Production and Characterization of Maize Chromosome 9 Radiation Hybrids Derived From an Oat-Maize Addition Line

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ABSTRACT

In maize (*Zea mays* L., $2n = 2x = 20$), map-based cloning and genome organization studies are often complicated because of the complexity of the genome. Maize chromosome addition lines of hexaploid cultivated oat (*Avena sativa* L., $2n = 6x = 42$), where maize chromosomes can be individually manipulated, represent unique materials for maize genome analysis. Maize chromosome addition lines are particularly suitable for the dissection of a single maize chromosome using radiation because cultivated oat is an allohexaploid in which multiple copies of the oat basic genome provide buffering to chromosomal aberrations and other mutations. Irradiation (gamma rays at 30, 40, and 50 krad) of a monosomic maize chromosome 9 addition line produced maize chromosome 9 radiation hybrids (M9RHs)—oat lines possessing different fragments of maize chromosome 9 including intergenomic translocations and modified maize addition chromosomes with internal and terminal deletions. M9RHs with 1 to 10 radiation-induced breaks per chromosome were identified. We estimated that a panel of 100 informative M9RHs (with an average of 3 breaks per chromosome) would allow mapping at the 0.5- to 1.0-Mb level of resolution. Because mapping with maize chromosome addition lines and radiation hybrid derivatives involves assays for the presence or absence of a given marker, monomorphic markers can be quickly and efficiently mapped to a chromosome region. Radiation hybrid derivatives also represent sources of region-specific DNA for cloning of genes or DNA markers.

ANALYSIS of plant genome organization, especially in grasses, is often complicated because of large genome sizes, a high proportion of repeated DNA sequences in a genome, and extensive gene or chromosome duplication or polyploidy (Flavell 1986; Lapitan 1992; Bennetzen and Freeling 1993; Gill and Gill 1994). These are also serious obstacles to map-based gene cloning and the construction of physical maps. Physical mapping and cloning in plants in general have focused on species such as Arabidopsis (*Arabidopsis thaliana* L.) and rice (*Oryza sativa* L.) that have relatively small genomes [150 and 600 Mbp/1C, respectively (Bennett and Smith 1976)]. The ability to construct physical genomic maps using overlapping cosmids, bacterial artificial chromosomes, and yeast artificial chromosomes (YACs) has been clearly demonstrated in Arabidopsis (Schmidt *et al.* 1995, 1997; Zachgo *et al.* 1996) and rice (Hong *et al.* 1997; Kurata *et al.* 1997) but has been severely restricted in species like maize (*Zea mays* L.), which has a large genome [2500 Mbp/1C (Bennett and Smith 1976)] and high amounts of repeated sequences (Edwards *et al.* 1992; Bennetzen *et al.* 1994). The use of comparative mapping based on the conservation of synteny and order of genetic loci between genomes of distantly related species is a promising approach for identifying gene positions and marker linkages in large genome species through comparisons with small genome model species like rice (Bennetzen and Freeling 1993; Flavell *et al.* 1993; Paterson *et al.* 1995; Han *et al.* 1998). However, there are several potential limitations involved in this approach, including the observation that many traits of economic interest like disease resistance may be species specific and therefore not detectable or even absent in the simpler genome; marker presence or order may not be conserved (Foote *et al.* 1997; Han *et al.* 1998) and marker polymorphism is often limited.

Various strategies have been necessary to build a physical map of the complex human genome. One approach has involved the use of YAC libraries to construct sequence-tagged-site (STS)-based maps of the entire genome. However, the construction of complete contigs using YACs and unordered STS markers has been difficult (Chumakov *et al.* 1995). The alternative has been to use an independently developed scaffold of ordered STS markers that in turn is used to order and align YAC contigs. A saturated human genetic map with ~5000

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simple sequence repeat (SSR) markers has been constructed (Dib *et al.* 1996). However, this and other genetic maps have not been sufficient for the construction of physical maps because these scaffolds are limited by the abundance of polymorphic markers and their resolution (1 Mb) is constrained by recombination rate. These obstacles and constraints are also expected in the construction of physical maps of complex plant genomes.

