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Genetics

Use of monosomics to map cloned DNA fragments in maize

(restriction fragment length polymorphism/aneuploids)

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ABSTRACT **Monosomic maize (*Zea mays* L.) plants were generated using the *r-X1* deficiency system, and the monosomy was confirmed both genetically and cytologically. Genomic DNAs prepared from a group of plants, each monosomic for one chromosome, were digested with restriction enzymes, electrophoresed in agarose gels, and blotted onto nylon membranes. Hybridization of labeled cloned DNA fragments to these blots proved efficient in assigning each fragment to the chromosome from which it originated. Cloned DNA has previously contributed to loci detection through the use of the restriction fragment length polymorphisms (RFLPs), these loci subsequently being arranged into linkage groups by segregation analysis. In this study, these linkage groups were assigned to specific chromosomes, facilitating the construction of a linkage map for maize containing 112 RFLP loci. An additional 35 loci were also assigned to chromosomes by this method; however, the linkage relationships of these loci to other RFLP loci on each chromosome remains undetermined.**

When a cloned fragment of genomic DNA hybridizes to a Southern blot containing genomic DNAs (prepared from related but distinct organisms) and a hybridization pattern difference appears, this difference is termed a "restriction fragment length polymorphism" (RFLP) (1). The potential application of RFLPs to plant genetics (2–4) and the variability among species discovered with cloned DNA probes have been reported (5, 6). These RFLPs have been used to detect loci that have led to the preliminary construction of linkage groups in maize and tomato (7); this process, however, could not identify the chromosomal locations of most of these linkage groups. A major difficulty in developing genetic maps based upon RFLPs is incorporating these results onto existing genetic maps that are drawn from cytological, morphological (8), and isozyme (9) data. Although conventional linkage analysis could be used to place the RFLP-identified loci on an existing genetic map, the task would be time-consuming and require the inheritance analysis of many RFLPs in several different populations segregating for known morphological markers or isozymes.

The *r-X1* deficiency system in maize produces both monosomic (lacking one chromosome) and trisomic plants (10–12). These plants, germinated from kernels carrying the submicroscopic *r-X1* deficiency on chromosome 10 that contains the *R* locus, typically include 10–18% monosomics—in instances in which the female parent does not supply the missing chromosome—and 10–18% trisomics. Most remaining plants are diploids, but some multiple aneuploids and partial chromosome deficiencies are also produced. Several thousand maize monosomic plants including monosomics for each of the 10 maize chromosomes, have been recovered using this system. It has also been used successfully to assign a biochemical marker [the benzoxazinless locus (13)], a histone electrophoretic variant (14), and an isozyme locus of peroxidase (15) to the respective chromosomes. Monosomics have been compared with diploid sibling plants in studies of gene dosage effects on factors controlling the level and composition of embryo lipids (16, 17), the free fatty acid composition in leaves (18), and recombination (19). In this report we show that the system also can be an efficient and economical means for assigning cloned DNA fragments to specific chromosomes through Southern analysis (20), an approach that has potential for solving several problems in plant molecular genetics.

