Note

Molecular mapping of the short awn 2 (*lks2*) and dense spike 1 (*dsp1*) genes on barley chromosome 7H

Shin Taketa^{*1}, Takahisa Yuo¹, Yukie Sakurai², Shoko Miyake² and Masahiko Ichii³

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

²) Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki, Kagawa 761-0795, Japan

³) Kagawa University, 1-1 Saiwai, Takamatsu, Kagawa 760-8521, Japan

The short awn 2 (*lks2*) and dense spike 1 (*dsp1*) genes are unique to East Asian barley. These two spike-related morphological genes are important because they may be involved in stable production and local adaptation. As a first step of their positional cloning, molecular mapping was conducted in 98 F₂ plants derived from a cross between Karafuto Zairai and Aizu Hadaka 3. The *dsp1* gene was mapped to the proximal region of the short arm of chromosome 7H. The *lks2* gene was located on the long arm of 7H and flanked by EST-based markers k04151 and k06123, with distances of 0.5 cM in the proximal side and 1.0 cM in the distal side. Both k04151 and k06123 shared homology to rice genes on chromosome 6 that were separated with the physical distance of 5.6 Mbp. In this interval, rice–barley microsynteny was exploited for marker enrichment. Of 57 rice genes attempted, 15 (26.3%) yielded polymorphic EST-based markers. Breakdown of collinearity was found in the candidate region of *lks2*, suggesting occurrence of structural changes in the chromosome region harboring *lks2* during divergence of barley and rice from a common ancestor.

Key Words: collinearity, dense spike, *Hordeum vulgare* L., marker, microsynteny, rice, short awn.

Introduction

The awn of barley (*Hordeum vulgare* L.) is a linear extension of the distal end of the lemma. It was shown to contribute to photosynthesis (Kjack and Witter 1974). Different estimates have been made as to its contribution to the grain yield. Grundbacher (1963) estimated that the awn of wheat (*Triticum aestivum* L.) and barley contributes more than 10 percent of the total kernel dry weight. In barley, Qualset *et al.* (1965) confirmed the significant contribution of the awn to the yield from the field tests using backcross-derived isogenic lines differing in the length of the awn (full-, half-, quarter-awned, and awnless); they reported that the respective yields of full-awned and half-awned lines were significantly higher than those of either the quarter-awned or awnless lines. Despite the prevalence of full-awned phenotype in the barley crop, short-awned accessions are frequently found in eastern Asia (Takahashi 1987). Many genes are known to condition awn development in barley (Lundqvist and Franckowiak 2003). Among others, the short awn 2 (*lks2*) gene on chromosome 7H is a spontaneous recessive mutation that is distributed widely among East Asian barley ac-

cessions, suggesting that it confers some agronomic advantages (Hayashi *et al.* 1972, Takahashi *et al.* 1953). It is particularly interesting that among East Asian barleys, which predominantly have six-rowed spikes, *lks2* is often associated with the dense spike 1 (*dsp1*) gene, which is also located on 7H (Takahashi 1987). Although many dense spike mutations of either natural or induced origin have been reported for barley, *dsp1* is a spontaneous recessive mutation that is unique to eastern Asia (Lundqvist and Franckowiak 2003). Takahashi (1987) reported favorable effects of *dsp1* for high productivity by increasing the number of flowers per spike, but the gene decreases kernel weight to some degree.

We attempted molecular mapping of two morphological mutant genes that are unique to eastern Asia, *lks2* and *dsp1*, with the ultimate goal of their positional cloning, particularly of *lks2*. Here, we summarize our results of rough mapping of these two spike-related morphological genes on chromosome 7H. We also describe exploitation of rice (*Oryza sativa* L.)–barley microsynteny for marker enrichment of the region surrounding *lks2*.

Materials and Methods

For mapping, 98 F₂ plants derived from a cross between Karafuto Zairai (OUJ301) and Aizu Hadaka 3 (OUJ323)

Communicated by T. Komatsuda

Received October 8, 2010. Accepted November 10, 2010.

