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STRUCTURE OF THE INTERGENIC SPACER OF BARLEY RIBOSOMAL DNA REPEAT UNITS: EVIDENCE FOR CONCERTED EVOLUTION

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Summary: The region from NOR 6H in barley rDNA flanked by 26S rRNA and 18S rRNA was sequenced (HQ825319). This region contains the IGS and ETS of the rDNA repeat unit; it was examined and compared to rDNA in NOR 5H (AF147501). In the IGS of both NORs two distinct repeat regions are present; one, composed of 79 bp repeat units and another, composed of 130 bp repeat units. The number of 79 bp units is six and a half in both NORs. However, NOR 5H and NOR 6H have different number of 130 bp units. Results suggest concerted evolution and homogenization within and between NORs.

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Abbreviations: ETS – external transcribed spacer; IGS – intergenic spacer; NOR – nucleolus organizer region; rRNA – ribosomal RNA; 5H and 6H – *Hordeum* chromosomes 5 and 6; rDNA – DNA coding pre-rRNA.

INTRODUCTION

The rRNA genes in *Hordeum vulgare* are clustered on chromosomes 5 and 6. In general the two NORs contain similar amounts of rDNA (with 5% more genes on NOR 6H). The haploid gene number (1880) and the rRNA gene distribution between NORs may vary in different accessions and from plant to plant (Zang et al., 1990).

As a rule in barley two IGS length variants are observed (Gerlach and Bedbrook, 1979). It is firmly established that short variants are present in NOR 5H, and longer variants are present in NOR 6H (Allard et al., 1990).

It is relevant to note that in barley two unique phenomena are observed. One is

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the dominance of NOR 6H over NOR 5H in nucleoli formation after mitosis if both NORs are situated on one chromosome (Nicoloff et al., 1979). It was speculated that this reflects the delayed reactivation of NOR 5H after mitosis (Karagyozov et al., 1986). This is supported by the finding that rDNA methylation pattern is not affected by changes in NOR chromosome position (Dimitrova et al., 2012). Another specific phenomenon is the observation that the length of IGS depends on habitat and presumably is subject to selective pressure (Saghai Maroof et al., 1984; Saghai Maroof et al., 1990). Until now there are no clues for understanding the prevalence of certain IGS lengths.

The sequence of the IGS from NOR 5H, which contains the short rDNA repeats, was released previously (AF147501).

Here we report on the sequencing of IGS from NOR 6H. Results show that rDNA repeat length variations are due entirely to differences in the number of 130 bp subrepeats.

MATERIALS AND METHODS

Clone R10 (Ananiev et al., 1986) includes one complete barley rDNA repeat unit (EcoRI-EcoRI fragment). Its length (9.8 kb) shows that it derives from NOR 6H, which harbors the long rRNA gene repeats (Gerlach and Bedbrook, 1979; Allard et al., 1990). EcoRI – XbaI fragment (4.7 kb) was subcloned in pBScript SK+ (Stratagene) and sequenced by primer walking. It contains the 3'-end of 26S rRNA, the non-transcribed IGS, the promoter, the ETS and the 5'-end of 18S rRNA (GenBank: HQ825319).

The nucleotide sequences were compared and managed by BLAST (http://blast.ncbi.nlm.nih.gov/) and Clone Manager Suite 8 (Scientific and Educational Software).

RESULTS

General structure

The main structural features of the EcoRI-XbaI fragment of barley rDNA are shown on Fig. 1. The 5'-end of the fragment is occupied by 488 bp coding the 3'-end of 26S rRNA. The 3'end of the large rRNA were determined by alignment with clone AK248318 derived from a full length *Hordeum* cDNA library (Sato et al., 2009) and *Oryza* sequences for 25S rRNA (M11585).

The 3'-end of the 26S rRNA is followed by a short C-rich sequence (CCCTCCCCCA), which is conserved between *Triticeae* species. It probably serves as a signal, which is relevant to transcription termination or processing (Barker et al., 1988).

Prominent in IGS are two repeat regions (R79 and R130). The 130 bp units share homologous regions with the 135 bp A-repeats in *Triticum aestivum* (Barker et al., 1988). The pattern, however, is a mosaic, apparently the relationship between the two type of repeats is complex.

The R79 and R130 regions are separated by 183 bp segment, large part of which (76 bp) is homologous to the 130 bp repeat unit (74%). The rDNA gene promoter is 15 bp downstream of R130; ETS and sequences of the mature 18S rRNA follow.

