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ORIGINAL PAPER

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Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (*nud*)

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Abstract To elucidate the origin of naked barley, molecular variation of the marker sKT7 tightly linked to the *nud* locus was examined. A total of 259 (53 wild, 106 hulled domesticated, and 100 naked domesticated) barley accessions were studied. Restriction analysis of the sKT7 PCR-amplified product revealed the alleles *I*, *II*, *III*, and *IV*. All four alleles were found in wild barley, but allele *IV* was found only in a single accession from southwestern Iran. Hulled domesticated accessions showed alleles *I*, *II*, or *III*, but all naked domesticated accessions had allele *IV*. The distribution of allele *IV* in wild barley and its pervasive presence in naked domesticated lines support the conclusion that naked barley has a monophyletic origin, probably in southwestern Iran. The available results suggest two scenarios for the origin of naked barley: either directly from a wild barley with allele *IV* or from a hulled domesticated line with allele *IV* that later became extinct. Naked domesticated accessions from different regions of the world have extremely homogeneous DNA sequences at the *sKT7* locus, supporting the monophyletic origin of naked barley. For allele *IV*, four haplotypes (IVb to IVe) were found in 30 naked accessions: IVb was predominant (66.7%) and widely distributed, while the other three haplotypes, differing by only one nucleotide at different positions relative to IVb, showed a localized distribution. The geographical distribution of the haplotypes of *sKT7* allele *IV* suggests migration routes of naked domesticated barley in central and eastern Asia.

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Introduction

Domesticated barley (*Hordeum vulgare* subsp. *vulgare*) is classified into two major categories according to the grain type, namely, hulled barley and naked barley. Hulled barley has caryopses with the husk adhering to the grain, while naked barley grows with easily separable husks upon threshing. This difference is controlled by a single locus, and the hulled caryopsis is dominant over the naked one. The gene for naked caryopsis, *nud*, is located on the long arm of chromosome 7H (Scholz 1955; Fedak et al. 1972). No other crops in the tribe Triticeae show such a differentiation in grain type. Thus, this character is not only unique to barley, but is also directly connected to its utilization. At present, most barley varieties are of the hulled form, and they are mainly used for brewing malt and animal feed. By contrast, naked barley is produced on a small scale and used mainly as human food because of the ease in processing and edibility. Recently in North America, naked barley is attracting attention as feed and as a healthy food because of the high feed value and abundance of dietary fiber, respectively (Liu et al. 1996). According to a survey by Takahashi (1955), naked barley is distributed widely, but its frequency greatly differs among regions; naked barley accounts for more than 95% of domesticated barley in the highlands of Nepal and Tibet, and almost 50% in China, Korea, and Japan, but the frequency decreases toward the west, becoming low in Europe. Such a skewed distribution of naked barley toward East Asia is likely to be established not only by natural factors, but also by human factors. Because the naked caryopsis is an easily recognizable character, it can become a subject of strong human selection. In fact, in the regions where naked barley is grown at high frequencies, it is an important staple food.

The widespread distribution of naked barley makes the hulled or naked caryopsis character a key trait to follow the origin and domestication process of barley (Harlan 1995; Salamini et al. 2002). It is generally accepted that barley was domesticated from the wild ancestor *H. vulgare* subsp. *spontaneum*, which has brittle rachises,

Table 1 Barley germplasms used in this study

Region	Wild	Domesticated		Country
		Hulled	Naked	
Ethiopia	–	7	6	Ethiopia
North Africa	2	1	–	Libya, Morocco
Europe	1	12	9	Czechoslovakia, Germany, Hungary, Italy, Russia, Spain, United Kingdom, Yugoslavia
Middle East	27	13	8	Armenia, Azerbaijan, Cyprus, Iran, Iraq, Israel, Syria, Turkey
South and central Asia	14	13	11	Afghanistan, India, Pakistan, Turkmenistan
Himalayas	8	3	11	Bhutan, Nepal, Tibet
East Asia	–	17	14	China, Korea
Japan	–	36	41	Japan
North America and unknown	1	4	–	Canada, United States
Total	53	106	100	

two-rowed spikes, and hulled caryopses. Harlan (1995) supposed that during the domestication of barley, the change to non-brittleness preceded the emergence of six-rowed spikes and naked caryopsis character. Most studies on the history of barley domestication have focused on the non-brittle rachis character (Takahashi 1955) and row-type (Harlan 1995; Zohary and Hopf 2000; Tanno et al. 2002). Little attention has been paid to the hulled or naked caryopsis character. According to the archeological evidence, the earliest domesticated barley grown in the Near East about 8000 B.C. was of the hulled form, and the naked form appeared by about 6500 B.C. (Zohary and Hopf 2000). This suggests that naked barley appeared after domestication of hulled barley. However, the following important questions about the origin of naked barley remain unanswered: Where and how did naked barley originate? How did naked barley migrate? Answers to these questions should contribute not only to the elucidation of the domestication history of barley, but also to barley breeding.

