Theor Appl Genet (2005) 110: 995–1002
DOI 10.1007/s00122-004-1839-x

ORIGINAL PAPER

N. Kubo · B. Salomon · T. Komatsuda · R. von Bothmer
K. Kadowaki

Structural and distributional variation of mitochondrial *rps2* genes in the tribe Triticeae (Poaceae)

Received: 12 May 2004 / Accepted: 10 October 2004 / Published online: 8 March 2005

Abstract The mitochondrial *rps2* gene from barley, like that of rice, wheat, and maize, has an extended open reading frame (ORF) at the 3′-region when compared to that from lower plants. However, the extended portions are variable among these cereals. Since barley and wheat belong to the same tribe (Triticeae), it would be interesting to know when and where the two types of *rps2* were generated during evolution. To determine this, we utilized the mitochondrial (mt) DNA sequence to examine variations of the *rps2* genes in the tribe Triticeae. By means of the variable 3′-region, the distribution of barley (B)-type and wheat (W)-type *rps2* sequences was studied in 19 genera of the tribe. The B-type sequence was identified in 10 of the 19 genera, whereas the W-type sequence was present in all 19 genera. Thus, ten of the examined genera have both types of *rps2* sequences due to the presence of two copies of the gene. The W-type sequence was also present in the tribe Bromeae and the B-type sequence was also found in Aveneae and Poeae. Phylogenetic trees based on the B-type and W-type sequences were different from those based on other molecular data. This suggests that the mitochondrial genome in Triticeae has a unique evolutionary history.

Electronic Supplementary Material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00122-004-1839-x

Communicated by R. Hagemann

N. Kubo · T. Komatsuda · K. Kadowaki
Genetic Diversity Department, National Institute of Agrobiological Sciences, Tsukuba 305-8602, Japan

B. Salomon · R. von Bothmer
Department of Crop Science, The Swedish University of Agricultural Sciences, 23053 Alnarp, Sweden

Present address: N. Kubo (✉)
Graduate School of Agriculture,
Kyoto Prefectural University,
Kyoto Prefectural Institute of Agricultural Biotechnology,
74 Oji, Kitainayazuma, Seika, Soraku 619-0244, Japan
E-mail: nk0103@kab.seika.kyoto.jp
Tel.: +81-774-933526
Fax: +81-774-933528

Introduction

Plant mitochondria have several unique characters compared to those of the vertebrates, such as a large genome, the presence of plasmid-like DNA, and specific modes of gene expression (for example, RNA editing and *trans*-splicing; see Binder and Brennicke 2003 for a review). The large genome size of plant mitochondria is due to the presence of repeated sequences and foreign DNA. Frequent recombination events among the repeated sequences have further increased the complexity of the genome, resulting in the generation of multiple subgenomic molecules, also called a ''multipartite structure''. Some genes acquire extra sequences through structural rearrangements (see Marienfeld et al. 1997 for an example).

In cereals, the gene for the ribosomal protein S2 (*rps2*) appears to have a sequence extension. The *rps2* gene has been isolated from the mitochondrial genomes of monocots such as rice, wheat, and maize (Itadani et al. 1994; Vaïtilingom et al. 1998; Perrotta et al. 2002), whereas it seems to be absent from the mitochondrial genomes of dicot plants, in which it is probably encoded by the nuclear gene (Adams et al. 2002). The *rps2* genes of monocots have 3′-extensions compared to that of liverwort (Oda et al. 1992). This 3′-extension shows no sequence homology among rice, wheat, and maize and, moreover, the 3′-extended region of the wheat and maize *rps2* genes has no homology to any other known sequences (Vaïtilingom et al. 1998; Perrotta et al. 2002), whereas that of the rice *rps2* gene contains a sequence homologous to a part of the *atpA*-coding region (Itadani et al. 1994). This suggests that these regions were acquired independently from different origins and that they have no important role in gene expression.

Therefore, it could be expected that the *rps2* genes of other grasses have novel extended sequences.

We previously examined the phylogeny of cultivated barley and related wild species using nuclear and chloroplast (cp)DNA (Komatsuda et al. 1999; Nishikawa et al. 2002). The tribe Triticeae includes some of the world's agriculturally most important cereals such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.). However, information on the evolution of these cereals at the molecular level is limited (see Judd et al. 1999 for a review). The objectives of the investigation reported here were: (1) to isolate the *rps2* gene from cultivated barley mitochondria and to see whether it has an extended sequence in the 3′-region like that of rice, wheat, and maize, and (2) to examine the distribution and diversity of the *rps2* sequences in other *Hordeum* species and in representatives of other genera of the Triticeae.

Materials and methods

Plant materials and DNA extraction

A cultivated barley Kanto Nakate Gold, 11 wild species, including the four basic genomes of the genus *Hordeum* (Table 1), 18 species of other genera found in the Triticeae, and five species representing the tribes Bromeae, Aveneae, Poeae, Oryzeae, and Andopogoneae (Table 2) were used in this investigation. Total DNA was isolated from young seedlings as described previously (Komatsuda et al. 1998). Voucher specimens and/or seeds are kept at the Department of Crop Science, The Swedish University of Agricultural Sciences.

