Theor Appl Genet (2011) 123:1089–1097
DOI 10.1007/s00122-011-1650-4

ORIGINAL PAPER

Selection for low-temperature germinability on the short arm of chromosome 3 in rice cultivars adapted to Hokkaido, Japan

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Received: 20 September 2010 / Accepted: 28 June 2011 / Published online: 9 July 2011

Abstract In plant breeding with intensive selection, the haplotype patterns in the targeted chromosomal regions may become monogenic among local populations with the most desirable combination of loci. This study demonstrated that the chromosomal region surrounding *qLTG3-1* was under selection during rice breeding programs in a local region of Japan, Hokkaido. *qLTG3-1* is a major quantitative trait loci controlling tolerance to low-temperature at the seed germination stage in rice, termed low-temperature germinability. A clear association between *qLTG3-1* alleles and low-temperature germinability was detected among 64 rice cultivars from Hokkaido. The allele with a loss-of-function mutation seemed to be selected during rice breeding programs. Comparison of haplotype patterns along with the short arm of chromosome 3 revealed that the selection of *qLTG3-1* alleles was focused on a distinct chromosomal region of at most 130 kb. In the short arm of chromosome 3, two major traits associated with the adaptability to local conditions have been identified; eating quality and heading date. This study demonstrated that recombinant haplotype patterns for these traits might shape the adaptability to local environmental conditions and market demands during rice breeding programs in addition to the selection of *qLTG3-1* alleles. The present results provide new opportunities for the design of hybridization combinations based on the haplotype patterns of chromosomal regions under selection during rice breeding programs in local regions.

Communicated by A. Paterson.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1650-4) contains supplementary material, which is available to authorized users.

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Introduction

Plant breeding programs generate intensive selection pressures focusing not only on shaping of adaptability to local environmental conditions but also on cultivation methods and market demands, and such programs have restricted the genetic diversity among local populations (Dilday 1990; Fu et al. 2003; Le Clerc et al. 2005; Roussel et al. 2005; Yamamoto et al. 2010). However, genetic diversity among local populations is an important component in the development of new cultivars. To achieve the objectives in plant breeding programs, desirable genes are continuously accumulated into cultivars by artificial selection after hybridization between parental cultivars with desirable traits. During the history of plant breeding in agriculture, two types of bottlenecks have restricted the genetic base of breeding populations; species domestication and the post-Mendelian adoption of breeding procedures (Morgante and Salamini 2003). An understanding of the genetic structure in local populations could provide strategies to broaden genetic diversity among local populations for breeding new cultivars with improved traits and help plant breeders choose parent lines or hybrid combinations by expanding the genetic diversity of the local gene pool.

Populations of plants differentiate as a consequence of spatial isolation, physical isolation, and local adaptation to natural environmental conditions. In addition, the adaptation to environmental conditions and cultivation methods has been the focus of plant breeding programs in local regions. Among landraces of rice, *Oryza sativa* L., in China, *japonica* appeared to differentiate between soil and watery ecotypes (upland–lowland), whereas *indica* was more clearly subdivided by seasonal ecotypes (early-, medium-, and late season) (Zhang et al. 2007, 2009). The different differentiation cues within each subpopulation may be attributed to their different environmental growth conditions and the corresponding cropping system. These results revealed that cultivation methods, cropping system, and man-made restricted-growth environments could be considered to be the main forces driving the intra-specific differentiation of cultivated rice.

The traits of interest in plant breeding programs mainly involve quantitative traits. For the control of such traits, identification of the most effective locus is useful. Furthermore, the molecular cloning of the major quantitative trait loci (QTL) for the target traits can open the door to new approaches for plant breeding programs. Once the gene responsible for the quantitative trait is identified, information on functional nucleotide polymorphisms (FNPs) and allelic variation of the gene are useful for marker-assisted selection (MAS) in plant breeding programs. In addition, surveys of germplasm with novel alleles and induced mutants are performed instead of phenotypic evaluation, which requires considerable time, cost and efforts.

Previously, we mapped three QTLs controlling tolerance to low-temperature at the seed germination stage in rice, termed low-temperature germinability, using backcrossed inbred lines derived from a cross between Italica Livorno from Italy and Hayamasari from Japan (vigorous and weak low-temperature germinability, respectively) (Fujino et al. 2004). Low-temperature-induced retardation of seedling growth is a common problem in temperate rice-growing areas, at high altitudes in tropical and sub-tropical areas, and in areas with a cold irrigation water supply. A major QTL for low-temperature germinability on chromosome 3, *qLTG3-1*, explained >30% of the total phenotypic variation in the mapping population (Fujino et al. 2004). Subsequently, *qLTG3-1* was cloned by a map-based strategy, and it was revealed that *qLTG3-1* encodes a protein of unknown function (Fujino et al. 2008). Genome-wide expression analysis demonstrated that *qLTG3-1* expression is required for the expression of defense response genes in low-temperature germinability in rice (Fujino and Matsuda 2010). In addition, the protein alignments of *qLTG3-1* among cultivated rice and its wild relatives are almost completely conserved (Fujino and Sekiguchi 2011). The results strongly suggested that *qLTG3-1* has an important role in not only seed germination but also rice growth.