We have been attempting positional cloning of the *nud* gene, with an ultimate goal to clarify the genetic and biochemical mechanisms underlying the differentiation of the hulled and naked caryopsis character in barley. Previously, we (Kikuchi et al. 2003) developed sequence-characterized amplified region (SCAR) markers closely linked to the *nud* gene, using the amplified fragment length polymorphism (AFLP) technique (Vos et al. 1995) and the high-efficiency genome scanning (HEGS) electrophoresis system (Kawasaki and Murakami 2000). Because we have not yet cloned the *nud* gene, we employed one closely linked SCAR marker, sKT7, for investigating molecular variation near the *nud* locus in barley. A total of 259 barley accessions, including both wild and domesticated (hulled and naked) forms, were investigated. The objective of this study was to obtain precise information about the origin and migration route of naked barley from detailed analyses of a tightly linked molecular marker, sKT7.

Materials and methods

Plant materials

The material analyzed included 53 wild (all hulled) and 206 domesticated (106 hulled and 100 naked) barley accessions (Table 1). These accessions were selected on the basis of collection sites and morphological characters so that they would represent genetic variations of barley. In wild barley, 41 accessions were two-rowed (*H. vulgare* subsp. *spontaneum*) and 12 accessions were six-rowed (subsp. *agriocrithon*). Six-rowed wild barley is currently considered a hybridization product between subsp. *spontaneum* and six-rowed domesticated barley (Zohary 1963; Bothmer et al. 1995), but is treated as a "wild" form in this study. The domesticated barley accessions were mostly landraces; 35 accessions were two-rowed and the remaining 171 accessions were six-rowed. All the barley germplasms were obtained from the Barley Germplasm Center, Research Institute for Bioresources, Okayama University, Kurashiki, Japan, except for six accessions, which were obtained from Shikoku National Agricultural Experiment Station, Kagawa, Japan. (Details on the materials are available upon request.)

PCR amplification and restriction analysis

sKT7 is a dominant SCAR marker converted from an AFLP marker KT7 (Fig. 1), and it is associated with the *nud* allele of the naked parent Kobinkatagi. A database search of the sKT7 sequence of Kobinkatagi using the BLAST program revealed no significant homology to any sequences in the DNA Data Bank of Japan (DDBJ). No recombination between sKT7 and the *nud* gene has been detected in 460 F_2 plants from the cross between Kobinkatagi and Triumph tested so far (Kikuchi et al. 2003). A total of 259 barley accessions were examined by the polymerase chain reaction (PCR) for the presence/absence or length polymorphisms at the *sKT7* locus. For PCR analysis, total DNA was extracted from young leaves of a single plant of each accession according to Komatsuda et al. (1998). PCR amplification was done in volumes of 15 μl, each containing 0.5 U of Ampli-*Taq* Gold DNA polymerase (Applied Biosystems), 0.2 μM of primers, 200 μM of dNTPs, 2.0 mM of $MgCl_2$, 20 ng of DNA, and reaction buffer. Primer pairs sKT7F1 (5′-CGATATGCTTGTCCGTGATG-3′) and sKT7R1 (5′-TGCT-CGTACTGCATCGACTC-3′) were used (Fig. 1). PCR was performed with the following cycling parameter: 9 min at 95°C; 30 cycles of 1 min at 95°C, 2 min at 60°C, 2 min at 72°C; 2 min at 72°C; and a final hold at 4°C. PCR products were digested with 2 U of *Nsp*I (New England Biolabs) and electrophoresed on 2% agarose gels.