Radiation hybrid (RH) maps, which are based on radiation-induced chromosome breakage in somatic cell hybrids, are suitable for the construction of physical maps. A high-resolution (100-kb) contiguous map of human chromosomes with ~41,000 ordered STSs that includes 30,000 unique human genes has been constructed using the RH mapping approach (Hudson *et al.* 1995; Schuler *et al.* 1996; Stewart *et al.* 1997; Deloukas *et al.* 1998). RH mapping uses radiation-induced breakage of chromosomes to order genetic loci, to estimate distance between them, and to build a map of the chromosome. Because chromosome breakage increases with radiation dose that is applied, RH panels with higher levels of mapping resolution than those obtained by meiotic mapping may be produced (Stewart *et al.* 1997). In one form of RH mapping, a rodent somatic cell hybrid containing rodent chromosomes and a single human chromosome of interest is lethally irradiated, inducing chromosome breakage. After irradiation, the human and rodent chromosomal fragments often rejoin with one another, resulting in complex chromosomal rearrangements. Such an irradiated cell is rescued by fusion with a nonirradiated rodent cell to form viable colonies. This results in the isolation of somatic hybrids with various human subchromosome fragments. These subchromosomal stocks are subsequently utilized for marker mapping (Goss and Harris 1975; Cox *et al.* 1990). Radiation hybrids with human subchromosome fragments have also been excellent vehicles for the production and characterization of libraries highly enriched in DNA markers and genes for a particular subchromosomal segment (Pritchard *et al.* 1989; Ledbetter *et al.* 1990). The success in using radiation hybrids in humans has fueled interest in applying this procedure for the analysis of the genomes of mouse (McCarthy *et al.* 1997), pig (Yerle *et al.* 1998; Hawken *et al.* 1999), dog (Priat *et al.* 1998; Vignaux *et al.* 1999), zebrafish (Kwok *et al.* 1999), cat (Murphy *et al.* 1999), and rat (Watanabe *et al.* 1999).

To increase the efficiency of mapping in an important large genome plant species, we have undertaken a project to explore a subchromosomal segment mapping for maize using oat as the host. This system is based on the use of maize chromosome addition lines of oat (Riera-Lizarazu *et al.* 1996) where a maize chromosome is present in addition to a full complement of oat chromosomes, being in this way comparable to human-rodent somatic cell hybrids. These maize chromosome addition lines were isolated from partially self-fertile oat-maize hybrids (Riera-Lizarazu *et al.* 1996). To date, self-fertilization of partial oat-maize hybrids has resulted in the production of fertile oat lines that contain an added maize chromosome or chromosome pair representing 7 of the 10 maize chromosomes. Disomic or monosomic addition lines with maize chromosomes 2, 3, 4, 6, 7, 8, and 9 have been identified (Riera-Lizarazu *et al.* 1996; Maquieira 1997). Besides mapping applications, maize chromosome addition lines of oat may be used for other studies. For example, Bass *et al.* (2000) used a maize chromosome 9 addition line to show that maize homologs lack premeiotic pairing but homologs do pair and synapse during the telomere bouquet stage during meiotic prophase. Other research uses of oat-maize addition lines currently underway include the study of the expression of a maize gene in an oat background (Muehlbauer *et al.* 2000) and the separation of oat and maize chromosomes by flow cytometric sorting (Li *et al.* 2000).

Although radiation has been used in cereals for the introgression of genes from related species (Driscoll and Jensen 1963; Islam *et al.* 1981; Riley and Law 1984), this approach has not been widely used for mapping or cloning experiments. This is partly due to the difficulty in identifying and separating materials that contain different portions of the chromosome of interest. We have overcome this limitation by using maize-specific dispersed repetitive sequences as probes for maize DNA identification in an oat background (Ananiev *et al.* 1997). Furthermore, the use of maize-specific DNA sequences to analyze the structure and composition of specific maize chromosome regions such as heterochromatic knobs (Ananiev *et al.* 1998a,c) and centromeres has been demonstrated (Ananiev *et al.* 1998b). Thus, oat-maize addition lines and maize-specific repetitive DNA sequences can be used to isolate, manipulate, and analyze DNA sequences from individual maize chromosomes or chromosome segments. In addition, maize chromosome addition lines of oat are particularly suitable for chromosome irradiation experiments because cultivated oat is an allohexaploid with multiple copies of the oat basic genome that provide buffering to chromosomal aberrations and other mutations.