Hokkaido is the most Northern region of Japan and represents one of the northern-limits of rice cultivation in the world. Rice cultivation in the region began in the late 1800s and during this initial period of rice cultivation which extended into the early 1900s, the pure line selection method was commonly used. Later, hybridization among local cultivars was used to develop improved cultivars with a very limited use of more exotic rice germplasm. The rice cultivars currently grown in Hokkaido exhibit key traits that appear to have maximized their genetic potential, but surprisingly, the newest registered cultivars still exhibit the improvement for all important agronomic traits when compared to the already registered cultivars. To date, the most important objective in the Japanese rice breeding programs has been eating quality, as epitomized by a cultivar Koshihikari. Rice breeding programs in local regions of Japan, including Hokkaido, aim to improve the adaptability of Koshihikari to local environmental conditions. Unfortunately, this intense focus for two decades could narrow the genetic diversity of local populations in Japan (Yamamoto et al. 2010). In order to achieve not only better eating quality but also improve important agronomic traits, broaden the genetic diversity using exotic germplasm in combinations with novel alleles is necessary to introduce alleles/genes lost in the process of developing modern cultivars.

A survey of *qLTG3-1* nucleotide sequences among cultivated rice revealed that the protein alignment is almost completely conserved (Fujino and Sekiguchi 2011). The distributions of FNPs indicated that mutation events causing FNPs occurred and were selected in Japan, specifically in Hokkaido. Based on the sequence alignments and functional analysis, the Hayamasari allele with a 71-bp deletion is a loss-of-function allele (Fujino et al. 2008) (Fig. 1). Another FNP was found in a cultivar Nipponbare that comprised a single amino acid substitution in a conserved amino acid motif among plant species (Fujino et al. 2008; Fujino and Sekiguchi 2011). It was strongly suggested that the Nipponbare allele has altered low-temperature germinability (Hori et al. 2010). Therefore, the functional or phenotypic difference among these alleles has not yet been determined. In Hokkaido rice breeding programs, tolerance to low-temperature during rice growth is the most important objective. *qLTG3-1* increases tolerance to not only low-temperature but also high salinity, high osmotic conditions, and high temperature at seed germination (Fujino et al. 2004, 2008). It is still unclear whether the alleles with these FNPs were selected as part of the rice breeding programs in Japan.

The selection of the FNPs at *qLTG3-1* might contribute to the adaptability of local cultivars to particular regions

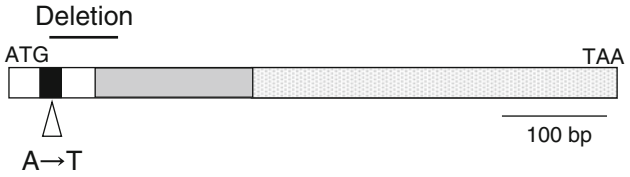

Fig. 1 Diagram of the structure of the *qLTG3-1* sequence. The *black*, *gray*, and *dotted* regions indicate the conserved amino acid motif, glycine-rich cell wall protein, and lipid transfer protein, respectively. The FNP in Hayamasari is a 71-bp deletion (*bar*). The FNP in Nipponbare is a non-synonymous substitution from A to T (*triangle*), generating an amino acid change of L to H

including cultivation methods and market demands. In this study, we identified the allelic variation of *qLTG3-1*, which is defined as the relationship between genetic variation in gene sequences and phenotypic variation in the trait. Then, we elucidated the genetic structure in a population from Hokkaido, including landraces and breeding lines, and the pattern and extent of selection on the chromosomal region surrounding *qLTG3-1* during rice breeding programs in Hokkaido.