*Corresponding author (e-mail: staketa@rib.okayama-u.ac.jp)

were used. The mapping parents are both six-rowed old Japanese varieties. Karafuto Zairai has lax spikes, long awns and covered caryopsis, whereas Aizu Hadaka 3 carries both *dsp1* and *lks2* along with the naked caryopsis (*nud1*) gene (Fig. 1). Here, *nud1* is used for the gene symbol for naked caryopsis according to the recommendation of Lundqvist and Falk (2005). Plants were grown in a greenhouse. For selected plants, F₃ progeny tests were conducted using 16 plants per line to infer their genotypes for *dsp1* and *lks2*.

DNA was extracted according to the methods of Komatsuda *et al.* (1998). A total of 15 molecular markers were used for initial mapping. Those included three sequence-characterized amplified region (SCAR) markers derived from amplified fragment length polymorphisms (AFLP) (Kikuchi *et al.* 2003, Taketa *et al.* 2006), eight simple sequence repeat (SSR) markers (Li *et al.* 2003, Ramsay *et al.* 2000), a sequence-tagged site (STS) marker *MWG511* (Künzel *et al.* 2000), and three expressed sequence tag (EST)-based markers (Sato *et al.* 2009). For *MWG511*, we originally selected a polymorphic enzyme *Hha*I; for EST-based marker k04151, we designed a new primer set (Table 1) and selected a polymorphic restriction enzyme (*Bsi* EI). Then PCR amplification was performed in volumes of 10 µl, each containing 0.5 U of Ampli-Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 µM primers, 200 µM of dNTP, 1.5, 2, or 2.5 mM of MgCl₂, 20 ng of DNA and reaction buffer. Subsequently, PCR was performed with the following cycling parameters: 9 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at annealing temperature of respective primers, 2 min at 72°C; 5 min at 72°C; with a final hold at 15°C. Restriction enzyme treatment of PCR products and electrophoresis were done according to the procedure described by Taketa *et al.* (2004, 2006). Recombination values were calculated using software (Mapmaker Macintosh Version 2.0; Lander *et al.* 1987); a genetic linkage map was constructed based on LOD scores greater than 3.0. Map distances were calculated using the Kosambi function (Kosambi 1944).

Based on rice–barley microsynteny, we originally developed 15 EST-based markers, which have a prefix OIS followed by numerals. For development of these markers, the rice annotation project database (RAP-DB, <http://rapdb.dna.affrc.go.jp/>) and TIGR rice genome annotation (<http://rice.plantbiology.msu.edu/>) database were used. Rice gene sequences on selected rice BAC/PAC clones were used as a query for the homology search in the DDBJ database (<http://www.ddbj.nig.ac.jp/Welcome-j.html>). Barley ESTs giving the best match to rice orthologues were selected for primer design. Then PCR primers of around 20 nt length were designed using Primer 3 (<http://frodo.wi.mit.edu/primer3/>) and were synthesized commercially. Amplified PCR products from both parental accessions were sequenced according to Taketa *et al.* (2004, 2006), and polymorphic markers were developed. For chromosome arm assignment of barley markers, wheat–barley chromosome addition lines (Islam 1983, Islam *et al.* 1981) were used as described by

Fig. 1. Spike morphology of full-awned variety Karafuto Zairai with normal spike density (left), and short-awned (*lks2*) variety Aizu Hadaka 3 with dense spike (*dsp1*), (right); side views are shown. Both varieties are six-rowed. Bar = 5 cm.

Taketa *et al.* (2010). Primer sequences, annealing temperature, amplicon size, and polymorphic restriction enzymes are summarized in Table 1.

Results

Although *dsp1* is known to slightly reduce awn length (Sameri *et al.* 2006), segregation of *lks2* in the mapping population was easily scored as either long or short by visual examination at maturity because *lks2* homozygous plants had shorter and thinner awns. Similarly, spike density in the mapping population was easily scored as either lax (normal) or dense. No segregation distortion was found for the phenotypic traits or molecular markers examined. Using public markers, the *lks2* gene was mapped on 7HL between EST-based markers k04151 and k06123 with the respective distance of 0.5 cM in the proximal side and 1.0 cM in the distal side (Fig. 2). Bmac0064 cosegregated with k06123, but the distal flanking marker of *lks2* was represented by k06123 because it was impossible to infer rice genes orthologous to this barley SSR marker. The *dsp1* gene cosegregated with three SSR markers (Bmag0359, BGMS0164, and Bmag0321) in the proximal regions of the short arm of 7H. In the middle of the *dsp1*–*lks2* interval, *nud1* was located 8.9 cM from *dsp1* and 8.2 cM from *lks2*. Takahashi *et al.* (1953) reported that the three genes were located on 7H in

