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Origins of functional nucleotide polymorphisms in a major quantitative trait locus, *qLTG3-1*, controlling low-temperature germinability in rice

Kenji Fujino · Hiroshi Sekiguchi

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Abstract *qLTG3-1* is a major quantitative trait locus (QTL) controlling tolerance to low-temperature at the seed germination stage (termed low-temperature germinability) in rice using a population derived from the cross between Italica Livorno from Italy and Hayamasari from Japan. Map-based cloning identified that *qLTG3-1* encodes a protein of unknown function. The molecular identification of this major QTL could make it possible to identify allelic variation and favorable alleles for rice breeding programs. The present study examined the identification of *qLTG3-1* alleles and their distribution among 62 landraces of Asian cultivated rice (*Oryza sativa* L.) collected from 19 different countries, termed the rice core collection. In the coding region, a single non-synonymous substitution and 3 in-frame insertion/deletion polymorphisms (indels) were detected. The almost completely conserved protein alignment of *qLTG3-1* was also identified among 5 *Oryza* species, suggesting that the function of *qLTG3-1* is critical for seed germination or for rice growth by pleiotropic effects of the gene. The functional nucleotide polymorphisms (FNPs), a 71-bp deletion found in Hayamasari and an amino acid substitution found in Nipponbare, was identified in varieties from Japan. These alleles with FNPs might be adapted to rice cultivation in specific local conditions. The present results may contribute to the utilization of favorable alleles of *qLTG3-1* for the improvement of low-temperature germinability in rice breeding programs.

Keywords Rice · Allelic variation · *qLTG3-1* · Natural variation · FNP

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K. Fujino (✉) · H. Sekiguchi
Agricultural Research Institute, HOKUREN Federation of Agricultural Cooperatives, Naganuma, 0691317 Hokkaido, Japan
e-mail: kfujino@affrc.go.jp

Present Address:
K. Fujino
National Agricultural Research Center for Hokkaido Region, Hitsujigaoka 1, 0628555 Sapporo, Japan

Introduction

It is important to understand the natural variation of genes and the selective forces on genes for adaptation to diverse environmental conditions in natural populations. Natural variation has been utilized to elucidate the functional role of candidate genes in a given process (Alonso-Blanco and Koornneef 2000; Mitchell-Olds and Schmitt 2006). However, genetically diverse germplasm as a source of natural variation exhibits diverse phenotypic variations for many traits, including the target trait. This makes it difficult to evaluate the desired trait in isolation. Furthermore, such traits with complex inheritance are governed by many genes and epistatic interactions among them, which are termed quantitative traits. It is quite difficult to find novel allelic variations based on the comparison of phenotypes in germplasm. The sequences of genes responsible for agronomic traits can lead to the identification of allelic variation in those genes. Sequence surveys of germplasm are independent of the effects of other traits. This strategy can contribute to not only understanding of the molecular mechanism of the trait but also the improvement of the trait in breeding programs.

Cultivated rice, *Oryza sativa* L., originated from tropical regions. Rice has been adapted to diverse environmental

conditions with considerable variations in adaptive traits. Due to the substantial efforts of breeding programs, rice is now grown all over the world from 53°N to 40°S (Lu and Chang 1980). The shaping of adaptability to local areas has an advantage for rice growth. Low-temperature-induced retardation of rice growth is a common problem in temperate rice-growing areas and at high altitudes in tropical and sub-tropical areas. In such areas, improvement of tolerance to low temperature at the seed germination stage, termed low-temperature germinability, is important in rice breeding programs. Due to its importance, many quantitative trait loci (QTLs) controlling low-temperature germinability have been identified using various mapping populations (Miura et al. 2001; Fujino et al. 2004; Zhang et al. 2005; Jiang et al. 2006; Chen et al. 2006; Ji et al. 2009).

Only a single gene controlling low-temperature germinability, *qLTG3-1*, has been identified at the molecular level (Fujino et al. 2008). *qLTG3-1* is a major QTL for low-temperature germinability on chromosome 3 that explained more than 30% of the total phenotypic variation in the mapping population derived from a cross between Italica Livorno and Hayamasari (vigorous and weak low-temperature germinability, respectively) (Fujino et al. 2004). The epistatic factor(s) for *qLTG3-1* make the inheritance of low-temperature germinability complex (Iwata and Fujino 2010). *qLTG3-1* was cloned by a map-based strategy and shown to encode a protein of unknown function (Fujino et al. 2008). The tissue-specific expression of *qLTG3-1* was tightly associated with vacuolation of cells in the tissues covering the embryo (Fujino et al. 2008). The function of *qLTG3-1* may be involved in tissue weakening, resulting in a reduction in mechanical resistance to the growth potential of the coleoptile. Genome-wide expression analysis suggested that genes involved in defense responses were up-regulated by *qLTG3-1* (Fujino and Matsuda 2010). However, the molecular function of *qLTG3-1* is still unclear.