Materials and methods

Plant materials

To identify the allelic variation of *qLTG3-1*, 64 *temperate japonica* rice cultivars from Hokkaido, Japan, including landraces and breeding lines, were used (Table S1). Previously, the *qLTG3-1* alleles among these cultivars were identified based on FNPs (Fujino and Sekiguchi 2011; Fig. 1). In addition, three cultivars from Europe were used for this analysis as references. For the comparison of genotype of SSR markers on the short arm of chromosome 3 surrounding *qLTG3-1*, a total of 30 cultivars were used (Table 1). Eighteen *temperate japonica* rice cultivars from Hokkaido were selected, which played an important role of the pedigree in this population. Eight *temperate japonica* cultivars from Honshu, three *temperate japonica* cultivars from Europe, and an *aus* cultivar, Kasalath, were selected as references, which were well known and used as representatives for each population. Cultivars used for the allelic variation analysis of *qLTG3-1* were cultivated in a paddy field at Hokuren Agricultural Research Institute (Naganuma, Hokkaido, Japan, 43°03′N latitude) under natural field conditions. The seeds provided from genebanks were sown without propagation. Seeds were harvested at maturity and then were kept at room temperature until use. Seeds were incubated at 15°C in the dark for the evaluation of low-temperature germinability (Fujino et al. 2004). Seeds were provided by Hokkaido Central Agricultural Experiment Station and National Institute of Agrobiological Sciences, Japan.

DNA analysis

Total DNA was isolated from young leaves using a CTAB method (Murray and Thompson 1980). PCR, gel electrophoresis, detection, and sequencing were carried out as described previously (Fujino et al. 2004, 2005). A total of 42 markers on the short arm of chromosome 3 surrounding *qLTG3-1* were examined among 30 cultivars. Genotypes of 40 SSR markers were determined using polyacrylamide gel electrophoresis with silver staining (Fujino et al. 2004). Among the 40 SSR markers, 33 were from the International Rice Microsatellite Initiative (McCouch et al. 2002; Gramene, http://www.gramene.org). The remaining seven SSR markers, including those developed previously (Fujino et al. 2004, 2008; Fujino and Sekiguchi 2008) and in this study, are listed in Table S2. In addition, the genotype of *qLTG3-1* and a sequence tagged site (STS) marker flanking *qLTG3-1*, STS73-28, were used (Fujino et al. 2008; Fujino and Sekiguchi 2011).

Data analysis

To determine polymorphisms in the 40 SSR markers, 26 cultivars from Japan with close genetic relationships, including landraces and breeding types, were used (Table 1). Polymorphism information content (PIC) was calculated for each marker according to Nei (1973): $PIC = 1 - \Sigma h_k^2$, where h_k is the frequency of the kth allele. The model-based STRUCTURE program (Pritchard et al. 2000; Falush et al. 2003; http://www.pritch.bsd.uchicago.edu/software.html) was used to determine the population structure using a burn-in length of 10,000 and run length of 100,000 with a model allowing for admixture and correlated allele frequencies. Thirty-three marker loci along the short arm on chromosome 3 were used to analyze the population structure of 30 cultivars. We ran the simulation 10 times independently for each K (the number of clusters) value from 1 to 20. We used both the LnP(D) value and Evanno's ΔK to estimate the K value (Evanno et al. 2005). LnP(D) in the STRUCTURE output is the log likelihood of the observed genotype distribution in K clusters. The true number of K is often identified using the maximal value of LnP(D) returned by STRUCTURE. However, it is sometimes difficult to determine the ideal K based on the value of LnP(D) because in most cases, once the real K is reached, LnP(D) at larger Ks plateaus or continues increasing slightly. ΔK is based on the rate of change in the log probability of the data between successive K values and usually can indicate the ideal K. The suggested $\Delta k = M(|L(k+1) - 2L(k) + L(k-1)|)/S[L(k)]$, where

Table 1 Alleles of *qLTG3-1* and population structure among 30 rice cultivars

Cultivar	Accession number	Origin	Breeding year[a]	Allele of *qLTG3-1*[b]	Population structure
Kamenoo	JP5903	Honshu	Landrace	HY	IV
Eiko	00041	Hokkaido	1942	HY	V
Koshihikari		Honshu	1956	HY	IV
Yukara	00079	Hokkaido	1962	HY	V
Kitahikari		Hokkaido	1975	HY	V
Hayamasari		Hokkaido	1988	HY	IV
Kirara397		Hokkaido	1988	HY	IV
Hakutyoumochi		Hokkaido	1989	HY	IV
Honoka224		Hokkaido	1990	HY	IV
Hoshinoyume		Hokkaido	1996	HY	IV
Asahi-a	JP10736	Honshu	Landrace	NP	III
Aikoku	JP6768	Honshu	Landrace	NP	V
Shinriki	JP7980	Honshu	Landrace	NP	III
Ginbouzu		Honshu	Landrace	NP	V
Wasefukoku	06493	Hokkaido	1936	NP	V
Nourin no. 15	01397	Hokkaido	1940	NP	V
Fukuyuki	00068	Hokkaido	1958	NP	V
Nipponbare		Honshu	1963	NP	III
Kamuimochi	00085	Hokkaido	1965	NP	V
Sorachi	00092	Hokkaido	1967	NP	V
Kitaake		Hokkaido	1983	NP	V
Aya		Hokkaido	1991	NP	III
Fanny		France	–	NP	II
Asahi-b	JP6761	Honshu	Landrace	IL	II
Akage		Hokkaido	Landrace	IL	II
Hokkaiwase		Hokkaido	Landrace	IL	II
Iburiwase		Hokkaido	Landrace	IL	II
Italica Livorno		Italy	–	IL	I
Arroz Da Terra		Portugal	–	IL	I
Kasalath		India	–	IL	I

