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ORIGINAL PAPER

Fine mapping of a gene for low-tiller number, *Ltn*, in japonica rice (*Oryza sativa* L.) variety Aikawa 1

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Abstract Tillering is one of the most important agronomic traits related to grain production in rice (*Oryza sativa* L.). A japonica-type variety, Aikawa 1, is known to have low-tiller number. The detailed location of a low-tillering gene, *Ltn*, which has been localized on chromosome 8 in Aikawa 1, was confirmed by molecular mapping. Using BC_5F_2 individuals derived from a cross between IR64 and Aikawa 1, the low-tillering gene was mapped to an interval defined by SSR markers *ssr5816-3* and *A4765*. This was designated as *Ltn* because there was no reported gene for tillering in the region of chromosome 8. Through high-resolution linkage analysis, the candidate region of *Ltn* was located between DNA markers *ssr6049-23* and *ind6049-1* corresponding to 38.6 kbp on the Nipponbare genome sequence. These DNA markers, which were tightly linked to *Ltn*, are useful for marker-assisted selection in breeding studies.

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Introduction

Tillering is one of the most important agronomic traits related to crop adaptation to target environments: high tillering is suitable for most favorable growing conditions; however, low tillering may be more desirable for stress conditions. Many breeders and scientists have been interested in controlling tillering to identify genotypes better adapted to the environment where they are grown. In irrigated lowland conditions, high tillering is preferable to achieve high yield. Under wet direct-seeding conditions where the seed rate is high to ensure good competitiveness against weeds, desirable genotypes will have less tillers because of high competition among plants in a field. In conditions, where drought events are expected to occur episodically during crop growth, desirable genotypes will produce few, but vigorous tillers which reduce water consumption while storing reserves to cope with unfavorable growing conditions. Tillering is also a key component in the expression of phenotype plasticity of the plant in response to transient changes in growing conditions. For these reasons, the control of tiller number in rice (*Oryza sativa* L.) has been an important focus of the research at the International Rice Research Institute (IRRI). Breeders developed new plant type (NPT) lines with low tillering and high productive tiller ratio to improve crop adaptation to direct-seeding conditions (Khush 2000).

These first-generation NPT lines did not have great success because of many undesirable factors that reduced

the productivity of the crop. Using a molecular (genotype-based) breeding approach to manipulate tillering by focusing on a small and well-identified genetic region will considerably reduce the undesirable effects that were expressed in the NPT lines that were developed through conventional (phenotype-based) breeding. Although tillering is an agronomically important trait for crop adaptation and phenotype plasticity, studies on the genetics of control of tillering have not been carried out in detail. Therefore, materials that are suitable for genetic analysis, such as mutant lines, near-isogenic lines, recombinant inbred lines, and double-haploid lines, are necessary.

Mutants derived from spontaneous mutations, γ-ray radiation, and ethyl methane sulfonate methods were used to identify genes controlling tiller number (Takamure and Kinoshita 1993; Takamure 1994; Iwata et al. 1995; Li et al. 2003; Jiang et al. 2006). Several genes for number of productive tillers at maturity have been cloned using mutants that were clearly different from the wild type. A mutant series of reduced culm number (rcn) at maturity, *rcn1–rcn9*, has been screened for low culm number (Takamure and Kinoshita 1993; Takamure 1994; Jiang et al. 2006). Among these genes, *RCN8* and *RCN9* have been mapped on the long arm of chromosome 1 and the short arm of chromosome 6, respectively. In addition, gene *MOC1* from a monoculm mutant that had only a single main culm was identified as a putative GRAS family nuclear protein that initiated axillary buds and promoted their outgrowth (Li et al. 2003). On the other hand, the high-tillering mutants were often recognized initially as dwarf mutants because of their short stature (Iwata et al. 1995). Two dwarf mutant genes for high tillering, *D3* and *Htd1*, have been identified. *D3* was orthologous to *Arabidopsis MAX2/ORE9* (Ishikawa et al. 2005), and *Htd1*, was orthologous to *Arabidopsis MAX3/CCD7* (Zou et al. 2005).

