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ORIGINAL PAPER

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On the origin of six-rowed barley with brittle rachis, *agriocrithon* [*Hordeum vulgare* ssp. *vulgare* f. *agriocrithon* (Åberg) Bowd.], based on a DNA marker closely linked to the *vrs1* (six-row gene) locus

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Abstract The origin of six-rowed cultivated barley has been revealed to be more complex since the discovery of *agriocrithon*, a six-rowed barley with brittle rachis. The present study investigates whether such six-rowed brittle barley is wild or hybrid in nature, by analyzing genetic diversity at the cMWG699 marker locus, which is closely linked to the *vrs1* (six-row gene) locus. DNA sequence analysis for 42 accessions showed only three types in six-rowed brittle barleys; in contrast, nine sequence types were found in ten wild barleys, ssp. *spontaneum*, in our previous study. Nucleotide diversities for the six-rowed brittle barley were 2.8–4.5 times lower than that for the ssp. *spontaneum* at this marker locus. The three sequence types found in the six-rowed brittle barley also appeared in the six-rowed cultivated barley. A cross-allelism test confirmed that the six-rowed character of the six-rowed brittle barley was controlled by the *vrs1* locus. The nucleotide diversity and genealogy demonstrated that f. *agriocrithon* does not have the same level of diversity as found in wild barley, ssp. *spontaneum*. Consequently, f. *agriocrithon* does not appear to represent genuinely wild populations, but more probably originated from hybridization between ssp. *spontaneum* and six-rowed cultivated barley.

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Introduction

There is no doubt that two-rowed cultivated barley was domesticated from wild barley, *Hordeum vulgare* ssp. *spontaneum*. However, the origin of six-rowed cultivated barley is unclear, because of the discovery of six-rowed barley with brittle rachis, viz. *H. agriocrithon* (Åberg 1938).

In 1938, Åberg found barley kernels in a wheat sample collected by H. Smith from Taofu in China and obtained six-rowed brittle barley after growing the kernels (Åberg 1938). He insisted that the six-rowed brittle barley was the progenitor of six-rowed cultivated barley and named it *H. agriocrithon* (Åberg 1940). This conclusion became widely accepted. This led to Freisleben's diphyletic hypothesis (Freisleben 1940), in which six-rowed cultivated barley was derived from six-rowed brittle barley in eastern Asia, while two-rowed cultivated barley was derived from hybridization between the cultivated six-rowed barley and ssp. *spontaneum* in western Asia. However, progeny of six-rowed brittle barley collected from Israel showed segregation in several morphological characters, suggesting high heterogeneity (Zohary 1959, 1960). Zohary (1963) insisted that six-rowed brittle barley was the result of a hybrid between domesticated six-rowed barley and wild two-rowed barley (ssp. *spontaneum*).

A few phylogenetic studies using biochemical and DNA markers were carried out for f. *agriocrithon*. Zhang et al. (1994) investigated esterase isozymes and rDNA variations in cultivated barley, *H. spontaneum* and *H. agriocrithon*. They found a differentiation between eastern and western populations and insisted that these results supported Freisleben's diphyletic hypothesis. Konishi (2001) insisted that the *H. agriocrithon* is a hybrid of ssp. *spontaneum* and six-rowed cultivated barley. His argument is based on an investigation of physiological and morphological characters and a combination of esterase alleles. Yin et al. (2003) analyzed Hordein variation and found that diversity in

Tibetan *H. agriocrithon* is almost equal to that of ssp. *spontaneum* in the Middle East, and they regard them as a wild relative of barley. In biochemical and molecular analysis, there is no congruence whether f. *agriocrithon* is wild or hybrid. For better understanding of the evolution of barley, it is necessary to clarify the origin of f. *agriocrithon*.

In the present study, we aimed to clarify the origin of the six-rowed character of f. *agriocrithon*. Since the six-row gene (the *vrs1* locus) has not yet been cloned, we analyzed nucleotide diversity of a DNA marker closely linked to the *vrs1* locus. In cultivated barley, we investigated the cMWG699 marker, which lies 0.1cM map distance from the *vrs1* locus (Komatsuda et al. 1999). The recombination rate is very low (about 1/1000 per one outcross), which indicates that cMWG699 and the *vrs1* locus appear to inherit together at high probability. Our previous DNA sequence study of the cMWG699 (Tanno et al. 1999, 2002) revealed considerably less variation in six-rowed cultivated barley compared with two-rowed cultivated barley and ssp. *spontaneum*, supporting the two-rowed progenitor hypothesis in which six-rowed barley was derived from a mutation from two-rowed barley (Harlan 1995). Therefore, if f. *agriocrithon* shows low variation as in six-rowed cultivated barleys, this would suggest that it was a hybrid; while if it had large variations similar to ssp. *spontaneum*, this would indicate that it is genuine wild barley.

