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5S-RNA genes of barley are located on the second chromosome

A. Kolchinsky, V. Kanazin, E. Yakovleva, A. Gazumyan, C. Kole* and E. Ananiev

Laboratory of Plant Molecular Genetics, N.I. Vavilov Institute of General Genetics of the USSR Academy of Sciences, Gubkin str. 3, Moscow B-333, USSR

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Summary. The genes coding for *5S* RNA in barley were cloned, sequenced, and their cluster was assigned to chromosome 2 using wheat-barley chromosome addition lines. High-resolution gel-electrophoresis of DNA and subsequent hybridization revealed new details of the organization of *5S* DNA both in wheat and barley. The in situ hybridization of the cloned *5S* gene with triploid endosperm nuclei also suggests that these genes are located in a single locus.

Key words: *5S* gene – Barley – Wheat – Addition lines – In situ hybridization

Introduction

The barley-wheat chromosome addition lines (AD-lines) were produced by Islam et al. (1981). Each of them contained one barley chromosome. However, we know of only a few cases where they were used for the localization of barley genes (e.g., Bohlmann et al. 1988; Belostotsky et al. 1989). This is due to the difficulties in growing AD-lines and in their cytological control, and also due to some technical problems. However, molecular markers now find wide use in genome studies, and AD-lines prove to be extremely useful for their mapping.

The cloning and analysis of the *5S* genes of barley were undertaken in an attempt to determine a new molecular marker based on the intervarietal polymorphism of the *5S* genes. With this in view, we used the AD-lines for the assignment of the cluster of *5S* genes to a specific barley chromosome. The high-resolution electrophoresis used in this work provided some additional data on the organization of *5S* genes in wheat and barley.

* On leave from: Department of Plant Breeding and Genetics, College of Agriculture, Orissa University of Agriculture & Technology, Bhubaneswar-751003, India

Materials and methods

The AD-lines were kindly sent to us by Dr. Islam. They were grown under constant control of caryotype and showed reasonable fertility only at relatively high temperatures and moderate photoperiod. DNA was isolated from 7- to 8-day-old shoots using (GK36) lysis in 50 m*M* TRIS-OH, 0.2 *M* EDTA, pH 9.0, 0.5% SDS, 200 μg/ml pronase at 55 °C, and was further purified using routine procedures. Barley genes coding for ribosomal *5S* RNA were cloned earlier in our lab (Khvyrleva et al. 1988). For this purpose, BamHI fragments of barley DNA were cloned in pUC19, and the library was screened with cloned *5S* wheat genes kindly donated by Vakhitov and Gimalov (1988).

The cloned *5S* gene was 301 bp in length and represented the shorter *5S* gene of barley. It was sequenced using the Maxam and Gilbert procedure (1980).

Total plant DNA was digested with BamHI or MvaI, according to the manufacturer's instructions ("Ferment", Vilnius), purified, concentrated, and applied on nondenaturing 5% polyacrylamide gel (TRIS-borate buffer). After the electrophoresis, DNA was transferred onto the membrane "Hybond" (Amersham) using the electro-blotting device LKB. DNA was cross-linked to the membrane by UV-treatment and hybridized with ^{32}P-labelled probe. In order to prepare probes, cloned fragments were cut out from the vector, isolated by electrophoresis, and 50–100 ng was labelled using the random primer procedure (Feinberg and Vogelstein 1983). The same procedure was used for the ^{3}H-labelling of the fragment for in situ hybridization. In this case, 500–1000 ng of DNA and 50 μCi of ^{3}H-TTP were used in one reaction. ^{3}H-labelled DNA was purified on a minicolumn with Sephadex G-50, ethanol-precipitated, dissolved in 5 × SSC at 2×10^4 cpm/μl, and used for in situ hybridization.

The chromosomes were prepared for hybridization according to published protocols (Appels and Moran 1984).

Results

The sequence of the cloned *5S* gene is presented in Fig. 1. Its structural region is 95%–99% homologous to the *5S* genes of wheat (Gerlach and Dyer 1980) and rye (Lawrence and Appels 1986) (1–6 changes when com-

pared with different copies). The spacer shows much less interspecific homology: 5′- and 3′-regions – 60%–75%, and the central part (50 nucleotides in length) has no homology with other species. In general, wheat and rye show more homology with one another than with barley.