In 1980, a spontaneous low-tiller mutation was identified in a farmer's field transplanted with the japonica-type variety, Akiyutaka. This mutant line has subsequently been released as the commercial variety 'Aikawa 1' in Japan (National Institution of Crop Science; http://www.ine-web.narcc.affrc.go.jp/index.html). Japanese breeders considered it as a useful variety for direct-seeding cultivation with high seeding rate to compete against weeds. The single gene, *Ltn*(t), that controlled low tillering in Aikawa 1 was mapped near the simple sequence repeat (SSR) marker *RM264* on the long arm of chromosome 8 by Araki et al. (2003).

An indica type rice, IR64, is a widely grown rice variety in the tropics. IR64 has many valuable agronomic traits, such as high yield, good grain quality, and tolerance for biotic and abiotic stresses (Khush and Virk 2005). Moreover, its grain quality was considered superior to that of other IRRI-bred varieties. The low-tillering trait of Aikawa 1 was introduced into the IR64 genetic background by backcross breeding, and introgression lines (INLs) (IR64-INLs) are developed as a breeding material suitable for tropical conditions. In this study, we analyzed the detailed chromosomal location of *Ltn* using BC_5F_2 and BC_5F_3 populations of these lines. A high-resolution linkage map of the target region on chromosome 8 was constructed for cloning of *Ltn* and elucidating the underlying mechanism of the low-tillering phenotype. In addition, IR64-INLs for low tillering using Aikawa 1 as a gene source are developed by marker-assisted selection (MAS) and phenotyping.

Materials and methods

Development of the backcross population

A BC_1F_1 population was developed from a cross between japonica variety Aikawa 1 and indica variety IR64 as a recurrent parent. The BC_1F_1 population was evaluated for tiller number at maturity and was subsequently used for linkage analysis in a previous study (Araki et al. 2003). A single semi-dominant gene, *Ltn*, that controlled low tillering in Aikawa 1 was localized near SSR marker *RM264* on the long arm of chromosome 8. Several BC_1F_1 plants that showed low tillering were repeatedly backcrossed with IR64 to develop a BC_5F_1 generation. The BC_5F_1 plants were then self-pollinated and 94 BC_5F_2 plants and BC_5F_3 lines were used to map the gene for tiller number. BC_5F_3 (3,550 plants) and BC_5F_4 lines were evaluated for tillering as a progeny test. INLs, LTK9 and LTK3, for *Ltn* were selected from the BC_5 progeny based on the tiller number.

Each line was represented by 2 rows of 12 individuals. Single plants were transplanted at 21 days after sowing at 20 cm between hills and at 30 cm between rows. At maturity stage, phenotypes for tiller number in BC_5F_3 and BC_5F_4 lines were classified based on visual observation as three types: low-tillering type, segregating type, and high-tillering type. Sixteen BC_5F_3 lines derived from heterozygous individuals at *Ltn* in the BC_5F_2 population were used to construct a high-resolution linkage map of *Ltn*.

Phenotypic evaluation of INLs for low-tillering gene

LTK9, LTK3, IR64, and Aikawa 1 were grown in the field at IRRI during the DS (DS: January–May) of 2008. A total of eight agronomic traits—culm length (CL), panicle length (PL), leaf length (LL), leaf width (LW), panicle number per plant (PN), total spikelet number per panicle (TSN), grain fertility (GF), and 100-grain weight (GW)—were evaluated in donor parents and the INLs at maturity. For each line, at least 20 individuals planted in the middle for each row were measured, and single values per plant for these traits were

scored. CL was measured from the soil surface to the neck of the tallest tiller in a plant. PL was measured from the panicle neck to the panicle tip of the tiller. LL and LW were measured on the second leaf under the flag leaf of the main tiller. PN was measured as the number of productive panicles per plant. TSN was calculated as the sum of filled and unfilled spikelets of a panicle. GF was calculated as the ratio of filled spikelets per total spikelet number of a panicle. One hundred filled grains were weighed as GW for each plant.

