RESEARCH PAPER

A SHORT INTERNODES (SHI) family transcription factor gene regulates awn elongation and pistil morphology in barley

Takahisa Yuo¹, Yuko Yamashita¹, Hiroyuki Kanamori², Takashi Matsumoto², Udda Lundqvist³, Kazuhiro Sato¹, Masahiko Ichii⁴, Stephen A. Jobling⁵ and Shin Taketa^{1,*}

¹ Institute of Plant Science and Resources, Okayama University, 2-20-1 Chuo, Kurashiki 710-0046, Japan

² National Institute for Agrobiological Sciences, Tsukuba 305–8602, Japan

³ Nordic Genetic Resource Center, Smedjevägen 3, PO Box 41, SE-230 53 Alnarp, Sweden

⁴ Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki, Kagawa 761-0795, Japan

⁵ CSIRO Plant Industry, GPO Box1600, Canberra, ACT 2601, Australia

* To whom correspondence should be addressed: E-mail: staketa@rib.okayama-u.ac.jp

Received 28 February 2012; Revised 16 May 2012; Accepted 22 May 2012

Abstract

The awn, an apical extension from the lemma of the spikelet, plays important roles in seed dispersal, burial, and photosynthesis. Barley typically has long awns, but short-awn variants exist. The short awn 2 (*lks2*) gene, which produces awns about 50% shorter than normal, is a natural variant that is restricted to Eastern Asia. Positional cloning revealed that *Lks2* encodes a SHI-family transcription factor. Allelism tests showed that *lks2* is allelic to *unbranched style 4 (ubs4)* and *breviaristatum-d (ari-d)*, for which the phenotypes are very short awn and sparse stigma hairs. The gene identity was validated by 25 mutant alleles with lesions in the *Lks2* gene. Of these, 17 affected either or both conserved regions: the zinc-binding RING-finger motif and the IGGH domain. *Lks2* is highly expressed in awns and pistils. Histological observations of longitudinal awn sections showed that the *lks2* short-awn phenotype resulted from reduced cell number. Natural variants of *lks2* were classified into three types, but all shared a single-nucleotide polymorphism (SNP) that causes a proline-to-leucine change at position 245 in the IGGH domain. All three *lks2* natural variants were regarded as weak alleles because their awn and pistil phenotypes are mild compared with those of the 25 mutant alleles. Natural variants of *lks2* found in the east of China and the Himalayas had considerably different sequences in the regions flanking the critical SNP, suggesting independent origins. The available results suggest that the *lks2* allele might have a selective advantage in the adaptation of barley to high-precipitation areas of Eastern Asia.

Key words: *Hordeum vulgare* L., IGGH domain, RING finger, stigma, varietal difference.

Introduction

Barley (*Hordeum vulgare* L.) inflorescences are characterized by stiff bristles, called awns, one on the tip of each spikelet. Botanically, the awn is an apical extension of the distal end of the lemma; it is probably a modified leaf blade (Dahlgren *et al.*, 1985). Awns are also found in other grass species such as wheat, rye, oats, sorghum, and rice. The awn is considered to play various biological roles (Grundbacher, 1963). In the wild, awns with barbs aid in seed dispersal by attaching to animal fur (zoochory) (von Bothmer *et al.*, 1995). The importance of the awn in seed

burial has been well studied in wild tetraploid wheat, which has a pair of awns in each spikelet; the awn movement driven by the daily humidity cycle propels the seeds into the ground (Elbaum *et al.*, 2007). The awn also protects seeds from predation by birds and animals (Grundbacher, 1963). Under agricultural conditions, however, the long and stiff awn with barbs has persisted as a nuisance to farmers because they hinder manual harvesting. More importantly for modern agriculture, the injurious awn reduces the feed value of straw for livestock (Takahashi, 1955). Consequently, during domestication and subsequent varietal differentiation, much selection pressure has been applied to reduce

Abbreviations: BAC, bacterial artificial chromosome; qRT-PCR, real-time quantitative reverse transcription-PCR; QTLs, quantitative trait loci; RACE, rapid amplification of cDNA ends; SNP, single-nucleotide polymorphism; UTR, untranslated region.

© The Author [2012]. Published by Oxford University Press [on behalf of the Society for Experimental Biology]. All rights reserved.

For Permissions, please e-mail: journals.permissions@oup.com

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.0/uk/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

awn length (Takahashi, 1955). Despite such selection pressure, most barleys have retained rather long awns. This is in contrast to other small grain crops, particularly rice (Takahashi *et al.*, 1986), where breeding has remarkably reduced awn length. This is probably because the awn in barley contributes significantly to photosynthesis (Kjack and Witters, 1974). In cross-sections, barley awns have a triangular shape with two zones of chlorenchyma cells and three well-developed vascular bundles inside and stomata on the surface (Reid, 1985). In contrast, rice awns are round-shaped in cross-section with one vascular bundle in the centre (Toriba *et al.*, 2010) and probably do not contribute as much to photosynthesis. Grundbacher (1963) estimated that photosynthate derived from barley awns contributes more than 10% of the total kernel dry weight. In field tests, Qualset *et al.* (1965) confirmed the significant yield advantage of barley accessions with full awns and half awns relative to those of quarter awns and awnless genotypes.

The molecular basis of awn elongation in grasses remains unclear because of a lack of cloned genes. In rice, many quantitative trait loci (QTLs) for awn length have been reported on all chromosomes except for chromosomes 1 and 5 (Cai and Morishima, 2002; Gu *et al.*, 2005). However, their causative genes have not yet been cloned. Barley provides excellent materials for such studies because many major genes subject to simple Mendelian inheritance are known to affect awn length in this species (Lundqvist and Franckowiack, 2003). In addition, genes pleiotropically affecting awn length and QTLs for awn length in barley have been reported (Sameri *et al.*, 2006; Gyenis *et al.*, 2007).

The *short awn 2* (*lks2*, for *length 2*) gene on the long arm of chromosome 7H is a spontaneous recessive mutation that reduces awn length by about 50%, as well as awn thickness (Fig. 1A). This natural mutant allele shows a unique geographical distribution restricted to Eastern Asia, including India, Nepal, China, Korea, and Japan; the frequencies of short-awned varieties in Nepal, China, Korea, and Japan were reported to be 22, 3.2, 25.8 and 13.6%, respectively (Takahashi, 1987). This restricted geographical distribution raises the possibility of a selective advantage of *lks2* in humid conditions. In light of the agricultural importance of *lks2* in Eastern Asia, Taketa *et al.* (2011) roughly mapped *lks2*. The present study reports the positional cloning and identification of the *Lks2* gene. From lines of experimental evidence such as the gene structure, allelic mutations, expression pattern, and histology of awns, our results revealed that *lks2* affects both awn elongation and pistil morphology. The origin of *lks2* in barley is discussed based on natural allelic variation of the gene.