The importance of utilizing natural variation for functional and evolutionary studies has been highlighted (Alonso-Blanco and Koornneef 2000; Kovach and McCouch 2008; Alonso-Blanco et al. 2009). Therefore, the identification of a QTL is defined as only the genetic difference between the parental varieties. For the efficient utilization of favorable alleles of a target gene, the identification of *qLTG3-1* alleles and their distribution would be useful for rice breeding programs. The present study examined the DNA sequences of *qLTG3-1* for natural variations among 62 landraces (partly modern cultivars) of cultivated rice, called the rice core collection (Kojima et al. 2005). The results indicated the almost complete conservation of the protein alignment of *qLTG3-1*, suggesting the importance of the function for *qLTG3-1* to rice growth. Mutation events causing a loss-of-function found in Hayamasari (Fujino et al. 2008) and an altered function found in Nipponbare (Hori et al. 2010) have occurred and been selected in the population of Japanese landraces. In contrast to the conservation of *qLTG3-1* among cultivated rice and Japanese landraces, the Hayamasari allele carrying the functional nucleotide polymorphism (FNP) was predominant in the population from Hokkaido, including landrace types and commercial varieties. The selection of the loss-of-function allele suggested that this allele is important for adaptability in this particular region.

Materials and methods

Plant materials

For the analysis of sequence diversity of *qLTG3-1* among cultivated rice, a set of 62 genetically diverse landraces were used, including some modern cultivars (Kojima et al. 2005) (Table 1). This population was collected from 19 different countries, mainly in Asia, and selected to represent the wide genetic diversity among cultivated rice varieties termed the rice core collection (Kojima et al. 2005). This population consisted of 3 genetically differentiated groups, group A, B, and C, which corresponded to cultivar groups of *japonica*, *aus*, and *indica*, respectively, based on the genome-wide RFLP analysis of 332 accessions (Garris et al. 2005; Kojima et al. 2005). In addition, 7 accessions of related wild species carrying the AA genome were used; *O. rufipogon* (W0106), *O. barthii* (W0652), *O. glumaepatula* (W1169 and W2199), *O. longistaminata* (W1413 and W1508), and *O. meridionalis* (W1625).

To identify the distribution of the *qLTG3-1* alleles, a set of 38 genetically diverse landraces from Japan were used, termed the Japanese rice core collection (Ebana et al. 2008) (Table S1). The Japanese rice core collection was selected to represent the wide genetic diversity among landraces from Japan based on genome-wide polymorphisms in 236 accessions (Ebana et al. 2008). In addition, 64 *temperate japonica* rice varieties from Hokkaido, Japan, including landrace and breeding types were used (Table S2). Seeds of wild relatives, varieties from Hokkaido, and the rice core collection and the Japanese rice core collection were provided by the National Institute of Genetics, Japan, Hokkaido Central Agricultural Experiment Station, and National Institute of Agrobiological Sciences, Japan, respectively.

DNA analysis

Total DNA was isolated from young leaves using a CTAB method (Murray and Thompson 1980). The provided seeds were sown without propagation. Leaf tissues from a single