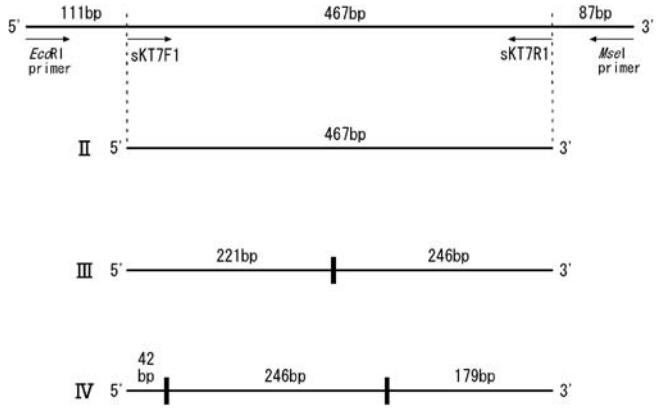

Fig. 1 Restriction site map of the three alleles, *II*, *III*, and *IV* at the *sKT7* locus. AFLP marker KT7 (665 bp) was a dominant marker, co-segregating with the *nud* gene (Kikuchi et al. 2003). PCR fragments (467 bp) were amplified with the internal primers sKT7F1 and sKT7R1 designed for SCAR marker sKT7. *Vertical bar* indicates *Nsp*I recognition site

DNA sequencing

Nucleotide sequences of the *sKT7* locus were determined in selected accessions. PCR products were directly sequenced using a BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems) and a DNA sequencer (Model 3100, Applied Biosystems). Both strands were sequenced using the sKT7F1 and sKT7R1 primers. Sequence data were aligned using the ClustalW multiple sequence alignment software on the Web server of the DDBJ.

Results

PCR amplification

In 133 (51.4%) of the 259 accessions examined, a single DNA fragment of about 470 bp was generated after the PCR amplification. The remaining 126 accessions (48.6%) produced no amplification products and are considered to have a null allele. Accessions with the null allele were named allele "*I*," and were not analyzed any further. Allele *I* was found only in hulled accessions of both wild and domesticated barley, and totally absent in naked barley accessions. The frequency of allele *I* was much higher in hulled domesticated accessions (90.6%) than in wild barley (56.6%). On the other hand, the 470-bp band was amplified in 23 accessions (43.4%) of wild barley, ten accessions (9.4%) of hulled domesticated accessions, and all naked domesticated accessions except one. The exceptional naked barley was an accession from Tibet (OUC652) and had a shorter band (about 400 bp). Sequence analysis revealed that this accession had an internal deletion of 65 bp (positions 180 to 244) compared to the 467-bp fragment of a standard naked barley Kobinkatagi, but otherwise had an identical DNA sequence. For this reason, this accession was included in the same group as the other naked barleys.

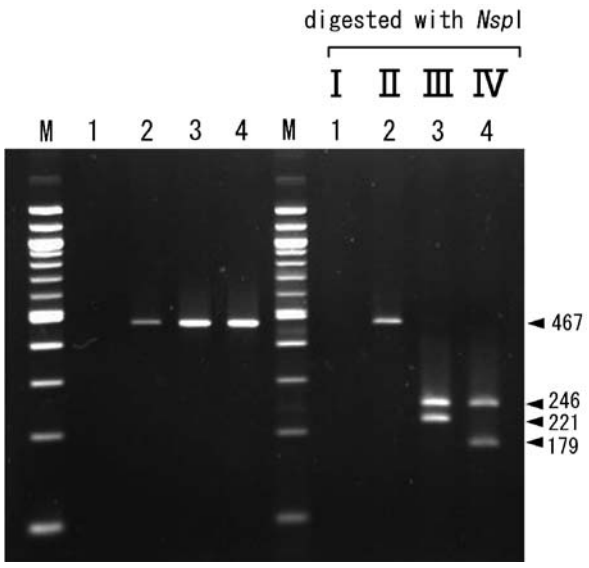

Fig. 2 Detection of the four alleles, *I*, *II*, *III*, and *IV*. The PCR fragments (467 bp) were amplified from: *1* Triumph; *2* Debre Zeit 40; *3* 70 g; and *4* Kobinkatagi, using the primers sKT7F1 and sKT7R1 (*left half*). Four alleles were revealed by the digestion with *Nsp*I (*right half*). No PCR fragment was amplified from Triumph. Allele *IV* has an additional fragment (42 bp), which does not appear in the figure. *M* 100-bp ladder size marker

Restriction analysis

To further differentiate the 133 accessions with the 470-bp band, we performed restriction analysis of the sKT7 PCR-amplified products with *Nsp*I. Three alleles of the 470 bp-band, named *II*, *III*, and *IV*, were revealed (Fig. 2). Their restriction site maps are depicted in Fig. 1. The sequence data indicated that the amplified fragments of the three alleles were all 467 bp long. Allele *II* had no restriction sites, but allele *III* was digested to 221 bp and 246 bp, and allele *IV* was digested to 42 bp, 246 bp, and 179 bp. It should be noted that the 246-bp fragments of alleles *III* and *IV* derived from different regions within the 467-bp fragment.