Materials and methods

Plant materials and allelism tests

For mapping of *lks2*, 1512 F₂ plants derived from a cross between Karafuto Zairai (*Lks2*, Okayama University accession no. OJ301) and Aizu Hadaka 3 (*lks2*, OJ323) were used (Fig. 1A). Aizu Hadaka 3 additionally carries the *dense spike 1* (*dsp1*) gene (Takahashi *et al.*, 1953a; Taketa *et al.*, 2011), but this gene did not affect phenotyping of *lks2*. For genetic analysis, each plant was scored qualitatively for awn phenotype as either long or short awn by visual inspection. F₃ progeny tests were carried out for selected recombinant plants.

Fig. 1. Morphology of long-awn and short-awn barley cultivars. (A) Spikes of long-awn *Lks2* (Karafuto Zairai, left) and short-awn *lks2* (Aizu Hadaka 3, right) cultivars used as mapping parents. Both cultivars are six-rowed. (B) Spikes of Bowman near-isogenic lines having the two-rowed genetic background. From left to right: wild-type cultivar Bowman, *lks2.b*-Bowman, *uba4.d*-Bowman, and *ari-d.15*-Bowman. (C) Pistils of Bowman near-isogenic lines arranged in the same order as in (B). Bars, 1 cm (A, B); 1 mm (C).

The following short-awn barley accessions were used for allelism tests with *lks2*. Short-awn mutant KM7 was induced by gamma-ray irradiation from Kanto Nijo 29 (a gift from Dr. N. Kawada, Tochigi Prefectural Agricultural Experiment Station, Japan). Bowman near-isogenic lines carrying *lks2.b*, *unbranched style 4.d* (*uba4.d*), or *breviaristatum-d.15* (*ari-d.15*) (Fig. 1B) were developed by back-crossing (a gift from Dr. H. Bockelman, National Small Grains Collection, USDA). Twenty-three lines of short-awn *breviaristatum-d* (*ari-d*) mutants and their wild types were used (a gift from Dr. F. Ottosson, NordGen, Alnarp, Sweden). The short-awn mutants used in this study are listed in Supplementary Table S1 at JXB online.

For allelism tests, short-awn mutant KM7 was crossed with Honen 6, a standard *lks2* line used by Takahashi *et al.* (1953a). Allelism tests among the three short-awn loci of *lks2.b*, *uba4.d*, and *ari-d.15* were also conducted using Bowman near-isogenic lines; F₁ hybrids and their parental lines were grown in a controlled environment room at a constant temperature of 15°C under natural light. About 3 weeks after flowering, awn phenotype was classified visually as long (wild-type Bowman), short (*lks2.b* type) or very short (*uba4.d* or *ari-d.15* type). Three plants per cross combination were tested. Quantitative data on awn length were also collected from measurements of at least five spikes per plant. Awn length was measured for samples on the second and third spikelets from the top of the spikes.

For studies of natural allelic variation, 39 *lks2* accessions and eight long-awn accessions were used (Supplementary Table S2 at JXB online). Nine of the 39 short-awn accessions were confirmed to carry *lks2* by crossing to Honen 6, a standard *lks2* line. The other 30 *lks2* carriers were selected based on phenotype. These 39 accessions were all six-rowed because spontaneous *lks2* mutation is not present among two-rowed barley landrace (Takahashi, 1983). These accessions were examined for the *Lks2* candidate gene by sequencing or marker genotyping of mutation points.

Genetic mapping

DNA was extracted according to methods described previously (Taketa *et al.*, 2012). PCRs were performed in 10 µl reaction mixtures containing 5 µl of Quick Taq™ HS DyeMix DNA polymerase (Toyobo Co.), 20 ng genomic DNA, and 0.2 µM of each specific primer using the following conditions: initial denaturation for 2 min at 94 °C, followed by 35 cycles of 0.5 min at 94 °C, 0.5 min at the annealing temperature of the respective primers, and 0.5 min at 68 °C, with a final extension for 7 min at 68 °C and a hold at 15 °C. For restriction assays, 10 µl of the PCR products was digested with 2 U polymorphic enzyme and buffer in a final volume of 15 µl. These fragments were separated on 1.5–2% agarose gels or 3% Metaphor agarose gels.

PCR markers were developed as described previously (Taketa *et al.*, 2011). PCR primers were designed using Primer 3 (<http://frodo.wi.mit.edu/primer3/>) and synthesized commercially. Amplified PCR products from both parental accessions were sequenced according to the method of Taketa *et al.* (2004, 2006), and polymorphic markers were developed. For chromosome arm assignment of barley markers, wheat–barley chromosome addition lines (Islam *et al.*, 1981; Islam 1983) were used. Primer sequences are presented in Supplementary Table S3 at JXB online.

Recombination values were calculated using MAPMAKER/EXP Macintosh version 2.0 (Lander *et al.*, 1987), and a genetic linkage map was constructed based on LOD scores greater than 3.0.

Chromosome walking

A barley bacterial artificial chromosome (BAC) library of cultivar Haruna Nijo (Saisho *et al.*, 2007) was used. BAC clones near the *lks2* locus were selected by PCR screening of DNA pools or by hybridizing the arrayed BAC colony filters with peroxidase-labelled probes. BAC DNA was extracted using a standard alkaline/SDS procedure. The size of the insert was analysed using pulsed-field gel electrophoresis after digestion with *NotI* or *AscI*. All BAC clones were end sequenced using M13 primers for designing PCR primers. Overlapping BACs were identified by Southern blot hybridization or by PCR marker analysis, and a BAC minimum tiling path was constructed.

BAC sequencing and annotation

Five BAC clones (HNB389J01, 573P19, 164O16, 719A14, and 507D16) were shotgun sequenced (tenfold coverage) according to the standard dideoxy terminator chemistry protocol of the Rice Genome Research Program (International Rice Genome Sequencing Project, 2005). Annotation analyses were conducted as reported by Taketa *et al.* (2008). The 519 kb BAC contig sequence reported in this paper has been deposited in EMBL/GenBank/DBJ under accession number AB678347.