Table 1. EST-based polymorphic markers on barley chromosome 7H developed from microsynteny with rice

Barley marker	Rice chr. 6 BAC/PAC	Rice gene (RAP-DB)	Barley EST	Forward primer (5' to 3')	Reverse primer (5' to 3')	Chromosome arm location	Tm (°C)	Amplicon size (bp)	Restriction enzyme
OIS21	AP003615	Os06g0603000	BJ450956	CAGGGTACCTTCCCACCTTGT	TCACACCATTCAGAAACCA	— ^a	60	650	<i>AclI</i>
<i>nudI</i>	AP003615	Os06g0604000	—	see Taketa <i>et al.</i> (2008)		7HL	55	857(A) ^b 785(B)	size polymorphism
OIS51	AP005446	Os06g0606800	AK249182	AAGGCGGAAAAGAGCAATTC	GGTAGCACGACAGCAAAACA	—	55	370	<i>PvuII</i>
OIS13	AP004792	Os06g0634300	AJ467612	CCTTCTGGTACAGCATGCAA	GGCATTAAACAACTTGATGG	7HL	56	446	<i>TaqI</i>
OIS29	AP004279	Os06g0608800	BU968266	CACAAAGCAGGCCCTTGAATCT	TAGACAGCGCGGTTATAGG	—	56	500	<i>MnlI</i>
OIS52	AP003616	Os06g0609700	AL504724	CACCAAATAATGCTGGCCAAAT	GGACACTGAAATTTGCAACAA	—	60	405	<i>AclI</i>
OIS23	AP003626	Os06g0614300	BG344141	CGTCAATAACTGGCGCTTCT	GCCACAATCCATTACCGTTC	7HL	56	701	<i>SacFI</i>
OIS35	AP003616	Os06g0610800	AV836965	CAGTTGGTGATACGCATTTC	GTTGACAAAGGTCATGTTGG	—	56	480	<i>XcmI</i>
k04151	AP003616	Os06g0611200	BJ478591	CAACGCCCATGCATTCTC	TCCACAATCACATTCCCAGA	7HL	56	426	<i>BstEI</i>
OIS34	AP003616	Os06g0611000	BJ462924	TAA TGGATGCGACTGTGGAG	CTTTTCTGCACGCTTGAGG	7HL	56	800	<i>HhaI</i>
k06123	AP003726	Os06g0710500	BJ448862	see Sato <i>et al.</i> (2009)		7HL	60	290(A) 310(B)	size polymorphism
OIS45	AP003634	Os06g0709000	AV943821	CGGCAGAGATACAAACTGGT	ATCAACACCAAGGGGTACGA	7HL	56	450(A) 459(B)	size polymorphism
OIS53	AP004278	Os06g0701100	AK251388	GAAGGTTCCCAGTTTGATGC	TGGCACTCAACCAAAACCATATA	7HL	58	534	<i>NdeI</i>
OIS26	AP004278	Os06g0700000	AV916542	TAGGTGCAGCTGGCTTTGTT	TCGATCGTGTGGCAGTAGT	7HL	56	1200	<i>AclI</i>
OIS25	AP004278	Os06g0699400	BQ464168	TCCTCAATGGTGTGTTGTC	CTCTCATATTTGTCAATCAATTGATG	7HL	56	300	<i>AclI</i> (dCAPS)
OIS7	AP003565	Os06g0653100	CV064036	TTGAGGTCAACACAGCAAGC	CTTCTGGCTTCAACGCTACC	—	58	397	<i>HaeIII</i>
OIS4	AP003517	Os06g0649500	BU989280	ATTCGGCTGTGGAGTACCAA	CAGCTGAAAGCAAGTGACCA	7HL	58	1183	<i>FokI</i>
OIS6	AP004008	Os06g0646900	BU969415	CTGGATGCAGCGAGGTTAAT	TCCGGAACGTATTCCTATGC	—	58	795	<i>TaqI</i>

^a Chromosome arm location was not confirmed using wheat-barley chromosome addition lines.^b Numbers followed by A and B in parentheses indicate the amplicon size of Karafuto Zairai and Aizu Hadaka 3, respectively.

the order of *dsp1*–*nud1*–*lks2*, and that the distances in the former and latter intervals are, respectively, 9.38% and 8.75%. The genetic distances by Takahashi *et al.* (1953) are based on observation of more than 2000 segregating plants of either F₂ or BC₁F₁ generations. Costa *et al.* (2001), based on 94 doubled haploid lines, reported that *nud1* and *lks2* are 12.1 cM apart on chromosome 7H. Consequently, the relative order and genetic distances of the three morphological genes in the present study based on 98 F₂ plants show good agreement with those of Takahashi *et al.* (1953) and Costa *et al.* (2001).

