8.9% of R^2. The Akihikari allele of this QTL reduced NSC content. A QTL on chromosome 11 for NSC content, explaining 9.3% of R^2, was only found at 14 d after heading.

Seven loci among nine genomic regions controlling leaf nitrogen content were detected only once during grain-filling. Three QTLs found at heading, which ranged between 7% and 12.3% of total variance, were clustered at the long arm on chromosome 1. Two different QTLs were detected on chromosome 2, once at the heading stage and 35 d after heading, accounting for 7.3% and 6.2% of R^2, respectively. A QTL was found on chromosome 5 only at 14 d after heading, which explained 7.3% of R^2. While one QTL was identified close to *XNpb13* on chromosome 9 once at 14 d after heading, the other QTL was repeatedly detected on the distal end of chromosome 9 at the heading stage, 21 d and 35 d after heading, ranging from 7–11% of total variance. The QTL close to *XNpb13* on chromosome 9 overlapped that for NSC content identified at heading. In addition, a putative QTL was continuously detected on chromosome 3 between 14 d and 21 d to heading, explaining 8.1% and 8% of total variance. This QTL also overlapped that for NSC content at heading.

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Table 2. *Putative QTLs for five traits associated with grain filling in the Milyang 23/Akihikari RILs*

* Putative QTLs with significant LOD scores on 1000 permutation tests at the 5% level.

Trait	Heading					14 d after heading					21 d after heading					35 d after heading				
	Chr.	Franking markers[a]	LOD	R^{2b}	A^c	Chr.	Franking markers	LOD	R^2	A	Chr.	Franking markers	LOD	R^2	A	Chr.	Franking markers	LOD	R^2	A
Days to heading	2	***RM6***	3.28*	7.8	1.71															
	10	***XNpb133***	2.54	6.0	−1.51															
	10	*RM228* − ***XNpb127***	3.30*	7.6	1.74															
Spikelet number per panicle																1	*XNpb90*	6.84*	16.5	12.28
																4	***XNpb161***	3.46*	7.0	−8.00
																4	***RM303*** − *RM255*	5.05*	11.6	−10.35
																4	***OSR15***	2.74	5.6	−7.28
																6	***XNpb172*** − *G2028*	2.71	6.6	8.27
																8	***XNpb321A***	3.51*	7.9	8.55
Filling percentage per panicle						9	*XNpb40* − ***XNpb103***	2.67	6.9	4.16	8	***RM44***	2.87	6.7	4.39	8	***RM44***	6.53*	15.2	5.68
						10	***RM271***	4.24*	9.6	−4.91	12	***C1069*** − *G1106*	4.72*	14.0	−6.51	12	***C1069***	6.79*	14.9	−6.80
						11	***RM206***	2.95	6.6	−4.06										
						12	***XNpb148*** − ***RM270***	6.54*	16.6	−7.22										
NSC content	1	***N079A***	2.69	6.3	−0.93	1	*R210B* − ***XNpb90***	2.87	8.9	−1.58	1	*R210B* − ***XNpb90***	5.47*	13.9	−2.37	1	***XNpb90***	3.42*	8.0	−1.46
	2	***G1327***	2.99	6.3	0.92	8	***RM44*** − *G2132B*	2.85	8.1	−1.54	5	***XNpb366***	3.10*	6.4	1.65	5	***XNpb366***	3.88*	8.9	1.58
	2	***G1314B***	2.91	14.0	1.38	11	*XNpb202* − ***XNpb257***	3.72*	9.3	1.67	6	***C235***	2.66	5.9	−1.75	6	***C235***−*C488*	3.57*	7.5	−1.58
	3	***XNpb249*** − ***RM143***	3.34*	8.8	1.10						8	***RM44***	7.03*	14.9	−2.54	8	***RM44***	3.00	6.7	−1.38
	6	***C488***	2.75	6.5	−1.00															
	9	*XNpb385* − ***XNpb13***	2.61	6.3	0.93															
Leaf nitrogen content	1	***XNpb147***	2.84	7.0	−0.11	3	*RM168* − ***XNpb249***	2.98	8.1	−0.12	3	***XNpb249*** − *RM143*	2.86	8.0	−0.12	2	***XNpb353***	2.74	6.2	−0.08
	1	***C86*** − *XNpb113*	3.94*	12.2	0.15	5	***C246***	2.98	7.3	−0.12	9	*RM245* −***G1445***	3.15*	8.3	−0.12	9	***OSR29*** − *XNpb293*	3.41*	8.8	−0.09
	1	***RM104*** − *XNpb393B*	4.39*	12.3	0.15	9	***XNpb13***	2.90	7.0	−0.11										
	2	*G1327* − ***XNpb227***	3.20*	7.3	−0.11															
	9	*OSR29* − ***XNpb293***	4.07*	11.0	−0.13															

[a] Bold indicates nearest marker of putative QTL.
[b] Percentage phenotypic variance explained by each QTL.
[c] Additive effect of the allele from Milyang 23 compared with Akihikari.

