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AT repeats in barley genome

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The DNA sequence $(AT)_{26}$ of barley genome has been cloned. This sequence is arranged in intraspecific locus and is repeated 1500 times per haploid genome. This fragment is not translated and can form cruciform structures in the AT region.

DNA simple sequence; Barley genome; Polymorphism

1. INTRODUCTION

Simple sequence repeats have been found as interspersed elements in and around genes from different eukaryotic species [1,2]. There is a number of speculations concerning their role in genome. Genome fragments containing simple sequences can be exposed to deletions and duplications [3]. DNA slippage reactions [4] that result in molecular variability. Some authors consider these sequences as a major source of genetic variation [5].

Simple sequences have been shown to be transcribed [6] and can regulate gene expression [7,8].

These sequences are known to influence the recombination process [9–12]. However, so far their direct participation in the definite functions is not considered to be proved.

As to plant genomes, only a few papers can be found dealing with DNA simple sequences. Sequences of alternating purine-pyrimidine residues have been detected in the nuclear DNA of wheat, radish and maize [13,14].

In this paper we present results of our investigation on a simple AT repetetive sequence isolated from barley genome by molecular cloning.

2. MATERIALS AND METHODS

2.1. *Plant material*

Seeds of different barley species (*H. spontaneum* ($2n=14$), *H. bulbosum* ($2n=28$) and *H. jubatum* ($2n=28$) were obtained from the collection of All-Union Institute of Plant Breeding, Leningrad. The rest was taken from the collection of our institute.

2.2. *Isolation of plant nuclear and plasmid DNA*

Plant DNA was prepared from nuclear pellet as described in [15]. DNA was purified by CsCl centrifugation because of the extraction of some nucleotide sequences by phenol treatment [16]. However, in the case of DNA isolation from individual seedlings the phenol method was used.

All plasmids were isolated according to [17].

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2.3. *Enzymatic treatment of DNA*

Restriction enzymes and *Bal*31 nuclease were obtained from Ferment, USSR. All other enzymes were purchased from Boehringer. All reactions were conducted in conformity with the firm's instructions.

2.4. *Cloning nuclear DNA of barley*

DNA fragments from *H. vulgare* were inserted to the *Hin*dIII site of pBR322. *E. coli* cells were transformed as described [18]. Recombinant clones were selected on the medium with tetracycline and ampicillin.

2.5. *Probe preparation*

Synthetic $(AT)_n$ co-polymer (Pharmacia) was labelled by incorporation of [α-^{32}P]dNTP as described by Greaves and Patient [2]. A cloned fragment of barley DNA was excised by *Bgl*II, isolated from agarose gel after electrophoresis and labelled by nick-translation [19].

2.6. *DNA hybridization*

In all experiments nitrocellulose membranes (Bio-Rad) were used.

To detect clones with AT-repeat, membranes were prepared according to Grunstein and Hognes [20]. Prehybridization and hybridization were performed for 3 h, washing for 30 min at room temperature in solution I (5× SSC; 0.5 M sodium phosphate, pH7.5; 0.1% polyvinylpyrrolidone and 0.35% sodium lauroylsarcosine).

Blot-hybridization with $(AT)_n$ probe was conducted under the same conditions. Membranes were washed in solution I at room temperature.

In the case of the DNA fragment from barley genome, prehybridization was conducted for 3 h, hybridization – 15 h at +38° C in solution I containing 50% formamide. Filters were washed by standard procedure.

2.7. *DNA sequencing and analysis of nucleotide sequence*

DNA sequencing was performed according to Maxam and Gilbert with the modifications of Chuvpilo and Kravchenko [21]. Computer analysis was carried out by software SAMSON in NI VC (Puschino, USSR).

3. RESULTS

3.1. *Cloning and nucleotide sequence of AT fragment*

Hybridization using the $(AT)_n$ probe was performed with barley genomic DNA library. One strongly

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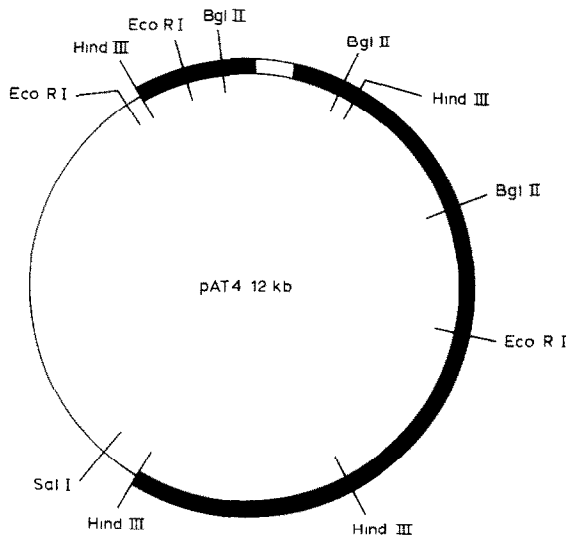