To enrich molecular markers near the k04151–k06123 interval, rice–barley microsynteny was exploited. A database search showed that two flanking markers for *lks2*, k04151 and k06123, share homology to rice genes Os06g0611200 and Os06g0710500 (respective e-values 2.0×10^{-93} and 1.8×10^{-66}). On the physical map of rice chromosome 6, these two genes were separated by about 5.6 Mbp. Consequently, first, four rice BAC clones (AP004008, AP003517, AP005453 and AP003565) that were located around the mid-point of this interval were chosen. Two randomly selected genes per BAC clone (a total of eight genes) were used as queries for search for homologous barley EST. Based on selected barley ESTs, primers were designed and sequences were compared between the mapping parents. In this way, three EST markers were developed: OIS4, OIS6, and OIS7 (Table 1). Unexpectedly, these markers were mapped on 7HL far distal to *lks2* (Fig. 2), suggesting disturbed microcollinearity between the barley chromosome region surrounding *lks2* and the corresponding rice chromosome 6 region. Similar attempts were continued, with specific attention to the rice chromosome regions that are close, respectively, to Os06g0611200 and Os06g0710500, because highly disrupted rice–barley collinearity was expected in the middle of the interval. From 49 attempts, 12 polymorphic markers were developed. Consequently, in all, 57 rice genes were attempted for rice–barley microsynteny-based marker development, and 17 out of 32 successfully sequenced for the barley parents were monomorphic, and 15 polymorphic EST markers (suffixed by OIS) were developed with the success rate of 26.3%. Then OIS35 and OIS34 occupied the same position as k04151, and OIS45 colocalized to k06123. However, no EST marker closer than the starting flanking EST markers was developed. In the current map (Fig. 2), all EST-based markers except for one share homology to rice genes on chromosome 6. A public marker, k07588 (Sato *et al.* 2009), which is 4.1 cM distal to *lks2*, shares homology to a rice gene on chromosome 8.

As the comparison between the barley genetic map and the rice physical map (Fig. 2) shows, breakdown of collinearity was observed in the region surrounding *lks2*; a large reversal of marker order was found between rice and barley in the region distal to *lks2*, suggesting an inversion in the orthologous region of barley (k06123–OIS6 interval) relative to the corresponding part of rice chromosome 6. In contrast, in the region surrounding the *nud1* gene, micro-

Fig. 2. Genetic map of barley chromosome 7H, in relation to a part of the rice chromosome 6 physical map. Lines connect barley EST markers with rice orthologues. C in the barley genetic map shows the location of the centromere.

collinearity is highly conserved, with the exceptions of OIS23 and OIS13.

Discussion

The *dsp1* gene cosegregated with three SSR markers that were located in the proximal region of the short arm of 7H. These SSR markers are assigned to 7HS using wheat–barley ditelosomic chromosome addition lines. However, it is uncertain whether *dsp1* is located on the proximal region of either the short arm or the long arm of 7H because *dsp1* maps close to the centromere. Sameri *et al.* (2006) mapped a QTL for spike density in the proximal region of the short arm of 7H, which is very close to the *cMWG704* molecular marker. Although the present map does not include this marker because of the unavailability of polymorphism, *dsp1* seems to occupy a similar location to the dense spike QTL on the 7HS

reported by Sameri *et al.* (2006). In a similar location, Shahinnia *et al.* (2010) reported the dense spike gene (*dsp.ar*) that was harbored in an X-ray induced mutant 7114. Although allelism of this dense spike gene with *dsp1* has not been tested yet, Shahinnia *et al.* (2010) claim that they occupy the identical locus. It is apparent that *dsp1* lies close to the centromere of 7H and that it is probably in the region of suppressed recombination according to the cytologically integrated molecular map of barley chromosome 7H (Künzel *et al.* 2000). Therefore, the *dsp1* gene appears to be only slightly accessible by map-based cloning approaches.