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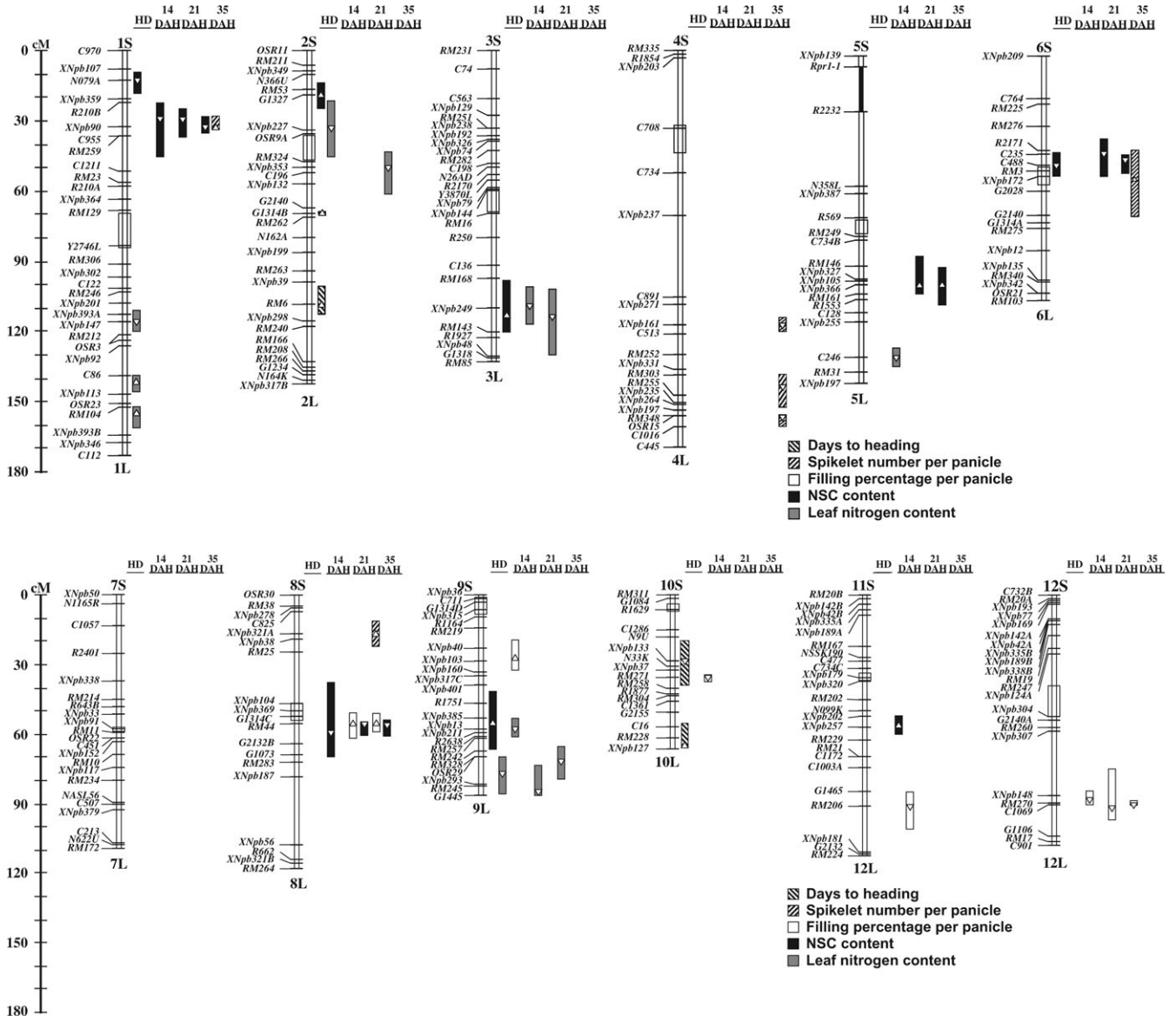

Fig. 3. Mapping of QTLs for five traits associated with grain-filling at heading stage (HD), 14, 21, and 35 d after heading (DAH) on the rice linkage map, respectively. The linkage map consisted of 192 RFLP markers and 81SSR markers (Fukuta *et al*., 2004). The rectangular boxes on chromosomes represent the estimated centromere regions (Singh *et al*., 1996). The numbers, S and L indicate each chromosome, short and long arm, respectively. Markers are located on the left of each chromosome. Triangles and boxes on the right of each chromosome represent LOD peaks of putative QTLs and their one-LOD support intervals (van Ooijen, 1992), respectively. Upward and downward triangles indicate that the Milyang 23 and Akihikari alleles increased each trait, respectively.

