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Physical mapping of four RAPDs in the B chromosome of maize

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Abstract Four DNA fragments were amplified specifically from the B chromosome by PCR using random 10-base oligonucleotides as primers. The location of the fragments in the B chromosome was determined based on whether or not they were amplified from the hypoploid DNA generated by four B-A translocations, three of which break in the proximal euchromatic region and the fourth in the distal one-third of the heterochromatic region on the B long arm. Since the hypoploid DNA carries the portion of the B chromosome distal to the breakpoint of a translocation, the presence of a fragment in the hypoploid DNA, but not in the control (which is devoid of any B chromatin), indicates that the fragments is located in the B region distal to the breakpoint in the B long arm. Two fragments were mapped to the euchromatic region and two others to either the distal portion of the euchromatic region or the proximal two-thirds of the heterochromatic region. These fragments in turn mapped three B-A translocations whose breakpoints were located in the euchromatic region.

Key words *Zea mays* · B chromosome · RAPD · B-A translocation

Introduction

In maize as in other plants, the B chromosome is also called extra, nonessential or supernumerary because of its genetic inertness. A plant may carry as many as 10 B chromosomes in addition to the normal complement and grow normally, but individual plants of higher B numbers express various morphological and physiological effects, including reduction in fertility, production of defective seeds, scarred endosperm, variation in pollen size and an increase in pollen abortion (Randolph 1941). Nevertheless, the B chromosome is not completely devoid of genes; it carries at least a gene(s) that causes chromosome breaks in knob regions (Rhoades and Dempsey 1973) and that enhances crossing-over in the normal chromosome complement (Roades 1968; Hanson 1969; Ward 1973b). It also carries genes conditioning its own behavior, i.e. nondisjunction, during the second pollen mitosis, which results in the formation of two sperm with different chromosome constitutions: one with two Bs and the other devoid of B (Roman 1947; Ward 1973a; Carlson 1970, 1973; Lin 1978, 1979; Carlson and Chou 1981).

The development of B-chromosome genetics is hindered by the fact that the effect of these genes is difficult to analyze, making the exploration of new genetic markers necessary. Toward this end, Alfenito and Birchler (1993) cloned eight DNA sequences that hybridized strongly or specifically with the genomic DNA containing the B chromosome. The fact that the Southern hybridization patterns of these sequences with genomic DNA are very similar and that they can hybridize strongly with each other suggests that the sequences are at least highly homologous and perhaps identical. Moreover, cytological mapping and *in situ* hybridization of B-chromosome derivatives using these sequences as probes resulted in an unexpected finding – all of the sequences are located in the centromeric region. Thus, the utility of these sequences for genetic analysis is rather limited expect for serving as landmarks for the centromere.

Other markers that can be exploited as B-chromosome markers are random amplified polymorphic DNA sequences (RAPDs). RAPDs are DNA fragments amplified from plant genomic DNA by the polymerase chain reaction (PCR) using a single 10-base oligonucleotide as primer (Williams et al. 1990). Since

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its inception, RAPDs have been widely used to produce molecular markers for studying genetic and breeding problems in various plants. Advantages include the requirement of only small amounts of DNA as template, simplicity of the technique, rapid performance and no involvement of radioactivity (Waugh and Powell 1992). It has been used to construct linkage maps (Devos and Gale 1992; Marsan et al. 1993), to tag genes of interest (Martin et al. 1991; Michelmore et al. 1991; Oh et al. 1994; Lorenz 1994), to map quantitative trait loci (Pe et al. 1993), to evaluate and conserve genetic resources (Waugh and Powell 1992) and to study phylogenic relationships (He et al. 1992; Assigbetse et al. 1994; Van-Buren et al. 1994).

The objective of the study presented here was to isolate B-specific RAPD fragments and to map them with B-A translocations.

Materials and methods

Genetic stocks

L289 carrying a single B chromosome was developed by J. B. Beckett from a B-containing plant that had been continuously backcrossed to L289. It was used to produce L289 carrying three B chromosomes (referred to as L289 + B in this report) and L289 without any B chromosomes. The genomic DNAs of these two L289 lines were used to screen the B-specific RAPD fragments. Four B-A translocations were used to map the B-specific RAPD fragments; all had a breakpoint in the B long arm and a second breakpoint in different members of the normal complement. They were introduced into L289 from translocation stocks by continuous backcrosses as female with L289 for at least five generations. The breakpoint of TB-1La is in the long arm of chromosome 1, TB-3Sb in the short arm of chromosome 3, TB-4Sa in the short arm of chromosome 4 and TB-7Lb in the long arm of chromosome 7 (Roman 1947; Beckett 1978).