Materials and methods

Plant materials

In this study, we analyzed 42 six-rowed brittle barleys (Table 1) that are germplasms of the Barley Germplasm Center, Research Institute for Bioresources, Okayama University, Japan.

Table 1 Plant materials and the result of sequence analysis of cMWG699 and cross-allelism test for the six-row allele

Accession	Classification	Origin	Sequence type[a]	Allele of the *vrs1* in F_1[b]
OUH641	*lagunculiforme*	Cyprus	I	NT
OUH662	*lagunculiforme*	Former Soviet Union	I	*vrs1a*
OUH663	*lagunculiforme*	Former Soviet Union	I	*vrs1a*
OUH765	*agriocrithon*	Tibet (China)	I I I	*vrs1a*
OUH768	*dawoense*	Tibet (China)	I I I	NT
OUH785	*agriocrithon*	Tibet (China)	I	*vrs1a*
OUH786	*agriocrithon*	Tibet (China)	I I I	NT
OUH787	*agriocrithon*	Tibet (China)	I	NT
OUH788	*agriocrithon*	Tibet (China)	I I	NT
OUH789	*agriocrithon*	Tibet (China)	I	*vrs1a*
OUH790	*agriocrithon*	Tibet (China)	I	*vrs1a*
OUH791	*agriocrithon*	Tibet (China)	I I I	*vrs1a*
OUH793	*dawoense*	Tibet (China)	I I I	NT
OUH795	*dawoense*	Tibet (China)	I I I	NT
OUH796	*dawoense*	Tibet (China)	I I I	NT
OUH797	*agriocrithon*	Tibet (China)	I	*vrs1a*
OUH799	*agriocrithon*	Tibet (China)	I	*vrs1a*
OUH800	*dawoense*	Tibet (China)	I I I	NT
OUH801	*dawoense*	Tibet (China)	I I I	NT
OUH802	*agriocrithon*	Israel	I	NT
OUH803	*agriocrithon*	Israel	I	*vrs1a*
OUH804	*agriocrithon*	Israel	I	*vrs1a*
OUH805	*agriocrithon*	Tibet (China)	I I	NT
OUH806	*agriocrithon*	Former Soviet Union	I	*vrs1a*
OUH807	*agriocrithon*	Former Soviet Union	I	*vrs1a*
OUH808	*paradoxon*	Tibet (China)	I I	*vrs1a*
OUH809	*paradoxon*	Tibet (China)	I	NT
OUH810	*paradoxon*	Tibet (China)	I	NT
OUH811	*paradoxon*	Former Soviet Union	I I I	NT
OUH812	*paradoxon*	Former Soviet Union	I I I	NT
OUH813	*paradoxon*	Former Soviet Union	I I I	NT
OUH814	*paradoxon*	Former Soviet Union	I	*vrs1a*
OUH815	*paradoxon*	Former Soviet Union	I I I	*vrs1a*
OUH816	*paradoxon*	Former Soviet Union	I I I	*vrs1a*
OUH817	*paradoxon*	Former Soviet Union	I I I	*vrs1a*
OUH818	*paradoxon*	Former Soviet Union	I I I	*vrs1a*
OUH819	*agriocrithon*	Former Czechoslovakia	I	*vrs1a*
OUH820	*agriocrithon*	Tibet (China)	I I I	*vrs1a*
OUH821	*agriocrithon*	Tibet (China)	I I	*vrs1a*
OUH822	*agriocrithon*	Tibet (China)	I I I	NT
OUH823	*agriocrithon*	Tibet (China)	I	*vrs1a*
OUH824	*agriocrithon*	Tibet (China)	I I I	*vrs1a*

NT Not tested
[a]Sequence types I and II are correspondent with our previous study (Tanno et al. 2002)
[b]Allelism of the *vrs1* is confirmed by cross to a six-rowed cultivar 'Akashinrik'. *vrs1a* is a six-rowed allele of the *vrs1* locus

Six-rowed brittle barley has been subdivided into five types: "*agriocrithon*", with sessile spikelets; "*lagunculiforme*", with pedicel spikelets; "*paradoxon*", without awns; "*dawoense*", having awns only on the central spikelets; and "*nudum*", with naked caryopses. However, taxonomists do not agree on the classification of these types. In some cases, all the six-rowed brittle barleys were incorporated into a single f. *agriocrithon* complex, which is the taxonomic level we chose for the purpose of this study.