Discussion

Conventionally, crop physiologists have scrutinized many characteristics for only a few cultivars, following their ontogeny. By contrast, plant geneticists and breeders focused on a few traits on a large number of lines (>100) of a segregating population at a fixed time or stage (Yin *et al*., 2003). Grain-filling is a crucial and dynamic process that determines the final grain yield in cereal crops. Despite the fact that physiologists directed their attention to grain-filling processes, there have been few genetic studies of grain-filling because of the complex and dynamic features. In the present study, time-course QTL mapping was carried out to understand genetic basis of dynamic grain-filling in rice using the Milyang 23/Akihikari RILs.

One of the most significant points in the field experiment is that variation in crop ontogeny, such as flowering time among cultivars, can cause environmental variation in their subsequent developmental stage. In such a case, the environmental variation may have a strong influence on phenotypic traits, making it hard to clarify the intrinsic genetic variation associated with the traits. In the present study, while variability in days-to heading was observed among

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155 RILs, variations in meteorological conditions such as solar radiation and temperature were relatively small among the 155 RILs during the grain-filling period (Fig. 1). Therefore, it was considered that grain-filling had proceeded under favourable and similar climate conditions among the 155 RILs.

Increasing spikelet number per unit land area is indispensable in order to enhance the yield potential of cereal crops. However, the success of sink size development often failed in poor grain-filling (Peng *et al*., 1999). In this study, Milyang 23 with a large number of spikelets per panicle also resulted in a lower filling percentage per panicle than Akihikari. Six QTLs were associated with spikelet number per panicle. Those QTLs have already been identified by previous studies (Yagi *et al*., 2001; Nagata *et al*., 2002*a*; Kobayashi *et al*., 2004), and some of them have been characterized, i.e. the QTL in the vicinity of *XNpb90* on chromosome 1 may hasten the differentiation of secondary rachis branches (Nagata *et al*., 2002*a*). QTLs controlling filling percentage per panicle were detected at five regions of the linkage map during grain-filling. It is of interest that no QTLs for filling percentage per panicle overlapped those for spikelet number per panicle (Fig. 3). No co-location of QTLs between filling percentage and spikelet number per panicle suggests that the two traits are controlled by different genes, conferring the possibility that the detected QTLs affecting filling percentage per panicle could be valuable for improving grain-filling regardless of sink size.

Among five loci associated with filling percentage per panicle, the QTL on chromosome 12 was detected as early as 14 d after heading and contributed to increase filling percentage per panicle with the Akihikari allele up to 35 d after heading. Recently, rice cultivar variation in grain-filling rate was studied and Yang *et al*. (2000*b*) reported that cultivars with fast grain-filling achieved a higher grain-filling percentage. That study agrees with the result that Akihikari, with rapid grain-filling, attained a higher filling percentage per panicle at maturity than Milyang 23, suggesting that the QTL on chromosome 12 may promote grain-filling from the early-filling stage to maturity. In addition, this QTL was not linked to any QTLs controlling source traits such as NSC content and leaf nitrogen content as well as sink size. Murchie *et al*. (2002) reported that there was no consistent relationship between the rate of grain-filling and photosynthetic capacity in rice varieties. It is assumed that the ability of spikelets' pumping up carbohydrate from leaves and stem, called sink activity, also influences grain-filling (Seo and Ota, 1982; Sumi *et al*., 1996). Further study is required to elucidate how the QTL on chromosome 12 can accelerate grain-filling. Another QTL for filling percentage per panicle on chromosome 8 was tightly linked to a QTL controlling NSC content in the culm and leaf sheaths. Co-location of QTLs associated with different traits can be explained by two possible reasons. One is that the QTLs are closely linked genetically but unrelated phenotypically. The second is that the two traits are affected by a single locus with pleiotropic effects. The peaks of LODs of two QTLs controlling filling percentage per panicle and NSC content exactly overlapped in the vicinity of *RM44* on the chromosome 8 (Figs 3, 4). This confers a strong possibility of pleiotropic effects by the same gene. In addition, the QTL for filling percentage per panicle was identified at 21 d after heading and strongly expressed at 35 d after heading, while the QTL for NSC content was continuously detected between 14 d and 35 d after heading with peak expression at 21 d after heading (Fig. 4). The Milyang 23 allele associated with increased filling percentage per panicle was associated with the reduction in NSC content in the culm and leaf sheath. These results suggest that the Milyang 23 allele of this QTL greatly accelerated NSC translocation from the culm and leaf sheath to the panicle at around 21 d after heading and raised the final grain-filling percentage. Negative correlations between NSC content and filling percentage per panicle during the grain-filling period (Table 1) and a large reduction in NSC content for Milyang 23 at 21 d after heading (Fig. 2) also support this hypothesis. Moreover, a QTL was detected that increased the number of ratoon with the Akihikari allele at a similar region on chromosome 8 using the same population (T Takai, unpublished data). Ratoon is regarded as an index of the regenerating ability of excised stem segment in rice (Oka, 1988). Since carbohydrate is required for plant growth and development, untranslocated NSC in the culms and leaf sheaths may have been used for ratoon development in RILs with the Akihikari allele of the QTL on chromosome 8. On the other hand, the Gramene database (http://www.gramene.org/) provides information on QTLs detected for days-to-heading or panicle length in the vicinity of *RM44* on chromosome 8 by some other populations (He *et al*., 2001; Brondani *et al*., 2002), while no QTLs have been detected for these traits in previous studies with the population from Milyang 23 and Akihikari (Fukuta *et al*., 1998; Kobayashi *et al*., 2003). Additional work will be needed to identify the association between QTLs detected in the populations used in this study and in some other ones.