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MATERIALS AND METHODS

Generation and Identification of Monosomic Plants. Plants monosomic for specific chromosomes were recovered from the following cross: An inbred line (W22) that is *R/r-X1* and also carries a dominant allele of a locus on each of the 10 maize chromosomes (*Bm2*, 1; *Lg*, 2; *A*, 3; *Su*, 4; *Pr*, 5; *Y*, 6; *Gl*, 7; *J*, 8; *Wx*, 9; and *G*, 10) was crossed as the female parent with another inbred, Mangelsdorf's multiple chromosome tester (MT), which is *r/r* and homozygous for the 10 corresponding recessive mutations (11). Colorless (*r/r-X1*) kernels from this cross were planted, and presumptive monosomics for chromosomes 2, 7, 8, and 10 were identified when they expressed either the recessive liguleless (*lg*), glossy (*gl*), japonica (*j*), or golden (*g*) plant phenotypes, respectively. Presumptive monosomics for chromosomes 3, 4, 6, and 9 were identified when they expressed the distinctive morphological phenotype characteristic of each of these monosomic types. No monosomic plants for chromosomes 1 or 5 were obtained in this particular screening. Presumptive diploid F_1 plants were of normal stature and maturity. Microsporocyte samples were taken from each analyzed plant: each presumptive diploid contained 20 chromosomes, while each presumptive monosome possessed 19 chromosomes at diakinesis. Because chromosome 6 carries the only nucleolar organizing region in maize, each presumptive monosomic 6 plant had only one chromosome associated with the nucleolus at diakinesis so that each monosomic 6 plant also was confirmed cytologically by this criterion.

DNA Preparation, Electrophoresis, Blotting, and Hybridization. Leaf tissue was obtained from the W22 and MT parental inbred lines, from the eight available F_1 monosomic types, and from diploid F_1 siblings of the monosomic plants and then lyophilized and stored at −20°C. The methods for subsequent isolation of plant genomic DNA from this material, restriction digestion, agarose gel electrophoresis, Southern blotting onto nylon membranes, and hybridization conditions have been previously described (6).

RESULTS

When a cloned DNA fragment originated from a maize chromosome that had been isolated in a monosomic plant, the resulting hybridization pattern with that plant's DNA differed

Abbreviation: RFLP, restriction fragment length polymorphism.

from the pattern obtained with any other monosomic plant type or diploid F_1 plant because it contained only the contribution of the male parent. This could be detected by a 50% decrease in the hybridization signal from that particular monosome as compared with other monosomes or diploids in which the parental hybridization patterns were identical. However, when the locus detected by this clone was polymorphic for the two parents and both DNAs were digested with a particular restriction enzyme, the loss of one of the two fragments (the fragment from the female parent) occurred in the corresponding monosomic F_1 plant. By Southern blotting with genomic DNAs prepared from 10 groups of plants each monosomic for one of the 10 chromosomes, a plant geneticist could unambiguously assign any cloned DNA fragment to its chromosome of origin through a single hybridization. We almost accomplished this theoretical goal with the exception that we were unable to obtain the monosomic plants for chromosomes 1 and 5. Consequently, we could not discriminate between these two chromosomes; clones originating from them were relegated to the category of "1 or 5."

To clarify results we first attempted to identify a restriction enzyme that exhibited a polymorphism for each clone in the material under analysis. Although one can detect the expected 50% signal reduction in nonpolymorphic F_1 plants monosomic for the chromosome of origin, the result is less ambiguous with a polymorphic locus when the W22 female parental fragment is absent from this monosome. To this end each clone was nick-translated and hybridized to blots containing genomic DNAs prepared from both parent plants and a diploid F_1 plant, all digested with one of three restriction enzymes. A typical result is shown in Fig. 1. This clone reveals one primary locus in each of the two inbreds. When *Hin*dIII was used to digest the DNAs, the fragments detected had essentially the same molecular weights. Thus, in an F_1 plant monosomic for the chromosome containing this clone, one would be able to detect only a signal-intensity reduction for the single fragment observed. However, use of either *Bgl* II or *Sst* I sharpens the interpretation because the detected fragments differ in molecular weights, and the W22 allele would be completely absent in the identifying F_1 monosomic individual. All clones to be evaluated were first tested in this manner and, whenever possible, a restriction enzyme and clone combination that yielded a polymorphism was subsequently used.