In order to assign the *5S* genes to a specific barley chromosome, we digested the DNA from AD-lines and from donor plants (the wheat cv Chinese Spring and the barley cv Betzes) with indicated restriction enzymes and analyzed them by hybridization as described in "Materials and methods". The result of this experiment is shown in Fig. 2. Here we used the restriction enzyme MvaI. It cuts the *5S* RNA genes of barley and most of the *5S* genes of wheat once per repeat and is not sensitive to methylation. DNA from different barley cultivars gives a known pattern (Khvyrleva et al. 1988); two major repeats, 301 and 450 bp long, are seen in the lower part of the lanes. Their relative content in cultivars studied varies significantly. Upper bands correspond to the repeats, which deviate from a canonical sequence; the shortest of them is 900 bp long.

The wheat cv Chinese Spring is a parental strain for AD-lines. It gives two sets of bands corresponding to unit repeats grouping around 410 and 500 bp (Gerlach and Dyer 1980). This fine heterogeneity of wheat *5S* within the 410-bp and 500-bp repetitive groups could not be shown earlier by agarose electrophoresis.

The picture clearly shows that only the AD-2 line contains the bands 301 and 900 bp long corresponding to barley *5S* genes. The 450-bp band of the barley moves too close to wheat bands and is probably masked by their hybridization.

Figure 3 presents the comparison of the digestion of *5S* genes with methylation-sensitive restriction enzyme EcoRII and with its isoschizomers BstNI and MvaI, which are not sensitive to methylation. The picture shows that the corresponding sites in *5S* genes are completely methylated both in wheat and in barley, and that the "EcoRII" site deviates from the sequence $CC^{A}/_{T}GG$ both in barley and in wheat. Some *5S* genes of wheat contain an additional "EcoRII" site and produce light bands on the gel (Fig. 1). It is to be noted that the degree of "EcoRII" degeneration in different AD-lines significantly varies, since the spectrum of oligomers is not conserved.

To check the identity of our AD-lines, the radioactive probe was washed away from the filter and was reprobed with a ^{32}P-labelled fragment of the repeat coding for the major ribosomal RNAs cloned earlier in our lab (Fig. 4) (Khvyrleva et al. 1987). We used the recloned fragment of a nontranscribed spacer that is known to be mostly species-specific. Although it shows some cross-hybridization with wheat DNA, there are barley-specific bands seen only in AD-lines 6 and 7. This experiment agrees well with the known localization of barley ribosomal genes on chromosomes 6 and 7 (Appels et al. 1980) and with the known fact of the different organization of ribosomal clusters in two nucleolar organizers (Appels and Moran 1984).

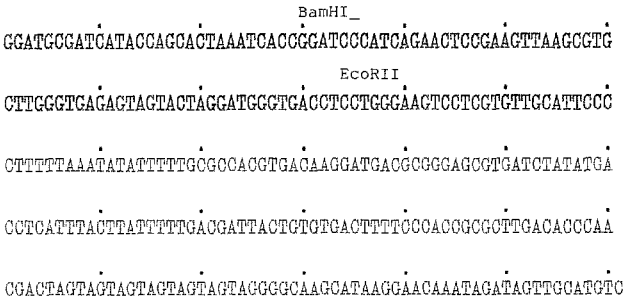

Fig. 1. The sequence of the 301-bp long repeat of barley coding for *5S* RNA. The coding region is indicated in boldface. Restriction sites used in this work are also indicated

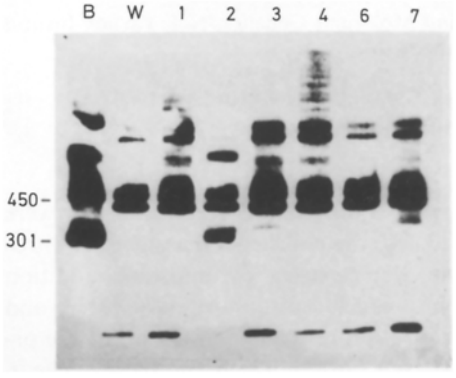

Fig. 2. Blot-hybridization of DNA isolated from AD-lines and their parental cultivars with the cloned *5S* gene. *B*–barley cv Betzes; *W*–wheat cv Chinese Spring; *1*, *2*, *3*, *4*, *6*, *7*–AD-lines. All DNAs were digested with MvaI. The length of some bands is indicated in base pairs

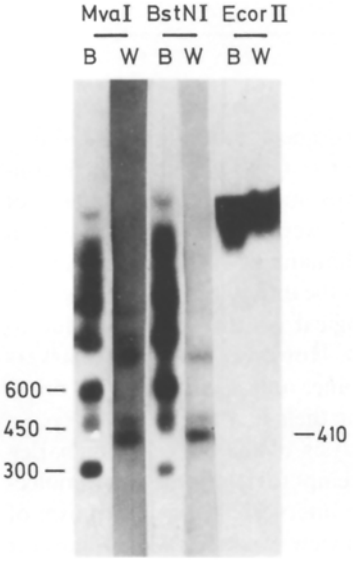

Fig. 3. Methylation of EcoRII sites in the *5S* genes of wheat and barley. The enzymes are indicated above the lanes. *B*–barley, *W*–wheat. The lengths of some fragments are also indicated