In addition to the QTL on chromosome 8, two QTLs on chromosomes 1 and 6 may also be of importance to NSC dynamics during grain-filling. Two QTLs detected near *XNpb90* on chromosome 1 and in the vicinity of the centromere on chromosome 6 were mapped to the same regions as QTLs for spikelet number per panicle, suggesting a sink size effect on translocation. The Milyang 23 alleles of both QTLs associated with increased spikelet number per panicle were associated with reduced NSC content in the culm and leaf sheaths, indicating that a large sink demands a large amount of carbohydrate even from temporary source organ. These results correspond to the negative correlation between the number of spikelets and NSC content during the grain-filling period (Table 1). The

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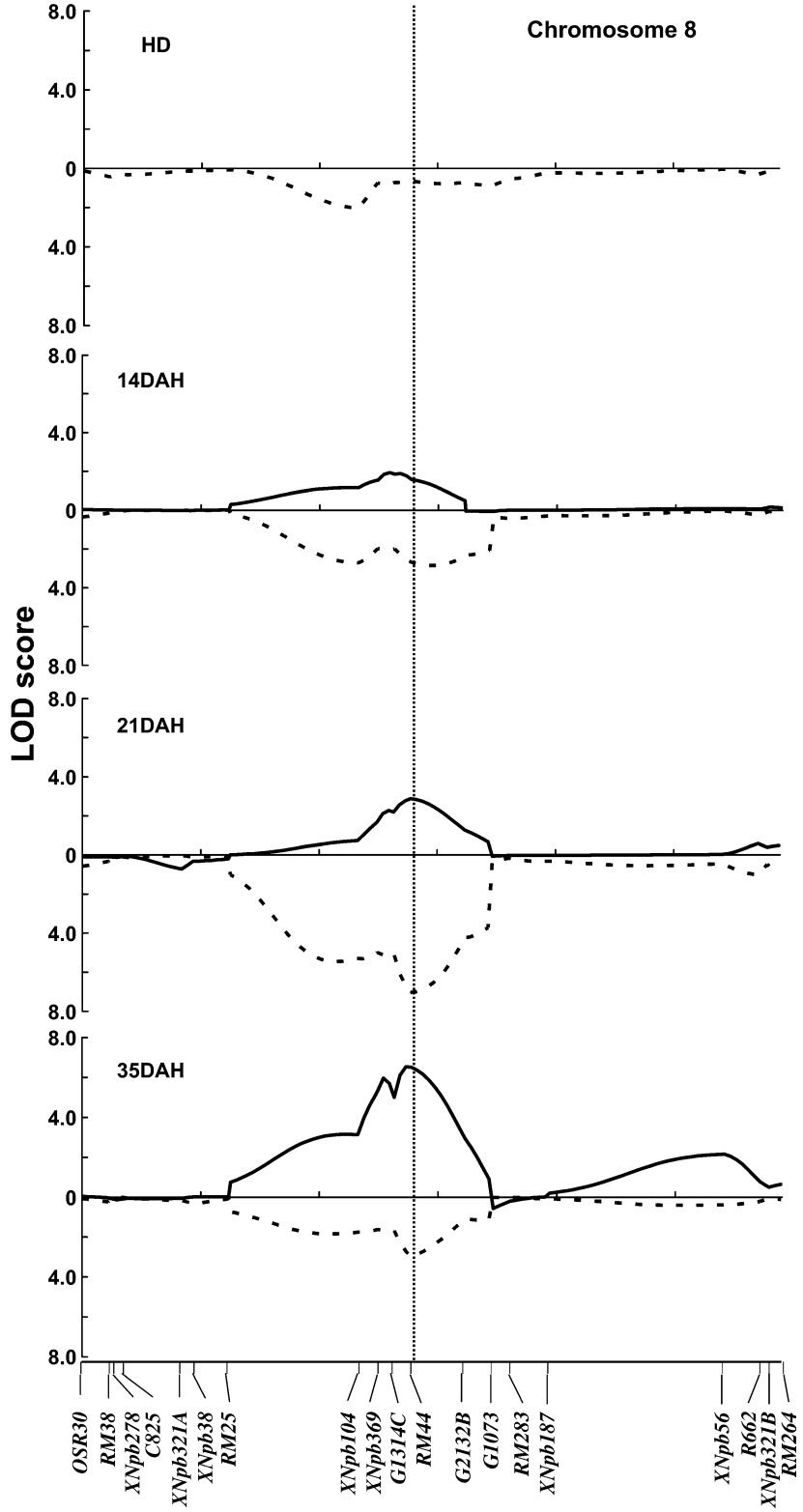