In this article, the effect of three dosages of gamma rays (30, 40, and 50 krad) on maize chromosome 9 breakage is documented toward the establishment of a subchromosomal segment mapping system for maize using oat as the host. The objectives of this study were: (1) to produce a set of oat lines with subchromosome fragments of maize chromosome 9 (radiation hybrids) by irradiation of a maize chromosome 9 monosomic addition line of oat, (2) to characterize the radiation-induced rearrangements of maize chromosome 9 by DNA marker analysis and fluorescence *in situ* hybridiza-

tion, and (3) to evaluate the usefulness of maize chromosome 9 radiation hybrids for maize genome analysis.

MATERIALS AND METHODS

Plant material: The oat line Starter-1, which served as the host parent genome for the maize chromosome 9 addition line, is a reselection from the oat cultivar Starter. The maize hybrid Seneca 60 was obtained from the Robson Seed Farm Corporation (Hall, NY). The maize chromosome 9 disomic addition lines of oat (42 oat + 2 maize chromosomes) were recovered following self-fertilization of the partial hybrid ST505-5 (21 oat + 1 maize chromosomes), which was obtained by crossing the oat line Starter-1 and the corn hybrid Seneca 60 (Riera-Lizarazu *et al.* 1996). Maize chromosome 9 disomic addition plants (42 oat + 2 maize chromosomes) were hand-emasculated and pollinated with freshly shed pollen of Starter-1 oat to produce maize chromosome 9 monosomic addition line seed (42 oat + 1 maize chromosomes).

Gamma-ray treatments: Prior to radiation treatments, seed moisture was equilibrated to ~12.5% in an airtight desiccator with a solution of 60% glycerol for 5 days (Conger 1972). Irradiation experiments with normal oat seed were carried out to determine the radiation dose needed to achieve maximum chromosome breakage while maintaining some plant viability. One hundred seeds from the oat line Starter-1 were used in each radiation treatment ranging from 5 to 100 krad. Equilibrated seeds that were not irradiated were used as controls (0 krad). Equilibrated oat seeds were exposed to gamma rays from a Cs^{137} source in the irradiator model 143-45 (J. L. Shepherd and Associates, San Fernando, CA). Seeds were planted immediately after irradiation in a 2:1 mixture of soil and potting mix and placed in a growth chamber or greenhouse. Plant survival was expressed as the proportion of surviving seedlings (%) from seeds treated at different radiation levels. Radiation doses higher than 50 krad were lethal (Figure 1). On the basis of these data, 30-, 40-, and 50-krad dosages were chosen as the levels at which to irradiate the oat-maize chromosome 9 monosomic seeds.

One hundred maize chromosome 9 monosomic addition line seeds were treated at 30 krad, 100 seeds at 40 krad, and 200 seeds at 50 krad. Seeds were planted immediately after irradiation and placed in a growth chamber with a 12-hr photoperiod and day/night temperatures of 20/15°. After 6–8 wk the photoperiod was increased to 14 hr to promote reproductive development. Plants were allowed to self-fertilize and at maturity each panicle was harvested individually. Seeds from harvested panicles were individually planted and placed in growth chambers in the same conditions as described earlier.

Identification of maize chromosome 9 radiation hybrids: We analyzed progenies from self-fertilization of maize chromosome 9 monosomic addition lines of oat that had survived seed treatment with gamma rays (30, 40, and 50 krads). Because materials with and without maize chromatin were expected to result from self-fertilization of irradiated maize chromosome 9 monosomic addition lines, three different DNA-based analyses were performed to identify plants that contained maize DNA. These analyses were Southern blot hybridization with the maize multiprobe (a mixture of maize-specific repetitive sequences; Ananiev *et al.* 1997), Southern analysis with the 180-bp maize knob-specific sequence (Peacock *et al.* 1981), and polymerase-chain-reaction (PCR) amplification of the long terminal repeat (LTR) sequence of the maize retrotransposon Grande-Zm1 (Sanmiguel *et al.* 1996; Ananiev *et al.* 1998a). Plants that were identified as containing maize chromatin in these tests are designated maize chromosome 9 radiation hybrids (M9RHs).