PCR amplification, DNA sequencing, and data analysis

The PCR amplification of cMWG699 was performed using the same method as in our previous study (Tanno et al. 2002). A dideoxytermination cycle sequence using the primers cMWG699T3-2 and cMWG699T7-2 was performed with an automated DNA sequencer ABI310 (Applied Biosystems, PerkinElmer).

All sequence data from the 42 accessions were used for a phylogenetic analysis, and sequences of 14 cultivated six-rowed barleys and ten examples of ssp. *spontaneum* obtained from Tanno et al. (2002) were added to the data of f. *agriocrithon* obtained in this study. The sequences were aligned manually in NEXUS files. Nucleotide diversities, Nei's π (Nei 1987) and Watterson's θ (Watterson 1975), were estimated using the DnaSP, version 3.52, software program (Rozas and Rozas 1999). The relationships between f. *agriocrithon*, six-rowed cultivated barleys, and ssp. *spontaneum* were obtained using the software program TCS (Clement), which uses a statistical parsimony approach to estimate gene genealogies (Posada and Crandall 2001). Because the peaks given by dye-deoxyterminator sequence were not stable near the primer region, 25 and 45 bases following the primer cMWG699T7-2 and T3-2, respectively, were deleted from the analysis. All the phylogenetic analyses for sequences were therefore performed with data from 823 bp.

PCR-RFLP analysis

In the sequence analysis, we found a new sequence type, named type III, which was the result of a single nucleotide polymorphism (SNP). Since this SNP exhibited a restriction enzyme *Bgl*II cleavage site, PCR-RFLP analysis was carried out on the 280 cultivated barleys that were used in the previous study (Tanno et al. 2002). The PCR product of cMWG699 was digested with restriction enzyme *Bgl*II and was observed under UV after 1.8% agarose gel electrophoresis containing ethidium bromide.

Cross-allelism test

To confirm that the six-rowed character of six-rowed brittle barley was really allelic to the *vrs1*, cross-hybridization was performed. The six-rowed brittle barleys sited in Table 1 were crossed with the pollen of six-rowed cultivated barley 'Akashinriki' (*vrs1a* allele of the *vrs1* locus). The fertility of the spikelets (row-type, two or six rowed) of F_1 plants was observed.

Results

DNA sequencing analysis

The results of sequence analysis (Table 1) showed three types in 42 accessions of f. *agriocrithon*. Two sequences were the same as for the types I and II in our previous study of six-rowed cultivated barley (Tanno et al. 2002). The new third sequence found in this study was named type III sequence and varied by only one SNP from the type I sequence. Figure 1 shows the nucleotide substitution site of the type III sequence with data from the previous study of six-rowed cultivated barley and ssp. *spontaneum* (Tanno et al. 2002). The SNP of the type III sequence was a transitional nucleotide substitution from A to G at the 730th nucleotide site.

The type III sequence was found in 19 accessions out of 42 f. *agriocrithon*, and its geographical distribution was limited to Tibet and what was recorded as the former Soviet Union (Table 1). Types I and II sequences were found in 19 and four accessions, respectively. Type I sequence was distributed widely from Tibet in the east to former Czechoslovakia in the west, which corresponded well with the geographical distribution of the type I sequence of the six-rowed cultivated barley in our previous study (Tanno et al. 2002). All four accessions of type II sequence were found in Tibet (Table 1).

SNP detection by PCR-RFLP analysis in cultivated barley

In our previous study of cultivated barley (Tanno et al. 2002), we did not find the sequence type III. Since the SNP found in the type III sequence created a restriction enzyme *Bgl*II cleavage site, PCR-RFLP analysis was carried out to investigate whether the SNP site is found in cultivated barley or not. We analyzed 280 cultivated barley accessions that are the same as those used in our previous study. The result showed that 23 out of 204 six-rowed cultivated barleys had a cleaved type of the SNP site that corresponded to the type III sequence. In two-rowed cultivated barley, there was no cleaved type among 76 accessions.