Accession numbers with 5-digit numbers and JP numbers are from Hokkaido Central Agricultural Experiment Station and from the genebank of NIAS, respectively

[a] Breeding cultivars are shown with their year of registration

[b] Alleles of IL, NP, and HY indicate wild-type of Italica Livorno, a single nucleotide substitution of Nipponbare, and a 71-bp deletion of Hayamasari, respectively

$L(k)$ represents the kth LnP(D), M is the mean of 10 runs, and S is their standard deviation.

Results

Allelic variation of *qLTG3-1*

To determine allelic variation among *qLTG3-1* alleles, an association study between *qLTG3-1* allele types and the phenotype of low-temperature germinability was performed using 69 rice cultivars (Fig. 2; Tables 2, S1). These cultivars showed a wide range of variation in their low-temperature germinability phenotype, from 2.3% for Yukimaru to 92.9% for Hokuto from Hokkaido or 100% for Dunghung Shali from Hungary after 6 days of incubation. A clear association was detected between *qLTG3-1* alleles and low-temperature germinability. Cultivars with the Italica Livorno allele (80.8%) showed more vigorous low-temperature germinability than those with the Hayamasari allele (30.7%), while cultivars with the Nipponbare allele (69.7%) showed slightly reduced low-temperature germinability compared with that of the Italica Livorno allele ($P = 0.043$) (Table 2). These results suggested that the amino acid substitution in the Nipponbare allele plays a significant role in the function of the gene.

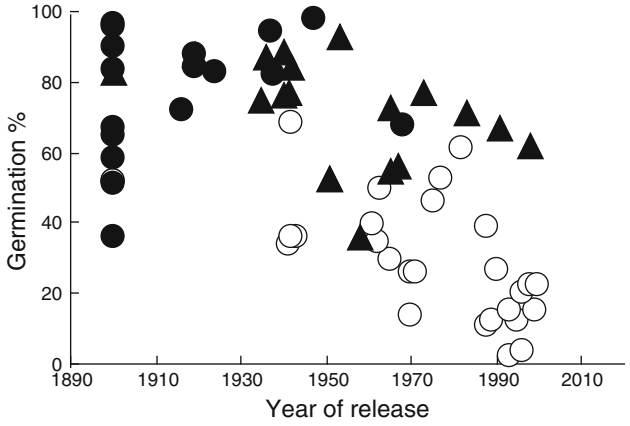

Fig. 2 Relationship between *qLTG3-1* alleles and low-temperature germinability among 69 cultivars in Table S1. *Closed* and *open circles* indicate the Italica Livorno and Hayamasari alleles, respectively. *Closed triangle* indicates the Nipponbare allele. The *X*-axis mentions the year of release

These results also revealed that *qLTG3-1* contributed strongly to the variation in low-temperature germinability among rice cultivars from Hokkaido. In addition, the relationships suggested gene(s) exhibiting an epistatic interaction with *qLTG3-1* and another locus(loci) controlling low-temperature germinability. Cultivars with the Italica Livorno allele showed varied low-temperature germinability of 36.0–97.8%, while those with the Hayamasari allele were 2.3–68.3%. Wasebouzu, Chinkomochi, and Kurogemochi carrying the Italica Livorno allele showed weak germinability, but they have an identical sequence with the Italica Livorno allele (data not shown). Nakateeiko and Igoshisoutou carrying identical sequences to the Hayamasari allele (data not shown) showed slightly more vigorous low-temperature germinability. These results indicated that a gene(s) other than *qLTG3-1* was involved in this variation in low-temperature germinability among rice cultivars from Hokkaido.