Expression analysis and rapid amplification of cDNA ends (RACE)

Long-awn cultivar Haruna Nijo was grown in the greenhouse. From immature 5 cm or 6 cm long inflorescences, awns, pistils, anthers, glumes, lemmas, paleas, and flag leaves were collected separately and used for RNA extraction. Experiments were done with three biological replications, and each replication included one immature inflorescence. Total RNA was extracted from various tissues using Sepasol RNA I Super G (Nakalai Tesque). Real-time quantitative reverse transcription-PCR (qRT-PCR) was performed as described previously (Taketa *et al.*, 2012). Briefly, RNA samples were treated with DNase I (Promega). For qRT-PCR, first-strand cDNA was synthesized with ReverTra Ace (Toyobo Co.). Quantitative analyses were carried out on Thermal Cycler Dice TP800 (TaKaRa) using SYBR Premix DimerEraser (TaKaRa) according to the manufacture's instructions. Normalization was carried out using the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene. For determination of 5'- and 3'-untranslated regions (UTRs), RACE was conducted as described by Taketa *et al.* (2008). The primer sequences are presented in Supplementary Table S3.

Histological observations

Awn samples for histological observation were collected from Bowman and an isogenic line of Bowman carrying *lks2.b* (*lks2.b*-Bowman) grown in a greenhouse. Awns were sampled from all positions of spikes at 3 weeks following flowering and were fixed with FAA solution (3.7% *p*-formaldehyde, 5% acetic acid) under low pressure, dehydrated, and embedded in Paraplast (McCormick Scientific). Using a microtome, 12 µm thick sections were prepared. Deparaffinization of the sections was conducted with xylene, with subsequent dehydration using a graded ethanol series. Staining was done with 1% safranin O (Waldeck GmbH and Co. KG). Awn dimensions (height: the distance from the mid-point of the longest side to the apex; and width: the length of the longest side) were measured in transverse sections prepared from the middle part of awns. In the longitudinal sections, the length of large parenchyma cells in the cell file adjacent to chlorenchyma tissues was measured. Measurements were made separately for the bottom, middle and top parts of awns. At least five awns were used for cell length measurement of each part of the awns. Cell numbers for entire length of an awn were estimated based on average lengths of awns measured at three positions within a spike: (i) awns on spikelets of the second and third spikelets from the top of spikes; (ii) those on two spikelets in the middle positions of the spikes; and (iii) those on the second and third spikelets from the bottom of spikes. This is because awns used for sectioning were the bulk of awns from all positions of the spikes. Student's *t*-test was used for statistical comparisons.

Phylogenetic analysis

The deduced amino acid sequences of barley *Lks2* homologues were compared with homologous proteins in *Arabidopsis*, wheat, and four grass species that possess known genome sequences (rice, maize, sorghum, and *Brachypodium distachyon*). Phylogenetic trees were generated by CLUSTALW sequence alignment of the complete proteins using the neighbour-joining method (Saitou and Nei, 1987) available in the MEGA4 software package (Tamura *et al.*, 2007). In tree construction, the gaps/missing data option was selected for the treatment of pairwise deletion.

Results

Positional cloning of *Lks2*

A total of 1512 F₂ plants from a cross between Karafuto Zairai and Aizu Hadaka 3 were screened using flanking markers developed in a previous report (Taketa *et al.*, 2011), and *lks2* was delimited to a 0.66 cM interval between expressed sequence tag markers k04151 and k06123. BLASTN analysis identified their respective rice homologues (Os06g0611200 and Os06g0710500) widely apart (5.6 Mb) on rice chromosome 6, because of disrupted microsynteny in this chromosomal region (Taketa *et al.*, 2011). Because marker saturation based on barley/rice microsynteny was difficult, chromosome walking was initiated from the closest distal marker, k06123 (0.27 cM from the locus), using a barley BAC library (Saisho *et al.*, 2007). Thereby, a 613 kb BAC contig was constructed (Fig. 2). Eight markers were developed on this BAC contig. Two recombination events between markers OIS56 and OIS55 correctly oriented the BAC contig relative to the barley genetic map. Database searches identified rice homologues for four other non-polymorphic markers. Six marker points on this 613 kb barley BAC contig corresponded in reverse order to the syntenic rice chromosome 6 segment, identifying an inversion distal to *lks2*. The next rice gene (Os06g0711800) was converted into a marker (OIS62). It was mapped 0.03 cM distal to

Fig. 2. Positional cloning of the barley *Lks2* gene. At the top, a high-resolution genetic map of barley based on 3024 gametes is shown. In the middle, a physical map of barley is presented. BAC clones representing the minimum tiling path are shown. Only the BAC contig shown in red was sequenced completely. At the bottom, a physical map of syntenic regions of rice chromosome 6 is shown. Identification numbers for each rice gene are those from the Rice Annotation Project Database, where the common prefix of Os06g has been omitted for brevity. Dotted lines connect common or homologous markers or genes. * denotes that the barley homologue was mapped to chromosome 4H; ** signifies that the barley homologue was mapped 5.7 cM proximal of *lks2*.

lks2. However, a marker (OIS63) from the second-next rice gene (Os06g0711900) was mapped 0.23 cM proximal of *lks2*. With one exception, the following four rice genes showed parallel correspondence between rice and barley. The barley homologue of the exceptional gene (Os06g0712500) was mapped on chromosome 4H. Two markers (OIS65 and OIS66) developed from rice genes Os06g0712300 and Os06g0712400 were mapped 0.2 cM proximal of *lks2*. Finally, the rice gene Os06g0712600 was converted into a marker (OIS71). It co-segregated perfectly with *lks2* in 3024 gametes. This gene encoding a *SHORT INTERNODES* (*SHI*)-family transcription factor is considered a strong candidate for *Lks2*. The barley homologue of the next distal rice gene (Os06g0712700) was mapped 5.7 cM proximal of *lks2*, which suggests a recurrence of disrupted barley/rice microsynteny. Taken together, the observations described above indicated substantial reshuffling of the corresponding syntenic blocks between barley and rice in the *Lks2* candidate region.

Starting from the *Lks2* candidate gene, an additional BAC contig of 519kb was developed (Fig. 2). Five BAC clones representing the minimal tiling path of the contig were selected for shotgun sequencing. The *SHI*-family gene is the sole non-transposon-based gene on this BAC contig; approximately 74% of the contig sequence was classified as repetitive elements. Seven markers were developed on this BAC contig, but they showed no recombination with *lks2*. Consequently, the 519kb BAC contig was shorter than the *lks2* candidate region (0.23 cM) that was delimited by markers OIS66 and OIS62 (Fig. 2). BAC contigs were also constructed starting from the flanking markers,

but these BAC contigs did not overlap with others. The current physical map near the *lks2* locus consists of five BAC contigs (about 1540kb in combined lengths) with four gaps.