Construction and screening of a mitochondrial DNA library

A library was constructed from mitochondrial (mt)DNA of barley, as described previously (Kadowaki et al. 1996). A 607-bp *Fok*I fragment of the rice *rps2* gene (Itadani et al. 1994) was used as a probe for screening. The final washing of membranes was performed with 0.5× SSC and 0.1% sodium dodecyl sulfate (SDS) at 42°C.

Table 1 List of *Hordeum* species and their accession numbers used for intrageneric analysis

Species	Accession or cultivar	Genome type[a]
H. vulgare ssp. *vulgare*	Kanto Nakate Gold	I
H. bulbosum	H3878	I
H. murinum ssp. *glaucum*	H10260	Xu
H. intercedens	H2310	H
H. bogdanii	H9215	H
H. brachyantherum		
ssp. *californicum*	H2419	H
ssp. *brachyantherum*	H2420	H
H. capense	H334	H
H. depressum	H2005	H
H. secalinum	H296	H
H. marinum		
ssp. *marinum*	H0547	Xa
ssp. *gussoneanum*	H2303	Xa

[a]Genome symbols from Wang et al. (1996)

Table 2 List of taxa analyzed, their accession numbers and result of PCR analysis

Tribes: Species:	Accession or cultivar	B-type[a]	W-type[a]
Triticeae			
Aegilops tauschii	H10257	–	+
Agropyron cristatum	H10154	–	+
Australopyrum pectinatum	H4202	–	+
Dasypyrum villosum	H3128	–	+
Elymus caninus	H3169	+	+
Eremepyrum bonaepartis	H10242	+	+
Henrardia persica	H5556	–	+
Hordeum vulgare ssp. *vulgare*	Kanto Nakate Gold	+	+
Leymus alaicus	H10272	+	+
Lophopyrum nodosum ssp. *dorudicum*	H3714	+	+
Peridictyon sanctum	H3841	–	+
Psammopyrum athericum	H3799	+	+
Psathyrostachys juncea	H10108	+	+
Pseudoroegneria libanotica	H3729	+	+
Secale cereale	H10207	+	+
Stenostachys gracilis	H10624	+	+
Taeniatherum caput-medusae	H10282	–	+
Thinopyrum bessarabicum	H6712	–	+
Triticum aestivum	Bando Wase	–	+
Bromeae:			
Bromus arvensis	NGB2364	–	+
Aveneae:			
Avena sativa	Haeibuki	+	–
Poeae:			
Lolium multiflorum	Dryann	+[b]	–
Oryzeae:			
Oryza sativa ssp. *japonica*	Nipponbare	–	–
Andopogoneae:			
Zea mays	Na23	–	–

[a]+, PCR product detected; –, no PCR product detected
[b]Nucleotide sequence was not determined

Northern and RT-PCR analyses

Total RNA was extracted from etiolated seedlings of barley, electrophoresed, and blotted onto a nylon membrane as described previously (Kadowaki et al. 1996). The 5′-portion of barley *rps2* was labeled with digoxigenin-dUTP (Roche Diagnostics, Indianapolis, Ind.) and hybridized at 42°C overnight. The final washing of the membrane was performed with 0.1× SSC and 0.1% SDS at 42°C.

cDNA was synthesized from DNase I-treated RNA as described previously (Kubo et al. 1996). Resultant cDNAs were used for PCR amplification with primer pairs P1/P3 and P6/P7. The amplified products were sequenced directly.

Amplification of mtDNA fragments

Parts of the *rps2*-coding region were amplified by PCR with primer pairs P2/P3, P2/P4 (specific for barley), and P2/P5 (specific for wheat). For phylogenetic analysis, primer pairs P1/P3, P1/P4, and P1/P5 were used to amplify larger parts of the *rps2* gene because of low levels of sequence divergence. The reaction was performed with AmpliTaq GOLD DNA polymerase (Applied Biosystems, Foster City, Calif.) in a GeneAmp 9700 thermal cycler (Applied Biosystems) as follows: pre-incubation at 95°C for 9 min followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 1 min, and extension at 72°C for 1 min. The final extension was performed at 72°C for 5 min. Amplified DNA fragments were sequenced directly.

Oligonucleotide primers

The primers used in this study are as follows:

P1: 5′-GTCTGTACTAAATTACTTTGTACGAATG-3′
P2: 5′-CTTCGGTCATACTGGAAGCT-3′
P3: 5′-TGCAAACTTAAAGGTATAGCTG-3′
P4: 5′-GTTCTCATGGGGTTCAGTCT-3′
P5: 5′-CGTCTTCCAAATCCAATTTCAG-3′
P6: 5′-CACTCCACCTCTTCAACCCGA-3′
P7: 5′-CCAACTAACTCTTCAAATGAAGAACT-3′

The locations of primers P1–P4, P6, and P7 are shown in Figs. 1 and 2a. The primer P5 was designed from the 3′-portion of the wheat *rps2* gene (positions 1,912–1,933) (Vaïtilingom et al. 1998; Fig. 2a).

DNA sequencing and data analyses

Nucleotide sequences were analyzed as described previously (Kadowaki et al. 1996). The nucleotide sequences reported in this paper have been deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under the accession nos. AB158193 (barley *rps2* gene), AB158194-AB158203 (part of B-type *rps2* sequences in Triticeae and *Avena sativa*), and AB158204-AB158223 (part of W-type *rps2* sequences in Triticeae and *Bromus arvensis*).