Synthesis of hypoploids

Hypoploids of a B-A translocation are plants that contain the A-B (carrying the A centromere) but not the B-A (carrying the B centromere) chromosome. They are deficient for the portion of the A chromosome arm that was carried on the B-A chromosome. They were produced by crossing inbred L289 carrying a B-A translocation as staminate plants to inbred B73. A B-A translocation, TB-1La for example, consists of two different chromosomes, 1-B and B-1 in this example. 1-B bears the short arm, the centromere and the proximal portion of the long arm of chromosome 1 attached to the distal portion of the B long arm. B-1 has the B short arm and centromere in addition to the proximal portion of the B long arm connected to the terminal portion of the long arm of chromosome 1. At maturity, B-1, but not 1-B, undergoes nondisjunction during the second pollen mitosis to result in two sperm with different genetic constitutions: one with 1-B B-1 B-1 (hyperploid) and the other with 1-B (hypoploid) (Roman 1947). As a consequences, when TB-1La plants were crossed as staminate plants with a pistillate plant devoid of any B-A translocation, two different kernel types were produced: one had a hypoploid embryo carrying only 1-B from the translocation in association with endosperm having 1-B B-1 B-1; the other kernel type had a reverse chromosome constitution. These two kernel types could be distinguished according to kernel size; the former being twice as large as the latter. The size reduction of the latter is due to its endosperm lacking a paternal endosperm factor(s) (*Ef*) located on the long arm of chromosome 1 that is carried on the B-1 chromosome (Lin 1982). Thus, the large kernels grew into a hypoploid plant, the chromosome constitution of which was further confirmed by the presence of 50% pollen sterility. Of the four B-A translocations used in this study, all were associated with the kernel size effect (Birchler and Hart 1987); therefore, hypoploid plants were isolated and identified as described above.

Genomic DNA isolation

Most genomic DNA was isolated by the method of Riedel et al. (1990) and some by the procedure outlined as Saghai-Maroof et al. (1984) with modifications. Briefly, frozen tissues were ground into powder which was placed into a 50-ml tube, mixed with 1.3 ml per gram of tissue of prewarmed (65°C) extraction buffer [100 m*M* Tris-Cl (pH 7.5), 0.7 *M* NaCl, 10 m*M* EDTA (pH 8.0), 140 m*M* β-mercaptoethanol, 10% CTAB (mixed alkyltrimethyl-ammonium bromide)], treated at 65°C for 60–90 min and cooled to RT. The DNA was then treated with 4.5 ml chloroform/octanol (24 : 1 v/v) twice, followed by treatment with RNase A (10 µg/ml) before precipitation with isopropanol. The precipitated DNA was spooled out using a glass hook and resuspended in TE [10 m*M* Tris-Cl (8.0), 1 m*M* EDTA]. Some genomic DNAs so prepared could be digested efficiently by restriction enzymes, but most required a phenol treatment before another chloroform extraction. For long-term storage, the isolated DNAs were further purified with cesium chloride centrifugation, dissolved in TE at about a 1 mg/ml final concentration and stored over chloroform at 4°C until used.

Primers

One hundred primers were from Operon Technologies (Calif., USA), and 200 others from the Oligonucleotide Synthesis Laboratory, the University of British Columbia (Vancouver, B.C., Canada). G + C content of the primer was 50%–70%, and they do not have internal repeats.

DNA amplification

The protocol outlined by Williams et al. (1990) was used to amplify DNA fragments using Super-*Taq* DNA polymerase (from HT Biotechnology) instead of *Taq* DNA polymerase. The amplification process was performed in a Ericomp DNA thermocycler for 40 cycles with 1 min at 94°C, 1 min at 36°C and 1 min at 72°C for each cycle. The reaction products were analyzed by electrophoresis in 1.2% agarose gels or in 5% polyacrylamide gels stained with ethidium bromide.