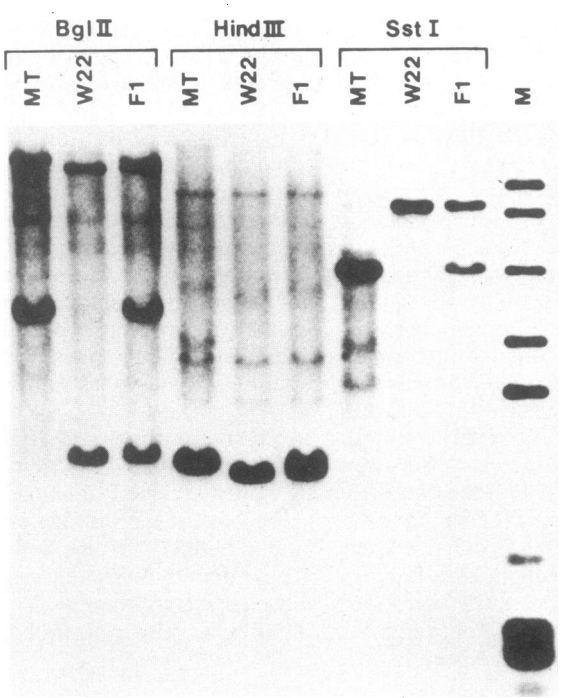

FIG. 1. Determination of clone-restriction endonuclease combinations that reveal polymorphisms between the W22 and MT parents. Genomic DNAs from the MT and W22 parents and their F_1 diploid progeny were each digested with one of three (*Bgl* II, *Hin*dIII, and *Sst* I) restriction enzymes and electrophoresed. A Southern blot was prepared and hybridized with a radioactively labeled clone as described. Lane M refers to molecular weight markers.

We then analyzed each clone with the total set of monosomics as shown in Fig. 2. The clone analyzed in *A* reveals one primary fragment of different molecular weights in the two parents and, as expected, both are also observed in the diploid F_1 plant. In the set of monosomic F_1s, the fragment from the MT male parent is found in each F_1 plant; however, the fragment from the W22 female parent occurs in all F_1 plants except the two monosomic for chromosome 4. Therefore this cloned DNA fragment originated from chromosome 4. A high percentage of the RFLP loci tested was assigned to specific chromosomes in this manner. In *B*, which also shows an analysis of a polymorphic locus, the fragments from both MT and W22 parents occur in all monosomes. According to our interpretation this clone did not originate from any of the eight chromosomes represented in our monosomic series. We conclude then that this cloned fragment originated from either chromosome 1 or 5. In *C*, a clone that revealed no polymorphism with any of the enzymes tested was hybridized to the monosomic set. The signal intensity of the fragment is fairly consistent across the set of F_1s except in both monosomic 4s, where it is distinctly less. From these data we would tentatively assign this clone to chromosome 4. Although the latter results are more ambiguous than when polymorphic loci are observed (*A* or *B*), this method proved accurate in every case; such assignments were later confirmed by testing with other closely linked and polymorphic loci or by linkage to morphological markers with known genomic locations.

We had previously analyzed the segregation of >100 loci detected by RFLPs in a single F_2 population, and these were subsequently grouped together by conventional linkage analysis into 15 linkage groups (7). This population was unrelated to the lines analyzed in the current study. Although these groups represented sections of the maize chromosomes, it was not possible to assign many of them to specific chromosomes. However, linkage groups containing cloned genes with known genomic locations, including bronze-1 (*bz*), shrunken-1 (*sh*), and waxy (*wx*)—all on chromosome 9—alcohol dehydrogenase-1 (*Adh1*) on chromosome 1, and anthocyaninless-1 (*a*) on chromosome 3, allowed these particular groups to be assigned by conventional methods. We first checked clones with identified origins from these linkage groups against the total monosomic set to confirm the accuracy of our procedure. All of the closely linked loci contained within these three identified linkage groups were located on the correct chromosomes by our analytic technique (data not shown). We next analyzed several clones from each of our other linkage groups for chromosomal origins.