Phylogenetic analysis

The nucleotide sequences were aligned with using CLUSTALX ver. 1.81 (Thompson et al. 1997). Parsimony and bootstrapping (1,000 replications) analyses were performed by using PAUP* ver. 4.0b10 (Swofford 2003) with strict conditions. The insertion-deletion characters were not included for this analysis. Due to the size of the data set, only a heuristic search procedure was performed. The most parsimonious trees were computed with simple addition sequence and the TBR branch-swapping algorithm. *A. sativa* and *B. arvensis* were used as outgroups for analyzing the barley and wheat types of *rps2* sequences, respectively, based on an earlier report (Judd et al. 1999).

Results

Isolation and characterization of the barley *rps2* gene

In order to test for the presence of the *rps2* gene in the barley mitochondrial genome, Southern analysis was performed using the rice *rps2* gene as a probe. Positive signals were obtained (data not shown), indicating the presence of the *rps2* gene in barley mitochondria. A DNA library was constructed from barley mtDNA and screened with the rice *rps2* probe to isolate the *rps2*-like sequence. Of the 392 clones made from the library, two were positive and the sequences of these two clones were identical. The complete nucleotide sequence is shown in Fig. 1. The part of the sequence from position 1,260 to position 1,915 was 98% similar to the wheat *rps2* sequence and 83% similar to the rice *rps2* sequence (data not shown). Thus, we concluded that the isolated sequence encodes the mitochondrial *rps2* gene of barley.

The barley *rps2* gene has a single open reading frame (ORF) capable of encoding 562 amino acids. The predicted amino acid sequence of the barley *rps2* gene showed 50–87% homology to RPS2 peptides from wheat, rice, maize, and liverwort (Fig. 2b). Compared with the liverwort *rps2* sequence, the *rps2* gene of barley, like that of rice, wheat, and maize, has a C-terminal extension. In barley, the extension corresponds to nucleotide positions 1,872–2,951, encoding 359 amino acids. However, the amino acid sequence of the C-terminal extension was entirely different among the four cereals (Fig. 2b). In the case of barley, a part of the extension (nucleotide positions 1,967–2,274) showed 67% identity to an *atpA–atp9* spacer region found in the mitochondrial genomes of wheat, durum, and rye (Bégu et al. 1989; Laser et al. 1995).

The 3′-untranslated region (UTR) of the barley *rps2* gene showed 97% homology to that of the wheat *rps2* gene (Fig. 2a, thick line). This homology extends to 670 bp downstream of the translational stop codon for the barley *rps2* gene. That is, the two *rps2* genes have a common sequence within the 3′-UTR and 5′-portion of the conserved region (Fig. 2a, grey zone).

Transcription and RNA editing of the barley *rps2* gene

Expression of the barley *rps2* gene was analyzed by Northern and cDNA sequencing analyses. The Northern analysis was conducted using the 5′-region of the barley *rps2* gene as a probe, and a 1.8-kb band was detected (data not shown). The size of the transcript was