LTK9 and donor parents were grown in a screen house at IRRI, Los Baños, Laguna, Philippines, during the dry season in 2009. More than 14 plants of each line were planted in pots. The tiller numbers of LTK9, IR64, and Aikawa 1 were counted every week from transplanting to heading.

Genotyping using DNA markers

The genomic DNA of 94 BC_5F_2 plants for gene mapping, 99 BC_5F_3 plants for recombinants in high-resolution linkage mapping, and LTK9 was individually extracted from freeze-dried leaves using the CTAB method (Murray and Thompson 1980). The genomic DNA of 3,550 BC_5F_3 plants for high-resolution linkage mapping was extracted from fresh leaves using a simple extraction method. The leaves of 3,550 BC_5F_3 plants were collected in 96-deep well plates that had 100-µl NaOH (0.25 M) and two stainless-steel beads in each well. The leaf samples in 96-deep well plates were crushed using a Geno/Grinder (Glen Mills). At 10 min after crushing leaf samples, 400 µl Tris–HCl (100 mM, pH 7.5) was put in each well and the plates were centrifuged at 1,500 rpm for 10 min. After the supernatants were diluted 20 times, 5-µl diluted supernatants were used as a PCR template. The genotypes of DNA markers in BC_5F_2 and BC_5F_3 individuals were then determined by PCR amplification in a DNA engine dyad peltier thermal cycler (Bio-Rad). The thermal cycler was programmed for a first denaturation step of 5 min at 95°C, followed by 35 cycles, each of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. The PCR products were resolved in 4.0% agarose gel by electrophoresis at 200 V for 1 h in 0.5× TBE buffer.

The whole genome of LTK9 was surveyed using 272 SSR markers that showed polymorphism between IR64 and Aikawa 1. The 272 SSR markers were evenly distributed across the 12 rice chromosomes (McCouch et al. 2002).

Marker development and linkage analysis

In the previous analysis, the single gene for low tillering in Aikawa 1, *Ltn*(t), was localized near *RM264* on the long arm of chromosome 8 (Araki et al. 2003). Therefore, 13 DNA markers near *RM264*, *ssr4762-1*, *ssr5245-1*, *ssr5816-3*, *ssr5816-9*, *RM23422*, *ssr6049-23*, *snp6049-1*, *ind6049-1*, *ind6049-3*, *ind6049-4*, *ssr6049-2*, *A4765*, and *RM5493* on chromosome 8, were used to make a linkage map and high-resolution linkage map (Table 1). The information on SSR markers *RM23422* and *RM5493* is available in the Gramene database (http://www.gramene.org). Other SSR markers were designed based on the Nipponbare genome sequence (IRGSP 2005). Three STS markers, *ind6049-1*, *ind6049-3*, and *ind6049-4*, were designed based on deletions between the japonica and indica genome sequences. Seven new SSR markers and three STS markers were designed using Primer3 (v. 0.3.0) (Rozen and Skaletsky 2000). The linkage analysis of *Ltn* in the BC_5F_2 population was conducted using the developed SSR markers. The genotypes of SSR markers in the BC_5F_2 population were detected by PCR and electrophoresis. The genetic distances between the SSR markers and *Ltn* were calculated based on the genotype and phenotype data for the BC_5F_2 population. The linkage map was constructed using Kosambi centimorgans (cM) to calculate the genetic distances.

PCR-based markers to genotype SNP

One single-nucleotide polymorphism (SNP) marker was developed based on the genome sequences of IR64 and Aikawa 1. A modified method for allele-specific PCR was used to improve the specificity of the traditional allele-specific PCR method (Kwok et al. 1990; Drenkard et al. 2000; Hayashi et al. 2004). The *snp6049-1* (Aikawa 1) and the *snp6049-1* (IR64) were Aikawa 1 and IR64 allele-specific primers, respectively, with artificial base pair mismatch in the 3rd base pair from the 3′ end of the reverse primer (Table 1). SNP genotypes were determined by the presence or absence of PCR products using *snp6049-1* (Aikawa 1) and *snp6049-1* (IR64).