Fig. 1. Recombinant plasmid pAT4. Thick closed bars indicate inserted DNA of barley, open bar indicates repeat AT.

hybridizing clone among 1000 was isolated (Fig. 1). The clone marked as pAT4 has a barley DNA insertion of about 8.0 kb. It was shown by restriction and hybridization that the smaller fragment (650 bp) excised by *Bgl*II and *Cfr*13I restriction enzymes contains AT repeat. After filling ends of this fragment it was inserted into the *Sma*I site of pUC19 (vector was incubated with alkaline phosphatase). A new construction was marked as pAT4-2. Later on a part of the fragment from pAT4-2 and the *Bgl*II-*Bgl*II fragment from pAT4 were sequenced:

5′ TTGGGTGACGTGAAACGACATTAAACCCGG-
TATATATATATATATATATATATATATATATA-
TATATATATATATATATATAAAGGACGACGAT-
AAAACAGTAATTAGTTCTATACGTAAACAATG-
TGTAAAAAAGCGGCAAAACTTAACGATTATT -
GATTAATTAACATATAGTATACAATTTTGATA-
TCTGTACATTTTACGGCTCTTAAAACACATAT -
AATTCTTATAGGTTAAAAATTGGTCTATTTTT -
ACAAATTTGAACAACAAATTAATTCAAACGTA-
TCTACGGTACATTTTTAATAAACAGAGTATTA-
ATTAATTGGCATCGAGGCTAATTTTGTA 3′

As can be seen, this cloning sequence contains AT repeated 26 times. Computer analysis of the sequenced fragment has demonstrated a lack of translation frames as well as the absence of any significant homology with published sequences.

3.2. *Polymorphism analysis of fragment containing AT repeat*

We tried to analyse polymorphism of barley $(AT)_{26}$ in cereals. Nevertheless it is hard to choose such conditions for hybridization which would allow us to distinguish sequences varying in length by a few

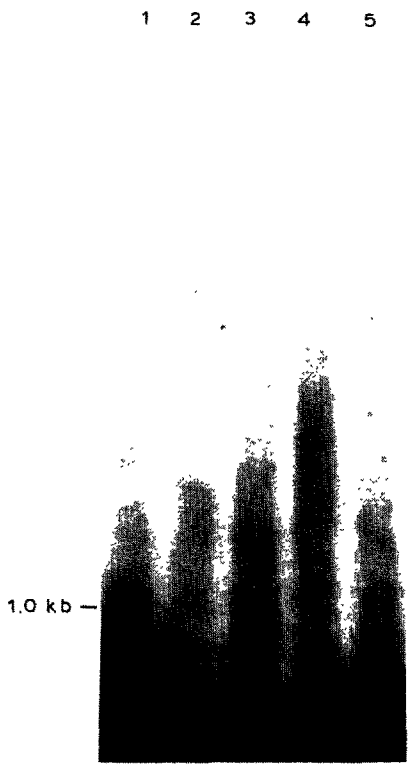

Fig. 2. Hybridization analysis of the nuclear DNA of different cereals using $(AT)_n$ probe. DNA is digested with *Bsp*RI. 1 = barley; 2 = oats; 3 = rye; 4 = wheat; 5 = maize.

nucleotides from $(AT)_{26}$. That is why $(AT)_n$ co-polymer labelled by nick-translation was used.

As a result $(AT)_n$ sequences were detected in the genome of oats, rye, wheat and maize as well as in barley genome (Fig. 2). As seen Fig. 2 separate bands are not observed. It means that $(AT)_{26}$ are interspersed sequences in genomes of the plants investigated.

Blot hybridizations using the 0.9 kb *Bgl*II-BglII fragment (Fig. 1) were fulfilled for searching polymorphism in fragments flanking AT repeat.

No DNA variation among individual plants, lines or varieties of barley was detected (Fig. 3). Using results of these hybridization experiments we have estimated that there are about 1500 copies of the *Bgl*II-*Bgl*II fragment per haploid barley genome.

Interspecific variation was found in barley (Fig. 4). Lack of hybridization is evident in the case of *H. jubatum*. Furthermore, the 1.0 kb hybridization band does not coincide with bands of other species. Moreover, an additional 0.2 kb fragment is present in *H. bulbosum* and a 7.0 kb fragment is absent in *H. vulgare*.

Polymorphism expressed among genera is stronger (Fig. 5). There are only some homologous sequences in barley, rye and wheat genomes and they quite lack in oat and maize genomes.