Fig. 4. Dynamic changes of logarithm of odds (LOD) score in QTLs controlling filling percentage per panicle (solid line) and NSC content (dotted line) in the culm and leaf sheaths on chromosome 8 during grain-filling. Upward and downward LOD score curves indicate the positive additive effects in the presence of Milyang 23 and Akihikari allele, respectively. HD: heading stage, DAH: days after heading.

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QTL on chromosome 1 was continuously detected between 14 d and 35 d after heading with a peak expression at 21 d after heading, which was also consistent with the result that Milyang 23 decreased a large amount of NSC in the culm and leaf sheaths at 21 d after heading (Fig. 2). On the other hand, the QTL on chromosome 6 was identified at heading stage as well as at 21 d and 35 d after heading. The Milyang 23 allele of this QTL was associated with a decreased NSC content in the culm and leaf sheaths even at the heading stage. This implies that the QTL with the Milyang 23 allele on chromosome 6 may not contribute to NSC accumulation at heading, although it can work for NSC dynamics during grain-filling.

In addition to the QTL on chromosome 6 already described above, five QTLs were also detected at the heading stage for NSC content in the culm and leaf sheaths on chromosomes 1, 2, 3, and 9. Nagata *et al*. (2002*b*) also detected two QTLs controlling NSC content in the culms and leaf sheaths on chromosomes 5 and 11 at the heading stage using a different population in a temperate climate. Interestingly, the location of those QTLs differed from ours. The process of NSC reservation is complicated and possibly affected by environmental conditions prior to heading (Rowland-Bamford *et al*., 1996; Nagata *et al*., 2001). Although differences in detailed climate conditions before heading between the two experiments were unclear, the QTLs identified for NSC content in both studies were not co-located with those for days-to-heading. Detected QTLs in two studies may play different roles in the process of NSC accumulation. In addition, those five QTLs detected for NSC accumulation were independent of those associated with NSC dynamics during grain-filling. Tsukaguchi (1999) and Yang *et al*. (2002) reported that some rice cultivars with a large amount of NSC reserves resulted in poor grain-filling due to little NSC translocation. The lack of alleles promoting NSC translocation may have brought about poor grain-filling. These results indicate that increasing NSC reserves may not be sufficient to enhance grain-filling.

Yang *et al*. (2000*a*) claimed that NSC translocation required the initiation of whole plant senescence, which suggests the mutual relationship between nitrogen and carbohydrate dynamics. In the present study, two QTL co-locations were observed between leaf nitrogen content and NSC content on chromosomes 3 and 9. The Milyang 23 alleles of these QTLs associated with increased NSC accumulation at heading were associated with nitrogen reduction in the middle of the grain-filling period. Negative phenotypic correlation between NSC content at heading and leaf nitrogen content during grain-filling supports this result. However, it is uncertain whether these two QTLs could contribute to increased grain-filling, because these QTLs were not linked to those for filling percentage per panicle on the linkage map.

