Mapping RFLP Loci in Maize Using B-A Translocations

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ABSTRACT

Plants hypoploid for specific segments of each of the maize (Zea mays L.) chromosomes were generated using 24 different B-A translocations. Plants carrying each of the B-A translocations were crossed as male parents to inbreds, and sibling progeny hypoploid or not hypoploid for specific chromosomal segments were recovered. Genomic DNAs from the parents, hypoploid progeny, and nonhypoploid (euploid or hyperploid) progeny for each of these B-A translocations were digested with restriction enzymes, electrophoresed in agarose gels, blotted onto reusable nylon membranes, and probed with nick-translated, cloned DNA fragments which had been mapped previously by restriction fragment length polymorphism (RFLP) analysis to the chromosome involved in the B-A translocation. The chromosomal segment on our RFLP map which was uncovered by each of the B-A translocations was determined. This work unequivocally identified the short and long arms of each chromosome on this map, and it also identified the region on each chromosome which contains the centromere. Because the breakpoints of the B-A translocations were previously known on the cytological and the conventional genetic maps, this study also allowed this RFLP map to be more highly correlated with these maps.

CONSIDERABLE progress has been made by our group and other groups in constructing restriction fragment length polymorphism (RFLP) maps for maize. The high levels of polymorphism in the maize genome (Burroughs et al. 1983; Rivin et al. 1983; Helentjaris et al. 1985; Helentjaris, Weber and Wright 1986) and the wealth of cytogenetic variants in maize (Carlson 1977) have allowed this work to progress at a remarkably rapid rate. Currently, RFLP maps are being developed in maize by our group (Helentjaris, Weber and Wright 1986, 1988), and by B. Burr and F. Burr (Burr et al. 1988), D. Hoisington (Hoisington, Coe and Neuffer 1988), Agrigenetics (Murray et al. 1988), and Pioneer Hi-Bred Company (D. Grant, personal communication).

The establishment of linkage groups on RFLP maps is an exceedingly efficient process because most RFLP loci can be analyzed in a single segregating F2 or testcross population. Furthermore, the chromosome associated with each linkage group can be efficiently determined utilizing monosomics (Helentjaris, Weber and Wright 1986). In fact, the number of RFLP loci mapped in maize by various groups now far exceeds the number of morphological and biochemical (isozymic) loci mapped on the conventional maize genetic map.

Unfortunately, RFLP maps in maize are poorly correlated with the conventional map at the present time. Certain maize loci, which were previously mapped on the conventional map, have been cloned by other investigators, and these cloned sequences have been used as probes and mapped on our RFLP map (Helentjaris, Wright and Weber 1986; Wright, Helentjaris and Kikuchi 1987). Also, certain morphological and isozymic loci have been mapped on our RFLP map by linkage analysis of segregating populations (Helentjaris, Wright and Weber 1986; Helentjaris, Weber and Wright 1988; M. Edwards, C. Stuber, T. Helentjaris and S. Wright, unpublished results). Information from these experimental approaches has enabled us to correlate our RFLP map to a certain extent with the conventional maize genetic map. Other investigators have also taken advantage of cloned genes and isozymes to correlate their maps with the conventional linkage map.

Several different translocations between supernumerary (B) chromosomes and normal (A) chromosomes (B-A translocations) have been recovered in maize. These B-A translocations involve each of the maize A chromosomes, and in many cases, several different ones are available which have breakpoints at different positions on the same A chromosome. The breakpoints of many of these B-A translocations have been determined on both the cytological and conventional genetic maps (Beckett 1978; Hoisington 1985).

The centromeres of B chromosomes often undergo nondisjunction at the second male gametophyte division (the second postmeiotic mitosis during pollen
grain formation in which the generative nucleus divides to produce two sperm). An A chromosome segment translocated onto a B chromosome (a B-A chromosome) will be present in zero and two copies in the two sperm nuclei of a pollen grain in which the B centromere nondisjoined at this division (Figure 1).

When haploid eggs produced by a diploid plant are fertilized by pollen grains of this type, kernels are produced containing embryos which are hypoploid or hyperploid for the translocated segment of the A chromosome. These embryos are accompanied by hyperploid and hypoploid endosperms, respectively. Kernels with euploid embryos and endosperms also are produced when normal disjunction of the B-A chromosome takes place. B-A translocations have been used extensively in maize to locate genes to chromosome arms and specific segments of arms (ROMAN and ULLSTRUP 1951; BECKETT 1978).

NEWTON and SCHWARTZ (1980), EVOLA, BURR and BURR (1986), and BURR et al. (1988) have demonstrated the usefulness of B-A translocations in mapping both isozymic and RFLP loci in maize. In the current study, the breakpoints of 24 different B-A translocations (involving 18 of the 20 chromosome arms) were determined on our RFLP map. The results (1) identify the short and long arms of each chromosome on the RFLP map, (2) identify the chromosomal regions which contain the centromeres on the RFLP map, and (3) allow a greater correlation of this RFLP map with the cytological and conventional genetic maps.