The selection of *qLTG3-1* alleles during rice breeding programs

The pedigree of Hayamasari clearly revealed the origin of the FNP in the Hayamasari *qLTG3-1* allele (Fig. 3). All *qLTG3-1* alleles were present in the progenitors of Hayamasari; the Italica Livorno allele in Akage, the Nipponbare allele in Aikoku, and the Hayamasari allele in Kamenoo. The FNP in Haymasari might be derived from both or either parental cultivars, Yukara and Ishikari/Koshihikari, respectively. The *qLTG3-1* alleles in Kirara397 and Hoshinoyume are also the progenies of that of Koshihikari. Therefore, the FNP in these cultivars was derived from Kamenoo. Previously, we demonstrated that the mutation event generating the FNP may have occurred in a population of Hiyadachito, and then it might have been selected from Kamenoo as a pure line breeding (Fujino and Sekiguchi 2011).

Selection of the *qLTG3-1* allele could mirror the history of rice breeding programs in Hokkaido. Almost all of the landrace type cultivars carried the Italica Livorno allele, while most cultivars in the current breeding programs carry the Hayamasari allele (Fig. 2, Table S1). The results strongly suggested that the selection pressure was focused on this gene during rice breeding programs in Hokkaido. It cannot be stated with certainty whether this selection was focused on *qLTG3-1* itself or a gene(s) tightly linked to *qLTG3-1*.

Haplotype patterns of the chromosomal region surrounding *qLTG3-1*

The pattern and extent of selection on the chromosomal region surrounding *qLTG3-1* were compared using 42 loci in 30 cultivars (Fig. 4, Table S3). Among the 40 SSR loci, eight markers showed monomorphic results among cultivars from Japan, while the others showed variations in the number of alleles and PIC values. The mean allele number was 3.26 and ranged from two to nine among cultivars from Japan. Nine SSR markers showed more than 10 alleles among all cultivars. These 17 SSR markers were not included for the determination of the haplotype patterns.

Diverse haplotype patterns were observed among landrace type cultivars carrying the Italica Livorno allele at *qLTG3-1*, while landrace type cultivars carrying the Nipponbare allele showed conserved patterns except for Asahi-a (Fig. 4). This trend was consistent with the Nipponbare allele being generated from a cultivar with the Italica Livorno allele in the early phase of rice cultivation in Japan (Fujino and Sekiguchi 2011). This selection of the Nipponbare allele at *qLTG3-1* may cause a bottleneck effect on diversity in the chromosomal region surrounding the gene. Kamenoo carrying the Hayamasari allele at *qLTG3-1* had different haplotype patterns from other landrace type cultivars, suggesting that the progenitor of Kamenoo, which carries the Italica Livorno allele, may have a unique haplotype pattern in this chromosomal region.

Next, we focused on changes in the haplotype patterns among rice cultivars from Hokkaido (Fig. 4). Among cultivars carrying the Nipponbare and Hayamasari alleles at *qLTG3-1*, several kinds of haplotype patterns were found as regions A (2 Mb) and B (8 Mb). These haplotype patterns were consistent with some of the linkage blocks that were shared with both *qLTG3-1* alleles or were allele-specific (Fig. 4). In region B, three haplotype patterns extending about 8 Mb in length were identified, while in region A, two major haplotype patterns extending about 2 Mb in length were identified, in addition to their

Table 2 Germination rates between *qLTG3-1* alleles among rice cultivars from Hokkaido

	Allele[a]		
	IL	NP	HY
No. of cultivar	21	20	28
Mean (%)	80.8	69.7[b]	30.7[c]
Range (%)	36.0–100	35.9–92.9	2.3–68.3

[a] Alleles of IL, NP, and HY indicate wild-type of Italica Livorno, a single nucleotide substitution of Nipponbare, and a 71-bp deletion of Hayamasari, respectively

[b] Significant difference from the Italica Livorno allele at 0.05% level by *t* test

[c] Significant difference from the Italica Livorno allele at 0.001% level by *t* test