The linkage map derived from these studies (Fig. 3) includes 112 loci that had been analyzed previously by linkage analysis and that were polymorphic in our original F_2 population (7). It also includes another 35 loci that could not be analyzed previously by genetic segregation because they were not polymorphic in the original F_2 population. This latter group of loci is set along the right side of the map and has been designated as "Assigned Clones." Eventually, linkage information for these loci could be obtained in another segregating population to determine the spatial arrangement in relation to other clones on the same chromosome. For example, locus 304, first identified by monosomic

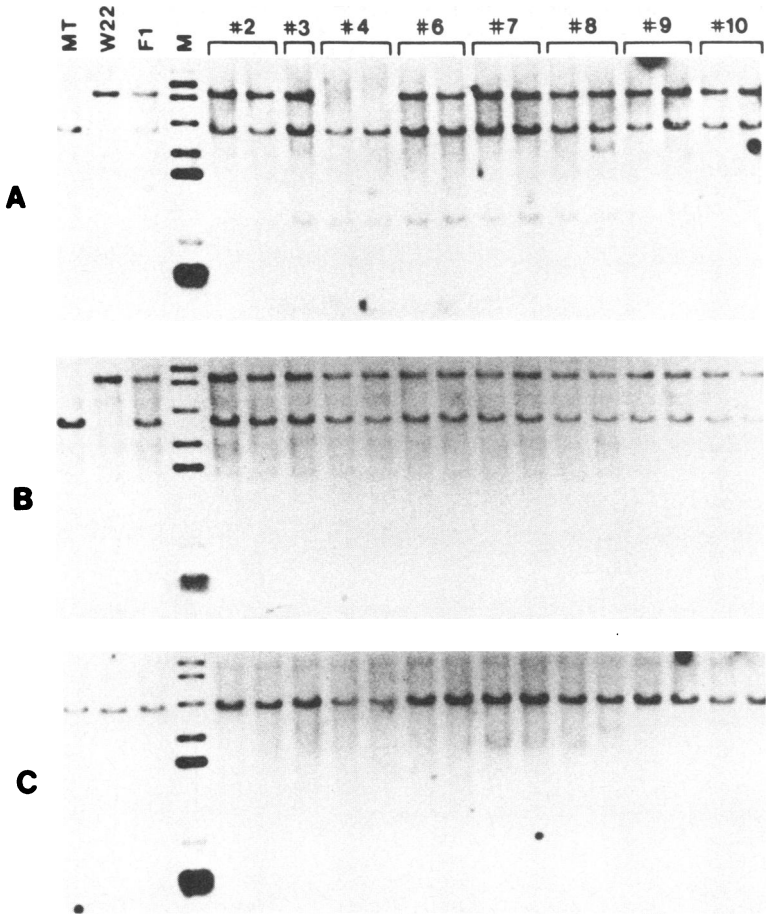

FIG. 2. Evaluation of clones with monosomics. Genomic DNAs from the MT and W22 parents and their diploid and monosomic F_1s were used to prepare Southern blots that were hybridized with three different clones in *A*, *B*, and *C*. Numbers at top refer to F_1s that were confirmed to be monosomic for the designated chromosome; only one monosomic was obtained for chromosome 3. (*A* and *B*) Clones that were polymorphic in the parents. The clone analyzed in *A* was assigned to chromosome 4 because the W22 allele was lost in the monosomic 4s. The clone analyzed in *B* was assigned to the "1/5" group because none of the available monosomics revealed a W22 allele loss. The clone analyzed in *C* was not polymorphic; however, it could be assigned to chromosome 4 because of the reduced signal intensity in the monosomic 4s. Lane M refers to molecular weight markers.

analysis as having originated from either chromosome 1 or 5, was later found to be tightly linked to locus 40 in the 1/5 group by inheritance analysis of a more informative F_2 population (data not shown). All of the boxed loci in Fig. 3 have been tested against the complete monosomic set to ascertain the linkage group origin. As is evident, a high percentage of the loci was tested to verify chromosomal assignment and resolve ambiguities created by those clones revealing loci located at different genomic sites. Through this analytic technique, all linkage groups have been assigned to a respective chromosome except those originating from chromosomes 1 and 5. We assigned linkage groups to chromosome 1, because the group included a locus identified with an *Adh1* clone that contains DNA known to be located on the long arm of chromosome 1. The remaining unassigned linkage groups were then allocated to the "1/5" group (containing all loci originating from either chromosome).