Geographical distribution of *sKT7* alleles

The results of restriction analysis of the sKT7 PCR-amplified products are summarized in Fig. 3, together with those of the null allele *I*. For simple presentation, barley accessions were divided into three categories (wild, hulled domesticated, and naked domesticated), and each category was subdivided into nine regional groups as shown in Table 1.

In wild barley, four alleles (*I–IV*) were detected. All four alleles were found in the wild accessions from the Middle East, indicating that this region is the center of genetic diversity in barley. In wild barley, allele *I* is predominant (56.6%), occurring in the highest frequencies in all regions except southern and central Asia, where allele *III* was predominant. Allele *II* was the second most

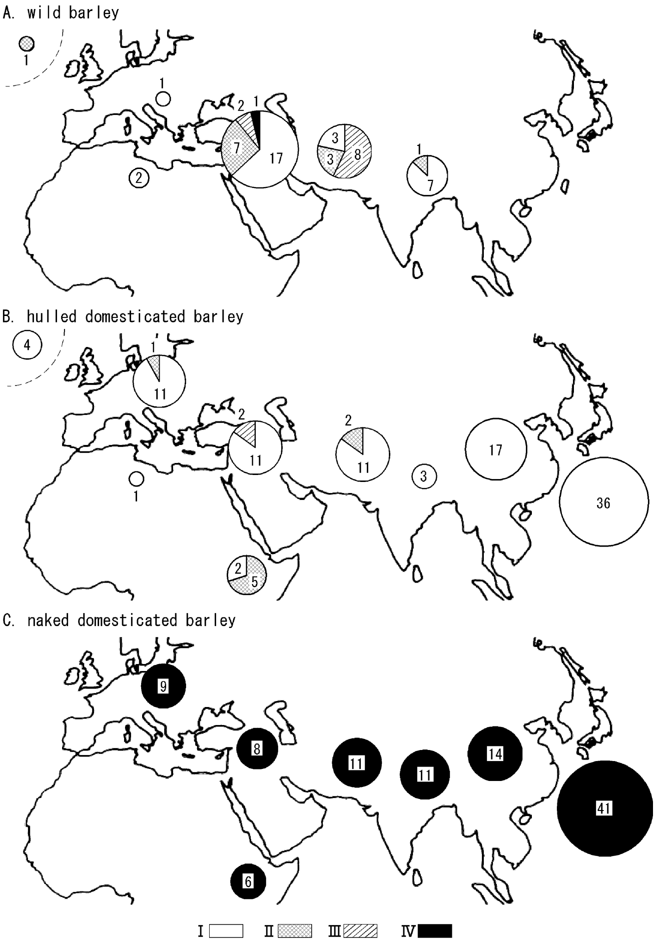

Fig. 3 Geographical distribution of the four alleles *I*, *II*, *III*, and *IV* at the *sKT7* locus for **A** wild barley, **B** hulled domesticated barley, and **C** naked domesticated barley. The figures show the numbers of accessions of respective alleles. The circle graphs of North American and unknown accessions are shown at the *upper-left corner* of each map

frequent (22.6%) and was distributed from the Middle East to the Himalayas, but its frequency decreased toward the east. Allele *III* was found in ten accessions (18.9%) and was distributed in the Middle East and southern and central Asia. Allele *IV* was rare (1.9%) and found only in one accession collected in southwestern Iran (OUH625).

In hulled domesticated barley, three alleles (*I–III*) were found. Allele *I* was predominant (90.6%), and alleles *II* (7.5%) and *III* (1.9%) appeared at low frequencies. Allele *II* was the most frequent (71.4%) in Ethiopian hulled domesticated barley, but it also appeared at a low frequency in Europe and southern and central Asia. Allele *III* was found only in accessions of the Middle East.

All the 100 accessions of naked domesticated barley had allele *IV*. One Tibetan accession (OUC652) with a slightly shorter band of 402 bp, was considered to have allele *IV* because it has a variant of allele *IV*, as mentioned before. This variant is regarded as a rare mutant because

Table 2 Nucleotide polymorphism within alleles *II*, *III*, and *IV* at the *sKT7* locus found in wild and domesticated barley