The 79 bp repeat region

The R79 region in NOR 6H and NOR 5H contains six full 79 bp tandem repeat units and a seventh unit, which is

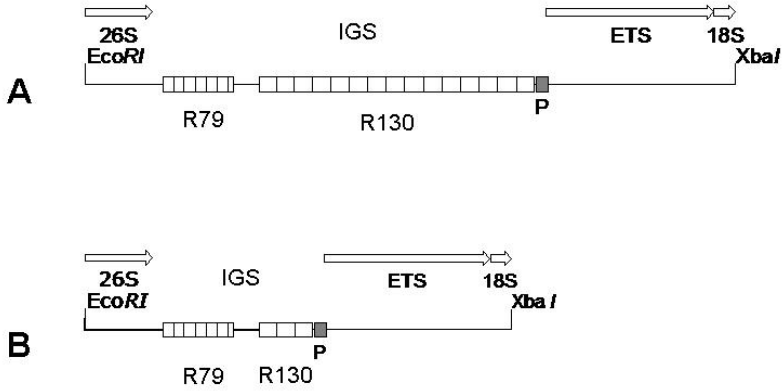

Figure 1. Structure of the EcoRI – XbaI region of barley rDNA.
Repeat units: A – NOR 6H (HQ825319), IGS – 2834 bp ; B – NOR 5H (AF147501), IGS - 1239 bp. R79 and R130 – subrepeat regions (average repeat unit sizes of 79 bp and 130 bp). R79 - 510 bp (5H) and 511bp (6H). R130 - 386 bp (5H) and 1980 bp (6H). P – promoter region (82 bp).

truncated after position 40. Comparison of the R79 regions in rDNA repeats from 5H and 6H shows high sequence similarity (97%). Comparison between the 79 bp repeat units from one array reveals great similarity of the units (Fig. 2).

The 130 bp repeat region

The R130 region on NOR 6H contains 15 tandem repeat units varying in length from 128 to 135 bp. Comparison of the 6H repeats with each other shows variability, which is more pronounced in the region 1- 30 nt (Fig. 3).

The R130 region on NOR 5H consists of three 130 bp repeat units. Comparison reveals high sequence similarity between R130 repeats 1 – 3 in NOR 6H and the R130 region in NOR 5H.

The promoter

The pre-rRNA transcription start site was determined by alignment of *Hordeum* rDNA with transcript AK251731 (Sato et al. 2009). The transcription start is +1GGGGAGGAGGGGG. It is positioned at the 3'-end of a 82 bp sequence, which is highly conserved among *Triticeae* species; presumably this sequence is the RNA polymerase I promoter.

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Repeat Pos=  10        20        30        40        50        60
Rep 1 GCTAAGTCCAACGGAAACCGTACGTGCCAAGTCAC-AAGAGATATGGTAAAGTCCGCCCTGGGAC
Rep 2 GCTAAGTCCAACAGAAACCATACGTGCCAAGTCG-GAAGAGATATGGTAAAGTCCGTCCTGGGAC
Rep 3 GCTAAGTCCAACGGAAACCATACGTGCCAAGTCA-GAAGACATATGGTAAAGTCCGTCCTGGGAC
Rep 4 GCTAAGTCCAACGGAAACTATACGTGCCAAGTCACGAAGAGATATGGTCAAGTCCGTCCCGGGAC
Rep 5 GCTAAATCCAACGGAAACGATACGTGCCAAGTCACGAAGAGATATGGTCAAGTCCGTCCCGGGAC
Rep 6 GCTAAGTCCAACGGAAACTATACGTGCCAAGCCACGAAGAGATATGGTTAAGTCCGTCCTGGGAC
Rep 7 GCTAAGTCCAACGGAAACTATACGTGCCAAGCCACGAAGA
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Figure 2. Side-by-side comparison of the 79 bp sub-repeats in NOR 6H (pos= 1 to pos=65).
Repeat No 1 is proximal to the 26S rRNA 3'-end, repeat No 7 is truncated.