Comparison between cDNA and genomic sequences in barley showed that the *Lks2* candidate gene (*SHI*-family transcription factor) harboured two introns (one in the 5'-UTR) and that the open reading frame encoded a deduced protein of 344 aa with a calculated molecular mass of 34.3 kDa (Fig. 3). This gene contained a zinc-binding RING-finger motif (Freemont, 1993) and an IGGH domain (Fridborg *et al.*, 2001).

Screening and analysis of allelic mutants

No alleles at the *lks2* locus were identified in previous studies of induced mutants (Kucera *et al.*, 1975; Franckowiack, 2007). In the present study, an induced short-awn mutant KM7 was crossed with Honen 6 (a standard *lks2* line), and their F₁ hybrids showed the short-awn phenotype, indicating that KM7 is allelic to *lks2*. KM7 also has reduced stigma hairs and partial female sterility. This phenotype is similar to that of *ubs4* (Takahashi *et al.*, 1953b), which was mapped only 1 cM distal to *lks2* (Franckowiack, 1997). The *ubs4* locus is known to be allelic to the *ari-d* short-awn mutant locus (Franckowiack and Lundqvist, 1997; Franckowiack, 2007). However, neither *ubs4* nor *ari-d* has yet been cloned. To test allelism, intercrosses were made among near-isogenic lines of *lks2.b*, *ubs4.d*, and *ari-d.15* that were bred in the genetic background of the cultivar Bowman (Fig. 1B, 1C). Regarding awn length (Fig. 1B),

those in *lks2.b*, *ubs4.d* and *ari-d.15* were 51, 28, and 29% of that in Bowman, respectively. In terms of pistil morphology (note, the term pistil will be used throughout the paper instead of gynoecium), *lks2.b* has slightly shorter styles, but has normal stigma hairs (Fig. 1C). However, *ubs4.d* and *ari-d.15* have almost no stigma hairs on reduced styles (Fig. 1C). In allelism tests, the awn lengths in F₁ hybrids between *lks2.b* and *ubs4.d* (62% of wild type) were almost comparable with that in the *lks2.b* parent (51% of wild type). The awn length in F₁ hybrids between *lks2.b* and *ari-d.15* (61% of wild type) was also comparable with that in the *lks2.b* parent. Therefore, it was concluded that *lks2*, *ubs4*, and *ari-d* occupy the same locus, and that *lks2* is dominant over *ubs4* and *ari-d*. The different locations of *lks2* and *ubs4.d* in the genetic map by Franckowiack (1997) can be probably attributed to phenotyping errors in the segregating populations.

Sequences of the *Lks2* candidate gene in 25 allelic mutants that had significantly shorter awns and reduced stigma hairs were compared with those of their respective wild type. All 25 mutants had lesions in the *Lks2* candidate gene (Supplementary Table S1); 11 revealed a single amino acid substitution, four revealed truncation of the protein by a premature stop codon, four showed altered splicing as a result of a nucleotide substitution in the exon–intron boundary (altered splicing was confirmed by transcript sequencing), two were a nucleotide substitution in the 5′-non-coding region, and four were a complete deletion of the gene based on analysis of many flanking markers (Fig. 3). Most mutants have lesions in the functionally important RING motif or IGGH domain (Fig. 3). The mutant allele sequence data showed unequivocally that the *SHI*-family transcription factor

gene comprised the *lks2* locus. Therefore, the gene is referred to as *Lks2* in the following text.

Natural variation of the *Lks2* gene

To assess natural allelic variation (Supplementary Table S2), 39 *lks2* accessions and eight long-awn (*Lks2*) accessions were examined. Of the eight long-awn accessions tested, six shared the same *Lks2* gene sequence (the ‘standard’ haplotype). Relative to the standard haplotype, long-awn barley cultivars Bonus and Kristina had two nucleotide substitutions in the coding sequence, one of which is a non-synonymous substitution, plus three single-nucleotide polymorphisms (SNPs) and one indel in the 5′- and 3′-non-coding regions (Table 1). This type was named the ‘Bonus’ haplotype. The ‘standard’ and ‘Bonus’ haplotypes were thus regarded as wild-type *Lks2* alleles (long awn). The sequences of *lks2* accessions differed from the wild-type sequences. Three natural *lks2* alleles were found: *lks2.b1*, *lks2.b2*, and *lks2.b3* (Table 1 and Fig. 3). Allele symbols were assigned according to Franckowiack (2007). They all shared a C-to-T nucleotide substitution leading to a proline (P) to leucine (L) change at position 245 in the IGGH domain, indicating the significance of this domain in determination of awn length. Both *lks2.b2* and *lks2.b3* had a second amino acid substitution at different sites outside the conserved regions. The *lks2.b2* allele had a C-to-T nucleotide substitution leading to an alanine (A) to valine (V) change at position 292. The *lks2.b3* allele had a G-to-A nucleotide substitution leading to an alanine (A) to threonine (T) change at position 29, but this amino acid substitution was also present in the Bonus haplotype. All three natural *lks2* alleles could be considered weak alleles because the plants had an awn length about half of the long-awn type and showed normal seed fertility. *lks2.b1*, *lks2.b2*, and *lks2.b3* were found, respectively, at frequencies of 12.8, 56.4, and 30.8% among the 39 *lks2*-carrying accessions tested. *lks2.b1* and *lks2.b2* were distributed in eastern China, Korea and Japan, whereas *lks2.b3* was localized in the Himalayas (regions including India, Nepal and Tibet) (Fig. 4 and Supplementary Table S2).

Fig. 3. Analyses of mutants allelic to *lks2*. Lesions detected in three natural variants and 25 mutants are summarized. The three thick downward arrows indicate amino acid substitutions in *lks2.b1*, *lks2.b2*, and *lks2.b3* (natural allelic variants). Others are mutant alleles. Black arrows pointing down signify an amino acid substitution. Black arrows pointing up with a solid line signify a frame shift. The two black arrows pointing up with a dotted line represent a nucleotide substitution in the 5′-UTR region. The white arrow pointing up with a solid line represents a premature stop codon. The white arrow pointing up with a dotted line represents altered splicing as a result of a nucleotide substitution in the exon–intron boundary.

Fig. 4. Geographical distribution of natural *lks2* alleles in Eastern Asia. Each symbol represents one accession. Circles indicate covered (hulled) barley, while triangles represent naked (hull-less) barley.