Allele	Haplotype	No. of accessions	Polymorphic site[a]																																						
			40	62	63	65	77	108	119	132	140	141	148	149	151	163	181	186	193	197	201	211	216	220	222	225	226	239	244	261	272	275	287	298	309	312	319	354	357	366	374	397	445
II	IIa	1	C	T	G	C	G	G	G	C	A	C	C	G	T	C	T	G	C	G	C	C	A	G	T	T	G	G	G	G	G	G	C	C	G	G	C	G	A	G	G	G	C
	IIb	1	*	C	T	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*	*	*	*	*	C	*	*	*	*
	IIc	1	*	*	*	A	*	*	*	T	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*
	IId	1	G	*	*	*	*	*	*	*	*	*	*	*	-	*	*	A	*	*	T	T	C	*	C	*	*	A	*	*	*	C	*	*	*	C	T	*	C	*	C	*	*
	IIe	2	*	*	*	*	*	*	*	*	G	*	*	*	*	*	*	*	*	*	*	*	C	*	C	A	*	*	*	*	*	C	*	*	*	*	T	*	C	*	C	*	*
III	IIIa	3	*	*	*	*	A	*	A	*	*	*	T	*	*	T	*	*	*	A	*	*	C	T	*	*	*	*	*	A	*	*	*	*	A	*	*	A	C	*	*	C	*
	IIIb	1	*	*	*	*	A	*	A	*	*	*	T	*	*	T	*	*	*	A	*	*	C	T	*	*	*	*	*	A	A	*	*	*	A	*	*	A	C	*	*	C	*
IV	IVa	1	A	*	*	*	*	*	A	*	*	*	*	T	*	*	*	*	G	*	*	T	C	*	*	*	C	A	*	*	A	*	T	A	*	*	*	*	C	T	*	*	*
	IVb	20	A	*	*	*	*	A	A	*	*	*	*	T	*	*	*	*	G	*	*	*	C	*	*	*	C	*	*	*	*	*	T	A	*	*	*	*	C	T	*	*	T
	IVc	7	A	*	*	*	*	A	A	*	*	*	*	T	*	*	*	*	G	*	*	*	C	*	*	*	C	*	A	*	*	*	T	A	*	*	*	*	C	T	*	*	T
	IVd	2	A	*	*	*	*	A	A	*	*	*	*	T	*	*	G	*	G	*	*	*	C	*	*	*	C	*	*	*	*	*	T	A	*	*	*	*	C	T	*	*	T
	IVe	1	A	*	*	*	*	A	A	*	*	T	*	T	*	*	*	*	G	*	*	*	C	*	*	*	C	*	*	*	*	*	T	A	*	*	*	*	C	T	*	*	T

[a] Underlined numbers indicate *Nsp*I recognition sites

* denotes the same sequence as that of haplotype IIa (OUH774), - denotes gap. The lengths of the aligned sequences are 467 nucleotides, including 20-base primer sequences on both ends

Table 3 Row-type and collection site of 41 accessions used for sequencing of the *sKT7* locus

Allele	Haplotype	Accession		Status	Row type	Locality
II	IIa	OUH774	ssp. *spontaneum*	Wild	2	Afghanistan
	IIb	OUI444	Qizil 2	Hulled domes.	6	Afghanistan
	IIc	OUH823	ssp. *agriocrithon*	Wild	6	Tibet
	IId	OUH639	ssp. *spontaneum*	Wild	2	Turkey
	IIe	OUE229	Debre Zeit 40	Hulled domes.	2	Ethiopia
		OUE215	Debre Zeit AES 1	Hulled domes.	6	Ethiopia
III	IIIa	OUH632	ssp. *spontaneum*	Wild	2	Iran
		OUT329	Turkey 86	Hulled domes.	6	Turkey
		OUI030	70g	Hulled domes.	6	Iran
	IIIb	OUH663	ssp. *agriocrithon*	Wild	6	Turkmenistan
IV	IVa	OUH625	ssp. *spontaneum*	Wild	2	Iran
	IVb	OUE312	Ethiopia 35	Naked domes.	2	Ethiopia
		OUE515	Debre Zeit AES 2	Naked domes.	2	Ethiopia
		OUE552	Addis Ababa 62	Naked domes.	2	Ethiopia
		OUE250	Addis Ababa 55	Naked domes.	6	Ethiopia
		OUE278	Dabat 1	Naked domes.	6	Ethiopia
		OUU324	Hungarie 929	Naked domes.	6	Hungary
		OUU648	Tuxsky nahy	Naked domes.	6	Czechoslovakia
		OUU612	2547	Naked domes.	6	Italy
		OUT046	Turkey 136	Naked domes.	2	Turkey
		OUT514	Turkey 642	Naked domes.	2	Turkey
		OUT247	Turkey 741	Naked domes.	2	Turkey
		OUT634	Turkey 102	Naked domes.	6	Turkey
		OUI026	H. nudum	Naked domes.	2	Iran
		OUI031	151	Naked domes.	6	Iran
		OUI744	Qiail 3	Naked domes.	6	Afghanistan
		OUI343	Happar 1	Naked domes.	6	Pakistan
		OUI629	Minapin	Naked domes.	6	India
		OUN005	Sama 1	Naked domes.	6	Nepal
		OUC050	Violaceum Sg Type 3	Naked domes.	6	Tibet
			TKB64	Naked domes.	6	Bhutan
	IVc	OUU063	Russia 9	Naked domes.	6	Russia
		OUI022	J. 4	Naked domes.	6	Afghanistan
		OUC329	Paishapu 2	Naked domes.	6	China
		OUK613	Gogseong Naked 4	Naked domes.	6	Korea
		OUJ369	Kobinkatagi	Naked domes.	6	Japan
		OUJ087	Shimabara	Naked domes.	6	Japan
			Chinkoichigou	Naked domes.	6	Japan
	IVd	OUU363	Russia 7	Naked domes.	2	Russia
		OUU048	Michalovicky nahy	Naked domes.	2	Czechoslovakia
	IVe	OUU601	Spain	Naked domes.	6	Spain