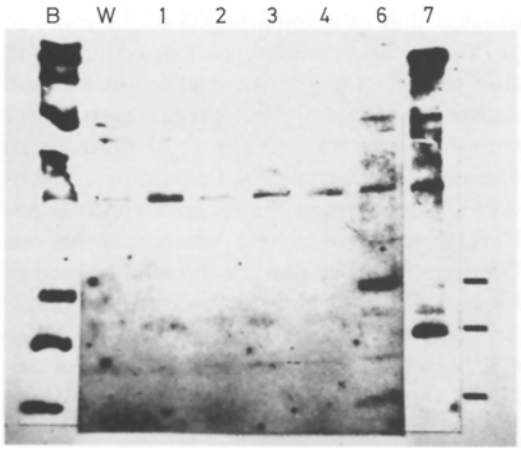

Fig. 4. The filter shown on Fig. 1 was reprobed with a fragmei of the repeat coding for 18 S and 28 S ribosomal RNAs. The ba indicate barley-specific bands

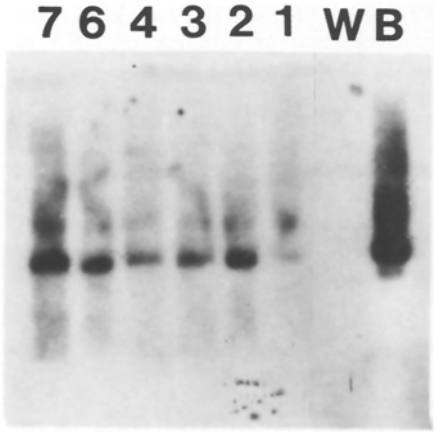

Fig. 5. Blot-hybridization of DNA preparations used for the localization of *5S* genes with barley-specific probe (see text). The designations are the same as in Fig. 1. DNA was digested with BspRI

Another way to show the presence of barley chromosomes in the AD-lines is to hybridize their DNA with a barley-specific repeat. One such repeat was cloned in the laboratory of Dr. Metzlaff (Halle, GDR) (Junghans and Metzlaff 1988) and kindly donated to us by the authors. The result of this experiment is shown in Fig. 5. It proves the presence of barley chromosomes in our stocks of AD-lines.

The in situ hybridization of cloned *5S* gene was carried out in order to estimate the number of loci containing these genes. We used triploid nucleus from the endosperm and Fig. 6 shows the results of this experiment. Three hybridizing sites are seen on some metaphase plates (maximum number) (Fig. 6A); the control hybridization with cloned major ribosomal repeat reveals six hybridizing sites (Fig. 6B).

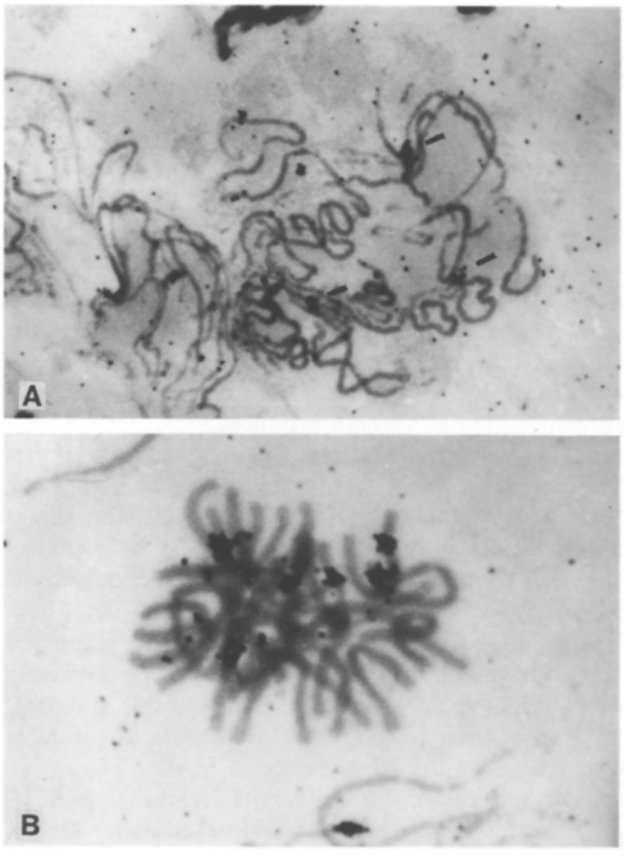

Fig. 6A and B. In situ hybridization of ^{3}H-labelled *5S* genes (**A**) and major ribosomal repeat (**B**) with triploid nuclei of barley endosperm. The *bars* on **A** indicate the sites of hybridization