Table 1 *qLTG3-1* alleles in the rice core collection

Code no.	Variety	Type	Origin	Group	Allele group	Allele
WRC1	NIPPONBARE	Breeding	Japan	*Japonica*	I	5
WRC2	KASALATH	Landrace	India	*Aus*	I	1
WRC3	BEI KHE	Landrace	Cambodia	*Indica*	II	7
WRC4	JENA 035	Landrace	Nepal	*Aus*	I	1
WRC5	NABA	Landrace	India	*Indica*	II	7
WRC6	PULUIK ARANG	Landrace	Indonesia	*Indica*	II	7
WRC7	DAVAO 1	Landrace	Philippines	*Indica*	II	7
WRC9	RYOU SUISAN KOUMAI	Landrace	China	*Indica*	II	7
WRC10	SHUUSOUSHU	Landrace	China	*Indica*	III	9
WRC11	JINGUOYIN	Landrace	China	*Indica*	II	7
WRC13	ASU	Landrace	Bhutan	*Indica*	I	1
WRC14	IR 58	Breeding	Philippines	*Indica*	I	2
WRC15	CO 13	Unknown	India	*Indica*	I	2
WRC16	VARY FUTSI	Landrace	Madagascar	*Indica*	II	7
WRC17	KEIBOBA	Landrace	China	*Indica*	III	9
WRC18	QINGYU(SEIYU)	Landrace	Taiwan	*Indica*	III	9
WRC19	DENG PAO ZHAI	Breeding	China	*Indica*	III	9
WRC20	TADUKAN	Landrace	Philippines	*Indica*	II	7
WRC21	SHWE NANG GYI	Landrace	Myanmar	*Indica*	I	1
WRC22	CALOTOC	Landrace	Philippines	*Aus*	II	7
WRC24	PINULUPOT 1	Landrace	Philippines	*Indica*	II	7
WRC25	MUHA	Unknown	India	*Aus*	I	4
WRC26	JHONA 2	Unknown	India	*Aus*	I	2
WRC27	NEPAL 8	Landrace	Nepal	*Aus*	I	1
WRC28	JARJAN	Landrace	Bhutan	*Aus*	I	1
WRC29	KALO DHAN	Landrace	Nepal	*Aus*	I	1
WRC30	ANJANA DHAN	Landrace	Nepal	*Aus*	I	1
WRC31	SHONI	Landrace	Bangladesh	*Aus*	II	7
WRC32	TUPA 121-3	Landrace	Bangladesh	*Aus*	I	1
WRC33	SURJAMUKHI	Breeding	India	*Aus*	I	1
WRC34	ARC 7291	Landrace	India	*Aus*	I	1
WRC35	ARC 5955	Landrace	India	*Aus*	II	7
WRC36	RATUL	Landrace	India	*Aus*	II	7
WRC37	ARC 7047	Landrace	India	*Aus*	r	10
WRC38	ARC 11094	Landrace	India	*Aus*	II	7
WRC39	BADARI DHAN	Landrace	Nepal	*Aus*	II	7
WRC40	NEPAL 555	Unknown	India	*Aus*	II	7
WRC41	KALUHEENATI	Landrace	Sri Lanka	*Aus*	I	2
WRC42	LOCAL BASMATI	Landrace	India	*Aus*	II	7
WRC43	DIANYU 1	Breeding	China	*Japonica*	I	5
WRC44	BASILANON	Landrace	Philippines	*Aus*	I	1
WRC45	MA SHO	Landrace	Myanmar	*Japonica*	I	1
WRC46	KHAO NOK	Landrace	Laos	*Japonica*	I	1
WRC47	JAGUARY	Unknown	Brazil	*Japonica*	I	1
WRC48	KHAU MAC KHO	Landrace	Vietnam	*Japonica*	I	1
WRC49	PADI PERAK	Landrace	Indonesia	*Japonica*	I	1
WRC50	REXMONT	Breeding	America	*Japonica*	I	1
WRC51	URASAN 1	Landrace	Japan	*Japonica*	I	1

Table 1 continued

Code no.	Variety	Type	Origin	Group	Allele group	Allele
WRC52	KHAU TAN CHIEM	Landrace	Vietnam	*Japonica*	I	3
WRC53	TIMA	Landrace	Bhutan	*Japonica*	I	1
WRC55	TUPA 729	Landrace	Bangladesh	*Japonica*	I	1
WRC57	MILYANG 23	Breeding	Korea	–	I	5
WRC58	NEANG MENH	Landrace	Cambodia	*Indica*	II	8
WRC59	NEANG PHTONG	Landrace	Cambodia	*Indica*	II	7
WRC61	RADIN GOI SESAT	Landrace	Malaysia	*Indica*	II	7
WRC62	KEMASIN	Landrace	Malaysia	*Indica*	II	6
WRC63	BLEIYO	Landrace	Thailand	*Indica*	II	7
WRC64	PADI KUNING	Landrace	Indonesia	*Indica*	II	7
WRC65	RAMBHONG	Landrace	Indonesia	*Indica*	II	7
WRC66	BINGALA	Landrace	Myanmar	*Indica*	II	7
WRC67	PHULBA	Landrace	India	*Japonica*	I	1
WRC68	KHAO NAM JEN	Landrace	Laos	*Japonica*	I	1

Information in the rice core collection is from Kojima et al. (2005)

plant were used for DNA extraction. PCR, electrophoresis, and sequencing were performed as described previously (Fujino et al. 2004, 2005, 2010). The 1,735-bp *qLTG3-1* region were sequenced, including the 933-bp 5′ upstream region, 555-bp coding region, and 296-bp 3′ downstream region except for a 49-bp region from position -433 to -384 around T mono- and TA di- nucleotide repeats. PCR products were sequenced directly using cycle sequencing with BigDye terminators (Applied Biosystems) on a Prism 3700 automated sequencer (Applied Biosystems). DNA sequences were aligned using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and then aligned visually. All polymorphisms were rechecked from the chromatograms with special attention to low-frequency polymorphisms. The sequences of *qLTG3-1* alleles in cultivated rice and wild relatives and that of a transposon, which was inserted into the 5′ upstream region of *qLTG3-1*, were deposited in GenBank as Accession Nos. AB433790 - AB433806.