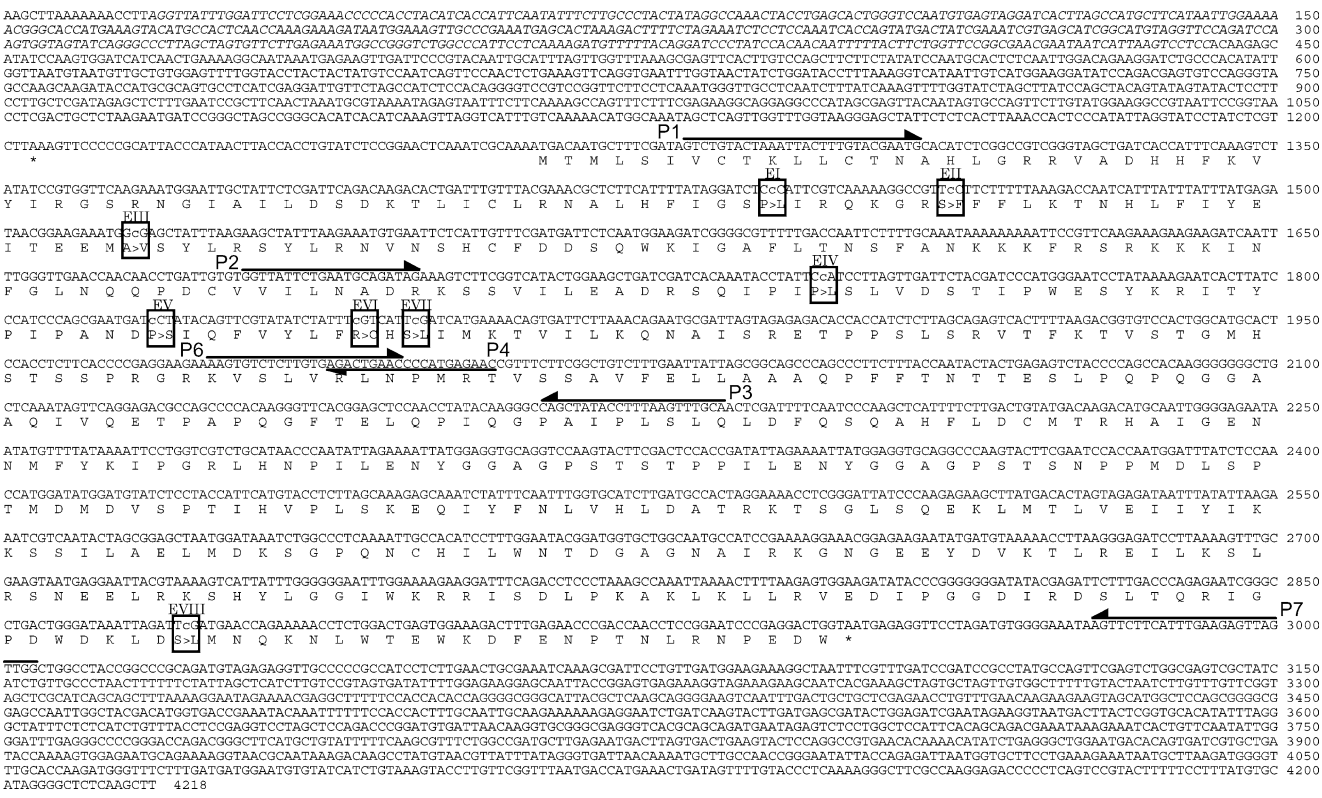

Fig. 1 Nucleotide sequence of the *rps2* gene from barley mitochondria. The deduced amino acid sequence is shown *below* the nucleotide sequence. A translational stop codon is indicated by an *asterisk*. RNA editing sites are indicated by *low-case letters*. The amino acids altered by the editing events are *boxed* and *numbered* (*EI–EVIII*). Primers used for PCR are shown by *horizontal arrows*. A chloroplast-like sequence upstream of the *rps2*-coding region is shown in *italics*

large enough to cover the entire coding region of the barley *rps2* gene (1,689 nucleotides).

The cDNA sequence was analyzed by reverse transcriptase (RT)-PCR to examine the status of RNA editing in the barley *rps2* gene. Nucleotide sequencing of the amplified cDNA and comparison to the corresponding genomic sequence identified eight C-to-T transitions within the *rps2*-coding region (Fig. 1, box), indicating the occurrence of RNA editing events in the barley *rps2* transcript. All eight editing sites were predicted to cause amino acid changes. Of these sites, seven (EI–EVII) were located within the 5′-conserved region, as is the case in the wheat and rice *rps2* genes (Vaïtilingom et al. 1998; Notsu et al. 2002), and one (EVIII) was located at position 2,872 within the 3′-extended region of barley *rps2* gene. Five of the editing sites in the 5′-region (EII, EIV–EVII) correspond to those of the maize *rps2* gene (Perrotta et al. 2002).

Distribution and characterization of the *rps2* sequence in the tribe Triticeae and other tribes

To assess the nucleotide diversity of the *rps2* gene within the genus *Hordeum*, we amplified part of the gene by PCR with the primer pair P2/P3 using template DNA of 12 *Hordeum* accessions (Table 1). The nucleotide sequences of these fragments were the same for all 12 species (data not shown), suggesting that the *rps2* sequence is highly conserved within the genus *Hordeum*. Subsequently, the same fragment was amplified from the DNA of 19 genera within Triticeae (Table 2) in order to determine the extent to which this sequence is conserved. A sequence homologous to the barley *rps2* gene was found in ten genera within the Triticeae (*Elymus*, *Eremopyrum*, *Hordeum*, *Leymus*, *Lophopyrum*, *Psammopyrum*, *Psathyrostachys*, *Pseudoroegneria*, *Secale*, and *Stenostachys*) (Table 2). No specific product was obtained from the remaining nine genera of Triticeae (*Aegilops*, *Agropyron*, *Australopyrum*, *Dasypyrum*, *Henrardia*, *Peridictyon*, *Taeniatherum*, *Thinopyrum*, and *Triticum*) despite the testing several other primer sets (data not shown).

Although another *rps2* gene has been isolated from a genus in Triticeae, namely *Triticum* (Vaïtilingom et al. 1998), it is unknown whether the structure of the wheat *rps2* gene is distributed in other species of Triticeae. Therefore, we also examined the distribution of sequences homologous to the wheat *rps2* gene. PCR amplification was performed with the primer pair P2/P5 using DNA samples from the 19 genera of Triticeae. The wheat type of the *rps2* sequence was amplified in all 19 genera (Table 2). These results demonstrate the presence