DNA isolation and analysis: Small- and large-scale DNA extractions from 100 mg to 3 g of leaf tissue from putative M9RHs, Starter-1 oat, oat-maize chromosome 9 monosomic addition lines, and Seneca 60 maize were carried out using a modified version of buffers described by Liu and Whittier (1994). The nuclei isolation and lysis buffer components were combined into a single DNA extraction buffer [10 mm Tris-HCl pH 9.5, 10 mm EDTA, 100 mm KCl, 0.5 m sucrose, 4 mm spermidine, 1.0 mm spermine, 0.1% (v/v) mercaptoethanol, 2% (w/v) sarkosyl]. Leaf tissues were ground to a fine powder in liquid nitrogen. Ice-cold DNA extraction buffer was then added to the powder and vortexed. All other manipulations thereafter were those described by Ananiev *et al.* (1997). Genomic DNA restriction endonuclease digestion, agarose electrophoresis, and Southern blot hybridization were performed as described by Riera-Lizarazu *et al.* (1996). The presence of maize DNA in a particular individual was determined by positive hybridization with the multiprobe and/or the 180-bp knob-specific sequence.

PCR amplification of the maize retrotransposon Grande-Zm1 LTR sequences was performed in 200-μl tubes using a Perkin Elmer 9600 thermal cycler (PE Applied Biosystems, Foster City, CA). The primers used were those described by Ananiev *et al.* (1998a). The 30-μl PCR reaction mix consisted of 0.5 μm of each primer, 1 unit of *Taq* DNA polymerase (Promega, Madison, WI), 0.2 mm of each dNTP, 1× *Taq* buffer, 2.5 mm $MgCl_2$, 10 to 50 ng of template DNA, and distilled H_2O. The reaction mix was overlaid with 25 μl of mineral oil. The PCR amplification consisted of an initial denaturation step of 7 min at 95°, followed by 30 cycles of three steps of 30 sec each: denaturation at 95°, annealing at 65°, and elongation at 72°. A final elongation step at 72° for 10 min was performed, and the program ended holding at 4°. After PCR, 3 μl of 10× loading buffer was added to each tube. PCR amplification products were electrophoresed in a 3% (w/v) agarose (Gibco/BRL Ultrapure, GIBCO Laboratories, Grand Island, NY) gel in 1× TAE containing 0.3 $\mu g\ ml^{-1}$ ethidium bromide. The presence of a 500-bp amplification product indicated presence of maize DNA.

Characterization of maize chromosome 9 in the M9RHs: The integrity of maize chromosome 9 in the radiation hybrids was determined using maize chromosome 9 DNA-based markers and by *in situ* hybridization of metaphase chromosomes using maize-specific probes. A set of 21 maize chromosome 9 specific restriction fragment length polymorphism (RFLP) probes was kindly provided by the maize RFLP laboratory of the University of Missouri, Columbia. The procedures for plasmid isolation and manipulation as well as Southern blot analysis were the same as described by Riera-Lizarazu *et al.* (1996). Each individual was scored for the presence or absence of hybridization signal with the maize chromosome 9 specific RFLP probes used. Faint bands were scored as missing data.

We also used primers for 12 SSR loci for maize chromosome 9. The primers were prepared by the Advanced Genetic Analysis Center (St. Paul) using primer sequences published in the Maize Database (http://www.agron.missouri.edu/). The PCR amplification conditions were the same as those described before, except that the annealing temperature was 70° for phi016 and 56° for all other SSR primers. Each individual was scored for the presence or absence of an amplification product for the SSR markers that were used. Weak amplification products were scored as missing data.

For *in situ* hybridization of chromosomes, the procedures for the root-tip mitotic chromosome spreads and slide preparation were the same as those described by Riera-Lizarazu *et al.* (1996). Three different probes were used alone or in combination for *in situ* hybridization of metaphase chromosomes of the M9RHs; these were total maize DNA cut with

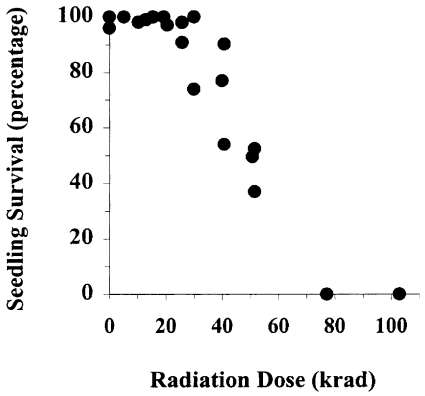

FIGURE 1.—Proportion of surviving seedlings from Starter-1 oat seeds treated at different radiation levels.