Figure 2 shows the geographical distribution of the *Bgl*II polymorphisms in the cultivated six-rowed barley. The cleaved type of *Bgl*II was distributed in the east of Nepal. This type was not common in Japan (20% of 30 accessions), while it was frequent in Tibet (27% of 11 accessions) and Nepal (43% of 21 accessions).

Fig. 1 Nucleotide substitution site at cMWG699 in f. *agriocrithon*. The data of six-rowed cultivated barleys and ssp. *spontanum* were taken from Tanno et al. (2002), except for two accessions of type III sequence

	Nucleotide substitution site
Sequence type	0 1 2 2 2 2 3 4 4 4 5 5 5 5 7 7 7 6 7 4 5 8 9 2 4 4 8 0 0 2 3 3 3 3 4 2 2 4 9 7 5 0 8 0 0 9 7 9 0 2 5
Consensus sequence	**C C C T A A C T G T C T C T A T T**
f. *agriocrithon*	
Type I (19 accessions)	. . . . .
Type II (4 accessions)	. . . . . **C A** . . . **T** . . . **C**
Type III (19 accessions)	. . . . . **G** . .
Six-rowed cultivated barley	
Type I (10 accessions)	. . . . .
Type II (3 accessions)	. . . . . C A . . . T . . . C
Type III (2 accessions)	. . . . . G . .
ssp. *spontaneum*	
OUH730 (Turkmenistan)	. . . . .
OUH644 (Turkmenistan)	. . . . . T . . C . C .
OUH630 (Afghanistan)	. T . . . . .
OUH729 (Iran)	. . . C . . . . .
OUH728 (Iran)	. . . . - . . . . .
OUH742 (Iraq)	. . . . . T . . . . .
OUH707 (Iraq)	T . . . . G . . . C . G . . . .
OUH726 (Turkey)	. . T . . . . C A . . . . .
OUH638 (Jordan)	. . . . - . . . . .
OUH777 (Morocco)	. . . . . C A . . . T . . . C

To confirm whether the sequence of the cleaved type of *Bgl*II in PCR-RFLP analysis is similar to the type III sequence found in f. *agriocrithon*, we analyzed the DNA sequence of 'Hayakiso 2' from Japan and 'Sama 1' from Nepal; both of them showed the cleaved type of *Bgl*II. The results show that their sequences were identical to the type III sequence. Therefore, all three types of sequences found in f. *agriocrithon* were also found in six-rowed cultivated barley.

Comparison with ssp. *spontaneum* and six-rowed cultivated barley

The relationships between the sequences of f. *agriocrithon*, ssp. *spontaneum*, and six-rowed cultivated barley were calculated, and the resulting genealogy is given in Fig. 3 (sequences of ssp. *spontaneum* accessions taken from Tanno et al. 2002). The 42 accessions of f. *agriocrithon* located at three points are denoted by circles