Data analysis

The functional *qLTG3-1* sequence from a *temperate japonica* Italica Livorno was used as the reference in this study (GenBank accession No. AB369214) (Fujino et al. 2008). Data analyses for sequence of *qLTG3-1* were performed as described previously (Fujino et al. 2010). DNA sequences were analyzed using the computer program DnaSP 4.0 (Rozas et al. 2003). Minimum spanning (allele/haplotype) networks, representing unique DNA sequences or alleles separated by mutational steps, were constructed using the computer program TCS (Clement et al. 2000) using statistical parsimony methods. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 (Kumar et al. 2004) to construct neighbor-joining (NJ) trees. The bootstrap values for nodes in the phylogenetic tree are from 1,000 replications. Insertion/deletion polymorphisms (indels) were included in the analysis, with each indel treated as a single character and nonsynonymous.

Genotyping of *qLTG3-1*

The genotype of *qLTG3-1* was determined using sequence tagged sites (STS) and cleaved amplified polymorphc sequence (CAPS) methods. The STS PCR using the primer set of S103 (Fujino et al. 2008) amplified the sequence surrounding the FNP of the 71-bp deletion found in Hayamasari. For the CAPS method for the detection of the FNP of a single nucleotide change found in Nipponbare, the PCR product produced using primer set of S103 was digested with restriction enzyme *Bse*RI. GAGGAG in the Italica Livorno allele was digested and GTGGAG in the Nipponbare allele was not digested by *Bse*RI.

Results

DNA polymorphisms of *qLTG3-1*

A total of 1,735 nucleotides of the *qLTG3-1* gene were sequenced. Compared with the sequence of Italica Livorno as a functional allele (allele 1), 34 mutation events at 32 sites, including indels and substitutions, were detected (Table 2). In the coding region, only 4 mutation events were detected; 2 deletions at positions +127 and +181, a single

Table 2 Nucleotide changes in the 1,784-bp region of *qLTG3-1* among the rice core collection

Allele group	Allele	Position																														
		−905	−845	−813	−674	−640	−637	−620	−598	−579	−562	−515	−493	−492	−492	−481	−480	−464	−459	−455	−444	−433	−217	−130	−97	−69	−41	+50	+127	+181	+190	+604	+627
I	1	C	G	C	C	–	C	G	C	T	A	G	–	–	–	T	–	C	G	G	C	–	C	–	C	C	T	T	–	–	–	–	–
I	2	C	G	C	C	–	C	G	C	T	A	G	–	–	–	1	–	C	G	G	C	–	C	–	C	C	T	T	–	–	–	–	–
I	3	T	G	C	C	–	C	G	C	T	A	G	–	–	–	T	–	C	G	G	C	–	C	–	C	C	T	T	–	–	–	–	–
I	4	C	G	C	C	–	C	G	C	T	A	G	–	–	–	T	1	C	G	G	C	–	C	–	C	C	T	T	–	–	–	–	–
I	5	C	G	C	C	–	C	G	C	T	A	G	–	–	–	T	–	C	G	G	C	–	C	–	C	C	T	A	–	–	–	–	–
II	6	C	A	T	G	1	C	G	C	A	A	G	1	–	1	T	–	C	A	A	C	–	T	12	G	G	A	T	18	9	9	11	–
II	7	C	A	T	G	1	C	G	C	A	A	G	1	–	1	C	–	C	A	A	C	–	T	12	G	G	A	T	18	9	9	11	–
II	8	C	A	T	G	1	C	G	C	A	A	G	1	–	1	C	–	T	A	A	C	–	T	12	G	G	A	T	18	9	9	11	–
III	9	C	G	C	C	–	A	A	T	T	T	A	1	–	–	C	–	C	G	G	T	–	C	–	G	G	A	T	18	–	36	11	10
r	10	C	G	C	C	–	A	A	T	T	T	A	–	1	–	C	–	C	G	G	C	6	C	12	C	C	T	T	18	9	9	11	–
	Category	N	N	N	N	D	N	N	N	N	N	N	D	I	D	N/D	I	N	N	N	N	D	N	I	N	N	N	R	D	D	I	D	D

The 49-bp region from −433 to −384 containing the (T)n(TA)n repeat was not sequenced