Table 1 Natural allelic variation in the *Lks2* gene. Bold letters show changes from the standard type.

Polymorphism	5'-UTR		2nd exon		3rd exon		3'-UTR	
Nt position	−563	−48	85	195	829	970	1185	1245
Nt change	C→G	C→T	G→A	T→C	C→T	C→T	A→G	ΔGA
Aa change	–	–	A→T	No	P→L	A→V	–	–
Aa position	–	–	29	65	245	292	–	–
Allele/haplotype	SNP-1	SNP-2	SNP-3	SNP-4	SNP-5	SNP-6	SNP-7	Indel-1
Standard type (<i>Lks2</i>)	C	C	G	T	C	C	A	No
<i>lks2.b1</i>	C	C	G	T	T	C	A	No
<i>lks2.b2</i>	C	C	G	T	T	T	A	No
Bonus type (<i>Lks2</i>)	G	T	A	C	C	C	G	ΔGA
<i>lks2.b3</i>	G	T	A	C	T	C	G	ΔGA

Phylogenetic analysis of *Lks2*

The *SHI*-family transcription factor encodes proteins with a RING finger-like zinc-finger motif and an IGGH domain. Most likely, this family is plant specific (Kuusk *et al.*, 2006). The *Arabidopsis SHI* gene family comprises ten members: *SHORT INTERNODES (SHI)*, *STYLISH1 (STY1)* and *STYLISH2 (STY2)*, *LATERAL ROOT PRIMORDIUM1 (LRP1)*, and *SHI-RELATED SEQUENCE 3–8 (SRS3–SRS8)*. In the rice genome, five *SHI* family members are present (Kuusk *et al.*, 2006). *B. distachyon*, sorghum, and maize have four, five, and nine members of this family, respectively. In barley, database searching identified three additional *LRP*-like genes, while in wheat, a single orthologue (GenBank accession no. AK335496) of *Lks2* was identified.

Phylogenetic trees of *SHI*-family proteins of *Arabidopsis* and six grass species were generated using the neighbour-joining method. The tree showed division into two major clades: one containing *Arabidopsis* and grass proteins, and the other containing only grass proteins (Supplementary Fig. S1 at *JXB* online). HvLks2 belonged to the grass-specific clade and was distinct from all ten members of the *Arabidopsis SHI*-family proteins. Within the grass-specific clade, HvLks2 formed a highly supported subclade with probable orthologues of other grass species. These results suggested that HvLks2 has no clear orthologue in *Arabidopsis*.

Expression of *Lks2*

Lks2 was highly expressed in pistils (including stigmas, styles, and ovaries), followed by awns; expression was weak in the anthers, glumes, lemmas, paleas, and flag leaves among the organs tested (Fig. 5). Gene expression data coincided with the organs of the types (pistils and awns) in which the phenotypes were clearly manifested in *lks2.b/ubs4.d/ari-d* mutants.

Histological analysis

To determine the basis of the short-awn phenotype in *lks2*, awn sections of Bowman and *lks2.b*-Bowman were compared (Fig. 6). In the transverse sections, the awns appeared triangular. Three vascular bundles (a large one in the centre and two smaller ones located in each peripheral corner) and two zones of chlorenchyma cells were observed in both genotypes, as reported for normal barley by Reid (1985).

Fig. 5. qRT-PCR analysis of *Lks2* expression in a long-awn cultivar, Haruna Nijo. Transcript levels were normalized to the *GAPDH* gene as a reference. Error bars indicate standard deviation ($n=3$).

Fig. 6. Histological observation of sections of long-awn (*Lks2*) Bowman and short-awn *lks2.b*-Bowman. (A, B) Transverse sections of awn in the middle part of Bowman (A) and *lks2.b*-Bowman (B) with the abaxial sides up. (C, D)

Longitudinal sections of awn in the bottom part of Bowman (C) and *lks2.b*-Bowman (D). p, Parenchyma cells; vb, vascular bundle; ch, chlorenchyma tissue; W, awn width; H, awn height. Arrows indicate the cell files used for parenchyma cell length measurements in longitudinal sections. Bars, 200 μ m.

Fig. 7. Measurement of awn sections from Bowman and *lks2.b*-Bowman. (A) Parenchyma cell length in the cell file adjacent to chlorenchyma tissues measured in longitudinal awn sections. (B) Longitudinal parenchyma cell number for entire lengths of awns. (C) Heights and widths of awns measured in transverse sections from the middle part of awns. Bars represent standard deviation. ns, Not significant; **, significantly different at the 1% level.

No apparent differences existed in the overall structures of the awns, but *lks2.b*-Bowman had significantly thinner awns both in height and width (Fig. 7C). No significant differences in longitudinal cell length existed between Bowman and *lks2.b*-Bowman at the three awn parts (bottom, middle, and top) measured (Fig. 7A). However, the longitudinal cell number constituting the awn in *lks2.b*-Bowman was reduced to 55.5% of that of Bowman (Fig. 7B). Therefore, it was concluded that the short-awn phenotype in *lks2* resulted from a reduced cell number.

Discussion

Positional cloning

Because the exploitation of barley/rice microsynteny in the search for an *Lks2* candidate gene was hampered by highly

disrupted collinearity, chromosome walking was initiated from the closest marker (k06123), 0.27 cM distal to *lks2*. A complete BAC contig spanning the *lks2* locus could not be constructed, mainly because of low gene density, a high proportion of repetitive DNA sequences, and suppressed recombination near the target locus. However, these attempts identified local inversions of the corresponding syntenic blocks between barley and rice in the *Lks2* candidate region. An *SHI*-family transcription factor gene was identified as a strong candidate for *Lks2* because of perfect co-segregation of its marker with the phenotype in 3024 gametes (Fig. 2). Gene isolation was validated by: (i) genetic lesions found in all 25 mutant alleles and in three natural allelic variants at the *lks2* locus; and (ii) strong gene expression in awns and pistils, the two tissues where strong visible phenotypes are observed. As pointed out previously by Pourkheirandish *et al.* (2007), the present study verifies the importance of implementing physical mapping strategies in the target species (barley) because of the possibility of a breakdown of microsynteny between barley and other related species.