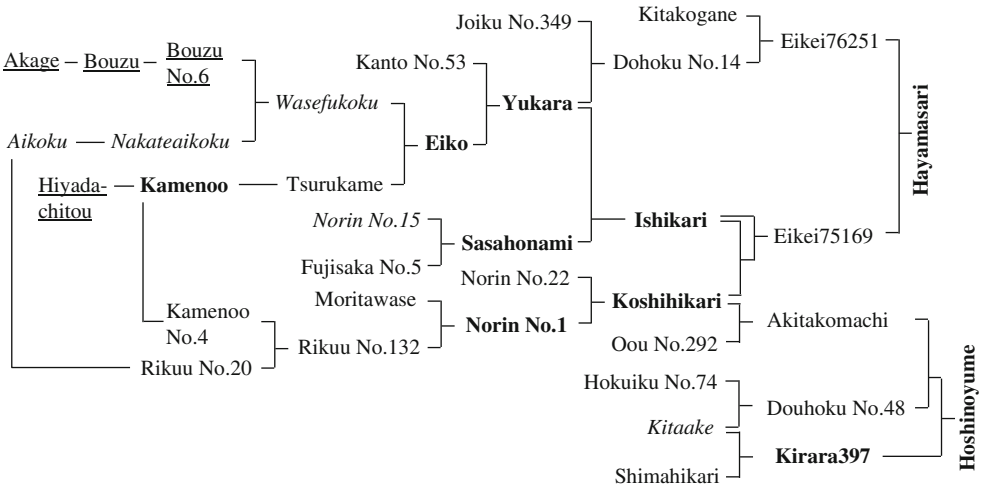

Fig. 3 The pedigree of Hayamasari and Hoshinoyume. *Bold*, *italic*, and *underline* indicate the Hayamasari, Nipponbare, and Italica Livorno alleles for *qLTG3-1*, respectively

recombinants. This indicated that recombinations between cultivars with each *qLTG3-1* allele generated new haplotype patterns along the short arm of chromosome 3, which could exhibit novel phenotypes.

However, there was no linkage block specific to *qLTG3-1* alleles among all the cultivars with each *qLTG3-1* allele. Furthermore, variation in genotypes in both flanking loci of *qLTG3-1* suggested that frequent recombination events occurred in this region. Kamenoo carries the haplotype pattern of B-HY-A in the order GBR3001-*qLTG3-1*-STS73-28 marker loci, while three patterns were detected among cultivars from Hokkaido with the Hayamasari allele at *qLTG3-1*; A-HY-A, C-HY-A, and C-HY-B. In the chromosomal region surrounding *qLTG3-1*, two to four alleles at each marker loci were identified (Fig. 4). Therefore, PIC values in cultivars with each *qLTG3-1* allele supported the suggestion of frequent recombinations along the short arm of chromosome 3 during rice breeding programs (Fig. S1). The results demonstrated that selection of the *qLTG3-1* allele was focused on *qLTG3-1* itself or tightly linked gene(s) on the distinct chromosomal region, at most 130 kb between the markers GBR3001 and STS73-28.

Population structure of cultivars from Hokkaido

The selection of *qLTG3-1* focused on the distinct chromosomal region surrounding *qLTG3-1* during rice breeding programs in Hokkaido. However, chromosomal blocks on the short arm of chromosome 3 were conserved beyond *qLTG3-1* alleles. Next, we focused on the association of selection of the *qLTG3-1* allele with selection along the short arm of chromosome 3. When we ran the STRUCTURE simulation using 30 cultivars with 33 marker loci along the short arm of chromosome 3, the LnP(D) value showed an evident peak at $K = 5$, indicating that the cultivars were classified into five distinctly divergent subpopulations (Fig. 5; Table 1).

The subpopulation classification was clearly associated with *qLTG3-1* alleles. The *aus* cultivar and the two European cultivars (subpopulation I) were clearly divergent from the Hokkaido cultivars. Subpopulations II and IV contained landrace types with the Italica Livorno allele and breeding types with the Hayamasari allele, respectively. Subpopulation III contained cultivars with the Nipponbare allele from Honshu. Subpopulation V mainly contained breeding types with the Nipponbare allele. Three cultivars