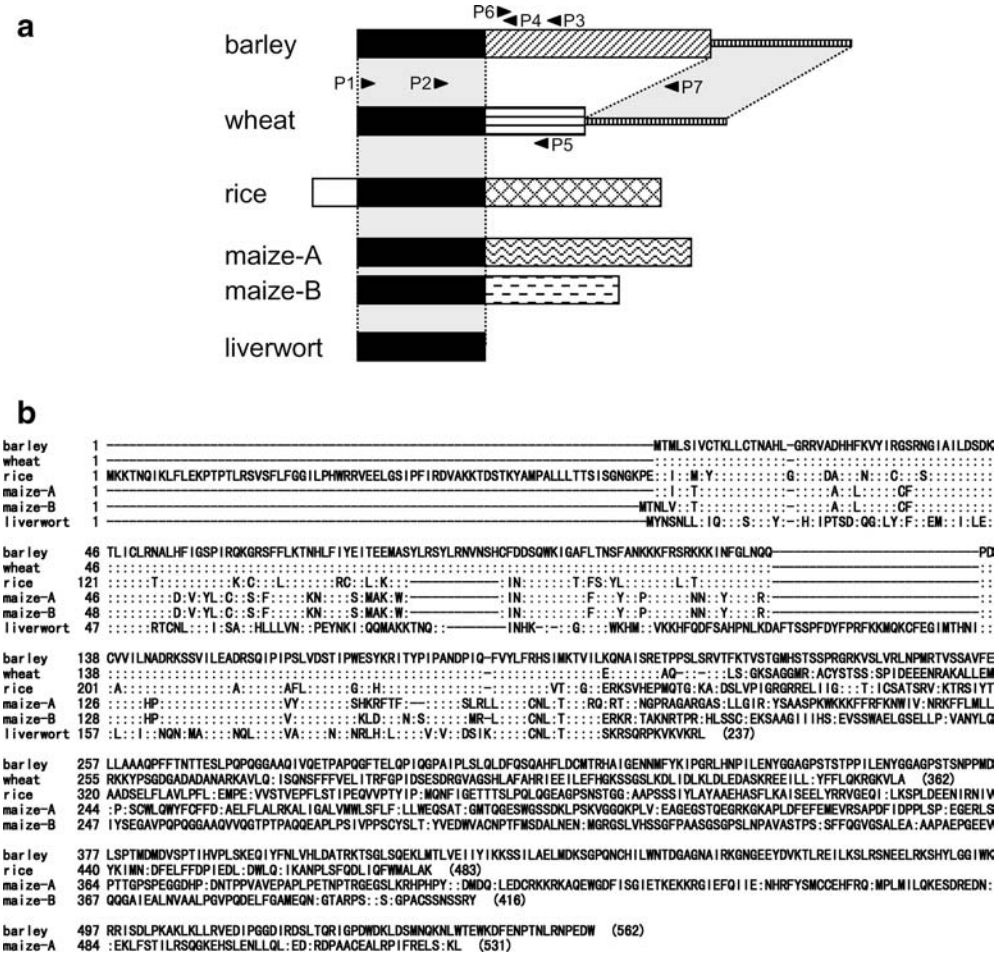

Fig. 2 **a** Schematic representation of mitochondrial *rps2* genes from barley (this study), wheat (Vaïtilingom et al. 1998), rice (Itadani et al. 1994), maize (Perrotta et al. 2002), and a lower plant, liverwort (Oda et al. 1992). An evolutionarily conserved region of the *rps2* gene is shown by a *black box*. The extension specific for each *rps2* gene is shown by a different pattern. A *thick line* indicates the 3′-UTR that is homologous between barley and wheat. Each homologous region is highlighted by a *grey zone*. Locations of primers used for PCR analysis are indicated by *arrowheads*. **b** Amino acid sequence alignment of plant *rps2* genes. Amino acid residues identical to barley RPS2 are indicated by *colons*. Gaps are shown by *dashes*. The total number of amino acids is indicated in *parenthesis*

of two types of *rps2* sequences in the Triticeae, one being a barley-type sequence and the other being a wheat-type sequence. Therefore, we named the barley and wheat types of the *rps2* sequences B-type and W-type, respectively.

We further examined other tribes of the family Poaceae for the presence of B-type and W-type sequences. For this purpose, five species belonging to different tribes were used for PCR amplification (Table 2). In *Bromus*, the primer pair P2/P5 yielded a product, but the primer pairs P2/P3 and P2/P4 did not. Conversely, the primer pairs P2/P4 and P2/P3 yielded products in *Avena* and *Lolium*, respectively, but the primer pair P2/P5 did not. In *Oryza* and *Zea*, no amplification was observed with any of these primer pairs.

Phylogenetic analyses of Triticeae based on *rps2* DNA sequences

The nucleotide sequences of PCR products (amplified with primer pairs P1/P3, P1/P4, and P1/P5) were determined and aligned. The B-type sequences showed 91% homology among genera within Triticeae. The B-type sequences of barley and *Avena* showed 84% homology. The homologies of W-type sequences were 95% within Triticeae and 97% between wheat and *Bromus* (the detailed data are not shown here, but alignments of all the *rps2* sequences are available from the journal Web site as Electronic Supplementary Material).

The data were subjected to phylogenetic analysis. In a tree based on the B-type sequence (Fig. 3a), four genera formed two subclades (*Hordeum-Psathyrostachys* and *Secale-Leymus*). No distinct subclade was formed because the bootstrap values were less than 50% for the other six taxa. In a tree based on the W-type sequence (Fig. 3b), *Hordeum*, *Psathyrostachys*, and *Stenostachys* formed one clade. The genera *Aegilops*, *Taeniatherum*, *Thinopyrum*, and *Triticum* made up a clade with *Elymus* and *Psammopyrum* as sister groups. *Leymus* appeared at the most basal position in Triticeae although this position was supported with less than a 50% bootstrap value. The remaining genera formed an unresolved polytomy. The topologies of the two cladograms are incongruent. Only the conclusion that *Hordeum* and *Psathyrostachys* are sister groups is explicitly supported.