Function of the barley *Lks2* gene

The present study revealed that barley *Lks2* is a *SHI*-family transcription factor that regulates awn elongation and pistil morphology. Four *SHI*-family genes have been found in barley, and mutation of *Lks2* showed prominent phenotypes in the awn and pistil. The *SHI*-family transcription factors are already well studied in *Arabidopsis*. The ten members of the *Arabidopsis SHI*-family are highly divergent in sequence except for the two conserved regions (the RING motif and the IGGH domain), but show partial redundancy in function (Fridborg *et al.*, 1999, 2001; Kuusk *et al.*, 2006). A loss-of-function *styl-1* mutant shows subtle morphological defects in the pistil, while mutations in seven other family members tested had no apparent effects on pistil development (Kuusk *et al.*, 2006). Therefore, among the *Arabidopsis SHI*-family, *STY1* is most similar to barley *Lks2* in terms of mutant phenotype affecting the pistil. Double or higher-order multiple mutants of *styl-1* with other members showed an enhanced mutant phenotype in pistil, stamen, and leaf development. *STY1* was expressed not only in the apical parts of the developing pistil but also in many other organs such as hypocotyls, cotyledons, leaf and root primordia, and leaf margins (Kuusk *et al.*, 2002).

The present histological observations revealed that the short-awn phenotype in *lks2.b* resulted from a reduced longitudinal cell number, which was likely to be due to reduced cell divisions (Figs 6 and 7). Similarly, reduced stigma hairs and style length could be caused by reduced cell divisions during pistil development. It has been reported that *Arabidopsis* homologue *STY1* protein regulates auxin homeostasis (Sohlberg *et al.*, 2006) by transcriptional activation (Eklund *et al.*, 2010), and, by analogy, barley *lks2* mutation may reduce cell divisions by affecting auxin levels in developing awns and pistils. This point needs to be examined in future studies.

The phylogenetic analyses of *SHI*-family proteins in *Arabidopsis* and six grass species showed that barley *Lks2* belonged to the grass-specific clade (Supplementary Fig. S1). This may coincide with the fact that barley *Lks2* is controlling

elongation of the awn, which is an organ unique to grasses. Thus, *Lks2* and its grass orthologues may have diversified functionally in the *SHI* family. However, barley *Lks2* and the *Arabidopsis SHI*-family genes (especially *STY1*) share a common function in regulation of pistil morphology. These findings suggest the hypothesis that, to some extent, the highly conserved RING motif and IGGH domain may play common roles among different plant species in the regulation of plant development including pistil morphogenesis. In contrast, other parts of the *SHI*-family proteins with relatively low homology among different plant species could be contributing to diversity in functions of the *SHI*-family proteins and their regulatory mechanisms.

Although *Lks2* orthologues were found in grass species whose genomes have been completely sequenced (Supplementary Fig S1), there is no report about their mutants and/or associated phenotypic changes. The barley *Lks2* appears to be orthologous to rice gene Os06g0712600, but no QTL for awn elongation has been reported in the syntenic region of rice chromosome 6 (Cai and Morishima, 2002; Gu *et al.*, 2005). According to the rice gene expression database RiceXPro, Os06g0712600 is highly expressed in pistils, inflorescences, and embryos of the awnless cultivar Nipponbare (Sato *et al.*, 2011). Whether this gene is expressed in rice awns needs to be tested using long-awn varieties. Because barley and rice largely differ in their awn morphology (Reid, 1985; Toriba *et al.*, 2010), different genes may control awn elongation in these species. Similarly, a database search identified an orthologue in wheat (GenBank accession no. AK335496), but no awn gene is documented for wheat homoeologous group 7 chromosomes (McIntosh *et al.*, 2003). Thus, a grass-wide survey is required to test whether control of awn elongation is specific to barley *Lks2*. It also remains to be tested whether *Lks2* orthologues in other grasses control pistil morphology.

In barley, the dominant *Hooded* mutation (*Kap*) transforms the awn into an extra flower of inverse polarity on the lemma; this homeotic *Hooded* mutation is known to be caused by a 305bp duplication in a homeobox gene intron (Müller *et al.*, 1995). Interestingly, recessive *lks2* is epistatic over *Kap* and suppresses the *Hooded* phenotype (Takahashi *et al.*, 1953a). This epistatic inhibition of *Kap* by *lks2.b* may be mediated by suppression of cell divisions in the awn, as was revealed by the present histological investigation (Figs 6 and 7). Suppressor mutant genes of *Kap* (*suK*), which show a short-awn phenotype, were induced, but they were non-allelic to *lks2* (Roig *et al.*, 2004). A QTL for awn length was also detected near the row-type locus (*vrs1*) (Sameri *et al.*, 2006), suggesting its effects on awn length. The molecular bases of the interaction of *lks2* with *Kap*, *suK*, and *vrs1* need to be clarified by future investigations.

Origin of natural *lks2* alleles

A total of 25 allelic mutants had severe reductive phenotypes in awns and stigmas accompanied by seed sterility. In contrast, barley accessions carrying one of the three natural *lks2* variants, *lks2.b1*, *lks2.b2*, and *lks2.b3*, have awns about half the length of the long-awn type, normal stigma hairs, and normal seed fertility. These natural variants share a P-to-L change at position 245 in the IGGH domain, which is regarded as key to producing mild

phenotypes. This critical mutation occurred within a short acidic cluster present in the IGGH domain (Fridborg *et al.*, 2001), where conservation is rather low among the *SHI*-family members of different species (Supplementary Fig. S2). Similar nucleotide sequences and geographical distribution suggest that the *lks2.b1* and *lks2.b2* alleles are closely related (Table 1, Fig. 4, and Supplementary Table S2). It is likely that *lks2.b1* is the ancestral allele and that its descendant, *lks2.b2*, became predominant in the east of China. The allele *lks2.b3*, which is localized in the Himalayas, differs from *lks2.b1* by having five SNPs and one indel in regions flanking the critical mutation. Creation of the *lks2.b3* allele by intragenic recombination after hybridization between the Bonus haplotype and *lks2.b1* is unlikely because it requires double crossovers within a small region. Gene conversion could be an alternative mechanism. However, no evidence of exchange of barley materials exists between the Himalayas and eastern China (Konishi, 1995; Taketa *et al.*, 2004). Therefore, it is proposed that critical mutations resulting in a P-to-L change at position 245 might have independent origins in the Himalayas and the east of China, although deeper sequence sampling is required in future to confirm this.

The short-awn trait conferred by the *lks2.b* allelic series is considered to be an example of varietal differences (Doebley *et al.*, 2006) that have occurred after domestication of the barley crop. This is because these mutant alleles are present among limited barley accessions from Eastern Asia (Takahashi, 1987). The frequency of varieties with *lks2.b* allele in Eastern Asia regions varies from 3.2 to 25.8% (Takahashi, 1987). The reasons why *lks2.b* established an ecological niche in Eastern Asia are not clear. It may be related to the Eastern Asian climate, which has high precipitation during the growing season of barley. Under such climatic conditions, the short-awn *lks2.b* allele may be advantageous in alleviating lodging, because short-awn spikes would hold less water during rain and would dry faster, as suggested by Grundbacher (1963). The effects of *lks2.b* on the degree of lodging need to be tested in future studies using isogenic lines.