Discussion

The first step in the investigation of *5S* barley genes as a potential molecular marker was their cloning and sequencing (Fig. 1). The comparison of barley gene with the corresponding genes of wheat and rye (Khvyrleva et al. 1988) shows that the homology between them is somewhat higher than with barley. This trend is manifested in their chromosomal localization: in rye and wheat, NOR-containing chromosomes bear *5S* loci, while in barley they do not.

The *5S* genes of wheat and rye were already assigned to the chromosomes carrying nucleolar organizers (NOR) some time ago (Appels et al. 1980; Lawrence and Appels 1986). These authors used in situ hybridization techniques and easily identified NOR-containing chromosomes. They also concluded that in barley the NOR-containing chromosomes 6 and 7 did not hybridize with the *5S* gene.

We used another approach to the localization of the *5S* cluster. It is based on the analysis of DNA from chromosome addition lines of wheat containing single barley chromosomes. Evidently, this approach requires

careful control of AD-lines, which tend to lose barley chromosomes spontaneously and frequently.

Figure 2 shows the result of the hybridization of the barley *5S* gene with DNA from six available AD-lines and their parental cultivars of barley (Betzes) and of wheat (Chinese Spring). High resolution of this picture is due to the use of PAGE and allows one to conclude that the cluster of *5S* genes is located on chromosome 2 of barley, and that the *5S* genes are absent from chromosomes 1, 3, 4, 6, and 7, because only AD-line 2 contains bands 300 and 900 bp in length that are characteristic for barley. On the basis of this experiment, no statement can be made concerning chromosome 5, because the corresponding AD-line was not produced.

Figure 2 suggests that the 301- and 450-bp repeates are not interspersed, because there is no trace of the 750-bp band which should arise in this case. It does not appear even in overexposed pictures.

The *5S* repeats of wheat are more variable than was reported earlier. In fact, the so called "410"- and "500"-bp long units are both families of repeats. These families consist of discrete members differing by approximately 10 bp from one another. There is also a class of repeats with an additional "EcoRII" (MvaI) site. It produces short bands on the gel.

Barley contains 3–5 times more *5S* repeats per similar amount of DNA than wheat (visual estimate). Hence, it contains some 10–15 times more genes per haploid genome (barley is diploid and wheat is hexaploid).

Figure 3 illustrates the methylation of the EcoRII site in the *5S* genes of wheat and barley. It shows that the cytosine in the sequence $C^A/_TG$ (a target of methylation in plants) is completely methylated at this site. It also shows that the heterogeneity of barley *5S* genes is much more pronounced than that of wheat. The restriction enzymes MvaI and BstNI are not sensitive to methylation. The MvaI site is located in the coding region of the *5S* gene; however, this site is often not cut by the enzyme due to nucleotide changes. In contrast to this, this site is rather conservative in wheat. It is possible that many *5S* repeats in barley are not functional. A similar phenomenon is known for wheat (Gerlach and Dyer 1980).

The reprobing of the filter used for the localization of *5S* genes with a fragment of the major ribosomal repeat clearly reveals chromosomes 6 and 7 of barley in the corresponding AD-lines (Fig. 4). It is quite evident that the repeats in two NORs of barley have a different organization (Appels and Moran 1984). Another proof of the presence of alien chromatin in AD-lines is presented in Fig. 5: a barley-specific repetitive probe hybridizes with AD-lines, but not with Chinese Spring itself.

It has not been possible to produce an AD-line containing chromosome 5 to date. Hence, the possibility cannot be excluded that there is another cluster of *5S* genes in this chromosome. Moreover, Appels et al. (1980) suggested that additional site(s) of *5S* genes in cereals exist. To test this possibility, we carried out in situ hybridization of the *5S* gene with triploid nuclei from barley endosperm (Fig. 6). The preparations never showed more than one clear-cut cluster of silver grains per haploid genome. Unfortunately, the cytological identification of chromosomes in these preparations is not possible. Nevertheless, we conclude that there is only one cluster of *5S* genes in barley genome. It could be used as a molecular marker in genetic studies since the minor bands hybridizing with the *5S* probe and the relative amount of 301- and 450-bp repeats vary in different cultivars (data not shown).

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Note added in proof

Recently we used the polymerase chain reaction with 5S gene specific primers. The results confirmed our conclusion: the barley 5S genes are on chromosome 2.