Results

Mapping of *Ltn*

The average for tiller number of Aikawa 1 was 5.0 and that of IR64 was 20.9 in the DS. The tiller number of the BC_5F_2 population showed a continuous distribution. However, phenotype of BC_5F_3 plants could be clearly differentiated into low-tillering and high-tillering types by visual observation. Among 94 BC_5F_3 lines tested, all plants in 19 lines showed high-tillering and 48 lines segregated for tillering, whereas 27 lines showed low tillering. This segregation showed a good fit to a 1:2:1 ratio ($\chi^2 = 1.40$, $P = 0.50$) indicating that a single gene controlled tiller number in the BC_5F_2 population. The low-tillering gene was designated as *Ltn*. By co-segregation

Table 1 DNA markers used for high-resolution linkage mapping of the low-tillering gene on chromosome 8

Marker	Forward primer sequence (5′ → 3′)	Reverse primer sequence (5′ → 3′)	Nipponbare BAC or PAC	$(Motif)_n$	Predicted size (bp)[d]
ssr4762-1	GAGATCAACCCACCACCAAC	GGCAGATTGGGAATCCAAG	AP004762	$(CT)_9$	126
ssr5245-1	CGACTGTTCAGCACACACAA	TAGATCCCCAGGAAGTGACC	AP005245	$(AT)_{24}$	137
ssr5816-3	CCACAGCGACTCTCTCCATT	ACATACCTCGTCGGAGATGC	AP005816	$(GCG)_6$	113
ssr5816-9	TGCAGCTCAATTTTGGTCAG	ATGGTAGCAACGGGGATGTA	AP005816	$(AAAG)_5$	145
RM23422	GTCGGTCACGAAGTTCAGATCC	TCAGGCAAAGTTGAAGATGGTAGC	AP005816	$(AAAG)_5$	186
ssr6049-23	TCATCCTTAAGACGGTATCACA	CTCCCTCTCCGTTTCATATTC	AP006049	$(AT)_{14}$	192
snp6049-1 (Aikawa 1)[a]	GGTGCACCTCATCAAGTGAGACTTC	ACCGACTCGAGCTGTGATA[c]	AP006049	–	256
snp6049-1 (IR64)[b]	GGTGCACCTCATCAAGTGAGACTTC	ACCGACTCGAGCTGTGATC[c]	AP006049	–	256
ind6049-1	GCTCCCCAAAAGACAACATC	TCTCACTCCCTTTCTTCTCTCA	AP006049	–	299
ind6049-3	CTGAAAACCACCGTTAGAGC	GGCTAGATTGGCCGGTTAAA	AP006049	–	181
ind6049-4	GGAGATTTTTCTGTCGAATATCAGG	GTTAAGTACACTCCTCCGTTCTAAA	AP006049	–	300
ssr6049-2	GAGTCGGAGACCATGACGAC	TACCGCGTATCATGTCCTTG	AP006049	$(GAG)_7$	147
A4765	CTGTCGAATTGGAGCAAAATTTGCC	CCAATTTCCCCATCCCAAGAACAG	AP004765	$(CT)_{22}$	126
RM5493	GCAGGACACAGTCACACAGG	AGATTCTTTCACCGGTGACG	AP003888	$(TC)_{24}$	154