This report is the first to describe the molecular isolation of a gene controlling awn elongation in grasses. The molecular cloning of *Lks2* in barley revealed that an *SHI* transcription factor in barley plays important dual roles in controlling awn length and pistil morphology. The isolation of *Lks2* and future analysis will give many insights into the control of floral morphology of both awns and pistils in grasses. In barley, smooth-awned mutant genes (*raw1* and *raw2*) also reduce the number of stigma hairs (Lundqvist and Franckowiack, 2003), indicating that a morphological link appears to exist between the awn and stigma hair. Further work to isolate other awn-related genes of barley is underway for a better understanding of the genetic network controlling awn elongation. As awns appear to have an effect on grain yield, such studies may enable the design of barley awns for improving productivity in response to various climatic conditions.

Supplementary data

Supplementary data are available at *JXB* online.

Table S1. List of mutants allelic to the *lks2* locus.

Table S2. Barley accessions used for natural allelic variation at the *lks2* locus.

Table S3. Primer sequences used in this study.

Fig. S1. Neighbour-joining tree based on deduced amino acid sequences of the *SHI*-family transcription factor genes. The tree was drawn using MEGA4 software. Confidence estimates for junction points are from bootstrap tests with 1000 replicates. Gene names are preceded by abbreviations of plant scientific names except for maize where GRMZM is used: Sb, *Sorghum bicolor*; Bradi, *Brachypodium distachyon*; At, *Arabidopsis thaliana*; Os, *Oryza sativa*; Hv, *Hordeum vulgare*. The species origin of full length cDNA is indicated in parenthesis.

Fig. S2. Alignment of deduced amino acid sequences of the *SHI*-family transcription factor genes from rice, *Arabidopsis*, and barley (HvLks2). Highly conserved amino acid residues in the RING motif and the IGGH domain are highlighted in pink and yellow, respectively. The acidic stretch is boxed. In HvLks2, amino acid substitutions were found in three natural alleles (*lks2.b1*, *lks2.b2*, and *lks2.b3*): proline (P) at position 245 is marked in red, alanine (A) at position 29 is marked in green, and alanine at position 292 is marked in blue.

Acknowledgements

The authors are deeply appreciative to Ms Y. Sakurai and Ms S. Miyake for technical assistance. The authors are grateful to Dr N. Kawada, Dr H. Bockelman, and Dr F. Ottosson for supplying materials. The barley resources were also provided by the National Bioresource Project – Barley, Japan. Dr J. Franckowiack is acknowledged for information and discussion. The authors thank Dr N. Suzuki, Dr G. Muehlbauer, Dr I. Galis, Dr K. Kakeda, and Dr D. Saisho for critical reading. This work was partly supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Genomics for Agricultural Innovation Grant TRC1007 and TRG1007).

References

- Cai HW, Morishima H.** 2002. QTL clusters reflect character associations in wild and cultivated rice. *Theoretical and Applied Genetics* **104**, 1217–1228.
- Dahlgren R, Clifford HT, Yeo PF.** 1985. *The families of the monocotyledons. Structure, evolution and taxonomy*. New York: Springer.
- Doebley JF, Gaut BS, Smith BD.** 2006. The molecular genetics of crop domestication. *Cell* **127**, 1309–1321.
- Eklund DM, Ståldal V, Valesecchi I, et al.** 2010. The *Arabidopsis thaliana* STYLISH1 protein acts as a transcriptional activator regulating auxin biosynthesis. *Plant Cell* **22**, 349–363.
- Elbaum R, Zaltzman L, Burgert I, Fratzl P.** 2007. The role of wheat awns in the seed dispersal unit. *Science* **316**, 884–886.
- Franckowiack JD, Lundqvist U.** 1997. Unbranched style 4. *Barley Genetics Newsletter* **26**, 56.
- Franckowiack JD.** 1997. Revised linkage maps for morphological markers in barley, *Hordeum vulgare*. *Barley Genetics Newsletter* **26**, 9–21.
- Franckowiack JD.** 2007. Short awn 2. *Barley Genetics Newsletter* **37**, 197–198.
- Freemont PS.** 1993. The RING finger. A novel protein sequence motif related to the zinc finger. *Annals of the New York Academy of Sciences* **684**, 174–192.
- Fridborg I, Kuusk S, Moritz T, Sundberg E.** 1999. The Arabidopsis dwarf mutant shi exhibits reduced gibberellin responses conferred by overexpression of a new putative zinc finger protein. *Plant Cell* **11**, 1019–1031.
- Fridborg I, Kuusk S, Robertson M, Sundberg E.** 2001. The Arabidopsis protein SHI represses gibberellin responses in Arabidopsis and barley. *Plant Physiology* **127**, 937–948.
- Grundbacher FJ.** 1963. The physiological function of the cereal awn. *Botanical Review* **29**, 366–381.
- Gu XY, Kianian SF, Hareland GA, Hoffer BL, Foley ME.** 2005. Genetic analysis of adaptive syndromes interrelated with seed dormancy in weedy rice (*Oryza sativa*). *Theoretical and Applied Genetics* **110**, 1108–1118.
- Gyenies L, Yun SJ, Smith KP, Steffenson BJ, Bossolini E, Sanguineti MC, Muehlbauer GJ.** 2007. Genetic architecture of quantitative trait loci associated with morphological and agronomic trait differences in a wild by cultivated barley cross. *Genome* **50**, 714–723.
- International Rice Genome Sequencing Project.** 2005. The map-based sequence of the rice genome. *Nature* **436**, 793–800.
- Islam AKMR, Shepherd KW, Sparrow DHB.** 1981. Isolation and characterization of euplasmic wheat–barley chromosome addition lines. *Heredity* **46**, 161–174.
- Islam AKMR.** 1983. Ditelosomic addition lines of barley chromosomes to wheat. In: Sakamoto S, ed. *Proceedings of the 6th International Wheat Genetics Symposium*. Kyoto: Maruzen, 233–238.
- Kjack JL, Witters RS.** 1974. Physiological activity of awns in isolines of Atlas barley. *Crop Science* **14**, 243–248.
- Konishi T.** 1995. *Geographical diversity of isozyme genotypes in barley*. Fukuoka: Kyushu University Press.
- Kucera J, Lundqvist U, Gustafsson Å.** 1975. Induction of brevistaratum mutants in barley. *Hereditas* **80**, 263–278.
- Kuusk S, Sohlberg JJ, Eklund DM, Sundberg E.** 2006. Functionally redundant *SHI* family genes regulate Arabidopsis gynoecium development in a dose-dependent manner. *The Plant Journal* **47**, 99–111.
- Kuusk S, Sohlberg JJ, Long JA, Fridborg I, Sundberg E.** 2002. STY1 and STY2 promote the formation of apical tissues during Arabidopsis gynoecium development. *Development* **129**, 4707–4717.
- Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L.** 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* **1**, 174–181.
- Lundqvist U, Franckowiack JD.** 2003. Diversity of barley mutants. In: von Bothmer R, Knüpfer H, van Hintum T, Sato K, eds. *Diversity in Barley (Hordeum vulgare)*. Amsterdam: Elsevier, 77–96.
- McIntosh RA, Devos KM, Dubcovsky J, Morris CF, Rogers WJ.** 2003. Catalogue of gene symbols for wheat: 2003 Supplement.