Results and discussion

Isolation of B-specific RAPD fragments

Each of the 300 10-base primers was singly used to amplify DNA L289 and L289 + B. Four UBC primers amplified a fragment from L289 + B but not from L289 DNA (lanes 2 and 3, Fig. 1A–D), and the remaining primers produced either the same fragment pattern for L289 and L289 + B DNAs (83% for Operon primers

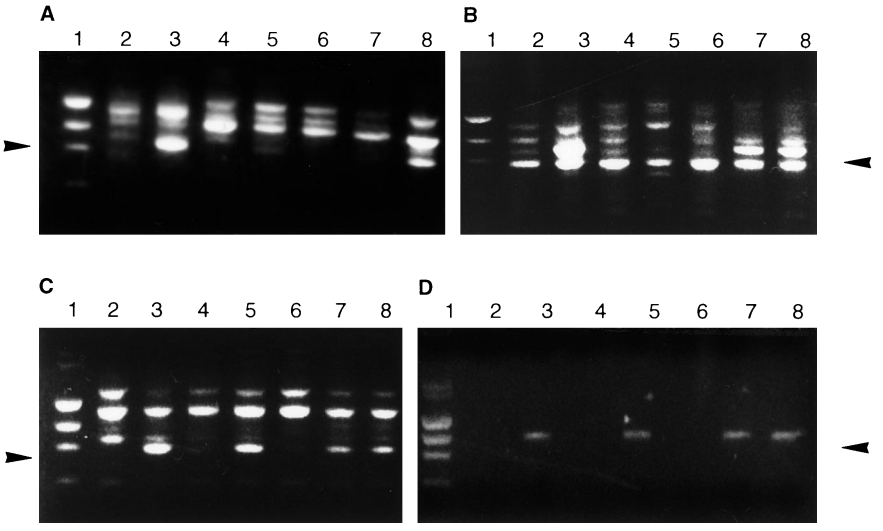

Fig. 1A–D Mapping of B-specific RAPD fragments by the breakpoints of four B-A translocations. The four 10-base primers used to amplify the B-specific fragments (*arrowed*) are UBC313 (**A**), UBC345 (**B**), UBC349 (**C**) and UBC426 (**D**). *Lane 1* Size markers (ϕ χ174-*Hae*III + λ-*Hin*dIII digest), *lane 2* L289, *lane 3* L289 + B, *lane 4* hybrid between L289 and B73, *lane 5*, hypoploid of TB-1La, *lane 6* hypoploid of TB-3Sb, *lane 7* hypoploid of TB-4Sa, and *lane 8* hypoploid of TB-7Lb

Table 1 Four 10-base primers used to amplify B-specific DNA fragments

Primer	Sequence	Number of major bands amplified	Size of B-specific fragment (kb)
UBC313	ACGGCAGTGG	4	0.83
UBC345	GCGTGACCCG	4	0.97
UBC349	GGAGCCCCCT	3	0.83
UBC426	TCTCCCGGTG	1	1.18

and 74% for UBC primers) or no fragment at all (data not shown). The experiments that produced the four B-specific fragments were repeated at least five times. The absence of the B-specific fragment occurred not only in L289 but also in the hybrid DNA between inbred L289 and inbred B73 as template (lane 4, Fig. 1A–D). None of the Operon primers produced any B-specific fragment. The 4 UBC primers that produced B-specific fragments had at least 60% G + C content. The B-specific fragments ranged from 0.83 kb to 1.18 kb and were named after the primer that was used to amplify them (Table 1). The UBC313, UBC349 and UBC426 fragments were very clear, for they were present only in L289 + B (lane 3, Fig. 1A–D) but not in the L289 template (lane 2, Fig. 1A–D). UBC345 differs in that while both L289 and L289 + B templates produce a fragment of about 0.97 kb, that from the L289 + B template is much more intense than that from L289 in agarose gels (lane 2, Fig. 1B). This difference persisted when DNA of a hybrid between inbred L289 and inbred B73 was used as the template (lane 4, Fig. 1A–D). The same reaction products were analyzed by 1.5% polyacrylamide gel electrophoresis. A 0.97-kb fragment was clearly present only in L289 + B and not in L289 (data not shown). Evidently, the faint band is an artifact.