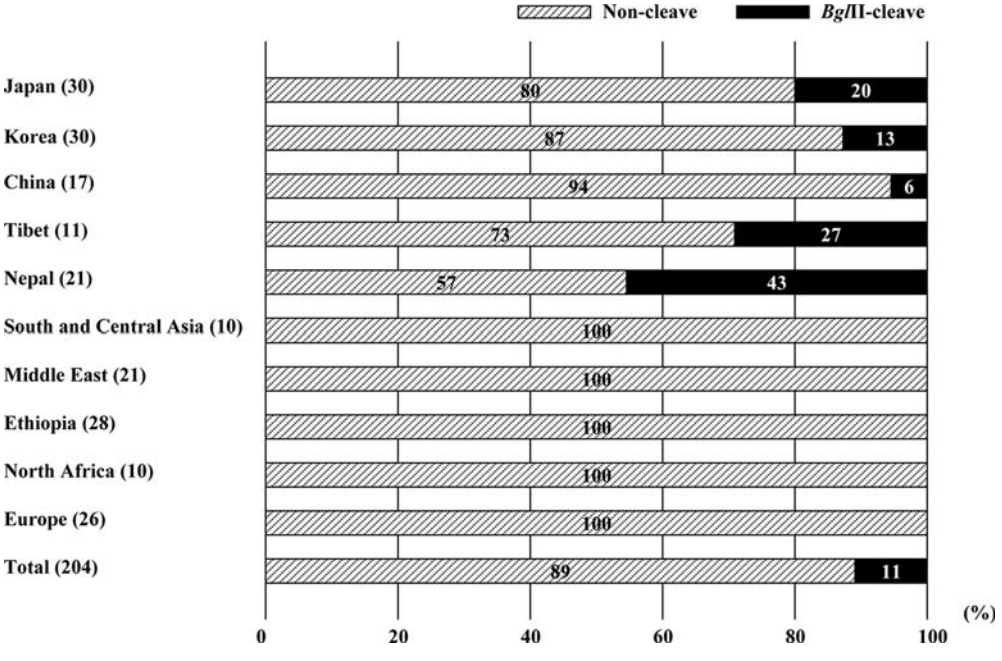

Fig. 2 Geographical distribution of *Bgl*II-cleave type of cMWG699 locus in six-rowed cultivated barley. The *Bgl*II-cleave type was distributed in the east of Nepal and not found in two-rowed cultivated barley. Tibet was dealt with separately from other areas of China

(types I, II, and III), and they occur at the same positions as the six-rowed cultivated barleys, denoted by squares (Fig. 3). On the other hand, ten accessions of ssp. *spontaneum*, denoted by triangles, show nine alleles and they are well scattered with maximum differences of seven nucleotide substitutions. The genealogy within f. *agriocrithon* was considerably different from that of ssp. *spontaneum*. The genealogy did not show a network distribution, which implies that recombination probably did not happen at this locus.

To evaluate the difference in variation between f. *agriocrithon* and ssp. *spontaneum*, the nucleotide diversity was calculated by Nei's π (Nei 1987) and Watterson's θ (Watterson 1975) estimations (the data of ssp. *spontaneum* was also from Tanno et al. 2002). As shown in Table 2, the values of f. *agriocrithon* were $\pi = 0.0014$, $\theta = 0.0014$, about 2.8 times and 4.5 times lower than those of ssp. *spontaneum* ($\pi = 0.0040$, $\theta = 0.0064$); therefore, f. *agriocrithon* did not have the same level of diversity as seen in the wild species. If we calculate the nucleotide diversity in six-rowed cultivated barley, assuming that all the 204 six-rowed cultivated barleys are classified in one of the three sequence types (i.e., 174, 7, and 23 accessions for the sequence type I, II, and III), then the diversity values are $\pi = 0.00057$, $\theta = 0.00103$. These values are considerably lower than those for ssp. *spontaneum* ($\pi = 0.00403$, $\theta = 0.00645$) and are closer to those of f. *agriocrithon* ($\pi = 0.00147$, $\theta = 0.00141$). As we have seen, both the genealogy (Fig. 3) and the nucleotide diversity (Table 2), demonstrate that f. *agriocrithon* does not have the same level of diversity as found in wild barley, ssp. *spontaneum*.

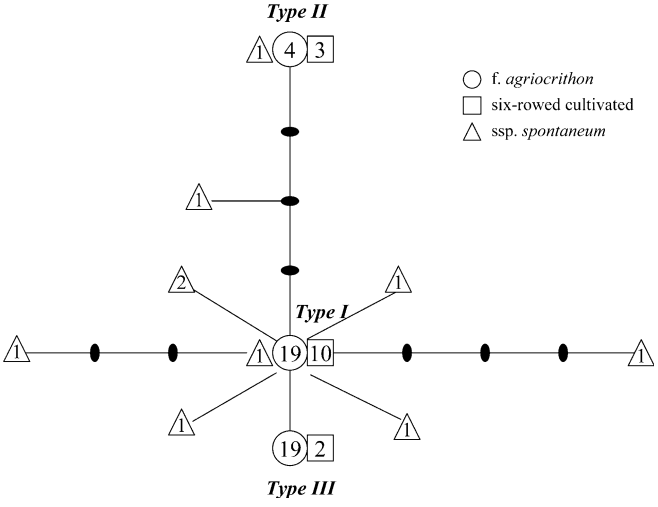

Fig. 3 Genealogy of f. *agriocrithon*, ssp. *spontaneum*, and six-rowed cultivated barley cMWG699 sequences. Sequence types are represented by *circles* (f. *agriocrithon*), *triangles* (ssp. *spontaneum*) and *squares* (six-rowed cultivated barley); the number of times each sequence type was observed is indicated by the *number inside the marks*. *Lines* connecting the sequence types represent a single nucleotide substitution with *filled circles* representing inferred mutational steps