DISCUSSION

The maize genetic linkage map derived in this study should be substantially correct in chromosomal assignments for three reasons: (*i*) clones with known chromosomal locations or other RFLP loci tightly linked to them were always appropriately assigned when analyzed by this monosomic technique, (*ii*) whenever possible we have used results from several clones before assigning a particular linkage group to a chromosome, and (*iii*) in subsequent verification of linkage of these loci with morphological markers or isozymes previously mapped (data not shown), the chromosomal assignments agreed for all of the tested linkage groups. Further extrapolation of this approach could employ the B-A translocations currently available in maize (21); such plants are either hyperploid or hypoploid for sections of chromosome arms and by comparing both hyperploid (three copies) and hypoploid (one copy) plants with the technique of monosomic analysis, one might not only assign markers to chromosomes but also to arms or even portions of arms, as the breakpoints become precisely known. This should allow a closer correlation of these maps with the conventional and physical maps to yield higher resolution of the maize genome.

Because lyophilized plant material, stable for years, can be an efficient source of genomic DNA, a few individuals could make field collections to provide material for many different researchers. This, together with the facts that less than 5 μg of DNA is required for one assay and Southern blots on nylon membranes can be reused a number of times, widens the uses of the described analytic approach in studies of isolated cloned DNA fragments.

A monosomic series could also find future use as a preliminary screen for RFLP loci with specific chromosomal locations. For example, the dearth of RFLP loci on chromosome 10 would make its monosome an attractive candidate for RFLP screening. Another use of this technique would be to assign identified genes with unknown genomic locations to chromosomes; using monosomic analysis we were able to assign a second sucrose synthase gene (provided by L. C. Hannah) to chromosome 9 and a heat shock gene (provided by D. Ho) to chromosome 8, results that were also confirmed by linkage analysis (data not shown). This method would also find use in assigning clones that possess multiple cross-hybridizing loci to their respective chromosomal locations. Maize clones often detect more than one locus on different chromosomes; for instance, loci 1 on chromosome 8 and 2 on chromosome 6 are both detected by a single cloned fragment, and loci 4 on chromosome 2 and 5 on chromosome 7 are detected by a different cloned fragment. Examination of a number of such loci has allowed the identification of large segments of chromosomes that appear to be duplicated in the genome (unpublished work) and provides information on the