[a] Dominant marker for amplifying Aikawa 1 allele

[b] Dominant marker for amplifying IR64 allele

[c] The underline shows the location of artificial base pair mismatch

[d] The PCR product size was estimated based on the Nipponbare genome sequence

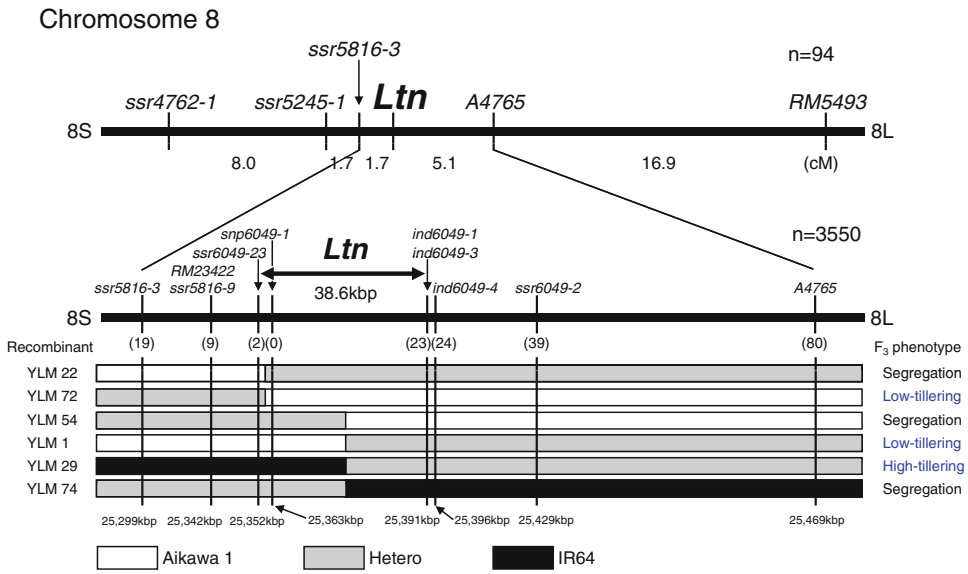

Fig. 1 Linkage map around the *Ltn* locus on chromosome 8. **a** The linkage map of *Ltn* using 94 BC_5F_2 individuals derived from a cross between IR64 (recurrent parent) and Aikawa 1. *Horizontal bars* represent the positions of SSR markers and *numbers* between markers indicate the genetic distance. **b** The high-resolution linkage map of *Ltn* using 3,550 BC_5F_3 individuals. The *numbers* in *parentheses* under DNA markers show the number of plants in which recombination occurred between the flanking marker and the *Ltn* locus. The physical distances at the *bottom* indicate the location of DNA markers on the Nipponbare genome sequence (build 4)

analysis, the linkage between *Ltn* and the SSR markers (*ssr4762-1, ssr5245-1, ssr5816-3, A4765*, and *RM5493*) near *RM246* on chromosome 8 were confirmed using the BC_5F_2 population. *Ltn* was located between *ssr5816-3* and *A4765*. The genetic distances between *ssr5816-3* and *A4765*, between *ssr5816-3* and *Ltn*, and between *Ltn* and *A4765* were 6.8, 1.7, and 5.1 cM, respectively (Fig. 1a).

High-resolution linkage mapping of *Ltn*

We used flanking SSR markers (*ssr5816-3* and *A4765*) to screen recombinant individuals from a large segregating population derived from a cross between IR64 and Aikawa 1. Among the 3,550 BC_5F_3 plants, 99 recombinants were identified by these markers. The BC_5F_4 lines derived from the 99 recombinant individuals were evaluated for tillering

phenotype to estimate the genotypes of the *Ltn* locus in these recombinants. In 22 out of 99 BC_5F_4 lines derived from the recombinants, all plants showed low tillering and the genotypes of the *Ltn* locus in these 22 lines were estimated as Aikawa 1 homozygous alleles; 19 out of 99 BC_5F_4 lines showed all high tillering and the genotypes were estimated as IR64 homozygous alleles. Among the 99 BC_5F_4 lines, 58 were segregating for tillering and the genotypes were estimated to be heterozygous. To construct a high-resolution linkage map of *Ltn*, the eight DNA markers (*ssr5816-9, RM23422, ssr6049-23*, *snp6049-1*, *ind6049-1*, *ind6049-3*, *ind6049-4*, and *ssr6049-2*) located between *ssr5816-3* and *A4765* were used. The candidate region of *Ltn* was specified between *ssr6049-23* and *ind6049-1*, representing 38.6 kbp based on the Nipponbare genome sequence (Fig. 1b). The SNP marker *snp6049-1* in 3,550 BC_5F_3 individuals completely co-segregated with the *Ltn* locus.