In this study, *lks2* mapped 8.2 cM distal to *nud1*. Recently, the *nud1* gene for naked caryopsis was isolated by our group through positional cloning (Taketa *et al.* 2008). Considering its more distal location than that of *nud1* (Fig. 2), positional cloning of *lks2* is expected to be feasible because of a favorable ratio of genetic/physical distance in the distal region of chromosome 7H (Künzel *et al.* 2000). The results reported herein showed that the *lks2* candidate region most likely corresponds to rice chromosome 6 (Fig. 2), but the breakdown of collinearity precluded accurate determination of the *lks2* candidate region on rice chromosome 6 based on rice–barley microsynteny. Structural changes are likely to include inversions and translocations occurring in the chromosome region harboring *lks2* during divergence of barley and rice from a common ancestor. Therefore, as Poukheirandish *et al.* (2007) reported, in the case of breakdown of collinearity, construction of a BAC contig-based physical map is necessary to identify a candidate gene(s) in the target species. High-resolution genetic mapping and physical mapping of the *lks2* gene is under way toward its positional cloning and functional analysis. We initiated chromosome walking from the closest flanking markers using the BAC library of a Japanese barley cultivar ‘Haruna Nijo’ (Saisho *et al.* 2007).

In wild barley, the awn facilitates seed dispersal and burial, so the long awn is considered adaptive (Harlan 1968). However, in domesticated barley, long awns may have some adverse effects. For example, under humid conditions, the awned varieties might be more susceptible to lodging than the short awned ones because the awned spikes accumulate excessive amounts of moisture during rain (Grundbacher 1963). Such lodging resistance might confer adaptive advantages to short awned varieties in eastern Asia, where precipitation is rather high because of monsoon climate zones. Molecular cloning of genes controlling the awn length might enable optimization of the barley awn length through genetic manipulation.

Acknowledgments

The authors are grateful to Dr. K. Sato for providing EST marker information before publication. Seeds of barley parental lines were provided by the National Bioresource Project – Barley, Japan. This work was partly supported by a grant from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Genomics for Agricultural Innovation Grant TRC1007).

Literature Cited

- Costa, J.M., A. Corey, P.M. Hayes, C. Jobet, A. Kleinhofs, A. Kopisch-Obusch, S.F. Kramer, D. Kudrna, M. Li, O. Riera-Lizarazu *et al.* (2001) Molecular mapping of the Oregon Wolfe Barleys: a phenotypically polymorphic doubled-haploid population. *Theor. Appl. Genet.* 103: 415–424.
- Grundbacher, F.J. (1963) The physiological function of the cereal awn. *Bot. Rev.* 29: 366–381.
- Harlan, J.R. (1968) On the origin of barley. *In: Barley: Origin, Botany, Culture, Winterhardiness, Genetics, Utilization, Pests. Agriculture Handbook No. 338* (U.S. Department of Agriculture, Washington, DC), pp. 9–31.
- Hayashi, J., I. Moriya and R. Takahashi (1972) Effects of the gene *lks2* for short awn on some agronomic characters in barley. *Jpn. J. Breed.* 22: (Suppl. 2) 100–101.
- Islam, A.K.M.R. (1983) Ditelosomic additions of barley chromosomes to wheat. *In: Sakamoto, S. (ed.) Proc. 6th Int. Wheat Genet. Symp., Maruzen, Kyoto*, pp. 233–238.
- Islam, A.K.M.R., K.W. Shepherd and D.H.B. Sparrow (1981) Isolation and characterization of euplasmic wheat–barley chromosome addition lines. *Heredity* 46: 161–174.
- Kikuchi, S., S. Taketa, M. Ichii and S. Kawasaki (2003) Efficient fine mapping of the naked caryopsis gene (*nud*) by HEGS (high-efficiency genome scanning)/AFLP in barley. *Theor. Appl. Genet.* 108: 73–78.
- Kjack, J.L. and R.S. Witters (1974) Physiological activity of awns in isolines of Atlas barley. *Crop Sci.* 14: 243–248.
- Komatsuda, T., I. Nakamura, F. Takaiwa and S. Oka (1998) Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*. *Genome* 41: 680–685.
- Kosambi, D.D. (1944) The estimation of map distance from recombination values. *Ann. Eugen.* 12: 172–175.
- Künzel, G., L. Korzun and A. Meister (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. *Genetics* 154: 397–412.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln and L. Newburg (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174–181.
- Li, J.Z., T.G. Sjakste, M.S. Röder and M.W. Ganal (2003) Development and genetic mapping of 127 new microsatellite markers in barley. *Theor. Appl. Genet.* 107: 1021–1027.
- Lundqvist, U. and J.D. Franckowiak (2003) Diversity of barley mutants. *In: Bothmer, R.v., T.v. Hintum, H. Knüpfer and K. Sato (eds.) Diversity in barley (Hordeum vulgare)*, Elsevier, Amsterdam, pp. 77–96.
- Lundqvist, U. and D. Falk (2005) Report of barley genetic coordination workshop at the IX International Barley Genetics Symposium in Brno, Czech Republic, June 19, 2004. *Barley Genet. Newslett.* 35: 100–102.
- Poukheirandish, M., T. Wicker, N. Stein, T. Fujimura and T. Komatsuda (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. *Theor. Appl. Genet.* 114: 1357–1365.
- Qualset, C.O., C.W. Schaller and J.C. Williams (1965) Performance of isogenic lines of barley as influenced by awn length, linkage blocks, and environment. *Crop Sci.* 5: 489–494.
- Ramsey, L., M. Macaulay, S. degli Iwanisovich, K. MacLean, L. Cardle,