MATERIALS AND METHODS

Stocks: Inbreds Mo17, B14, B73 and B57 were obtained from the USDA Plant Introduction Center, Ames, Iowa. Stocks containing the B-A translocations analyzed in this study, and tester lines used to confirm each B-A translocation were generously provided by J. KERMICLE, University of Wisconsin, Madison; E. PATTERSON, University of Illinois, Urbana; and the Maize Genetics Cooperation Stock Center, Urbana, Illinois. The source of each B-A translocation utilized in this study is indicated in Table 1.

Crosses: Plants containing 22 of the B-A translocations (all except TB-5Sc and TB-9Sd) were crossed as male parents to various lines by the individuals who provided them, and progeny of these crosses were the starting material for this study. Fifteen sibling progeny of crosses with each of these B-A translocations were planted as a family in a winter nursery in 1985–1986. Five nonhypoploid plants in each family were identified and crossed as male parents to plants carrying a recessive mutation located distal to the breakpoint of the B-A translocation in the A chromosome.

TABLE 1

<table>
<thead>
<tr>
<th>Translocation</th>
<th>Breakpoint(s)</th>
<th>Source</th>
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</tr>
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<tr>
<td>TB-1Sb</td>
<td>1S.05</td>
<td>PATTERSON</td>
<td>v4</td>
</tr>
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<tr>
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<td>PATTERSON</td>
<td>c2</td>
</tr>
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<td>PATTERSON</td>
<td>a1</td>
</tr>
<tr>
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<td>3L.10–60, 2S.46</td>
<td>Stock center</td>
<td>lg1</td>
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<tr>
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<td>3S.50</td>
<td>Stock center</td>
<td>h1</td>
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<tr>
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<td>4S.25</td>
<td>PATTERSON</td>
<td>su1</td>
</tr>
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</tr>
<tr>
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<tr>
<td>TB-9Sc</td>
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<td>11L.36</td>
<td>KERMICLE</td>
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</table>

Stock center

KERMICLE

Urbana; and the Maize Genetics Cooperation Stock Center, Urbana, Illinois. The source of each B-A translocation utilized in this study is indicated in Table 1.

Crosses: Plants containing 22 of the B-A translocations (all except TB-5Sc and TB-9Sd) were crossed as male parents to various lines by the individuals who provided them, and progeny of these crosses were the starting material for this study. Fifteen sibling progeny of crosses with each of these B-A translocations were planted as a family in a winter nursery in 1985–1986. Five nonhypoploid plants in each family were identified and crossed as male parents to plants carrying a recessive mutation located distal to the breakpoint of the B-A translocation in the A chromosome.
of these crosses were analyzed to (1) establish that the B-A translocation was correct and (2) to identify plants that were heterozygous for the complete B-A translocation. These same plants were also crossed as male parents to three genetically distinct inbreds (Mo17, B14 or B73, and B57). Plants were identified which contained each of the 22 B-A translocations, and progeny of these plants crossed as male parents to each of the three inbreds were grown in a summer nursery in 1986. Plants with 50% or greater pollen abortion and a smaller stature and/or a distinctive abnormal morphology were identified as hypoploids. The hypoploid plants were compared with their sibling plants that were not hypoploid (euploids or hyperploids) in each family.

Also, a plant heterozygous for TB-5Sc which carried a2 on chromosome 5 and A2 on the B-A chromosome was crossed as a male parent by an inbred female parent that was a2. Kernels from this cross with colorless endosperm and a purple embryo contained hyperploid embryos and had 22 chromosomes while colored kernels contained hypoploid or euploid embryos. Both kernel types were grown, and plants from kernels of the latter type with 20 chromosomes (hypoploids) and were compared with sibling hyperploids and a2 tester plants which were siblings of the plant used as the female parent. Similarly, a plant heterozygous for TB-9Sd which carried cl on chromosome 9 and Cl on the B-A chromosome was crossed as a male parent by an inbred female that was cl. Kernels from this cross with colorless endosperm and a purple embryo were hyperploid and contained 22 chromosomes. Sibling colored kernels with 20 chromosomes were hypoploids. Hyperploids, hypoploids, and siblings of the cl plant used as the female parent were sampled and analyzed.

Sources of samples: leaf samples from each of the inbreds (Mo17, B14, B57 and B73) and from each B-A-containing plant crossed as a male parent were harvested. Hypoploid and nonhypoploid (euploid or hyperploid) sibling progeny from crosses of B-A translocation-containing plants by inbreds were also harvested. All leaf samples were freeze-dried soon after they were harvested and stored at -20°C until analyzed. Plants for analysis of TB-5Sc and TB-9Sd were grown in a greenhouse in 1988.