In conclusion, several QTLs, which can promote grain-filling independently of the number of spikelets per panicle, were detected, conferring the possibility of improving grain-filling together with an enlargement of sink size. In particular, two loci on chromosomes 8 and 12 were strongly associated with increased filling percentage per panicle. One, on chromosome 12, accelerated grain-filling from the early-filling stage. The other, on chromosome 8, contributed to increased grain-filling by translocating NSC from the culm and leaf sheaths to the panicle. The NSC dynamics during grain-filling was also affected in part by spikelet number per panicle (QTLs on chromosomes 1 and 6). The NSC accumulation at the heading stage was mainly controlled by QTLs that did not affect the NSC dynamics during grain-filling. Nitrogen dynamics during grain-filling may be involved in stored NSC content at heading. However, it should be noted that the present work was based on a single field experiment, which does not exclude the possibility that there may be more QTLs related to grain-filling under different environmental conditions. Further investigations are necessary to collect more information on the genetic basis of the grain-filling mechanism. Nevertheless, this study confirmed that united efforts between crop physiologists and geneticists should be essential to enhance an understanding of grain-filling. The current identification of QTLs controlling grain-filling is a step towards an increased rice yield potential. Further studies with near isogenic lines for targeting QTLs will determine how the detected QTLs could influence final grain yield.

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References

Ajmone-Marsan P, Monfredidni G, Ludiwig WF, Melchinger AE, Franceschini P, Pagnotto G, Motto M. 1995. In an elite cross of maize a major quantitative trait locus controls one-fourth of the genetic variation for grain yield. *Theoretical and Applied Genetics* **90,** 415–424.

Austin DF, Lee M. 1996. Comparative mapping in $F_{2:3}$ and $F_{6:7}$ generations of quantitative trait loci for grain yield and yield components in maize. *Theoretical and Applied Genetics* **92,** 817–826.

Basten CJ, Weir BS, Zeng ZB. 2002. *QTL cartographer. Version 1.16*. A reference manual and tutorial for QTL mapping. Raleigh, North Carolina: Department of Statistics, North Carolina State University.

Bremner JM, Mulvaney CS. 1982. Nitrogen-total. In: Page AL, Miller RH, Keeney DR, eds. *Methods of soil analysis*, 2. Madison, Wisconsin: American Society of Agronomy, 595–624.

Brondani C, Rangel PHN, Brondani RPV, Ferreira ME. 2002. QTL mapping and introgression of yield-related traits from *Oryza*

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glumaepatula to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theoretical and Applied Genetics* **104,** 1192–1203.

Campbell BT, Baenziger PS, Gill KS, Eskridge KM, Budak H, Erayman M, Dweikat I, Yen Y. 2003. Identification of QTLs and environmental interactions associated with agronomic traits on chromosome 3A of wheat. *Crop Science* **43,** 1493–1505.

Churchill GA, Doerge RW. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* **138,** 963–971.

Concibido VC, La Ballee B, Mclaird P, Pineda N, Meyer J, Hummel L, Yang J, Wu K, Delannay X. 2003. Introgression of a quantitative trait locus for yield from *Glycine soja* into commercial soybean cultivars. *Theoretical and Applied Genetics* **106,** 575–582.

Cock JH, Yoshida S. 1972. Accumulation of ^{14}C-labelled carbohydrate before flowering and its subsequent redistribution and respiration in the rice plant. *Proceedings of the Crop Science Society of Japan* **41,** 226–234.

Cui KH, Peng SB, Xing YZ, Yu SB, Xu CG, Zhang Q. 2003. Molecular dissection of the genetic relationships of source, sink and transport tissue with yield traits in rice. *Theoretical and Applied Genetics* **106,** 649–658.

Doerge RW, Churchill GA. 1996. Permutation tests for multiple loci affecting a quantitative character. *Genetics* **142,** 285–294.

Fukuta Y, Araki E, Uga Y, *et al*. 2004. Development of recombinant inbred lines and chromosome segment substitution lines derived from a wide cross between Indica and Japonica type rice (*Oryza sativa* L.). *World Rice Research Conference 2004, abstract*, 285.

Fukuta Y, Sato T, Ogata T, Morita S, Nagamine T, Matsue Y, Yagi T. 1998. Genetic and breeding analysis using molecular marker 9. QTL analysis for heading date using recombinant inbred lines in rice (*Oryza sativa* L.). *Japanese Journal of Breeding* **48,** (extra issue 1), 84 (in Japanese).

Gebbing T, Schnyder H. 1999. Pre-anthesis reserve utilization for protein and carbohydrate synthesis in grains of wheat. *Plant Physiology* **121,** 871–878.