- J.Fuller, K.J.Edwards, S.Tuvesson, M.Morgante, A.Massari *et al.* (2000) A simple sequence repeat-based linkage map of barley. *Genetics* 156: 1997–2005.
- Saisho,D., E.Myoraku, S.Kawasaki, K.Sato and K.Takeda (2007) Construction and characterization of a bacterial artificial chromosome (BAC) library from the Japanese malting barley ‘Haruna Nijo’. *Breed. Sci.* 57: 29–38.
- Sameri,M., K.Takeda and T.Komatsuda (2006) Quantitative trait loci controlling agronomic traits in recombinant inbred lines from a cross of Oriental- and Occidental-type barley cultivars. *Breed. Sci.* 56: 243–252.
- Sato,K., N.Nankaku and K.Takeda (2009) A high-density transcript linkage map of barley derived from a single population. *Heredity* 103: 110–117.
- Shahinnia,F., A.Druka, R.Waugh, M.Morgante and N.Stein (2010) Positional cloning of the gene dense spike (*dsp1*) on barley chromosome 7H by utilization of next generation grass-genomic tools. *Plant and Animal Genomes XVIII Conference abstract* p. 319.
- Takahashi,R. (1987) Genetic features of East Asian barleys. *In*: Yasuda,S. and T.Konishi (eds.) *Barley Genetics V.*, Maruzen, Okayama, pp. 7–20.
- Takahashi,R., J.Yamamoto, S.Yasuda and Y.Itano (1953) Inheritance and linkage studies in barley. *Ber. Ohara Inst. Landw. Forsch.* 10: 29–53.
- Taketa,S., S.Kikuchi, T.Awayama, S.Yamamoto, M.Ichii and S.Kawasaki (2004) Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*). *Theor. Appl. Genet.* 108: 1236–1242.
- Taketa,S., T.Awayama, S.Amano, Y.Sakurai and M.Ichii (2006) High-resolution mapping of the *nud* locus controlling the naked caryopsis in barley. *Plant Breed.* 125: 337–342.
- Taketa,S., S.Amano, Y.Tsujino, T.Sato, D.Saisho, K.Kakeda, M.Nomura, T.Suzuki, T.Matsumoto, K.Sato *et al.* (2008) Barley grain with adhering hulls is controlled by an ERF family transcription factor gene regulating a lipid biosynthesis pathway. *Proc. Natl. Acad. Sci. USA* 105: 4062–4067.
- Taketa,S., K.Matsuki, S.Amano, D.Saisho, E.Himi, N.Shitsukawa, T.Yuo, K.Noda and K.Takeda (2010) Duplicate polyphenol oxidase genes on barley chromosome 2H and their functional differentiation in the phenol reaction of spikes and grains. *J. Exp. Bot.* 61: 3983–3993.