Replacement from Leu to His occurred in allele 5

The R, N, I, and D categories indicate replacement, noncoding site, insertion, and deletion, respectively

r in allele group means recombination type between allele groups I, II, and III

Numbers indicate the base number of insertions or deletions compared with allele 1

Position: −933 to 0, 5′ upstream region; +1 to +555, coding region; +556 to +851, 3′ downstream region; −648 to −403, transposon

insertion at position +190, and a single nonsynonymous substitution at position +50. These indels occurred in-frame in the conserved glycine-rich cell wall protein (GRP) domain, which contains Gly repeats with a Ser residue. In the results of mutation events of these indels, the repeat number varied, alleles 6-10 (Fig. S1). A single nonsynonymous substitution was detected only in Nipponbare, allele 5. The T to A substitution generated an amino acid substitution, Leu to His. This substitution occurred in the conserved amino acid motif among plant species, LLALNLLFFT (Fujino et al. 2008). The FNP of the 71-bp deletion found in Hayamasari did not detected in this population.

In contrast, 27 mutation events occurred at 26 sites in the 933-bp 5′ upstream region. Among them, 17 mutation events at 17 sites occurred in the 241-bp region from position -648 to -403, which corresponds to a transposon. This transposon had 10-bp terminal inverted repeats (TIR), TTCCCTCCGT, and a 2-bp target-site duplication (TSD), TA. Based on these structural features, this transposon belongs to the *Stowaway*-like miniature inverted-repeat transposable elements (MITEs). This transposon was named *Maoi*. A BLAST search using the *Maoi* sequence as the query identified at least 330 and 910 sequences with high similarity (>1E-35) in the rice genomes of *temperate japonica* Nipponbare and *indica* 93-11, respectively. However, the autonomous element was not detected in either genome.

Allele network of *qLTG3-1*

To elucidate the molecular evolution of *qLTG3-1* among cultivated rice, an allele network was constructed from the nucleotide variations in the entire *qLTG3-1* sequence (Fig. 1a). Based on these mutations, 10 different alleles were found among the rice core collection (Table 2). Because allele 10 was generated from intragenic recombinations between allele groups (Fig. 2), this allele was not included the network. The network consisted of 3 allele groups, I, II, and III (Fig. 1a). Allele group I included alleles 1-5 from 31 varieties, while allele group II included alleles 6–8 from 25 varieties. These allele groups were separated from each other by 18 mutational steps. Allele group III was a minor group and involved a single allele, allele 9, from 4 varieties. The NJ tree for *qLTG3-1* strongly supported the results of the allele network (Fig. 1b).

The allele groups of *qLTG3-1* were clearly correlated with the cultivar group classification by genome-wide polymorphisms (Kojima et al. 2005). All 14 *japonica* varieties were in allele group I. Among 25 *indica* varieties, 17 and 4 were in allele groups II and I, respectively. The remaining 4 *indica* varieties were in allele group III. These results indicated that the ancestor types of *japonica* and *indica* varieties carried allele groups I and II, respectively. Two differentiated allele groups were detected in an *aus* cultivar group, with 13 and 8 varieties in allele groups I and II, respectively. It was unclear which allele group was in the ancestor type of *aus*. One of them might be introgressed into the ancestor type of *aus*. *Indica* varieties with allele group I strongly suggested that the chromosomal region surrounding *qLTG3-1* in *japonica* or *aus* was introgressed into *indica*. In addition, allele 2 was introgressed between *aus* and *indica*. At least 3 introgression events between cultivars groups occurred during rice domestication. This frequency was low compared with that identified in chromosomal region surrounding *Hd1* on chromosome 6 controlling flowering time (Fujino et al. 2010).