Candidate genes in the 38.6-kbp region

Based on the available sequence annotation, RiceGAAS (http://www.ricegaas.dna.affrc.go.jp/), there were six predicted genes (Predgene06-Predgene11 on a BAC clone of the Nipponbare genome, AP006049) in the 38.6-kbp target region. Among these genes, four had unknown functions and two had functional annotations: (1) Predgene06 (gene ID) was a gene with a transcript length of 1,503 bp containing 11 exons, classified as a putative *β*-glucosidase isozyme 2 precursor; (2) Predgene08 was a gene with a transcript length of 1,254 bp containing three exons, classified as a putative SBP-domain protein (Table 2).

Characteristics of INL with *Ltn*

Tiller numbers of IR64, Aikawa 1, and LTK9 were evaluated weekly (Fig. 2). Tiller development of LTK9 was similar to that of Aikawa 1, while it was clearly different from that of IR64. The introgressed segments from Aikawa 1 in LTK9 were detected on chromosomes 1 and 8 using 272 SSR markers (Fig. 3). Although the genome composition of LTK9 was similar to that of the recurrent parent IR64, several agronomic characteristics (PL, PN, LL, and TSN) of LTK9 were different from those of IR64 as well as Aikawa 1. PN of LTK9 was significantly lower than that of IR64 though higher than that of Aikawa 1 in the DS of

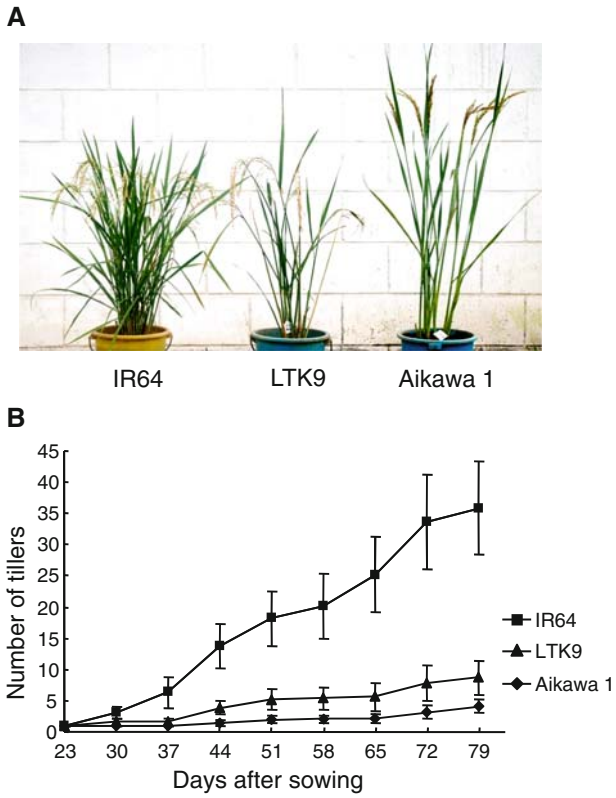

Fig. 2 Phenotypic characteristics of an introgression line for *Ltn* (LTK9). **a** Photo of IR64, LTK9, and Aikawa 1 at maturity stage. **b** Weekly changes in tiller numbers of IR64, LTK9, and Aikawa 1. *Tiller numbers* were counted weekly before heading and shown as mean ± SD

Table 2 Candidate genes that co-segregated with *Ltn* in the region between *ssr6049-23* and *ind6049-1*

Predgene	GenBank CDS feature	Full-length cDNA	Number of exons	Gene size (bp)	GC (%) content	Physical distance (bp)[a]	
						Start	End
Predgene06	Putative beta-glucosidase isozyme 2 precursor	AK059210, AK098938, AK105908	11	1,503	59.4	25,348,142	25,354,940
Predgene07	Unknown protein	AK068450	3	744	66.4	25,358,063	25,361,633
Predgene08	Putative SBP-domain protein	AK107191	3	1,254	64.0	25,362,546	25,366,701
Predgene09	Hypothetical protein	–	1	702	68.0	25,367,043	25,367,744
Predgene10	Hypothetical protein	–	2	291	52.7	25,368,974	25,369,384
Predgene11	Hypothetical protein	–	1	330	55.9	25,389,965	25,390,294