Cross-allelism test

If the six-rowed trait of f. *agriocrithon* is not controlled by the *vrs1* locus, the present study of the linkage marker cMWG699 will not be viable. We therefore crossed brittle six-rowed with cultivated six-rowed barleys to confirm the allelism of the six-row gene using a Japanese six-rowed cultivated barley 'Akashinriki' as a paternal parent. The results are given in Table 1; all the F_1 plants which were crossed had six-rowed spikes. Therefore, the row-type of the f. *agriocrithon* tested here was under the control of the same gene, *vrs1a* gene.

Discussion

In the present study, f. *agriocrithon* presents low nucleotide diversity compared with that of ssp. *spontaneum* (Table 2). This result disagrees with those obtained from morphological study (Konishi 2001), from biochemical study using Esterase (Zhang et al. 1994), and from electrophoresis of Hordein (Yin et al. 2003), which showed high variation in both f. *agriocrithon* and ssp. *spontaneum*. On the other hand, low genetic variation was found in both f. *agriocrithon* and ssp. *spontaneum* in rDNA loci compared with cultivated barley (Zhang et al. 1994). The results of rDNA analyses correspond to our present study in that f. *agriocrithon* had very low variation. The low diversity found in the present study is probably due to the hitchhiking effect of the bottleneck at the *vrs1* locus in six-rowed cultivated barley. If f. *agriocrithon* was truly a wild species, it should have more variation in the cMWG699 marker locus. As the result was the opposite, it suggests that f. *agriocrithon* is not a genuine wild barley.

In our present study, gene genealogy of cMWG699 (Fig. 3) indicated that f. *agriocrithon* shared the same three alleles with six-rowed cultivated barleys, while ssp. *spontaneum* showed considerable divergence. So far, many researchers working on barley have pointed out that six-rowed brittle barley occurred as weeds in cultivated fields (Bothmer et al. 1995; Shao and Li 1987; Zohary 1960). The three alleles of cMWG699 in f. *agriocrithon* were probably introgressed from six-rowed cultivated barley accompanied by the six-row character. The hybrid nature of f. *agriocrithon* was also demonstrated from esterase isozyme by Konishi (2001).

Table 2 Nucleotide diversity of f. *agriocrithon* and ssp. *spontaneum* in cMWG699 marker locus (nucleotide length = 823 bp)

	No. of accessions	No. of alleles	No. of segregating sites	Nucleotide diversity	
				π/bp[a]	θ/bp[b]
f. *agriocrithon*	42	3	5	0.0014	0.0014
ssp. *spontaneum*	10	9	15	0.0040	0.0064

[a] π Nei's estimate
[b] θ Watterson's estimate

In his study, alleles of *Est1*, *Est2*, and *Est4* showed particular haplotypes (*Est1-Est2-Est4*: *Ca-Un-Nz*, *Pr-Fr-At*, and *Pr*-it Fr-*Su*) in f. *agriocrithon* in Tibet, and the same haplotypes were also found in six-rowed cultivated barley in this area but not found in ssp. *spontaneum*. This agrees with the conclusions of our present study, which indicate that the genotype of f. *agriocrithon* is similar to six-rowed cultivated barley and differs from ssp. *spontaneum*.

For cultivated barley, the type II sequence was found in the western Mediterranean coastal area, and not found in East Asia in our previous study, which had a good sample size (280 cultivated barley accessions, Tanno et al. 2002). In this study, the type II sequence was found in the f. *agriocrithon* population from Tibet. Why the Tibetan f. *agriocrithon* had the type II sequence (Table 1) was unknown. Out of four Tibetan f. *agriocrithon* accessions, two were from very old collections (OUH808 collected at Lhasa in 1938 and OUH788 collected at Tsela Dzong in 1947), although for two (OUH805 and OUH821), the collection sites and year were not known.