the same variant has not been detected in other wild or domesticated barley accessions.

Sequence analysis of *sKT7* alleles

Sequence analysis was carried out to detect nucleotide variation within each of the three alleles (*II–IV*). A total of 41 accessions were analyzed. Table 2 summarizes the sequence polymorphisms at the *sKT7* locus. In this table, the sequence of an accession from Afghanistan (OUH744, haplotype IIa) was used as a standard, and only polymorphic sites are presented. Except for haplotype IId, all haplotypes had the same fragment size of 467 bp. Haplotype IId had the fragment size of 466 bp as a result of one base deletion at position 151. Nucleotide polymorphisms were found at 41 sites of the 467-bp region sequenced. Altogether, 12 haplotypes were detected. Sequence data of the haplotype IIa (excluding those of the primers) have been deposited in the DDBJ database under the accession number AB128163. Table 3 presents detailed information about the 41 accessions sequenced.

In allele *II*, five haplotypes were found in six accessions analyzed. Among these five haplotypes, nucleotide polymorphisms were found at 20 sites. In allele *III*, two haplotypes were found in four accessions sequenced, and these differed by one single nucleotide polymorphism (SNP). In allele *IV*, 31 accessions were analyzed. Allele *IV* included five haplotypes, but all of these five shared seven nucleotide polymorphisms specific to this allele (positions 40, 149, 193, 226, 287, 298, and 366) and constituted a homogeneous group. Haplotype IVa found in wild barley was rather different from the other four haplotypes found in naked barley (IVb to IVe), having five nucleotide changes unique to it. The four haplotypes (IVb to IVe) were mutually so similar that haplotypes IVc, IVd and IVe each had only one SNP compared to the most predominant haplotype IVb. Thus, at the sequence level, allele *II* included a high level of

nucleotide polymorphisms, while alleles *III* and *IV* were homogeneous.

In the four haplotypes (IVb to IVe) found in naked barley, haplotype IVb was considered as the prototype because of its predominance (66.7%) and wide distribution in all regions except China, Korea, and Japan (Table 3). The other three haplotypes (IVc, IVd, and IVe) are considered as derivatives because they appeared at low frequencies and showed a localized distribution. The second most frequent haplotype, IVc (33.3%), was distributed in central Asia (Russia and Afghanistan) and eastward from China to Japan. Haplotype IVd was localized in Eastern Europe. Haplotype IVb included both two- and six-rowed spikes, while haplotype IVc was all six-rowed, and haplotype IVd included only two-rowed spikes. Haplotype IVe was found in a Spanish, six-rowed naked barley.