Thus we find that AT repeat is arranged in intraspecific locus of barley genome that has homology

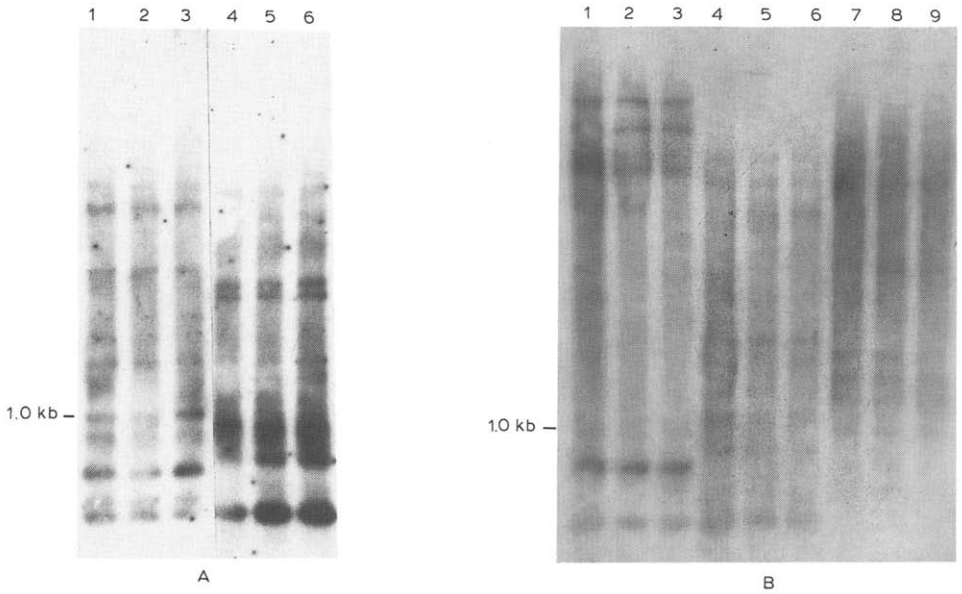

Fig. 3. Detection of polymorphism among individual plants or varieties of *H. vulgare* by their homology to the fragment *Bgl*II-*Bgl*II. (A) Hybridization pattern of DNA isolated from individual seedlings; 1,2,3 = DNA digested with *Cfr*13I; 4,5,6 = *Bsp*RI (B.) Hybridization pattern of DNA from Nadia cultivar (1,4,7); Min90 line (2,5,8); Waxy line (3,6,9). 1,2,3 = DNA digested with *Cfr*13I; 4,5,6 = *Bsp*RI; 7,8,9 = *Rsa*I.

with other sequences of such related cereals as rye and wheat.

3.3. *AT fragment is sensitive to nucleases*

As is already known, DNA segments containing simple sequences can form different uncanonical structures. For example $(AT)_{34}$ from the *Xenopus* genome adopts cruciform formation which is sensitive to different nucleases [22].

It was interesting to clarify the possibility of isolated $(AT)_{26}$ repeat to form such a structure. For this purpose supercoiled pAT4 was incubated with S1 and *Bal*31

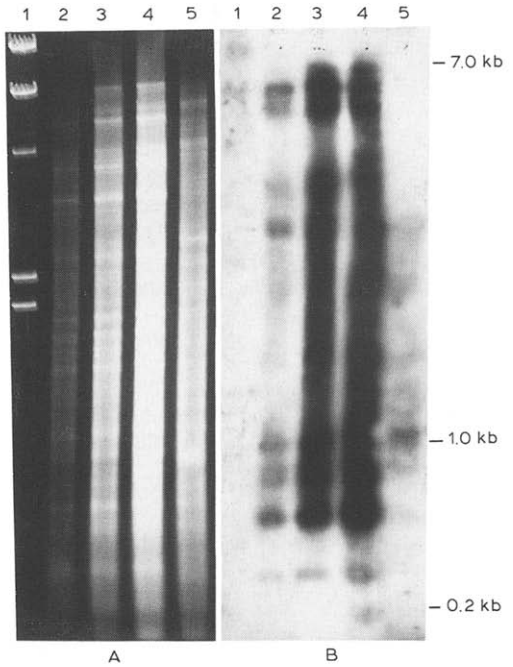

Fig. 4. Gel electrophoresis (A) and autoradiograms (B) showing polymorphism in *Cfr*13I fragments hybridizing cloned fragment in different species of *Hordeum*: 1 = λDNA digested with *Hin*dIII; 2 = *H. vulgare* DNA; 3 = *H. spontaneum*; 4 = *H. bulbosum*; 5 = *H. jubatum*.

