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Restriction site polymorphism in rDNA repeat unit of *Hordeum vulgare* for some restriction endonucleases

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Abstract

Restriction fragment length polymorphism in rDNA repeat unit for eight restriction endonucleases was studied in two genotypes of barley (*Hordeum vulgare* L.). Both genotypes contained restriction sites for EcoRI, EcoRV, PvuII and Sau3239I in rDNA repeat unit and contained two different rDNA repeat unit length variants 9.7 kbp and 9.1 kbp.

Introduction

Ribosomal RNA (rRNA) is a structural part and frame of ribosomes. Structural genes coding rDNA are organized in tandem repetitions of DNA sequences. The size of these repeat units in plants ranged between 7 - 15 kbp and rDNA locus can contain over 15.10^6 kbp (Flavell 1986).

The number of these repeat units in plant genomes is very large, they contain 500 - 40.000 copies in diploid genome. The plant nuclear rDNAs are located at the nucleolar organizer region (NORs) of chromosomes (Rogers and Bendich 1987). Every repeat unit of rDNA is arranged from coding region for 18S, 5.8S and 26S rRNA, two small spacers connected the gene for 5.8S rRNA with genes for 18S and 26S rRNA and from large intergenic spacer region (IGS), that contains several subrepetitions and separates repeat units of coding regions. Further repeat unit contains DNA sequences for the promotor region, enhancers of transcription, transcription rDNA processing and possibly for rDNA replication and unequal crossing-over (Hemleben *et al.* 1988). No other genes are known to interrupt the tandem arrays of these repeat units (Flavell 1989). Coding sequences for rRNAs are highly conserved among various organisms but large spacer regions are highly variable in length and primary structure among orgnisms and individuals (Kato *et al.* 1990).

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Material and methods

Total plant DNA were isolated from *Hordeum vulgare* L. genotypes - Kř M7/2 and Kř 1629. DNA was isolated from leaves by method of Dellaporta *et al.* (1983). Leaves were homogenized in liquid nitrogen, extraction buffer was modified - 100 mM TRIS, pH 8.0, 50 mM EDTA, 2 M NaCl and 10 mM mercaptoethanol. Isolated DNA was purified with 10 M LiCl. Plant DNA was digested with restriction endonucleases EcoRI, EcoRV, MluI, SalI, PvuII, SmaI, XbaI and Sau 3239I. 10 units of enzyme per 1 μg DNA were used for 6 - 8 h. Electrophoretic running of digested DNA in 0.8 % agarose gels was made overnight at 1.5 V cm^{-1} of gel length. Blotting of DNA was made to membranes *Hybond-N* (*Amersham*) according to Sambrook *et al.* (1989). Prehybridization and hybridization to membranes was made according to Sambrook *et al.* (1989) at temperature 68 °C. Washing of membranes in solution 2× SSC, 0.1 % SDS, theree times in 5 min at room temperature, 90 min in 0.1× SSC, 0.1 % SDS at 68 °C and additionaly in the same solution 30 min at 68 °C. As DNA probe was used plasmid pHV014, which included complete rDNA repeat unit of barley cloned in pBR325 (Ananiev *et al.* 1986). The probe was labeled by nick-translation according to Sambrook *et al.* (1989). The size of DNA fragments from autoradiographs was determined by the computer program "*GEL*" (Albert 1991, unpublished).

Results and discusion

Restriction sites polymorphism in rDNA repeat unit of barley for eight restriction endonucleases was studied.

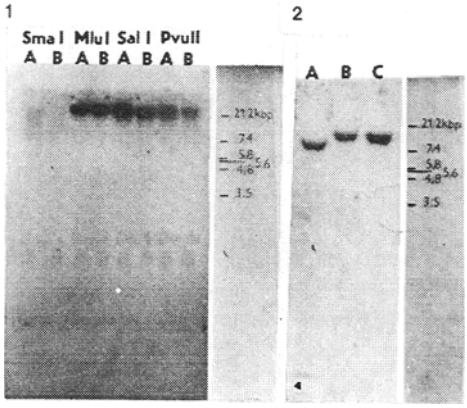

Fig. 1. *H. vulgare* DNA digested with SmaI, MluI, SalI and PvuII and probed with pHV014. Kř M7/2 (*A*), Kř 1629 (*B*) (*on the left*).
Fig. 2. *H. vulgare* genotypes Kř 1629 (B) and Kř M7/2 (C) and *H. bulbosum* DNA (A) digested with EcoRI (*on the right*).

The used probe pHV014 hybridized only to high molecular mass DNA (over 25 kbp) after digestion with MluI, SalI, and PvuII restriction endonucleases (Fig. 1). This indicates that in rDNA repeat unit of these genotypes of barley no restriction sites for these enzymes are present. After digestion of barley DNA with SmaI, the autoradiograph indicated that this restriction endonuclease had probably a low quality and an endonuclease activity.

For EcoRI digestion *H. vulgare* and *H. bulbosum* was used (Fig. 2). The autoradiograph indicated that *H. vulgare* and *H. bulbosum* possessed in rDNA single restriction site for EcoRI. Simultaneously both *Hordeum* species are differ in the rDNA repeat unit length and rDNA repeat unit length variability occurred with *H. vulgare* too. From Sai 3239I digest we can indicated, that *H. vulgare* contains two rDNA repeat unit, first 9.7 kbp and second 9.1 kbp. rDNA repeat unit length of *H. bulbosum* was 8.5 kbp (Fig. 3).

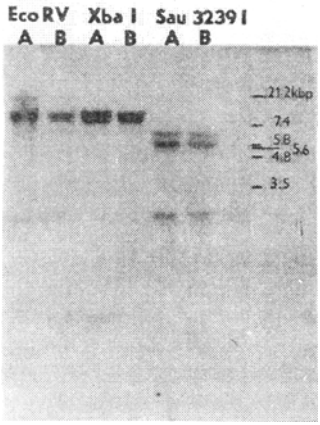

Fig. 3. EcoRV, XbaI and Sau3239I restriction patterns of *H. vulgare* DNA - Kř 1629 (A) and Kř M7/2 (B).

Hybridization patterns of pHV014 with Southern blots of EcoRV and XbaI digest indicated, that rDNA repet unit of barley possessed one restriction site for these restriction endonucleases (Fig. 3). In digestiion with EcoRV appeared fragment of length 18.9 kbp too, that indicated loss or methylation some cleavage sites for EcoRV. After digestion with Sau3239I three fragments 6.6 kbp, 6.0 kbp and 3.1 kbp appeared on autoradiograph. The repeat unit of *H. vulgare* contained two cleavage sites for this enzyme. The fragments of 6.6 kbp and 6.0 kbp together with fragment of 3.1 kbp demonstrate presence of two rDNA repeat unit length variants.

The length heterogeneity of large spacer (IGS) often caused the length polymorphism of rDNA repeat units. High variability in length of rDNA repeat unit were documented for *H. vulgare* (Saghai-Maroof *et al.* 1984), and it is known that *Hordeum* species are highly polymorphic for rDNA repeat unit length (Molnar and Fedak 1989) and length of rDNA repet unit vary between 8.5 - 10.7 kbp (Molnar *et al.* 1989). Similarly Yakura and Tanifuji (1981) reported that rDNA repeat units of

Vicia faba ranged from 8.3 to 12.9 kbp. In some plant species none or only minimal variability in rDNA repet unit length was found.

The rDNA polymorphism can be used as genetic marker for example in plant species evolution studies, extra- and intraspecific variability studies, homogeneity tests, *etc.*

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