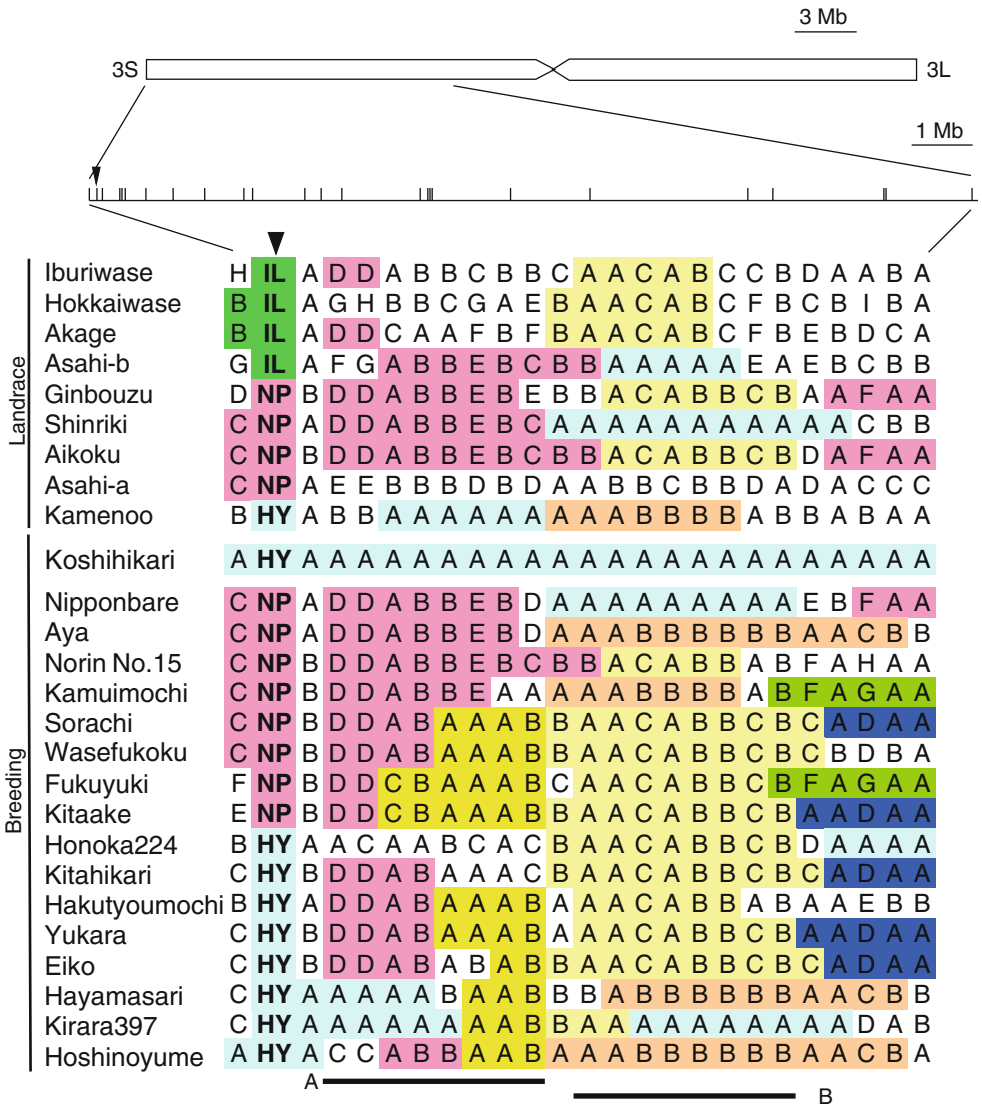

Fig. 4 Haplotype patterns of 25 SSR loci along the short arm of chromosome 3. *Letters* indicate the different haplotypes at these loci. Conserved linkage blocks are indicated by *different colors*. An *arrowhead* indicates the position of *qLTG3-1*. The position of each locus is referenced from Table S3. Regions A (2 Mb) and B (8 Mb) below haplotype patterns indicate the conserved linkage blocks (color figure online)

with the Hayamasari allele were classified into subpopulation V as a minor group due to recombination between cultivars with each *qLTG3-1* allele. In addition, admixtures of the genetic structure between subpopulations were frequently observed in all subpopulations, except for the subpopulation I. These results supported that recombinations on this chromosomal region were selected during rice breeding programs.

Discussion

In plant breeding programs in local regions, it is important to understand which gene is under selection for the target traits and how the selection extends to the chromosomal region of the target genes in order to improve strategies for creating new cultivars. *qLTG3-1* is a major QTL controlling low-temperature germinability in rice (Fujino et al. 2004, 2008). The distributions of FNPs indicated that the mutation events causing the FNPs occurred and were selected in Japan, especially in Hokkaido (Fujino and Sekiguchi 2011). In this study, we identified allelic variation of *qLTG3-1*, between *qLTG3-1* allele types and low-temperature germinability among the population from Hokkaido. Comparison of haplotype patterns along the chromosomal region surrounding *qLTG3-1* revealed that the selection of *qLTG3-1* alleles with the FNPs during rice breeding programs was focused on a distinct chromosomal region, at most 130 kb. Beside the selection of *qLTG3-1* alleles, the recombinant haplotype patterns might shape the adaptability to local environmental conditions during rice breeding programs.