the enzyme *Hae*III, the multiprobe (ANANIEV *et al.* 1997), and the 180-bp knob-associated sequence (PEACOCK *et al.* 1981). Unlabeled oat DNA cut with *Hae*III was used as the hybridization competitor or block at 10-fold higher concentration than the probes. The probes were labeled by random primed incorporation of a fluorochrome-linked deoxyuridine-triphosphate [fluorescein (fluor-12-dUTP) or rhodamine (tetramethyl-rhodamine-6-dUTP, Boehringer Mannheim, Indianapolis) using a commercial kit (Prime-It Fluor, Stratagene, La Jolla, CA)]. Following dehydration of the slide preparations, a 30-μl solution of probe (3 μg ml^{-1}), unlabeled blocking genomic DNA of oat (30 μg ml^{-1}), sonicated salmon sperm DNA (0.6 mg ml^{-1}), sodium dodecyl sulfate [SDS, 0.1% (w/v)], dextran sulfate [10% (w/v)], and deionized formamide [50% (v/v)] in 2× SSC was applied to each slide and covered with a coverslip. Slides were incubated in a humidity chamber at 37° overnight. After hybridization, slides were washed twice in 20% (v/v) formamide in 0.1× SSC at 42° for 5 min each time, twice in 2× SSC at 42° for 5 min each time, and 2× SSC at room temperature for 10 min. Slides were mounted in an antifade solution (Vectashield, Vector Laboratories, Inc., Burlingame, CA) with 0.5 μg ml^{-1} 4′,6-diamidino-2-phenylindole (DAPI) and analyzed on a Zeiss Axioskop microscope equipped with an epifluorescence attachment (Zeiss filter sets 02, 09, and 15). Fluorescence images from DAPI, fluorescein, and rhodamine were photographed separately using Kodak slide film (400 ASA). Photographic slides were scanned and images from the same preparation were digitally merged using PhotoShop 5.0 (Adobe Systems Incorporated, San Jose, CA).

Statistical analyses: Chi-square contingency table analysis was used to test for differences in marker retention frequencies and the proportion of individuals that had all DNA markers tested in materials belonging to different radiation dose groupings. A chi-square homogeneity test for marker retention across radiation dose groupings was used to evaluate whether or not radiation-induced chromosome breakage was random along each chromosome arm.

RESULTS

Effect of gamma radiation on seed germination and plant survival: The sigmoidal curve based on seedling survival data (Figure 1) after the irradiation of seeds of Starter-1 oat indicates a cumulative effect of radiation on plant survival. Radiation doses higher than 40 and 50 krad resulted in a dramatic decrease in plant survival (Figure 1) as well as initial plant vigor. The viability of the maize chromosome 9 monosomic addition line seeds irradiated at 30, 40, and 50 krad was 65, 52, and 27%, respectively (Table 1). The proportion of plants that were self-fertile among the surviving plants was high in the three radiation treatments (97, 92, and 93% for the 30-, 40-, and 50-krad treatments, respectively; Table 1). At maturity, 715 panicles tracing back to 161 plants were individually harvested.

Identification of M9RHs: We planted 702 seeds tracing back to different panicles, and 648 plants (92%) were generated for analysis. These 648 plants, representing selfed progenies of irradiated maize chromosome 9 monosomic addition line plants, were screened for the presence or absence of maize DNA. Among the 648 progenies screened, 64 (10%) were found to contain maize DNA using the maize-specific multiprobe (Figure 2 and Table 2). Among the 64 M9RHs, seven plants did not show hybridization with the 180-bp maize knob-specific sequence (individuals M9RH0524 and M9RH0526 from the 40-krad treatment and M9RH0864, M9RH-0872, M9RH1008, M9RH1105, and M9RH1126 from the 50-krad treatment; Figure 2).