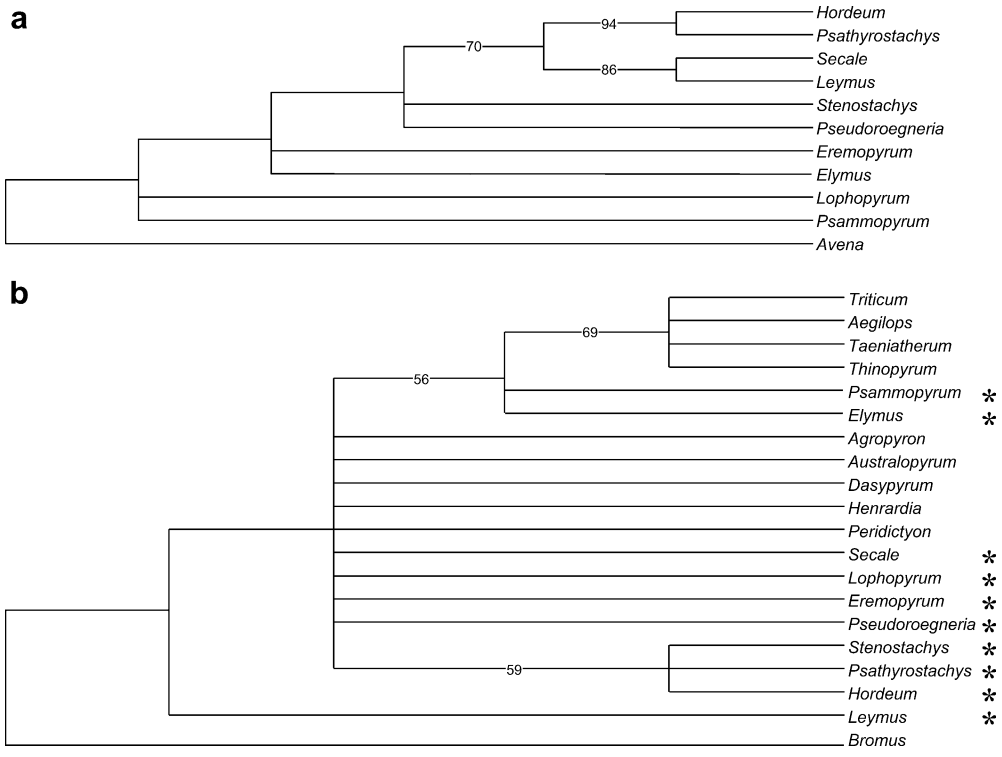

Fig. 3 Phylogenetic tree of the genera in the Triticeae based on mitochondrial *rps2* sequences. **a** A consensus tree of two equally parsimonious trees based on the B-type sequence [124 steps long, consistency index (CI) = 0.944, retention index (RI) = 0.750]. **b** A consensus tree of 244 equally parsimonious trees based on the W-type sequence (75 steps long, CI = 0.636, RI = 0.826). The *numbers* on the branches are bootstrap values (> 50%) from 1,000 replicates. Genera having both types of *rps2* genes are indicated by *asterisks* in the W-type cladogram

Discussion

The mitochondrial *rps2* gene sequences of monocots analyzed to date have different 3′-extensions. Our results revealed that the *rps2* gene from barley mitochondria has a 3′-extension and that this extension is completely different from that of rice, wheat, and maize (Itadani et al. 1994; Vaïtilingom et al. 1998; Perrotta et al. 2002). This suggests that the 3′-extensions were independently acquired from different origins. Since barley and wheat belong to the same tribe (Triticeae), the different 3′-extensions must have been acquired in the relatively recent past. Interestingly, the *rps2* genes of barley and wheat share the 5′-region and the 3′-UTR (Fig. 2a), suggesting that the 3′-extension was acquired by a single recombination or insertion event. Since the B-type *rps2* sequence was highly conserved within the genus *Hordeum*, this sequence would seem to be the more appropriate one for studying the relationship among genera or more distantly related taxa.

We surveyed the distribution of the two types of *rps2* sequences and found that all of the examined genera of Triticeae had at least one of the two types of *rps2* DNA, and interestingly, that ten genera had both types (Table 2). This result was confirmed by the finding that primer pair P2/P7 resulted in amplification of both types of *rps2* products in these genera (data not shown). However, we could not find any apparent pattern for their distribution in the Triticeae (Fig. 3b, asterisks). In the case of the consensus tree of Triticeae constructed by Kellogg et al. (1996), six of the genera were shown in the present study to have B-type sequences (Fig. 4,

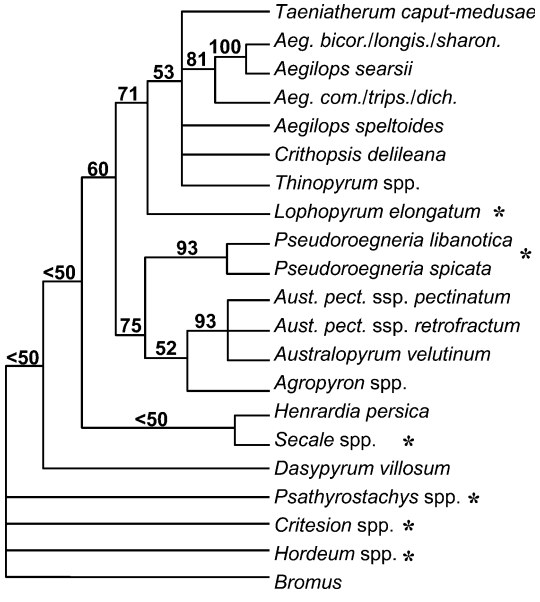

Fig. 4 Distribution of the B-type sequence in the consensus phylogenetic tree of Triticeae. The consensus tree of 5S short-spacer, 5S long-spacer, and ITS (internal transcribed spacer) sequences (Kellogg et al. 1996) was used with a slight modification. Genera having the B-type sequence are indicated by *asterisks*