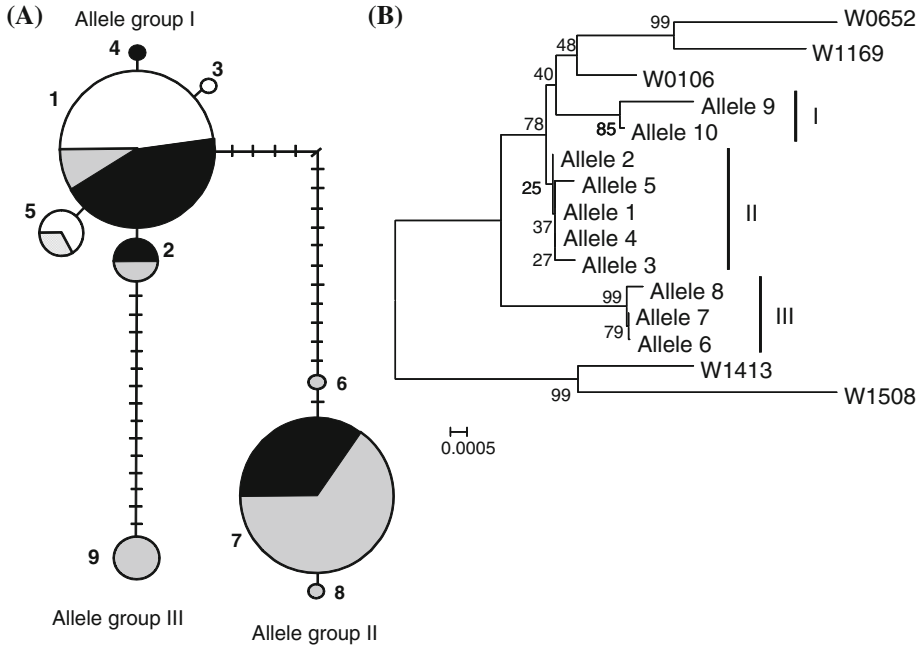

Fig. 1 Relationships between *qLTG3-1* alleles. (**a**) Allele network constructed using the TCS program. *Letters* beside the circles indicate the allele. The *size* of the *circle* corresponds to the number of varieties. *White*, *black*, and *gray* sectors indicate the cultivar groups of *japonica*, *aus*, and *indica*, respectively. (**b**) Neighbor-joining (NJ) tree of ten alleles of cultivated rice and five accessions of wild relatives carrying AA genomes; *O. rufipogon* (W0106), *O. glumaepatula* (W1169), *O. barthii* (W0652), and *O. longistaminata* (W1413 and W1508). Allele groups I-III of *qLTG3-1* are shown by *bars*

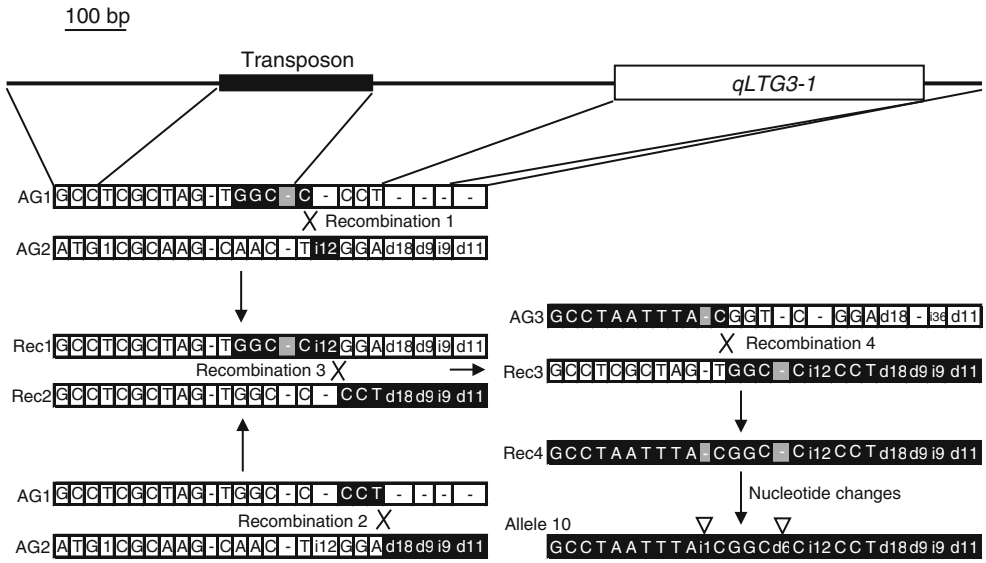

Fig. 2 Recombinations in intra-genic regions of *qLTG3-1*. Representative polymorphisms of the alleles are shown. Recombination sites are shown by X. Allele 10 found in ARC7047 from the *aus* subpopulation might be derived from four intragenic recombination events between three allele groups. Firstly, recombination 1 occurred between allele groups I and II at positions −217 to −130, generating allele Rec1. On the other hand, allele Rec2 was generated from the recombination between allele groups I and II at positions −69 to +127. Recombination between alleles Rec1 and Rec2 at positions −130 to −97 generated allele Rec3. Finally, recombination between allele Rec3 and allele group III at positions −480 to −444 generated allele Rec4. Then, allele 10 was derived from allele Rec4 with 2 mutations, a 1-bp insertion and a 6-bp deletion

Table 3 Distibutions of *qLTG3-1* genotypes in different populations

Population[a]	Genotype[b]		
	IL	NP	HY
World	60	3	0
	95.2	4.8	0
Japan	22	14	2
	57.9	36.8	5.3
Hokkaido	18	18	28
	28.1	28.1	43.8