Published online at <http://wheat.pw.usda.gov/ggpages/wgc/2004upd.html>.

- Müller KJ, Romano N, Gerstner O, Garcia-Maroto F, Pozzi C, Salamini F, Rohde W.** 1995. The barley *Hooded* mutation caused by a duplication in a homeobox gene intron. *Nature* **374**, 727–730.
- Pourkheirandish M, Wicker T, Stein N, Fujimura T, Komatsuda T.** 2007. Analysis of the barley chromosome 2 region containing the six-rowed spike gene *vrs1* reveals a breakdown of rice–barley micro collinearity by a transposition. *Theoretical and Applied Genetics* **114**, 1357–1365.
- Qualset CO, Schaller CW, Williams JC.** 1965. Performance of isogenic lines of barley as influenced by awn length, linkage blocks, and environment. *Crop Science* **5**, 489–494.
- Reid DA.** 1985. Morphology and anatomy of the barley plant. In: Rasmusson DC, ed. *Barley*. Wisconsin: American Society of Agronomy, 73–101.
- Roig C, Pozzi C, Santi L, Müller J, Wang Y, Stile MR, Rossini L, Stanca M, Salamini F.** 2004. Genetics of barley Hooded suppression. *Genetics* **167**, 439–448.
- Saisho D, Myoraku E, Kawasaki S, Sato K, Takeda K.** 2007. Construction and characterization of a bacterial artificial chromosome (BAC) library from the Japanese malting barley ‘Haruna Nijo’. *Breeding Science* **57**, 29–38.
- Saitou N, Nei M.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425.
- Sameri M, Takeda K, Komatsuda T.** 2006. Quantitative trait loci controlling agronomic traits in recombinant inbred lines from a cross of oriental- and occidental-type barley cultivars. *Breeding Science* **56**, 243–252.
- Sato Y, Antonio B, Namiki N, Takehisa H, Minami H, Kamatsuki K, Sugimoto K, Shimizu Y, Hirochika H, Nagamura Y.** 2011. RiceXPro: a platform for monitoring gene expression in japonica rice grown under natural field conditions. *Nucleic Acids Research* **39**, D1141–D1148.
- Sohlberg JJ, Myrenås M, Kuusk S, Lagercrantz U, Kowalczyk M, Sandberg G, Sundberg E.** 2006. *STY1* regulates auxin homeostasis and affects apical–basal patterning of the *Arabidopsis* gynoecium. *The Plant Journal* **47**, 112–123.
- Takahashi N, Alwan H, Alterfa H, Sato T.** 1986. Significant role of awn in rice plants (1). A survey of agricultural value of rice awn. *Reports of the Institute for Agricultural Research, Tohoku University* **35**, 21–31.
- Takahashi R, Yamamoto J, Yasuda S, Itano Y.** 1953a. Inheritance and linkage studies in barley. *Berichte des Ohara Instituts für landwirtschaftliche Forschungen* **10**, 29–53.
- Takahashi R, Yamamoto J, Yasuda S, Itano Y.** 1953b. Inheritance of semi-sterility due to defects of stigmatic structure in barley. *Nogaku Kenkyu* **41**, 69–78.
- Takahashi R.** 1955. The origin and evolution of cultivated barley. In: Demerec M, ed. *Advances in genetics*, Vol. 7. New York: Academic Press, 227–266.
- Takahashi R.** 1983. *Catalogue of barley germplasm preserved in Okayama University*. Okayama University, Kurashiki, Japan.
- Takahashi R.** 1987. Genetic features of East Asian barleys. In: Yasuda S, Konishi T, eds. *Barley genetics V*. Okayama: Maruzen, 7–20.
- Taketa S, Amano S, Tsujino Y, et al.** 2008. Barley grain with adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid biosynthesis pathway. *Proceedings of the National Academy of Sciences, USA* **105**, 4062–4067.
- Taketa S, Awayama T, Amano S, Sakurai Y, Ichii M.** 2006. High-resolution mapping of the *nud* locus controlling the naked caryopsis in barley. *Plant Breeding* **125**, 337–342.
- Taketa S, Kikuchi S, Awayama T, Yamamoto S, Ichii M, Kawasaki S.** 2004. Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*). *Theoretical and Applied Genetics* **108**, 1236–1242.
- Taketa S, Yuo T, Sakurai Y, Miyake S, Ichii M.** 2011. Molecular mapping of the short awn 2 (*lks2*) and dense spike 1 (*dsp1*) genes on barley chromosome 7H. *Breeding Science* **61**, 80–85.
- Taketa S, Yuo T, Tonooka T, Tsumuraya Y, Inagaki Y, Haruyama N, Larroque O, Jobling SA.** 2012. Functional characterization of barley betaglucanless mutants demonstrates a unique role for CslF6 in (1,3;1,4)- β -D-glucan biosynthesis. *Journal of Experimental Botany* **63**, 381–392.
- Tamura K, Dudley J, Nei M, Kumar S.** 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.
- Toriba T, Suzuki T, Yamaguchi T, Ohmori Y, Tsukaya H, Hirano HY.** 2010. Distinct regulation of adaxial–abaxial polarity in anther patterning in rice. *Plant Cell* **22**, 1452–1462.
- von Bothmer, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I.** 1995. *An ecogeographical study of the genus Hordeum, systematic and ecogeographic studies on crop gene pools*, Vol. 7, 2nd edn. Rome: International Plant Genetic Resources Institute.