asterisks). Although three of the genera (*Hordeum*, *Critesion* (synonym of wild *Hordeum* species), and *Psathyrostachys*) are located in basal positions of the tree, each of the other three genera was in a different subclade. A similar result was obtained with phylogenetic trees based on different data sets (data not shown). Based on the above results, we propose that the loss of

the B-type sequence from certain genera occurred at random during the course of evolution in Triticeae. The redundancy of the *rps2* sequence owing to the presence of the W-type sequence may have permitted such a gene loss.

The finding of a W-type sequence in *Bromus* (tribe Bromeae) and B-type sequences in *Avena* (tribe Aveneae) and *Lolium* (tribe Poeae) raises the question of which is older, the B-type or the W-type sequence. Our results suggest that the B-type sequence occurred earlier than the W-type sequence because Aveneae and Poeae form a sister group to Bromeae plus Triticeae (Judd et al. 1999) and because the sequence homology among B-type sequences (84–91%) is lower than that among W-type sequences (95–97%). However, a greater number of species in the Bromeae, Aveneae, and Poeae will need to be examined to confirm the above hypothesis.

The phylogeny of Triticeae has been the focus of several studies during the last decade, and various morphological and molecular methods have been used for this purpose (see Catalán et al. 1997; Hsiao et al. 1995; Kellogg et al. 1996; Mason-Gamer and Kellogg 1996, 1998; Mason-Gamer et al. 1998; Petersen and Seberg 1997, 2000, 2002; Seberg and Frederiksen 2001). The present study differs from these previous studies in that it uses mtDNA for phylogenetic studies of Triticeae. Our cladograms based on the B-type and W-type sequences did not clearly show the relationships among most taxa because of the paucity of phylogenetically informative sites. However, both trees showed a close association between the genera *Hordeum* and *Psathyrostachys* (Fig. 3). In the studies of Petersen and Seberg (2000, 2002) and Seberg and Frederiksen (2001), *Hordeum* and *Psathyrostachys* also appear as sister clades deeply embedded in the Triticeae. However, in most of the reports preceding their investigations, they appear at a basal position and often *Psathyrostachys* is placed as a sister group to *Hordeum* and the rest of the Triticeae. This suggests that the mitochondrial genome in Triticeae has a unique evolutionary history. In higher plants, some rearranged mtDNA molecules, called "sublimons", are present at low levels, and the molecular ratio of the sublimons can change (Small et al. 1989). On the other hand, horizontal gene transfer events have recently been reported in flowering plant mitochondria (Bergthorsson et al. 2003). Although such events probably occur only rarely, they might account for the discrepancy between the phylogenetic analyses based on the mitochondrial *rps2* sequences and those based on other data. To explain the discrepancy, intensive analysis using more sequence information, ideally with a comparison of entire mitochondrial genome sequences, would be helpful.

In conclusion, the present study showed structural variation in the mitochondrial *rps2* gene and how the different types are distributed in Triticeae and other related tribes. Our results demonstrate the presence of a dynamic variation in *rps2* gene structure that involves the entire 3′-region and not just nucleotide substitutions. This diversity in quality (nucleotide difference) and quantity (occurrence or loss) of the mitochondrial genes could cause variation in the cytoplasm of Triticeae species. Further analyses are required to resolve these discrepancies and to accurately describe the evolution of nuclear and cytoplasmic genomes of Triticeae.

Acknowledgements We thank Dr. M. Shahid Masood and T. Nishikawa for valuable discussions. We are grateful to Dr. T. Tominaga and T. Senda for providing *L. multiflorum* DNA, and to Dr. A. Stewart for providing seeds of *Stenostachys*. This work was partly supported by the Bilateral International Joint Research with Special Coordination (13073-2125-14) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) to TK and KK, and by a Grant-in-Aid for Young Scientists (B) (15780215) from MEXT to NK.

References

Adams KL, Qiu Y-L, Stoutemyer M, Palmer JD (2002) Punctuated evolution of mitochondrial gene content: high and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. Proc Natl Acad Sci USA 99:9905–9912

Bégu D, Graves P-V, Litvak S, Araya A (1989) Nucleotide sequence of the F_0-ATPase subunit 9 genes from two lines of wheat. Nucleic Acids Res 17:9491

Bergthorsson U, Adams KL, Thomason B, Palmer JD (2003) Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature 424:197–201

Binder S, Brennicke A (2003) Gene expression in plant mitochondria: transcriptional and post-transcriptional control. Philos Trans R Soc London Ser B 358:181–189

Catalán, P, Kellogg EA, Olmstead RG (1997) Phylogeny of Poaceae subfamily Pooideae based on chloroplast *ndh*F sequences. Mol Phylogenet Evol 8:150–166

Hsiao C, Chatterton NJ, Asay KH, Jensen KB (1995) Phylogenetic relationships of the monogenomic species of the wheat tribe, Triticeae (Poaceae), inferred from nuclear rDNA (internal transcribed spacer) sequences. Genome 38:211–223