Upper and lower rows indicate the number of varieties and their frequency, respectively

[a] World, the rice core collection of cultivated rice (Kojima et al. 2005); Japan, the Japanese rice core collection (Ebana et al. 2008); Hokkaido, varieties of landrace and breeding types from Hokkaido

[b] Genotypes of IL, NP, and HY indicate wild-type, a single nucleotide substitution found in Nipponbare, and a 71-bp deletion found in Hayamasari, respectively

Sequence diversity of *qLTG3-1* in wild relatives

Many nucleotide changes, including indels and substitutions, were detected in the sequences of *qLTG3-1* of wild relatives in the AA genome, which were not detected in cultivated rice (Table S3). In the coding region, a single nonsynonymous substitution, 13 synonymous substitutions, 6 deletions, and 11 insertions were detected (Table S2). This nonsynonymous substitution at position +193 found in *O. glumaepatula*, G to A, generated an amino acid substitution Gly to Ser in the GRP (Fig. S1). All indels occurred in-frame in the region of the GRP. Therefore, amino acid alignments of these wild relatives were almost completely conserved compared with allele 1.

Distribution of the FNPs in *qLTG3-1*

Allele 5 with the FNP of a single amino acid substitution in Nipponbare was found within a limited set of varieties from Asia, while the FNP of the 71-bp deletion in Hayamasari has not been detected among the rice core collection (Table 1). The 71-bp deletion causes loss-of-function of the gene (Fujino et al. 2008), while the substitution in the Nipponbare allele may cause an alteration in the function of the gene (Hori et al. 2010). To determine the distribution of these FNPs, 38 varieties from the Japanese rice core collection was genotyped (Tables 3, S1, S2). The Nipponbare and Italica Livorno genotypes were predominant among this population, 36.8 and 57.9%, respectively, while the Hayamasari genotype was detected in only 2 varieties (5.3%).

Mansaku and Nagoyashiro carrying the Hayamasari genotype originated from Yamagata, a northern-region of Japan (Table S1). The rice variety Kamenoo originated from Yamagata and is known to be a progeny selected from a rice variety Hiyadachitou as a spontaneous mutant or by pure line selection. Hiyadachitou carries the Italica Livorno

genotype (Table S1), while Kamenoo carries the Hayamasari genotype. These results strongly suggested that the mutation event causing the FNP of the 71-bp deletion occurred in the population of Hiyadachitou. Only 3 varieties, Nipponbare, Dianyu 1, and Milyang 23, carried allele 5 in the rice core collection (Table 1). Nipponbare is from Japan. Dianyu 1 from China is a progeny of Norin No. 34 from Japan, which carries the Nipponbare genotype (Fig. S2, Table S2). Milyang 23 from Korea is the progeny of Ginbouzu from Japan, which carries the Nipponbare genotype (Fig. S2). The Nipponbare genotype was predominant in the Japanese rice core collection (Table 3), suggesting that the mutation event causing the FNP occurred in the early phase of rice cultivation in Japan.

To identify the distribution of these FNPs in a local region of Japan, 64 rice varieties from Hokkaido, including landrace type and commercial varieties, were examined (Tables 3, S2). The frequency of each genotype was different from those in the rice core collection and the Japanese rice core collection. The Hayamasari genotype was predominant in this population at 43.8%. These results suggested that the alleles with these FNPs might be adapted to the rice cultivation in local conditions of Japan or more specifically Hokkaido.

Discussion

DNA sequence variation in natural populations has been exploited for the improvement of objective traits in crop breeding. Such an approach has made it possible to achieve stable crop production to meet the demands of people around the world. The importance of the utilization of natural variation for functional and evolutionary studies has been highlighted (Alonso-Blanco and Koornneef 2000; Kovach and McCouch 2008; Alonso-Blanco et al. 2009). Recently, substantial progress has been made in elucidating the molecular basis of genetic variation for agronomically important traits for rice production (Yamamoto et al. 2009). Seed germination is an early life-history developmental transition and has a requirement for responses to environmental conditions. The control of seed germination under environmental conditions, where plants will grow, is important for the adaptability of plants. In *Arabidopsis*, genes associated with natural variation in seed germination are likely to be under strong natural selection (Donohue et al. 2005; Donohue 2009; Wilczek et al. 2009). This study demonstrated the allelic variation of a major QTL, *qLTG3-1*, controlling low-temperature germinability among cultivated rice.