DNA-based marker characterization of maize chromosome 9 in the M9RHs: The integrity of maize chromosome 9 in the radiation hybrids was analyzed using 33 DNA-based markers (12 SSRs and 21 RFLPs; Figure 2). Because 9 individuals were analyzed with SSR markers alone, these individuals were not included in our overall analysis of maize chromosome 9 rearrangements. Out of 55 individuals, 25 (45%) contained all the DNA-based markers tested (Table 3). Chi-square analyses indicated that the proportion of individuals that had all of the tested DNA markers was significantly

TABLE 1

Plant survival and panicle production following irradiation of maize chromosome 9 monosomic addition line seeds with three levels of gamma rays

Radiation dose (krad)	Seeds irradiated	Viable plants (%)	Fertile plants (%)	Panicles harvested
30	100	65 (65)	63 (97)	334
40	100	52 (52)	48 (92)	274
50	200	54 (27)	50 (93)	107
Total	400	171 (43)	161 (94)	715

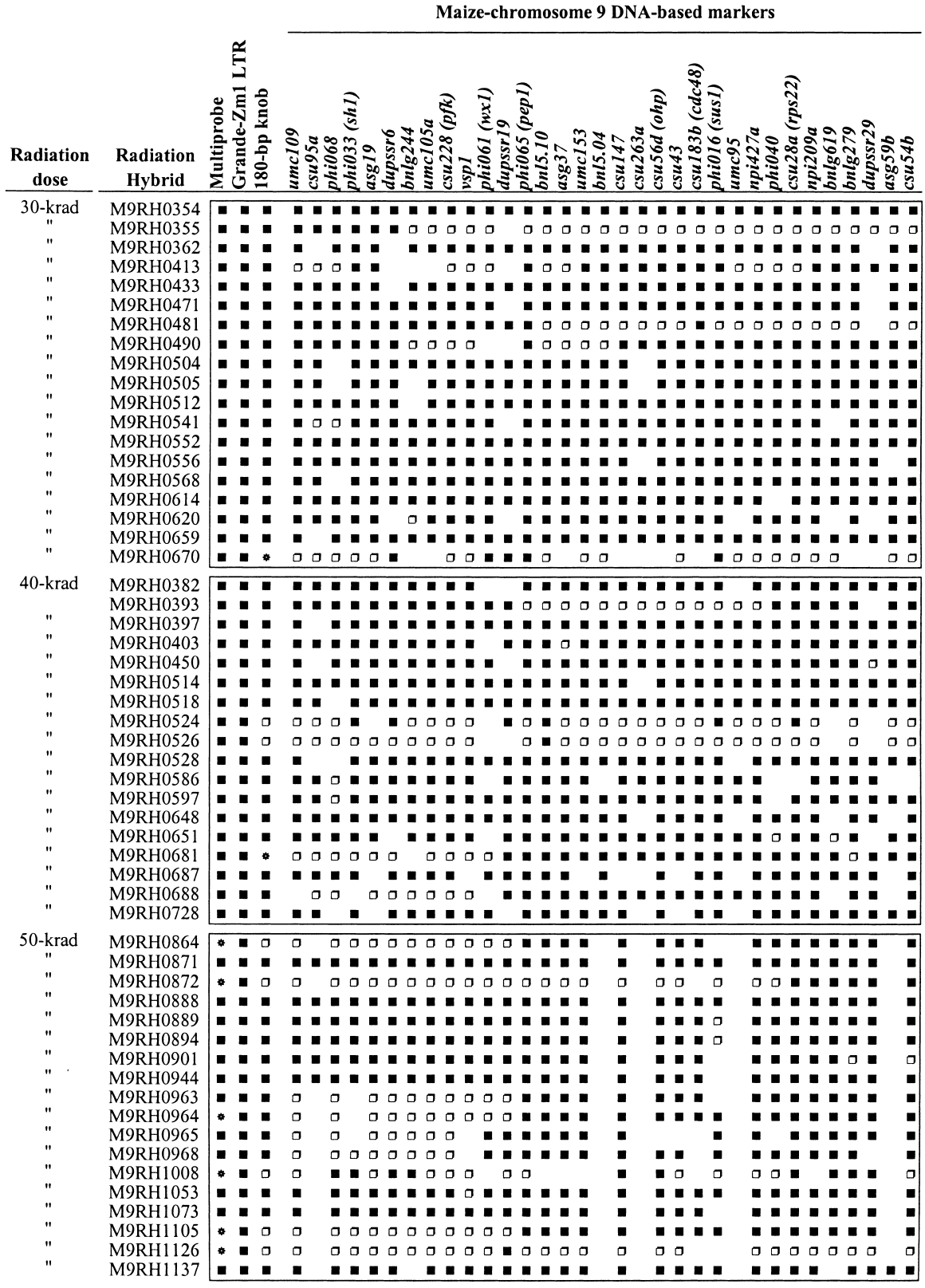

FIGURE 2.—DNA-based marker characterization of M9RHs. The order of DNA-based markers is based on the 1998 UMC maize bin map (Maize DB http://www.agron.missouri.edu). The gamma-ray dose corresponds to the treatments applied to the maize chromosome 9 monosomic addition line seed that gave rise to the M9RHs. Solid squares indicate that the marker was present in a given M9RH. Open squares indicate that the marker was absent in a given M9RH. Asterisks indicate that a marker was present with diminished hybridization intensity. Empty space indicates missing data.

TABLE 2

Plants with maize chromosome 9 rearrangements in radiation hybrids that originated from plants treated with three levels of gamma rays

Radiation dose (krad)[a]	Plants screened	Plants with maize DNA	Plants analyzed for maize chr. 9 rearrangements	Analyzed plants with:		Plants with various rearrangements		
				Apparently normal maize chromosome 9 (%)	Rearranged maize chromosome 9 (%)	Intergenomic translocation	Single or multiple deletions	Deletions plus intergenomic translocations
30	261	20	19	11 (58)	8 (42)	2	4	2
40	194	25	18	5 (28)	13 (72)	2	6	5[b]
50	193	19	18	4 (22)	14 (78)	4	4	6
Total	648	64	55	20 (36)	35 (64)	8	14	13

[a] Gamma-ray dose that was applied to the maize chromosome 9 monosomic addition line seed (Table 1).

[b] Includes maize radiation hybrids M9RH0681 and M9RH0687. M9RH0681 is an isochromosome for the long arm of maize chromosome 9 including a small deletion. M9RH0687 is chromosomally chimeric where all the DNA markers checked were present, but different maize chromosome 9 rearrangements were found in different cells of the same plant.