Discussion

The restriction analyses of the sKT7 PCR-amplified products revealed four alleles, *I–IV*. All four alleles were found in wild barley, but allele *I* was predominant (56.6%) followed by alleles *II* (22.6%) and *III* (18.9%), and allele *IV* was found only in one (1.9%) accession. In domesticated barley, hulled accessions had either allele *I* (90.6%), *II* (7.5%), or *III* (1.9%), but all naked accessions had allele *IV*. This clear separation in domesticated barley suggests that naked forms and hulled forms of domesticated barley independently originated from different wild barley ancestors. Allele *IV* wild barley (subsp. *spontaneum*), possible ancestor of naked domesticated barley, was found only in a single accession from southwestern Iran (OUH625, collected in Shushtar). The distribution of allele *IV* in wild barley and its fixation in naked domesticated accessions indicate that naked barley has a monophyletic origin, probably in southwestern Iran. This estimate coincides with the archeological evidence that the oldest carbonized naked domesticated barley (before 6000 B.C.) was found in the remains at Ali Kosh (southwestern Iran), Tell Abu Hureyra, and Tell Aswad (both Syria) within the Fertile Crescent (Zohary and Hopf 2000). In barley, natural crosses between wild and domesticated forms are rather common (Helbaek 1959), and this complicates the studies on domestication history of barley. However, the *sKT7* allele *IV* in the Iranian wild accession was not probably introgressed from naked domesticated barley because there are as many as five nucleotide differences between them. This DNA sequence dissimilarity may make it less likely that the Iranian wild accession is the direct ancestor of naked domesticated barley. Although further search may reveal wild barley accessions having identical or more similar DNA sequences at this locus, direct derivation of naked domesticated barley from wild barley may also be excluded for the following reasons. So far, no naked wild form has been found in nature (Harlan 1995) or in archeological remains (Zohary and Hopf 2000). This is probably because the naked seeds unprotected by the husks are non-adaptive in the wild. In the absence of wild naked forms, the emergence of naked domesticated barley from wild barley would have required two independent mutations, i.e., one from hulled to naked caryopses and the other from brittle to non-brittle rachises. Such double mutations would be rare.

An alternative possibility is that naked domesticated barley derived from hulled domesticated barley that later became extinct. This hypothesis seems to be more likely because it requires only a single mutation from hulled to naked caryopses in hulled domesticated forms. The archeological evidence (Zohary and Hopf 2000) indicates that domestication of hulled barley precedes the appearance of naked barley. However, the present molecular data contradict to this hypothesis because of the absence of hulled domesticated accessions with allele *IV*. If we assume that a naked mutant had occurred in a small population of a hypothetical hulled domesticated form with allele *IV*, then such a naked mutant could have quickly replaced its original hulled form because of its improved edibility. This may explain the absence of ancestral hulled domesticated barley with allele *IV*. The second possibility may be tested if DNA samples of carbonized seeds from archeological remains could be analyzed with our molecular marker. No matter which of the above two hypotheses is correct, the extremely homogeneous DNA sequences at the *sKT7* locus among naked barley accessions in the world indicates their monophyletic origin. Our conclusion disagrees with the generally accepted opinion that naked barley has multiple independent origins (Helbaek 1959). Based on morphological observations, Orlov (1929, cited in Takahashi and Yamamoto 1950) implied that the naked barley had appeared independently both in Ethiopia and East Asia. However, the present results unequivocally show that both Ethiopian and Himalayan naked barleys have an identical *sKT7* sequence, rejecting the possibility of their independent origins.

The presence of several haplotypes of allele *IV* within naked barley allows us to infer its migration routes to the east. Naked barley in China, Korea, and Japan were fixed with haplotype IVc, while those in the Himalayan regions (Nepal, Bhutan, and Tibet) were fixed with haplotype IVb. Fixation at the *sKT7* locus with a specific haplotype in restricted regions is probably due to a founder effect. This regional difference indicates that Himalayan naked barley did not migrate to China and eastward. Similar conclusions were obtained from the studies of esterase isozymes (Konishi 1995). Naked barley in China, Korea, and Japan was probably introduced from Afghanistan through the Silk Road, independently of the migration of naked barley into the Himalayan regions.

By using random amplified polymorphic DNA (RAPD) markers, Strelchenko et al. (1999) studied the genetic diversity of 303 barley accessions, including 43 naked ones. Statistical analyses of 93 polymorphic band data revealed three major groups: (1) “occidental,” (2) “oriental,” and (3) a third group mainly consisted of

naked barley from central Asia and the Caucasus region. The first and second groups confirm the oriental-occidental differentiation of domesticated barley proposed by Takahashi (1955), but each of them included both hulled and naked forms without clear clustering. Thus, the genome-wide RAPD analysis did not provide clues to the origin of naked barley. Their RAPD data suggest that the naked caryopsis gene (*nud*) had introgressed into the genetic background of local hulled domesticated barley through spontaneous or artificial hybridization, as naked barley widened its distribution. From genome-wide AFLP analysis of 317 wild and 57 domesticated accessions, Badr et al. (2000) concluded that domesticated barley has a monophyletic origin in the Israel-Jordan area. In combination with the molecular data on the homeobox gene *BKn-3*, they further pointed out that during the migration of barley to the east, hybridization between wild and domesticated accessions probably occurred in southwestern Iran, leading to the formation of the unique Himalayan domesticated varieties, frequently including naked forms. This agrees with our finding that a subsp. *spontaneum* accession from southwestern Iran is the most closely linked with naked domesticated barley.