Groos C, Robert N, Bervas E, Charmet G. 2003. Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. *Theoretical and Applied Genetics* **106,** 1032–1040.

He P, Li JZ, Zheng XW, Shen LS, Lu CF, Chen Y, Zhu LH. 2001. Comparison of molecular linkage maps and agronomic trait loci between DH and RIL populations derived from the same rice cross. *Crop Science* **41,** 1240–1246.

Horie T, Lubis I, Takai T, Ohsumi A, Kuwasaki K, Katsura K, Nii A. 2003. Physiological traits associated with high yield potential in rice. In: Mew TW, Brar DS, Peng S, Dawe D, Hardy B, eds. *Rice science: innovations and impact for livelihood*. Los Banos, Philippines: IRRI, 117–145.

Kobayashi S, Fukuta Y, Sato T, Osaki M, Khush GS. 2003. Molecular marker dissection of rice (*Oryza sativa* L.) plant architecture under temperate and tropical climates. *Theoretical and Applied Genetics* **107,** 1350–1356.

Kobayashi S, Fukuta Y, Yagi T, Sato T, Osaki M, Khush GS. 2004. Identification and characterization of quantitative trait loci affecting spikelet number per panicle in rice (*Oryza sativa* L.). *Field Crops Research* **89,** 253–262.

Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW. 1997. Epistasis for three grain yield components in rice (*Oryza sativa* L.). *Genetics* **145,** 453–465.

Mae T, Ohira K. 1981. The remobilization of nitrogen related to leaf growth and senescence in rice plants (*Oryza sativa* L.). *Plant and Cell Physiology* **22,** 1067–1074.

Matsushima S, Wada G. 1958. Analysis of developmental factors determining yield and its application to yield prediction and culture improvement of lowland rice. XLVIII. Studies on the mechanism of ripening (9). Relation of the percentage of ripened grains and the yield of grains to the amount of carbohydrates stored by the time of heading, that of carbohydrates accumulated after heading and the nitrogen content at heading time. *Japanese Journal of Crop Science* **27,** 201–203 (in Japanese with English summary).

Maughan PJ, Saghai Maroof MA, Buss GR. 1996. Molecular-marker analysis of seed-weight: genomic locations, gene action, and evidence for orthologous evolution among three legume species. *Theoretical and Applied Genetics* **93,** 574–579.

Murchie EH, Yang J, Hubbart S, Horton P, Peng S. 2002. Are there associations between grain-filling rate and photosynthesis in the flag leaves of field-grown rice? *Journal of Experimental Botany* **53,** 2217–2224.

Nagata K, Fukuta Y, Shimizu H, Yagi T, Terao T. 2002*a*. Quantitative trait loci for sink size and ripening traits in rice (*Oryza sativa* L.). *Breeding Science* **52,** 259–273.

Nagata K, Shimizu H, Terao T. 2002*b*. Quantitative trait loci for non-structural carbohydrate accumulation in leaf sheaths and culms of rice (*Oryza sativa* L.) and their effects on grain-filling. *Breeding Science* **52,** 275–283.

Nagata K, Yoshinaga S, Takanashi J, Terao T. 2001. Effects of dry matter production, translocation of non-structural carbohydrates and nitrogen application on grain-filling in rice cultivar Takanari, a cultivar bearing a large number of spikelets. *Plant Production Science* **4,** 173–183.

Ntanos DA, Koutroubas SD. 2002. Dry matter and N accumulation and translocation for Indica and Japonica rice under Mediterranean conditions. *Field Crops Research* **74,** 93–101.

Oka HI. 1988. The ancestors of cultivated rice. In: *The origin of cultivated rice*. Tokyo: Japan Scientific Societies, 15–24.

Peng S, Cassman KG, Vinmani SS, Sheehy J, Khush GS. 1999. Yield potential trends of tropical rice since the release of IR8 and the challenge of increasing rice yield potential. *Crop Science* **39,** 1552–1559.

Rowland-Bamford AJ, Baker JT, Allen Jr LH, Bowes G. 1996. Interactions of CO_2 enrichment and temperature on carbohydrate accumulation and partitioning in rice. *Environmental and Experimental Botany* **36,** 111–124.

Samonte SOPB, Wilson LT, McClung AM, Tarpley L. 2001. Seasonal dynamics of non-structural carbohydrate partitioning in 15 diverse rice genotypes. *Crop Science* **41,** 902–909.