higher in plants from the 30-krad treatments (63%) than in plants from the 50-krad (28%) treatments ($P = 0.031$; Table 3). There was no significant difference in the proportion of individuals that had all of the tested DNA markers when materials from the 30-krad and 40-krad or 40-krad and 50-krad treatments were compared. Chi-square contingency table analyses indicated that the average marker retention frequency varied significantly among the radiation treatment groups ($P < 0.001$). The average marker retention frequency of maize DNA markers tested was significantly reduced from the 30-krad treatment (85%) to the 50-krad treatment (75%) ($P < 0.001$). Similarly, the average marker retention frequency was significantly reduced from the 40-krad treatment (83%) to the 50-krad treatment (75%; $P = 0.001$). On the other hand, average marker retention frequencies were not significantly different between the 30-krad (85%) and 40-krad (83%) treatments (Table 3). The marker retention frequencies in individual M9RHs ranged from 3 to 100% (Table 3). About 21% of the radiation hybrids from all gamma-ray treatment categories retained 60% or less of the markers tested (Figure 3). A chi-square homogeneity test showed that the marker retention or loss in the long ($P = 0.74$) and short ($P = 0.96$) arms of maize chromosome 9 was homogeneous (Figure 4).

Cytological evaluation of the maize chromosome 9 rearrangements: *In situ* hybridization with maize-specific probes (total genomic DNA, the multiprobe, and the 180-bp knob-specific sequence) complemented the DNA-based marker analyses on the radiation hybrids. Whenever a M9RH line had a maize chromosome 9 with an apparently normal morphology similar to that of a monosomic maize chromosome 9 addition line (Figure 5A) and the M9RH line was also positive for all the DNA-based markers tested, the line was assumed to have a normal maize chromosome 9. If a M9RH line did not meet these criteria, the line is described as having a rearranged maize chromosome 9. Among the 55 M9RHs studied in detail, 20 (36%) had an apparently normal maize chromosome 9 (Table 2). Maize chromatin was not cytologically detected in M9RH0524, M9RH0526, M9RH0670, M9RH1008, and M9RH1126 even though maize chromatin was detected by Southern blot and PCR-based assays. Our failure to detect maize chromatin by *in situ* hybridization may be an indication of somatic instability and chimerism in these individuals. Alternatively, our failure to detect maize chromatin in these individuals might represent instances where fragments of maize chromosome 9 were smaller than the size limit of resolution by standard genomic *in situ* hybridization [estimated to be ≥10 Mb (Simpson and Savage 1996; Kodama *et al.* 1997)]. The use of more sensitive *in situ* hybridization protocols and analysis of progenies from these individuals might help clarify this situation.