Other methods: All other methods for the preparation of plant DNA, Southern blots, and hybridization (aqueous with 5X SSC and wash (0.25X SSC at 60°C) at 60°C conditions were as described previously (Heleintjaris et al. 1985; 1986).

RESULTS AND DISCUSSION

Plants with each of the 24 B-A translocations were crossed as male parents to inbreds, and F1 sibling hypoploid and nonhypoploid (euploid or hyperploid) progeny produced by these crosses were selected. DNAs were prepared from freeze-dried leaves of the parents and their hypoploid and nonhypoploid sibling progeny. These DNAs were analyzed by restriction fragment length analysis to determine the breakpoints of each of the B-A translocations on our RFLP map.

An example of such an analysis is shown in Figure 2. A plant containing TB-8La (which has its breakpoint at 0.70 in the long arm of chromosome 8) was crossed as a male parent to inbred Mo17. The parents, hypoploid progeny, and hyperploid progeny from this cross were analyzed. DNAs from each of these plant types were cut with the restriction enzyme, SstI, and analyzed using clone #107, which had been previously mapped by monosomic and linkage analysis on chromosome 8. This clone hybridizes with a slowly migrating restriction fragment in the parent carrying the B-A translocation and a more rapidly migrating fragment in the inbred, Mo17. Hyperploid F1 progeny display both fragments while sibling hypoploid F1 progeny (which lack the chromosomal segment from the male parent distal to the breakpoint in the long arm of chromosome 8) only display the fragment which was present in the female parent. Because the fragment from the male parent is not present in hypoploid progeny, the DNA sequence homologous to this probe must be located distal to the breakpoint in the long arm of chromosome 8. If it had been located proximal to this breakpoint on the long arm (between the breakpoint and the centromere), in the other arm of this chromosome, or in an other chromosome, the fragment from the male parent would have been present in both hypoploid and nonhypoploid progeny. To be consistent with descriptions used for morphological loci, we will indicate that this locus was "uncovered" by this B-A translocation because the
allel from the male parent is absent in plants hypoploid for this B-A translocation.

Analyses of this type were carried out using 87 RFLP probes and 24 B-A translocations. Nineteen of the B-A translocations analyzed in this study were simple B-A translocations and five were compound B-A translocations (TB-3La-2S6270, TB-15b-2L4464, TB-1La-4L4692, TB-7Lb-4L4698, and TB-9Sb-4L-6504). A simple B-A translocation is produced by an exchange between an A and a B chromosome while a compound B-A translocation is produced by crossing an appropriate reciprocal translocation (between two A chromosomes) with a B-A translocation and selecting a crossover derivative (RAKHA and ROBERTSON 1970). A plant hypoploid for a compound B-A translocation is hypoploid for segments of two different A chromosomes; it is hypoploid for the region between the two breakpoints on the chromosome arm in the original B-A translocation and also hypoploid for the region distal to the breakpoint in the translocated A chromosome. For example, TB-3La-2S6270 produces plants that are hypoploid for a segment of the long arm of chromosome 3 from 0.10 to 0.60 and also hypoploid for a segment of the short arm of chromosome 2 from 0.46 to the end of the arm. Data for these compound B-A translocations are also included in this study.

Figure 3 shows the loci on our RFLP map which were analyzed using each of the B-A translocations. All tested adjacent loci at one end of a linkage group were uncovered in the A chromosome translocated in each simple B-A translocation while those at the other end were not uncovered. This result is expected if the linear order of RFLP loci on the chromosome is correct. Clearly, the uncovered loci are located distal to the breakpoint in the A chromosome. Compound B-A translocations, as expected, uncovered loci on two different chromosomes. For example, TB-3La-2S6270 uncovered loci in the middle of chromosome 3 but not at either end, and it also uncovered loci at one end of chromosome 2.

Each B-A translocation uncovered RFLP loci on the linkage group that we had originally associated with that B-A translocation; this confirmed that the linkage groups on our RFLP map were assigned correctly. The current study also identified the long and short arms of each chromosome on our RFLP map. The chromosome arm involved in each of these B-A translocations was previously known, and each B-A translocation indeed uncovered RFLP loci at only one end of the RFLP linkage group associated with that chromosome. Clearly, the uncovered loci identify the chromosome arm that was involved in the B-A translocation. In our current map (Figure 4), the short arms of each linkage group are shown to the left and the long arms to the right.

The breakpoints of each of the B-A translocations tested are also shown in Figure 3. The region from the distal-most locus uncovered by each B-A translocation to the end of each chromosome is indicated with a solid line, and the region between that locus and the next closest locus which was not uncovered is indicated with a slashed line. The breakpoint of each B-A translocation, therefore, is within the region identified by the slashed line.