The genes on a BAC clone of the Nipponbare genome, AP006049 (OSJNBa0016N23), were predicted in RiceGAAS

[a] The physical distance shows the location of the predicted gene in the Nipponbare genome sequence (build 4)

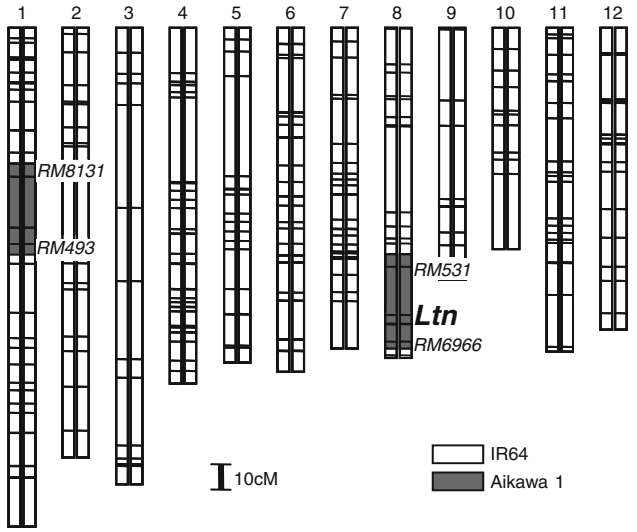

Fig. 3 Graphical genotype of an introgression line for *Ltn* (LTK9). The 12 pairs of *bars* represent the chromosomes, numbered at the *top*. The *horizontal lines* on the *bars* show positions of marker loci. *White squares* show the chromosomal segment of IR64, while *gray squares* show that of Aikawa 1

2008 (Table 3). PL and LL of LTK9 were significantly longer than those of parents, and TSN of LTK9 was significantly higher than that of IR64 and not different from that of Aikawa 1. As for CL, GF, and GW, LTK9 was similar to the recurrent parent IR64 in the 2008 DS. The agronomic traits of LTK9, IR64, and Aikawa 1 in the wet season were similar to those in the dry season (data not shown).

Discussion

The genes for tiller number of rice at maturity have been identified on a linkage map using DNA markers and several genes have already been cloned in different populations. *MOC1*, which encoded a putative GRAS family nuclear protein, was located on the long arm of chromosome 6 (Li et al. 2003). The genes for rice culm number, *RCN8* and *RCN9*, were located around *RM3411* on the long arm of chromosome 1 and around *RM6836* on the short arm of chromosome 6, respectively (Jiang et al. 2006). *rcn1*, *rcn2*, and *rcn5* were analyzed using marker genes for identifying chromosomal location (Takamure and Kinoshita 1993; Takamure 1994). *rcn1* from N-133 and *rcn5* from N-185 were linked to the marker gene of chromosome 6 and *rcn2* was linked to the marker gene of chromosome 4. In this study, *Ltn* from Aikawa 1 was located between *ssr6049-23* and *ind6049-1* on the long arm of chromosome 8. *Ltn* was a new gene for tiller number on chromosome 8 because the chromosomal locations of the reported genes, *MOC1*, *rcn1*, *rcn2*, *rcn5*, *RCN8*, and *RCN9* (excluding QTLs), were different from those of *Ltn*.

Using molecular genetic analysis, many QTLs for tiller and panicle numbers have been identified on rice chromosomes (Yan et al. 1998; Hemamalini et al. 2000; Xing et al. 2002; Brondani et al. 2002; Miyamoto et al. 2004). Three QTLs for tiller numbers have been detected on the long arm of chromosome 8 using DNA markers (Yan et al. 1998; Hemamalini et al. 2000; Miyamoto et al. 2004), and also two QTLs for panicle number have been detected in the same regions (Ishimaru et al. 2001; Lanceras et al. 2004).

