Distribution and clustering of two highly repeated sequences in the A and B chromosomes of maize*

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Summary. Clones from a family of highly repeated sequences present in a heterochromatin rich maize line have been characterized by sequencing and chromosome location. The repeats differ from each other in length and degree of sequence homology, and show areas rich in purine and pyrimidine. In situ hybridization experiments indicate that the repeats are mainly located in the knob heterochromatin of the A chromosomes and the centromeric heterochromatin of the B chromosome. However, in addition to previously published data, some copies are also distributed in euchromatic regions of the A chromosomes and in the distal heterochromatic block of the B chromosome. The results are discussed in relation to the centromeric activity of maize heterochromatin.

Key words: Maize – Repetitive DNA – A and B chromosomes – Centromere activity

Introduction

The genome of Zea mays is largely constituted by repetitive DNA (60–80%) mainly dispersed with unique or different repetitive sequences (Flavell et al. 1974; Hake and Walbot 1980). Studies on the genome organization of maize by DNA reassociation kinetics (Cot analysis) showed that short repetitive sequences of 500–1,000 bp are interspersed with unique sequences 2,100 bp in average length; middle repetitive sequences are also interspersed with highly repetitive sequences (Hake and Walbot 1980).

In most plant and animal genomes highly repetitive DNA is clustered at specific chromosomal sites, the heterochromatic regions (Jones 1970; Pardue and Gall 1970; Gall 1973; Flavell 1980). In maize, four types of heterochromatin can be recognized by their appearance, chromosomal location, replication pattern and genetic effects: (i) knob heterochromatin, (ii) NOR heterochromatin, (iii) centromeric heterochromatin and (iv) block heterochromatin of the B chromosome. Heterochromatic knobs are sharply delimited and are located at an appreciable distance from the centromere; moreover both number and size of knobs are not constant from variety to variety. The heterochromatin at the nucleolus organizer region (NOR) is morphologically similar to knob heterochromatin but it is always located at the secondary constriction on chromosome 6; hybridization studies localized most of the rDNA cistrons within the NOR heterochromatin (Givens and Phillips 1973). Most of the B heterochromatin is located in few large cushion-like blocks in the distal half of the long arm. Finally, centromeric heterochromatin is distinguishable from the adjacent chromatin only by the degree of heteropycnosis and not by its peculiar shape. The centromeric heterochromatin of the B chromosome, although similar in its appearance to knob heterochromatin, differs from it for its pericentric location.

Recently we have cloned several repetitive sequences from DNA fragments obtained by digestion with restriction enzymes that cut frequently. The distribution on A and B chromosomes and the reiteration frequency in maize lines with different heterochromatin and B chromosome content for three of these clones are reported in the present work.

Materials and methods

Maize and teosinte stocks

Stocks of maize with varying number of knobs and B chromosomes were used in the experiments. Three stocks of Black
Mexican with 0, 2 and 6 B chromosomes, respectively were used; all were knobless except for two small knobs on chromosome 6. The inbred lines KYS and W64A showed five and six knobs, respectively. Teosintes, *Zea mays* spp. *mexicana*, var. 'Chalco' and *Zea mays* spp. *parviglumis*, var. 'Batan', have 11 very large knobs.

**Purification of DNA and cloning**

DNA extracted from unfertilized ears of maize and seedlings of teosinte was purified as previously described (Viotti et al. 1982). For cloning, the DNA was digested with restriction enzymes, electrophoresed on 5% acrylamide gel and stained with ethidium bromide. The DNA of the more heavily stained bands in the heterochromatin rich line (W64A) was eluted from the gel slices and cloned in the pUC8 plasmid vector linearized by Hinc II, Acc I or BamHI restriction enzymes.

**Southern hybridization and DNA sequencing**

Three µg of DNA from each maize line were digested with ten units of restriction enzymes for 3 h at 37°C. The digested DNA was electrophoresed on 0.7% agarose, 2.5% acrylamide, composite gel. 2 h at 10 V/cm in Tris-borate buffer (Tris 90 mM, EDTA 2 mM, boric acid, pH 8.2). After staining, the gel (2 mm tick) was treated for 30 min with NaOH 0.4 M, NaCl 0.5 M and then blotted with 20×SSC (NaCl 3 M, sodium citrate 0.3 M, pH 7.2) on Biodyne A membrane (Pall) for 3 h at room temperature. The gel was then stained to control the transfer. Filters were treated as usual for Southern blot and hybridized as previously described (Viotti et al. 1982). Washing of the filters was carried out under stringent conditions, 0.2×SSC at 68°C for 2 h. The DNA sequences on both strands were determined on fragments labelled at the 5' end by polynucleotide-kinase and purified by strand separation, by the chemical degradation reactions G, G + A, T + C, C and A + C (Maxam and Gilbert 1980).

**“In situ” hybridization**

Recombinant plasmid labelled at a specific activity of 1–3× 10⁷ dpm/µg were obtained by nick translation using ³²P-TTP (90 Ci/m mole, NEN). "In situ" hybridization experiments were made essentially as described in a previous paper (Viotti et al. 1982). Denaturation was carried out in 90% deionized formamide, 0.1×SSC for 150 min at 65°C, according to Singh et al. (1977). The slides were hybridized for 24–36 h at 68°C and washed in cold 0.1×SSC three times for 10 min each, then in 1×SSC at 65°C for 30 min, and finally in 0.2×SSC at 65°C for 20 min. The slides were dipped in a Kodak NTB-2.