Breakpoints of B-A translocations

The B chromosome of maize has a very short arm and a long arm, and its centromere is closely associated with a large knob (Lin 1979). The long arm consists of three regions: the proximal euchromatic region (about one-third of the long arm), the distal heterochromatic region (about two-thirds of the long arm) and a small distal euchromatic tip. The distal heterochromatic region includes four subregions of about the same size (Lin 1979). The breakpoint in TB-1La is in the proximal euchromatic region such that the B-1 chromosome carries the proximal portion of the B chromosome including – from proximal to distal – the short arm, the centromere, the proximal portion of the euchromatic region of the B long arm (Fig. 2A); however, the 1-B chromosome carries the distal portion of the B chromosome, which includes the distal portion of the euchromatic region, the entire distal heterochromatic region and the small euchromatic tip (data not shown). Likewise, the breakpoints in TB-4Sa and TB-7Lb are in the proximal euchromatic region (Fig. 2C, D). TB-3Sb is different; it breaks in the distal one-third of the distal heterochromatic region such that the B-3 chromosome carries the entire B chromosome except for the distal one-third of the distal heterochromatic region and the small euchromatic tip (Fig. 2B). In short, of the four translocations used in this study, three break in the proximal euchromatic region and the other in the distal heterochromatic region.

Mapping RAPD fragments by B-A translocations

The B-specific fragments were mapped by the breakpoints of four B-A translocations. The rationale for the mapping is as follows. The A-B chromosome of each translocation carries the portion of the B long arm

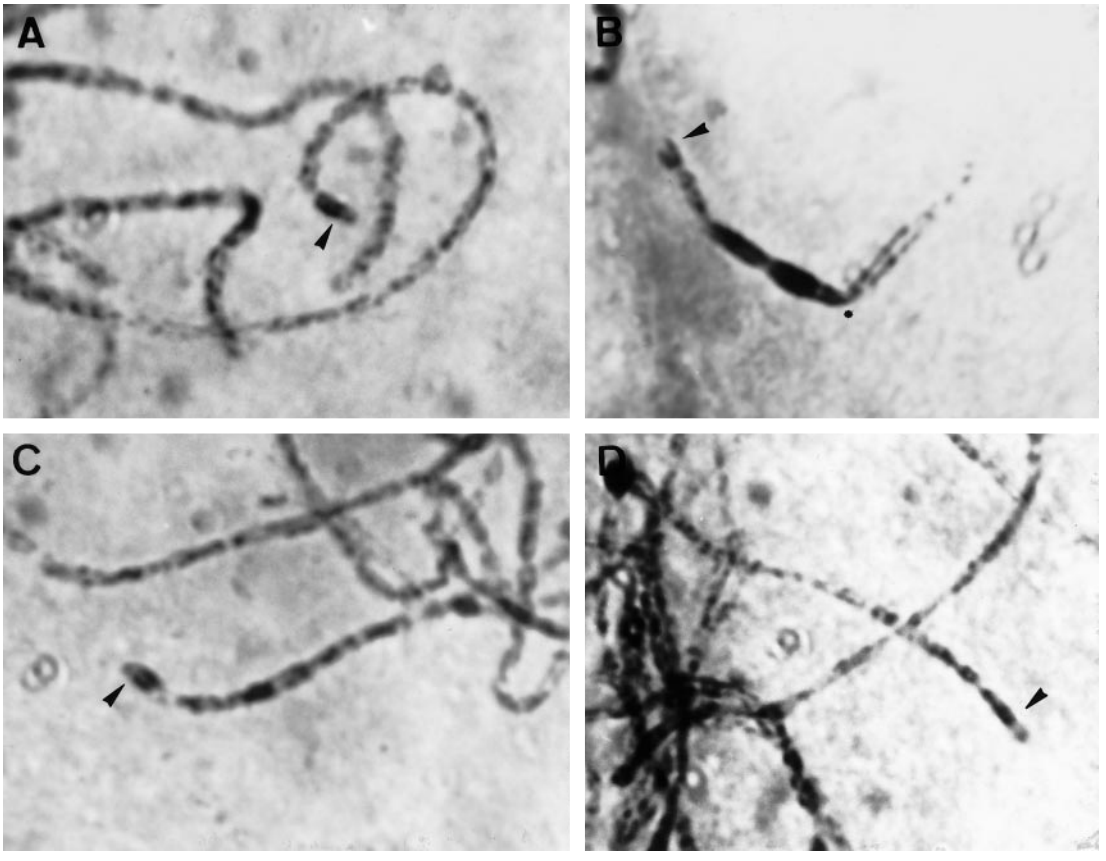

Fig. 2A–D Pachytene structure of four synapsed B-A chromosomes. The B centromere is marked by an *arrow*. The four translocations used to generate the B-A chromosomes were TB-1La (**A**), TB-3Sb (**B**), TB-4Sa (**C**) and TB-7Lb (**D**). The breakpoint of TB-3Sb is marked by an *asterisk*. The breakpoints for the other translocations could not be determined since the euchromatic region of the B chromosome is cytologically indistinguishable from that of the A chromosome