These QTLs have not been mapped as single Mendelian factors, and their precise chromosomal locations are unknown. Although *Ltn* might have corresponded to these QTLs for tiller and panicle numbers in previous studies, we designated the low-tillering gene from Aikawa 1 as a new gene, *Ltn*. Genetic information, such as the high-resolution linkage map for *Ltn* and tightly flanking DNA markers in this study, is useful for breeding using MAS. The SNP marker *snp6049-1*, which co-segregated with *Ltn* in high-resolution linkage analysis, is a valuable tool for introducing *Ltn* to cultivated varieties by MAS. In a future study, the identification of the encoding gene in *Ltn* will be useful in elucidating tiller development.

The substituted segments of Aikawa 1 in LTK9 were detected using SSR markers (Fig. 3). Although the genome composition of the INL was almost the same as that of IR64, several agronomic traits were different (Table 3). PN of LTK9 was much lower than that of IR64 although it was

Table 3 Agronomic traits of a low-tillering introgression line (LTK9) derived from a cross between Aikawa 1 and IR64 as a recurrent parent in the dry season of 2008

Line	Mean of agronomic traits[a]							
	CL (cm)	PL (cm)	LL (cm)	LW (cm)	PN	TSN	GF (%)	GW (g)
LTK9	65.1 ± 6.4 b	27.6 ± 1.3 a	38.8 ± 5.1 a	1.1 ± 0.1 b	10.4 ± 2.6 b	187.8 ± 41.0 a	72.4 ± 11.0 b	2.4 ± 0.2 b
IR64	63.4 ± 2.8 b	24.2 ± 1.1 b	34.1 ± 2.7 b	1.2 ± 0.1 ab	20.9 ± 4.3 a	109.2 ± 21.5 b	74.8 ± 5.0 b	2.6 ± 0.1 b
Aikawa 1	71.2 ± 4.2 a	21.7 ± 1.0 c	33.9 ± 6.2 b	1.2 ± 0.1 a	5.0 ± 1.4 c	176.8 ± 27.7 a	88.0 ± 4.7 a	2.8 ± 0.2 a

Means denoted with different letters are significantly different at the 1% level according to the Tukey–Kramer test

CL culm length, *PL* panicle length, *LL* leaf length, *LW* leaf width, *PN* panicle number per plant, *TSN* total spikelet number per panicle, *GF* grain fertility, *GW* 100 grain weight

[a] Average ± standard deviation of more than 20 plants in each line

not as low as that of Aikawa 1. This difference of PN between INL and Aikawa 1 might be the effect of genetic background. TSN of LTK9 was much higher than that of IR64 and was not different from that of Aikawa 1. The increased TSN in LTK9 could be explained mostly by a compensative reaction of reduced tiller number. To confirm the effect of introgressed segment on phenotype, a sister line of LTK9, LTK3 was compared. Agronomic traits (such as TSN, PL, and PN) of LTK3 were similar to those of LTK9. The introgressed segments from Aikawa 1 in LTK3 were detected on chromosomes 1, 4, and 8. A substituted segment was commonly detected on the long arm of chromosome 8 in LTK3 and LTK9 (data not shown). The result suggested that the substituted segment on the long arm of chromosome 8 was related to the agronomic traits, including increased TSN other than low tillering. Therefore, in addition to a compensative reaction, this result might be explained by the following reasons: (1) a pleiotropic effect of *Ltn* on TSN; or (2) a gene for TSN is tightly linked to *Ltn*. PL of LTK9 tended to be longer than that of both parents, IR64 and Aikawa 1. This was also thought to be caused by the introgressed segment of Aikawa 1, including the *Ltn* region.

Since the 1960s, many IRRI-bred varieties have been released and distributed worldwide and used by farmers and breeders as important parental varieties in breeding programs. In particular, IR64, which was released in 1985, has been widely accepted as a high-quality rice variety in many countries (Khush 1987). In our study, *Ltn* of Aikawa 1 was genetically identified and introduced into the IR64 genetic background. The INLs for *Ltn* probably had the elite agronomic characteristics of IR64, such as superior grain quality and enhanced resistance to several diseases and pests. These INLs are useful breeding materials for rice improvement. The INLs are also useful for understanding the development of plant structure and the relationship between yield components and grain yield of rice. Furthermore, these INLs could be used to identify suitable plant type under various field conditions.

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