The genetic structure of rice cultivars from Hokkaido revealed that *qLTG3-1* alleles show a correlation with haplotype patterns along the short arm of chromosome 3 only in landraces. The correlation was disappeared among

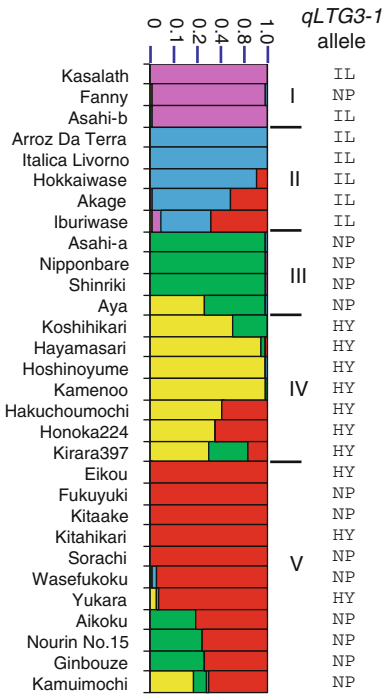

Fig. 5 The distribution of rice cultivars into five subpopulations. The proportion of alleles for each variety that is shared with each subpopulation is represented by *different colors*, subpopulation I (*purple*), II (*blue*), III (*green*), IV (*yellow*), and V (*red*). The *qLTG3-1* alleles of IL, HY, and NP indicate the Italica Livorno, Hayamasari, and Nipponbare alleles, respectively (color figure online)

breeding lines. These results suggested that recombination events between cultivars with different *qLTG3-1* alleles have generated new haplotype patterns, which could exhibit novel phenotypes. The most desirable haplotype patterns combined with a *qLTG3-1* allele and other loci along the short arm of chromosome 3 would be selected as new rice cultivars. This trend was supported by the comparison of pedigree haplotypes with genome-wide single nucleotide polymorphisms (SNPs) among rice cultivars from Japan (Yamamoto et al. 2010). In contrast to the decreasing genetic diversity of the gene pool of local populations during breeding programs, the distal end of the short arm of chromosome 3 showed an increased number of haplotypes. The results suggested that a new haplotype block might have been created by selection during rice breeding in Japan (Yamamoto et al. 2010). The present results could provide new opportunities for the design of hybridization combinations based on the haplotype patterns of chromosomal regions under selection during rice breeding programs in local regions.

In the chromosomal region targeted by this *qLTG3-1* selection, two major traits associated with the adaptability to local conditions have been identified: eating quality and heading date. A major QTL controlling eating quality of Koshihikari was mapped near to *qLTG3-1* (Kobayashi and Tomita 2008; Takeuchi et al. 2008; Wada et al. 2008). However, identical haplotype patterns to Koshihikari were limited among the cultivars from Hokkaido; about a 1.2 Mb region in Hayamasari and a total of 7.5 Mb regions in Kirara397 (Fig. 4). No region in Hoshinoyume, which showed the best eating quality among cultivars from Hokkaido used in this study, was identical to Koshihikari. The control of heading date during the limited growing period is important in rice breeding programs. Cultivars grown in Hokkaido have a unique genotype for heading date as an adaptation to being grown at the northern-limits of rice cultivation (Fujino and Sekiguchi 2005a, b; Nonoue et al. 2008). *qDTH3* on the short arm of chromosome 3 has been identified using a population derived from the crosses between landraces and breeding lines from Hokkaido (Fujino and Sekiguchi 2008; Fujino and Iwata 2011). In addition to *qLTG3-1*, these two traits might be under selection during rice breeding programs in Hokkaido.

The haplotype patterns along the short arm of chromosome 3 demonstrated that combinations between linkage blocks found in cultivars with the Hayamasari and Nipponbare *qLTG3-1* alleles were generated during rice breeding programs. Linkage blocks found in cultivars with the Italica Livorno *qLTG3-1* allele were eliminated in the early phase of rice breeding programs due to the selection for *qLTG3-1*, such as a bottleneck. To broaden the combinations of genes located on this chromosome region, these landrace type cultivars carrying the Italica Livorno *qLTG3-1* allele are useful.

In this study, a clear association was detected between *qLTG3-1* alleles and low-temperature germinability among a population from Hokkaido, called allelic variation. *qLTG3-1* was identified as the major QTL between Italica Livorno from Italy and Hayamasari from Japan (Fujino et al. 2004). These two cultivars show relatively wide genetic diversity among *temperate japonica* (Mackill et al. 1996; Fujino unpublished data). Therefore, *qLTG3-1* played a major role in the variation of low-temperature germinability among a population from Hokkaido. In addition, variation of low-temperature germinability among each varietal group with the *qLTG3-1* alleles indicated that the existence of other gene(s) than *qLTG3-1* also controlled this trait, which showed genetically narrow relationships. These results were in agreement with our previous report evaluating the effects of *qLTG3-1* in different genetic backgrounds (Iwata and Fujino 2010). These genes could make the genetic basis of a low-temperature germinability complex among a local population with genetically narrow relationships.

Acknowledgments This work was supported in part by grants from the Ministry of Agriculture, Forestry and Fisheries of Japan (Integrated research project for plant, insect and animal using genome

technology QT-3007 and Genomics for Agricultural Innovation QTL-1005).

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