Itadani H, Wakasugi T, Sugita M, Sugiura M, Nakazono M, Hirai A (1994) Nucleotide sequence of a 28-kbp portion of rice mitochondrial DNA: the existence of many sequences that correspond to part of mitochondrial genes in intergenic regions. Plant Cell Physiol 35:1239–1244

Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999) Plant systematics: a phylogenetic approach. Sinauer Assoc, Sunderland

Kadowaki K, Kubo N, Ozawa K, Hirai A (1996) Targeting presequence acquisition after mitochondrial gene transfer to the nucleus occurs by duplication of existing targeting signals. EMBO J 15:6652–6661

Kellogg EA, Appels R, Mason-Gamer RJ (1996) When genes tell different stories: the diploid genera of the Triticeae (Gramineae). Syst Bot 21:321–347

Komatsuda T, Nakamura I, Takaiwa F, Oka S (1998) Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*. Genome 41:680–685

Komatsuda T, Tanno K, Salomon B, Bryngelsson T, von Bothmer R (1999) Phylogeny in the genus *Hordeum* based on nucleotide sequences closely linked to the *vsr1* locus (row number of spikelets). Genome 42:973–981

Kubo N, Ozawa K, Hino T, Kadowaki K (1996) A ribosomal protein L2 gene is transcribed, spliced, and edited at one site in rice mitochondria. Plant Mol Biol 31:853–862

Laser B, Oettler G, Kück U (1995) RNA editing of the mitochondrial *atpA*/*atp9* co-transcript of Triticale, carrying the *timopheevi* cytoplasmic male sterility cytoplasm from wheat. Plant Physiol 107:663–664

Marienfeld JR, Unseld M, Brandt P, Brennicke A (1997) Mosaic open reading frames in the *Arabidopsis thaliana* mitochondrial genome. Biol Chem 378:859–862

Mason-Gamer RJ, Kellogg EA (1996) Chloroplast DNA analysis of the monogenomic Triticeae: phylogenetic implications and genome-specific markers. In: Jauhar JJ (eds) Methods of genome analysis in plants. CRC Press, Boca Raton, pp 301–325

Mason-Gamer RJ, Kellogg EA (1998) Phylogenetic analysis of the Triticeae using the starch synthase gene, and a preliminary analysis of some north American *Elymus* species. In: Jacobs SWL, Evetett J (eds) Grasses: systematics and evolution. CSIRO, Melbourne, pp 102–109

Mason-Gamer RJ, Weil CF, Kellogg EA (1998) Granule-bound starch synthase: structure, function, and phylogenetic utility. Mol Biol Evol 15:1658–1673

Nishikawa T, Salomon B, Komatsuda T, von Bothmer R, Kadowaki K (2002) Molecular phylogeny of the genus *Hordeum* using three chloroplast DNA sequences. Genome 45:1157–1166

Notsu Y, Masood S, Nishikawa T, Kubo N, Akiduki G, Nakazono M, Hirai A, Kadowaki K (2002) The complete sequence of the rice (*Oryza sativa* L.) mitochondrial genome: frequent DNA sequence acquisition and loss during the evolution of flowering plants. Mol Genet Genomics 268:434–445

Oda K, Yamato K, Ohta E, Nakamura Y, Takemura M, Nozato N, Akashi K, Kanegae T, Ogura Y, Kohchi T, Ohyama K (1992) Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA: a primitive form of plant mitochondrial genome. J Mol Biol 223:1–7

Perrotta G, Grienenberger JM, Gualberto JM (2002) Plant mitochondrial *rps2* genes code for proteins with a C-terminal extensions that is processed. Plant Mol Biol 50:523–533

Petersen G, Seberg O (1997) Phylogenetic analysis of the Triticeae (Poaceae) based on *rpo*A sequence data. Mol Phylogenet Evol 7:217–230

Petersen G, Seberg O (2000) Phylogenetic evidence for excision of stowaway miniature inverted-repeat transposable elements in the Triticeae (Poaceae). Mol Biol Evol 17:1589–1596

Petersen G, Seberg O (2002) Molecular evolution and phylogenetic application of DMC1. Mol Phylogenet Evol 22:43–50

Seberg O, Frederiksen S (2001) A phylogenetic analysis of the monogenomic Triticeae (Poaceae) based on morphology. Bot J Linn Soc 136:75–97

Small I, Suffolk R, Leaver CJ (1989) Evolution of plant mitochondria genomes via substoichiometric intermediates. Cell 58:69–76

Swofford DL (2003) PAUP*: Phylogenetic analysis using parsimony (*and other Methods), version 4. Sinauer Associates, Sunderland

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTALX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Vaïtilingom M, Stupar M, Grienenberger J-M, Gualberto JM (1998) A gene coding for an RPS2 protein in the mitochondrial genome of several cereals, but not in dicotyledons. Mol Gen Genet 258:530–537

Wang RR-C, von Bothmer R, Dvorak J, Fedak G, Linde-Laursen I, Muramatsu M (1996) Genome symbols in the Triticeae (Poaceae). In: Wang RR-C, Jensen KB, Jaussi C (eds) Proc 2nd Int Triticeae Symp. Utah State University Publication, Design and Production, Logan, pp 29–34