distal to its B breakpoint, and the hypoploid progeny derived from a translocation contains the A-B but not the B-A chromosome (see Materials and methods). When a B-specific fragment is amplified by a primer from a hypoploid DNA but not from the control DNA devoid of the translocation, the fragment must be located in the portion of the B long arm that is distal to the translocation breakpoint. On the other hand, when the B-specific fragment is not amplified by a primer from either the hypoploid or the control DNAs, it should be located in the B region proximal to the breakpoint of the translocation. Thus, the absence or presence of the B-specific fragment in the amplification products of a hypoploid DNA indicates whether it is located in a B region proximal or distal to the breakpoint of the translocation. When tested against all four B-A translocations by this system, a B-specific fragment will be mapped to a B region delimited by the breakpoints of two adjacent translocations.

Figure 1A shows the result of mapping analysis of the B-specific UBC313 fragment (0.83 kb). It is present in L289 + B (lane 3) and the hypoploid DNA of TB-7Lb (lane 8) but not in either the hypoploid DNA of the three other translocations or the control DNA (lanes 2, 4–7). Thus, UBC313 should be located in a region distal to the breakpoint of TB-7Lb but proximal to that of the three other translocations. However, UBC345 (0.97 kb) had a different map position; as shown in Fig. 1B; it is present not only in the products of the hypoploid DNA of TB-7Lb but also in that of TB-4Sa, indicating that it is distal to the breakpoint of TB-7Lb and TB-4Sa and proximal to that of TB-1La and TB-3Sb. Therefore, the position of UBC313 is between the breakpoints of TB-7Lb and TB-4Sa in the proximal euchromatic region.

As shown in Fig. 1C, the B-specific UBC349 fragment (0.83 kb) appeared in the products of hypoploid DNAs of TB-7Lb, TB-4Sa and TB-1La (lanes 5, 7 and 8) but not in that of TB-3Sb (lane 6) as template. This result in conjunction with that of Fig. 1B demonstrates that the position of UBC345 is between the breakpoints of TB-4Sa and TB-1La in the proximal euchromatic region and that UBC349 is located between the breakpoints of TB-1La and TB-3Sb, either in the euchromatic region or in the proximal two-thirds of the distal heterochromatic region. Also mapped to the same region was UBC426 (1.18 kb), which was similarly

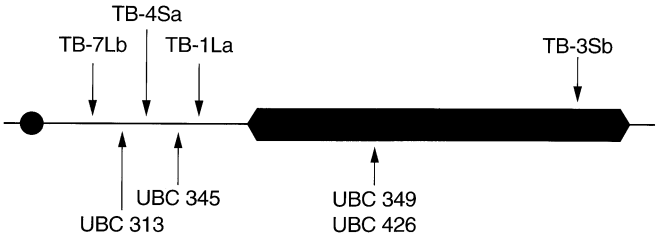

Fig. 3 Map position of four B-specific RAPD fragments in relation to the breakpoints of four B-A translocations

present in the products of hypoploid DNAs of TB-7Lb, TB-4Sa and TB-1La but not TB-3Sb (lanes 2, 5, 7 and 8, Fig. 1D).

The physical map of the four B-specific fragments in relation to the breakpoints of four B-A translocations is: centromere-TB-7Lb-UBC313-TB-4Sa-UBC345-TB-1La-(UBC349, UBC426)-TB-3Sb-the distal end of the B long arm (Fig. 3). The breakpoints of four B-A translocations mapped two (UBC313 and UBC345) of the four B-specific fragments in the proximal euchromatic region, and two others (UBC349 and UBC426) either in the distal portion of the proximal euchromatic region or in the distal heterochromatic region. In turn, these B-specific fragments mapped the breakpoint of three B-A translocations in the proximal euchromatic region of the B long arm, with TB-7Lb having the most proximal and TB-1La the most distal position. The relative position of the three breakpoints can not be determined by cytological analysis, as the pachytene structure of the proximal euchromatic region of the B long arm is indistinguishable from that of the A chromosomes.

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