This type of analysis turned out to be quite helpful in clarifying certain aspects of our RFLP linkage map. For example, the order of RFLP loci on chromosome 3 near the centromere had previously been determined to be ...249-219-89-83... (HELENSTJARIS, WEBER and WRIGHT 1988). However, because TB-3Sb uncovers 249 and 89 but not 219, there appears to have been a mistake in interpreting the recombinational data or the Southern blot data may have been misread. It is possible that the stock containing TB-3Sb and the RFLP mapping population differ by a small inversion; however, this is probably not the case because there is very little inversion polymorphism in maize. Clearly, 89 must be distal to 219, and this information is reflected in the current map. This type of mistake is not surprising because relatively small segregating populations are used to develop RFLP linkage maps, and data from four different segregating populations were used to assemble this map. Linkage data for closely linked clones are far less reliable than linkage data for more distantly located clones because recombinants between closely linked clones are infrequent. Data from B-A translocations are more reliable than recombinational data because B-A data are qualitative while recombinational data are quantitative in nature.

Another example of this type involves analysis of several RFLP loci which mapped near the centromere of chromosome 9. We had previously been unable to unambiguously determine the order or spatial relationship of these loci using only recombinational analyses for the reasons mentioned above. We found that TB-9Sb uncovered loci 253, 211, 343, sh1, and 266;
Figure 4.—Our current maize RFLP linkage map. Horizontal lines denote the chromosomes, and numbers above the lines represent RFLP loci. Where the exact location of a locus is unknown, this ambiguity is denoted as an underlined segment. Loci along the right side of the figure have been assigned to chromosomes by monosomic analysis, but linkage data are not available. Loci in brackets were detected as morphological markers or isozymes, while all other loci were detected by RFLP analysis. The region which must include the centromere of each chromosome is indicated with a dashed line.
thus, these loci must be located distal to the breakpoint of this B-A translocation on the short arm. TB-9Sd, which has its breakpoint more proximal than TB-9Sb, also uncovers loci B3.06 and 300; thus, these loci are also in the short arm and proximal to loci 253, 211, 343, sh1, and 266. Another group was uncovered by TB-9Lc (B5.10, B7.13, 454, 416, 222, Gs2, U20, 80, 293, 97); therefore, these loci are located on the long arm distal to the breakpoint of this B-A translocation. Locus Pep1 was not uncovered by any of these B-A translocations and must be located between the breakpoints of TB-9Sd and TB-9Lc near the centromere. Thus, B-A translocation analysis provided additional information about the order of loci tightly clustered around the centromeric region of chromosome 9. Subsequent recombinational analysis of all of these loci in the same F2 population confirmed this general order. It was previously known that TB-9Sb, TB-9Sd, and TB-9Lc uncover sh1, wx1, and Gs2 respectively, and B3.06 was found to be distal to wx1 by recombinational analysis (HOISINGTON 1985; BURR et al. 1988). The results obtained in the current study confirmed and extended these observations.

This study also provides information about the locations of centromeres on this RFLP map. Each centromere must be located between the most proximal RFLP locus uncovered in each arm by B-A translocations. The region on each chromosome which must include the centromere is indicated in Figure 4 by a dashed line. In some cases, additional information is available which allows more precise placement of the centromeres on the map. For example, because the y locus is uncovered by TB-6Lc (see HOISINGTON, COE and NEUFFER 1988), the centromere must be located proximal to this locus on our RFLP map. Similarly, because the P1, B1, and wx loci on chromosomes 1, 2, and 9 are uncovered by TB-1Sb, TB-3La-2S6270, and TB-9Sd (see HOISINGTON, COE and NEUFFER 1988), the centromeres on each of these chromosomes must be located proximal to these loci. This information is reflected in this figure. In certain cases, centromeres are very precisely defined on our RFLP map. For example, on chromosome 10, loci 105 and 85 map very close to each other; however, TB-10Sc uncovers 105 and TB-10L19 uncovers 85. Clearly, the centromere must lie between these two closely-linked RFLP loci.

The positions of the breakpoints on the cytological and conventional genetic map are also known for most of these B-A translocations (HOISINGTON, COE and NEUFFER 1988). The determination of the breakpoints on the RFLP map in the current study; therefore, allows these three types of maps (cytological, conventional, and RFLP maps) to be better correlated.

Clearly, B-A translocations are an exceedingly powerful tool in RFLP mapping studies, and they have provided much additional information about this RFLP map. Certain probes analyzed in this study are being used by others as their RFLP maps are being constructed, and this will enable them to identify the short and long arms of their linkage groups. Analysis of additional loci and B-A translocations will provide still additional information regarding these maps.

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LITERATURE CITED


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