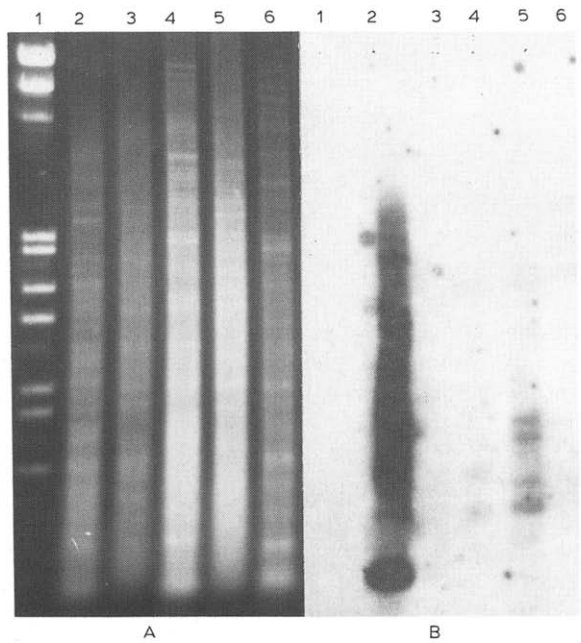

Fig. 5. Gel electrophoresis (A) and autoradiograms (B) showing polymorphism in *Bsp*RI fragments hybridizing cloned fragment in different cereals: 1 = λDNA digested with *Hin*dIII and *Eco*RI; 2 = barley DNA (Nadia cultivar); 3 = oats (Bug); 4 = rye (Voschod); 5 = wheat (Kupalinka); 6 = maize (606 5S line).

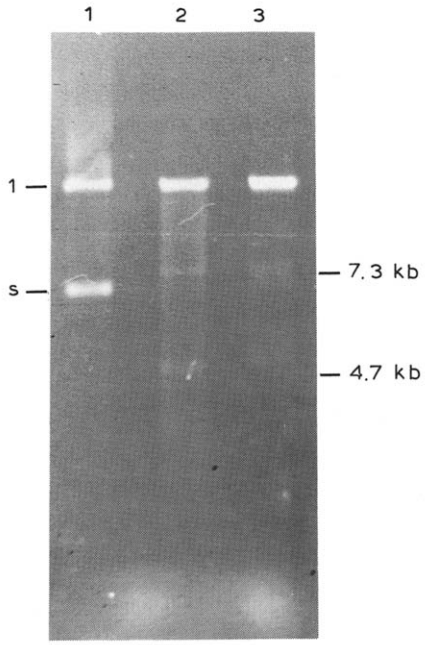

Fig. 6. Gel electrophoresis of supercoiled pAT4 cleaved by nucleases: 1 = native plasmid (l = linear form; s = supercoiled); 2 = pAT4 digested by restriction enzyme *SalI* and S1 nuclease; 3 - *SalI* and *Bal*31 nuclease.

nucleases which can recognize cruciform formations. Both nucleases together with the *SalI* restriction enzyme digest supercoiled pAT4 into 4.7 and 7.3 kb fragments (Fig. 6). Therefore at the level of resolution of agarose gel electrophoresis, we see that two single-strand specific nucleases appear to recognize and cleave a structural feature at, or close to, the $(AT)_{26}$ run in supercoiled pAT4. This AT repeat should therefore possess potential for cruciform formation.

4. DISCUSSION

So far, any published evidence concerning the role of simple sequences in genomes is unknown. However, as mentioned above, some authors consider them to be an important functional element of the genome. We therefore decided to investigate the AT repeat of barley genome.

It has been observed previously that genome segments containing simple sequences can be exposed to different changes that should result in restriction fragment length polymorphisms (RFLPs) of these sequences in genomes. An RFLPs study of the isolated AT locus shows its stability within species. But nevertheless it is not conservative within of Hordeum genus and especially Gramineae family. The cloned fragment can be used for identification of different barley species and for studies of filogenetic relationships in Gramineae too.

Absence of translation frames in the AT repeat region indicates its rearrangement in the intergenic region or region of big intron. Similar results have been obtained for the $(AT)_{34}$ repeat from *Xenopus* that was discovered in the first intron of the tadpole αT1 globin gene [2].

AT locus is sensitive to nucleases. That is why $(AT)_{26}$ can promote formation of cruciform structures as has been shown for *Xenopus* [22]. In turn these DNA structures can be different recognition sites for any proteins and regulator segments [23–25].

So the data obtained are indicative of the functional significance of the AT repeat in the genome. Probably $(AT)_{26}$ serves as a regulator sequence. However, further experiments are necessary to prove this.

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