Seo SW, Ota Y. 1982. Role of the hull in the ripening of rice plant. VI. Translocation of carbohydrates into fertile and sterile grains. *Japanese Journal of Crop Science* **51,** 570–576 (in Japanese with English summary).

Singh K, Ishii T, Parco A, Huang N, Brar DS, Khush GS. 1996. Centromere mapping and orientation of the molecular linkage map of rice (*Oryza sativa* L.). *Proceedings of the National Academy of Sciences, USA* **93,** 6163–6168.

Song X, Agata W, Kawamitsu Y. 1990. Studies on dry matter and grain production of F_1 hybrid rice in China. III. Grain-production character from the view point of time changes in non-structural carbohydrate and nitrogen contents during the yield production. *Japanese Journal of Crop Science* **59,** 107–112 (in Japanese with English summary).

Sumi A, Hakoyama S, Weng J, Agata W, Takeda T. 1996. Analysis of plant characteristics determining ear weight increase during the ripening period in rice (*Oryza sativa* L.). II. The role of the reserved carbohydrate at heading stage upon the receptive efficiency of assimilation products in spikelets. *Japanese Journal of Crop Science* **65,** 214–221 (in Japanese with English summary).

Tanksley SD. 1993. Mapping polygenes. *Annual Review of Genetics* **27,** 205–233.

Tsukaguchi T, Horie T, Ohnishi M. 1996. Filling percentage of rice spikelets as affected by availability of non-structural carbohydrates

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at the initial phase of grain-filling. *Japanese Journal of Crop Science* **65,** 445–452 (in Japanese with English summary).

Tsukaguchi T, Sheehy JE, Ito O, Horie T. 1999. Analysis of grain-filling of individual tillers of rice based on their emergence time and NSC content in genotypes IR72 and new plant type. In: Horie T, Geng S, Amano T, Inamura T, Shiraiwa T, eds. *Proceedings of international symposium 'World food security and crop production technologies for tomorrow'*. Kyoto, Japan: Kyoto University, 298–299.

van Ooijen JW. 1992. Accuracy of mapping quantitative trait loci in autogamous species. *Theoretical and Applied Genetics* **84,** 803–811.

Weng J, Agata W, Takeda T. 1986. Studies on dry matter and grain production of rice plants. IV. Varietal difference of total carbohydrate content at heading stage. *Japanese Journal of Crop Science* **55,** 201–207 (in Japanese with English summary).

Weng J, Takeda T, Agata W, Hakoyama S. 1982. Studies on dry matter and grain production of rice plants. I. Influence of the reserved carbohydrate until heading stage and the assimilation products during the ripening period on grain production. *Japanese Journal of Crop Science* **51,** 500–509 (in Japanese with English summary).

Wu WR, Li WM, Tang DZ, Lu HR, Worland AJ. 1999. Time-related mapping of quantitative trait loci underlying tiller number in rice. *Genetics* **151,** 297–303.

Xiao J, Li J, Yuan L, Tanksley SD. 1996. Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a subspecific rice cross. *Theoretical and Applied Genetics* **92,** 230–244.

Xing YZ, Tan YF, Hua JP, Sun XL, Xu CG, Zhang Q. 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.

Yagi T, Nagata K, Fukuta Y, Tamura K, Ashikawa I, Terao T. 2001. QTL mapping of spikelet number in rice (*Oryza sativa* L.). *Breeding Science* **51,** 53–56.

Yang J, Peng S, Visperas RM, Sanico AL, Zhu Q, Gu S. 2000*b*. Grain-filling pattern and cytokinin content in the grains and roots of rice. *Plant Growth Regulation* **30,** 261–270.

Yang J, Peng S, Zhang Z, Wang Z, Visperas RM, Zhu Q. 2002. Grain and dry matter yields and partitioning of assimilates in Japonica/Indica hybrid rice. *Crop Science* **42,** 766–772.

Yang J, Zhang J, Huang Z, Zhu Q, Wang L. 2000*a*. Remobilization of carbon reserves is improved by controlled soil-drying during grain-filling of wheat. *Crop Science* **40,** 1645–1655.

Yano M, Sasaki T. 1997. Genetic and molecular dissection of quantitative traits in rice. *Plant Molecular Biology* **35,** 145–153.

Yin X, Stam P, Kropff MJ, Schapendonk AHCM. 2003. Crop modeling, QTL mapping, and their complementary role in plant breeding. *Agronomy Journal* **95,** 90–98.

Yoshida S. 1972. Physiological aspects of grain yield. *Annual Review of Plant Physiology* **23,** 437–464.

Zeng ZB. 1994. Precision mapping of quantitative trait loci. *Genetics* **136,** 1457–1468.

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