The type III sequence differed from the type I sequence by a SNP. The results of PCR-RFLP analysis in cultivated barleys (Fig. 2) showed clear geographical distribution of the SNP of the type III sequence. Since this type was not found in two-rowed cultivated barley, the SNP of the type III sequence was probably an acquired nucleotide substitution from the type I sequence of six-rowed cultivated barley in these areas (from Nepal to Japan). The geographical distribution of *Bgl*II-cleave type in cultivated barley corresponded to that of ''Oriental'' characters given by Takahashi (1955). In particular, the characters of reduced lateral awns correlated to the distribution of the *Bgl*II-cleave type, and it is reported as an allelic of the *vrs1* locus (*vrs1c*, Lundqvist et al. 1997). We therefore observed the ears of the six-rowed cultivated accessions, but no relationship was seen between the awn type and the *Bgl*II-cleave type.

According to Dr. G. Willcox, an archaeobotanist in France, ssp. *spontaneum* from Djebel Bishiri, Syria, showed segregation with the appearance of six-rowed brittle types in experimental plots (Dr. G. Willcox, personal communication). The present author observed the kernels of the segregated plants, which were sessile kernels and therefore of f. *agriocrithon* type. This appears to be good evidence for the hybrid nature of six-rowed brittle barley. Thus, there is actually a potential to for the appearance of six-rowed brittle barley in nature.

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References

Åberg E (1938) *Hordeum agriocrithon* nova sp., a wild six-rowed barley. Ann Agric Coll Sweden 6:159–212

Åberg E (1940) The taxonomy and phylogeny of *Hordeum* L. Sect. Cerealia Ands. Symbolae Bot Upsalienses 4:1–156

Bothmer R von, Jacobsen N, Baden C, Jørgensen RB, Linde-Laursen I (1995) An ecogeological study of the genus *Hordeum*. Systematic and ecogeographic studies on crop genepools 7, 2nd edn. International Plant Genetic Resources Institute, Rome

Freisleben R (1940) Die phylogenetische Bedeutung asiatisher Gersten. Zuchter 12:257–272

Harlan JR (1995) Barley. In: Smartt J, Simmonds NW (eds) Evolution of crop plants, 2nd edn. Longman, London, pp 140–147

Komatsuda T, Li W, Takaiwa F, Oka S (1999) High resolution map around the *vrs1* locus controlling two- and six-rowed spike in barley, *Hordeum vulgare*. Genome 42:248–253

Konishi T (2001) Genetic diversity in *Hordeum agriocrithon* E. Åberg, six-rowed barley with brittle rachis, from Tibet. Genet Res Crop Evol 48:27–34

Lundqvist U, Franckowiak J, Konishi T (1997) BGS6; six-rowed spike 1. Barley Genet Newsl 26:49–50

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Posada D, Crandall KA (2001) Intraspecific gene genealogies: trees grafting into networks. Trends Ecol Evol 16:37–45

Rozas J, Rozas R (1999) DnaSP version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15:174–175

Shao Q, Li A (1987) Unity of genetic population for wild barley and cultivated barley in Himalaya area. In: Yasuda S, Konishi T (eds) Barley genetics V. Sanyo, Okayama, pp 35–41

Takahashi R (1955) The origin and evolution of cultivated barely. In: Demerec M (ed) Advances in genetics 7. Academic, New York, pp 227–266

Tanno K, Takaiwa F, Oka S, Komatsuda T (1999) A nucleotide sequence linked to the *vrs1* locus for studies of differentiation in cultivated barley (*Hordeum vulgare* L.). Hereditas 130:77–82

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

Watterson GA (1975) On the number of segregating sites in genetical models without recombination. Theor Popul Biol 7:256–276

Yin YQ, Ma DQ, Ding Y (2003) Analysis of genetic diversity of hordein in wild close relatives of barley from Tibet. Theor Appl Genet 107:837–842

Zhang Q, Yang GP, Dai X, Sun JZ (1994) A comparative analysis of genetic polymorphisms in wild and cultivated barley from Tibet using isozyme and ribosomal DNA markers. Genome 37:631–638

Zohary D (1959) Is *Hordeum agriocrithon* Åberg the ancestor of six-rowed cultivated barley? Evolution 13:279–280

Zohary D (1960) Studies on the origin of cultivated barley. Bull Res Counc Israel 9D:21–42

Zohary D (1963) Spontaneous brittle six-rowed barley, their nature and origin. Barley genetics I. In: Proceedings of the 1st international barley genetics symposium, Wageningen, pp 27–31