Sequence variation of a gene responsible for a trait among populations could elucidate the selection pressure on the alleles as a result of their allelic variation. Studies of single nucleotide polymorphisms (SNPs) provide a framework for examining how population history, breeding system, and selection affect variation at genetic loci (Nordborg and Innan 2002; Palaisa et al. 2004). Recently, allelic variations causing desirable phenotypes for rice production have been identified in rice, including *semi-dwarf 1* (*sd1*) conferring high-yield (Ashikari et al. 2002; Asano et al. 2007), *Grain number 1* (*Gn1*) enhancing grain yield (Ashikari et al. 2005), the *waxy* gene controlling amylose content for eating quality (Sano 1984; Heu 1986; Mikami et al. 1999; Sato et al. 2002), and *Ghd7* contributing to adaptability to a wide geographic range across Asia (Xue et al. 2008). In addition, introgression events for desirable alleles between cultivar groups have played an important role in the adaptation (Sweeney et al. 2007; Mikami et al. 2008; Fujino et al. 2010).

In contrast, the almost completely conserved protein sequence of *qLTG3-1* was observed among cultivated rice and wild relatives in this study, suggesting that the function of *qLTG3-1* may be critical for seed germination under diverse environmental conditions. Despite the conservation of the coding region of *qLTG3-1*, many substitutions and indels occurred in the 5′ upstream region. The 2-kb 5′ upstream region of Italica Livorno was responsible for the tissue specificity of the gene expression of *qLTG3-1* (Fujino et al. 2008). Italica Livorno carries allele 1 in allele group I of *qLTG3-1*. Fifteen mutations were identified in the 933-bp 5′ upstream region between allele groups I and II. Further study to identify the phenotypic differences among *qLTG3-1* alleles will be necessary to elucidate the desirable allele for the improvement of low-temperature germinability.

The other possibility is for pleiotropic effects of *qLTG3-1* on rice growth. *qLTG3-1* contributes to tolerance to several kinds of stress at the seed germination stage: low and high temperature, high salt, and high osmotic conditions (Fujino et al. 2008). Gene expression of *qLTG3-1* was detected in not only the embryo of the germinating seed but also the shoot of the seedling and young panicle, indicating that *qLTG3-1* may play a role in several biological aspects during rice growth (Fujino et al. 2008). Transcriptome analysis demonstrated that *qLTG3-1* up-regulated the expression of genes involved in defense-related processes during seed germination (Fujino and Matsuda 2010).

Mutation events causing the FNPs occurred in Japan and alleles with these FNPs are predominant in a population from a particular region, Hokkaido. Hokkaido is one of northern-limits of rice cultivation in the world and is distinctive with the respect to the environmental conditions for rice cultivation, such as low temperature and long natural daylength during the rice-growing season. Only extremely early-heading varieties carrying novel genotype for flowering time are adapted to this region (Fujino 2003;

Fujino and Sekiguchi 2005a, b, 2008). The shaping of adaptation to local environmental conditions is an important objective in breeding programs. In addition, mutations that are favorable for cultivation practice are selected and utilized during breeding programs in current agriculture systems. Selection for commercial varieties in a particular region is strongly influenced by local preferences and culture. In crops, the adaptation is defined by not only environmental conditions but also cultivation methods or human demands. *qLTG3-1* increases tolerance to several kinds of abiotic stresses (Fujino et al. 2008), which is desirable for adaptation to diverse environmental conditions. The selection of alleles with the FNPs suggested that the molecular function of *qLTG3-1* could be associated with traits for cultivation methods or human demands in Japan.

It has been shown that there is considerable variation in low-temperature germinability in rice. It is very useful to improve this trait in rice breeding programs. Many QTLs for low-temperature germinability has been identified in various populations (Miura et al. 2001; Fujino et al. 2004; Zhang et al. 2005; Jiang et al. 2006; Chen et al. 2006; Ji et al. 2009). On the distal end of the short arm of chromosome 3, 2 QTLs, *qLTG3-1* from a *temperate japonica* Italica Livorno and *qLTG-3* from a *indica* Zhenshan 97B, were detected in the analyzed populations (Fujino et al. 2004; Chen et al. 2006). Only *qLTG3-1* from Italica Livorno showing vigorous low-temperature germinability was molecularly identified (Fujino et al. 2008). It was unclear whether the collocation of the QTL identified in different populations indicates an allele at the same locus. There still remains the possibility that one gene was tightly linked to another from a different population. Sequence comparisons of *qLTG3-1* in the parental varieties of these populations could resolve these problems. Functional analyses of *qLTG3-1* alleles identified in this study may be useful to control low-temperature germinability in rice breeding programs. Furthermore, DNA markers based on the structural changes could be effective for selecting for the desirable allele as the functional marker.

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