In conclusion, our molecular analyses of the *sKT7* locus tightly linked to the *nud* gene indicate that naked barley today can be traced back to a single, naked mutation. Although the available results cannot unequivocally decide whether naked barley derived directly from wild barley or from hulled domesticated barley, we suggest southwestern Iran as the most likely site of origin. More concrete answers will be obtained once the *nud* gene has been cloned. Our conclusion provides an important implication for naked barley breeding, because naked barley has a very restricted level of genetic diversity around the *nud* locus. Such local genetic uniformity could be associated with the intrinsic difference between hulled barley and naked barley in some agronomic characters, such as reduced straw strength of naked barley (Rossnagel 2000). Although we cannot exclude the pleiotropic effects of the *nud* gene (Takahashi et al. 1962), efforts to enhance the genetic variation around the *nud* locus may significantly improve naked barley. Use of artificially induced naked mutants (Scholz 1955) may be helpful for this purpose.

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References

Badr A, Miller K, Schafer-Pregl R, El Rabey H, Effegen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol 4:499–510

Bothmer R von, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1955) An ecogeographical study of the genus *Hordeum*, 2nd edn. Systematic and ecogeographic studies on crop genepools 7. International Plant Genetic Resources Institute, Rome

Fedak G, Tsuchiya T, Helgason SB (1972) Use of monotelotrisomics for linkage mapping in barley. Can J Genet Cytol 14:949–957

Halbaek H (1959) Domestication of food plants in the old world. Science 153:365–372

Harlan JR (1995) Barley. In: Simmonds N (ed) Evolution of crop plants, 2nd edn. Academic Press, London, pp 140–147

Kawasaki S, Murakami Y (2000) Genome analysis of *Lotus japonicus*. J Plant Res 113:497–506

Kikuchi S, Taketa S, Ichii M, Kawasaki S. Efficient fine mapping of the naked caryopsis gene (*nud*) by HEGS (high-efficiency genome scanning)/AFLP in barley. Theor Appl Genet 108:73–78. DOI 10.1007/s00122-003-1413-y

Komatsuda T, Nakamura I, Takaiwa F, Oka S (1998) Development of STS markers closely linked to the *vrs*1 locus in barley, *Hordeum vulgare*. Genome 41:680–685

Konishi T (1995) Geographical diversity of isozyme genotypes in barley. Kyushu University Press, Fukuoka, Japan

Liu CT, Wesenberg DM, Hunt CW, Branen AL, Robertson LD, Burrup DE, Dempster KL, Haggerty RJ (1996) Hulless barley: a new look for barley in Idaho. Resources for Idaho http://info.ag.uidaho.edu/

Rossnagel BG (2000) Hulless barley—western Canada's corn. In: Logue S (ed) Barley genetics VIII. Proceedings of the 8th International Barley Genetics Symposium, pp 135–142

Salamini F, Özkan H, Brandolini A, Schäfer-Pregl R, Martin W (2002) Genetics and geography of wild cereal domestication in the Near East. Nat Rev Genet 3:429–441

Scholz F (1955) Mutationsversuche an Kulturpflanzen. IV. Über den züchterischen Wert zwei röntgeninduzierter Gerstenmutanten. Kulturpflanze 3:69–89

Strelchenko P, Kovalyova O, Okuno K (1999) Genetic differentiation and geographical distribution of barley germplasm based on RAPD markers. Genet Resour Crop Evol 46:193–205

Takahashi R (1955) The origin and evolution of cultivated barley. Adv Genet 7:227–266

Takahashi R, Yamamoto J (1950) Studies on the classification and the geographical distribution of the Japanese barley varieties. 13. The hulled and naked caryopsis character (in Japanese). Nogaku Kenkyu 39:32–36

Takahashi R, Inamura H, Matsumoto T (1962) Effects of the genes for covered and naked kernels on some agronomic characters in barley. I. Comparison of two covered barleys and their naked mutants. Ber Ohara Inst lndw Biol Okayama Univ 11:385–392

Tanno K, Taketa S, Takeda K, Komatsuda T (2002) A DNA marker closely linked to the *vrs1* locus (row-type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theor Appl Genet 104:54–60

Vos P, Hogers R, Bleeker M, Reijans M, Lee TVD, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Zohary D (1963) Spontaneous brittle six-rowed barley, their nature and origin. Barley genetics I. Proceedings of the 1st International Barley Genetics Symposium, Pudoc, Wageningen, The Netherlands, pp 27–31

Zohary D, Hopf M (2000) Domestication of plants in the old world: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley. Clarendon Press, Oxford