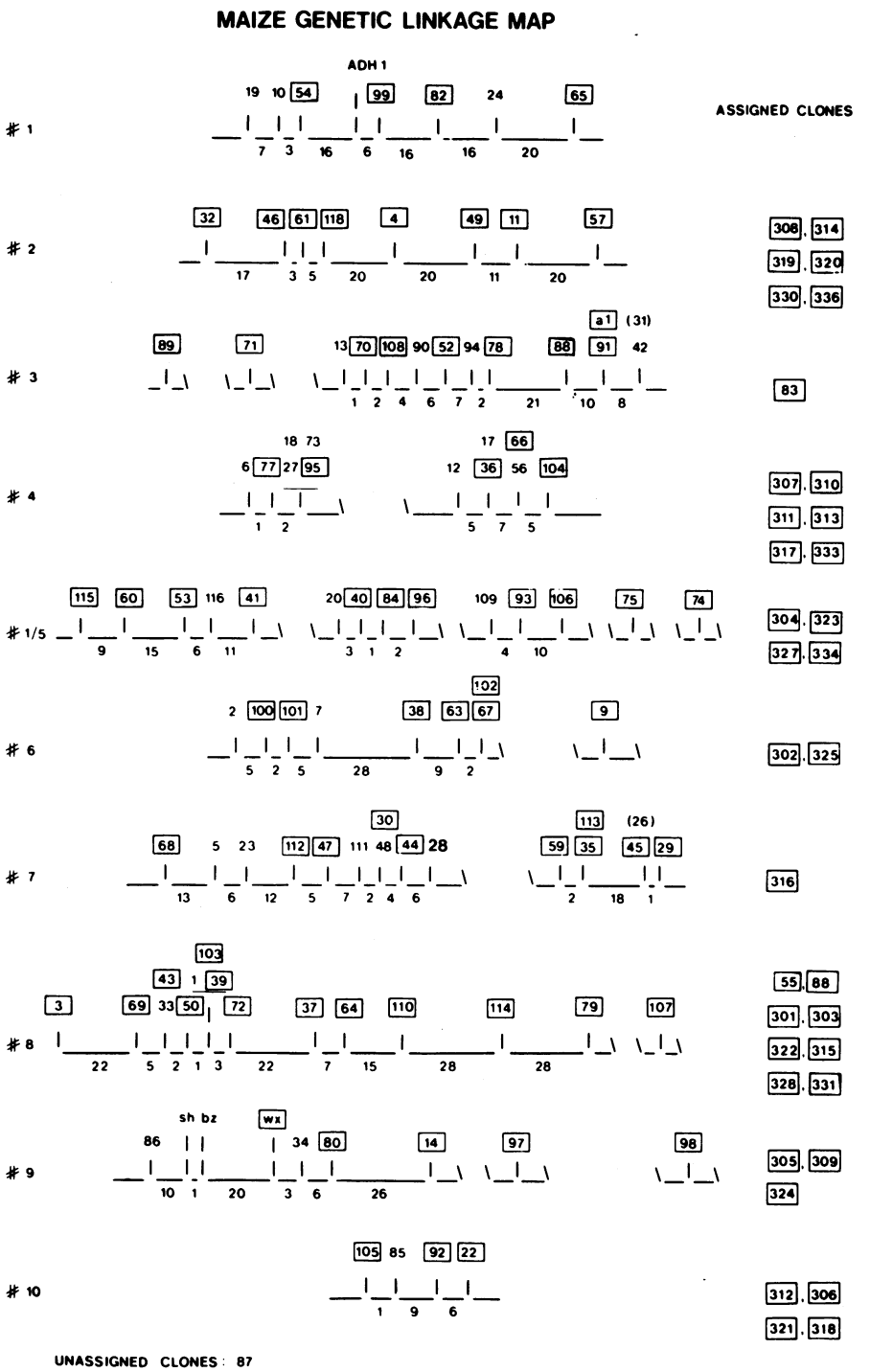

FIG. 3. Maize genetic linkage map based upon monosomic assignments. Linkage groups as determined previously (6) were assigned to chromosomes based upon the monosomic test results. Numbers above the horizontal lines designate RFLP loci; numbers below the lines designate the inter-locus distances in map units. Boxed loci are those that were placed by monosomic analysis. Loci along the right side, for which there are not yet any linkage data, were assigned by monosomic analysis.

distribution of redundant segments and the possible derivation of the maize genome.

Finally, an extremely important application of monosomic analysis could be in those gene cloning protocols that involve the isolation of many presumptive clones. For example, *Mu* [a transposable element that exists in multiple copies in maize, (22)] might be used to induce an insertion mutation into a mapped locus. Screening through several presumptive clones for the *Mu* sequence to find the desired insertion could be time-consuming. Using monosomic analysis, however, the clones could first be screened for chromosomal locations before devoting more intensive study to the entire set of *Mu* insertion clones, thus expediting recovery of the target locus. Monosomic analysis clearly has characteristics that should increase its power as a tool in a variety of maize molecular studies.

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