A

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  1 GAAACGGGNA AAAACSGGGT ACGRCGGCCG TGTTGCAAAA AACTGGGCGC
 51 GCACCATGGA AAACDGGTGA AAACCATGTG CGTGGCATGG ACGGATGCAC
101 GTACGGGCAC ACGGGCCAAA AAACGTGAAC GTGAG
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B

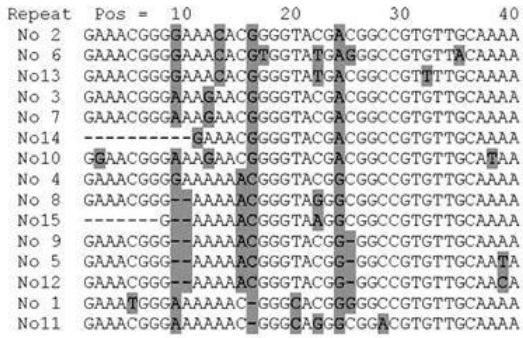

Figure 3. The 130 bp repeat units in rDNA on barley chromosome 6.
A. The 130 bp repeat unit consensus. R – purine; S – C or G; D – A, G or T.
B. Comparison of the 5'-end of the 130 bp repeat units. Repeat unit 1 is proximal to the promoter, repeat unit 15 is nearest to R79. Repeat units 15 and 14 have 5'-end deletions. The order of the repeat units is changed to maximize similarity.

The external transcribed spacer

The external transcribed spacer in *Hordeum vulgare* is 1206 bp. This length is close to the length of ETS in *Triticum*, *Aegelops* and *Secale* (1121 -1151 bp) (Sallares and Brown, 2004). Sequence comparisons reveal minor variations between ETS sequences, transcribed from NORs on chromosomes 5H and 6H.

Nearly 700 bp upstream of the 5'-end of 18S rRNA in *Hordeum bulbosum* are also sequenced (accession X53792). Comparison of the homologous ETS regions of *H.vulgare* and *H.bulbosum* reveals substantial 5'-end differences and increased homology in the 300 bp region preceding the 5'end of 18S rRNA.

DISCUSSION

Studies of individual barley plants demonstrated the presence of twenty distinct IGS length variants in *Hordeum* rDNA. It was shown that these variants fall into two classes: short variants, specific for NOR 5H, and longer variants, specific for NOR 6H (Allard et al. 1990). Earlier the sequence of the IGS from NOR 5H was released (AF147501), showing the presence of two regions formed by tandem 79 bp and 130 bp repeats. Previous studies (Allard et al., 1990) claimed that the IGS length variants differ by a fixed increment of 115 bp, forming a ladder. Thus, it was more likely to consider as a possibility that IGS length variation depends on changes in both R79 and R130 regions. Results presented here, however, prove convincingly that changes in IGS length are due only to changes in the R130 region.

Homogenization of the subrepeat regions

Our results show high degree of similarity between the 79 bp repeat units either from a single array or from the

arrays in different NORs. High degree of similarity is revealed also when the 130 bp repeat units are compared. This demonstrates the existence in barley IGS of high degree of inter- and intra-NOR rDNA homogenization.

The suggested mechanisms for rDNA homogenization include unequal crossing over and gene conversion (Barker et al., 1988; Naidoo et al., 2013). The contribution of these processes to homogenization in not easy to determine. However, detailed examination of the variation between repeat units at homologous positions may give a clue.

In IGS some variations are unique, they occur only once in the repeat units of the array. Apparently they are due to mutations not affected by homogenization. It should be emphasized that the unique, unit specific non-similarities are not frequent (less than 2 differences per repeat in R79 and R130). More common are the shared variations, which are present in two or more repeats. In the R79 region these variations often occur in neighboring repeat units (Fig. 2, positions 20, 50, 61}. However, in the R130 6H region the homologous variations are frequently held by non-neighboring repeats (Fig. 3). For example, in pos = 16 there is a G to C change in units 4, 8, 9, 12 and 15. The presence of homologous variations in non-neighboring units may be explained by the effect of gene conversion-type processes and imply that in *Hordeum* gene conversion is common.

It is relevant to note that examples of similar type of variations are observed also in the array of *Triticum* A-repeats (Barker et at., 1988).

In *Hordeum* repeat unit variations show additional unusual feature. In both R79 and R130 one position in the homologous repeat units may be occupied by three different bases (or two bases and a gap). For example, C, T and G are present in R79 in pos=20. This observation has no obvious explanation and emphasizes the complexity of co-evolution processes in repeated gene families.

In conclusion, sequencing the IGS in a repeat unit from the second NOR revealed the causes for rDNA repeat unit length differences in barley. The possibility is opened to understand the unique prevalence of specific rDNA repeat lengths under different environmental conditions.

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