Thirty-five (64%) M9RHs were identified to contain various maize chromosome 9 rearrangements based on

TABLE 3

DNA marker retention in maize chromosome 9 radiation hybrids

Radiation dose (krad)[a]	Marker retention % per plant (range)	% of individuals containing all the tested markers
30	85 (19–100)	63
40	83 (3–100)	44
50	75 (4–100)	28
Mean	81 (3–100)	45

[a] Gamma-ray dose that was applied to the maize chromosome 9 monosomic addition line seed (Table 1).

cytological analyses (Table 2). Examples of observed rearrangements included intergenomic translocations of chromosome segments such as those observed in M9RH0355 (Figure 5B), M9RH0901 (Figure 5C), and M9RH0872 (Figure 5D). Examples of ones with simple and multiple deletions of a nontranslocated maize chromosome include M9RH0393 (Figure 5E) and M9RH0413 (Figure 5F), respectively. More complex rearrangements such as a combination of deletions with translocations and multiple intergenomic translocations are exemplified by M9RH0481 (Figure 5G), M9RH0964 (Figure 5H), and M9RH0362 (Figure 5I), respectively. Complex rearrangements that involved at least one deletion, an inversion plus an intergenomic translocation were observed in M9RH0688 (Figure 5J) and M9RH0968 (Figure 5K). One individual (M9RH0681, Figure 5L) with an apparent isochromosome for the long arm of maize chromosome 9 also was the product of a complex rearrangement. DNA marker analysis of M9RH0681 (Figure 2) indicated the presence of a small deletion in each of the duplicated arms. This observation suggested that this isochromosome formed by misdivision of a chromosome 9 addition containing a radiation-induced deletion in the long arm. M9RH0687 (Figure 6) was chimeric for its maize chromosome 9 composition. Some cells in the same root tip contained variable numbers of maize chromosomes or chromosome fragments. This condition might have resulted from the transmission of a dicentric, ring chromosome, or some other complex rearrangement with segregation of chromosomes and/or chromosome fragments in subsequent mitotic divisions.

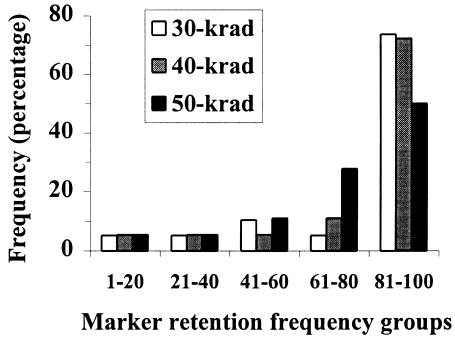

FIGURE 3.—Histogram showing the distribution of marker retention frequencies among M9RHs produced from materials treated at 30, 40, and 50 krad.

There was an increase in the proportion of plants with maize chromosome 9 rearrangements as the radiation dose was increased (42% at 30 krad, 72% at 40 krad, and 78% at the 50-krad treatment, respectively) as well as variation in the types of chromosome rearrangements present. Single intergenomic translocations, single and multiple interstitial deletions, and other complex rearrangements such as interstitial deletions plus intergenomic translocations involving maize chromosome 9 were similarly frequent among progenies of plants from 30-krad treatments (Table 2). Single and multiple interstitial deletions of maize chromosome 9 and complex rearrangements were the predominant forms of maize chromosome 9 aberrations observed in progenies of materials treated at 40 and 50 krad (Table 2). Overall, single or multiple interstitial deletions of the maize chromosome 9 and intergenomic translocations of a maize chromosome 9 piece with interstitial deletions were more predominant over simple intergenomic translocations in the M9RHs studied.

Identification of M9RHs with unique marker retention patterns: For mapping purposes, an informative M9RH was defined as an individual that contained a unique pattern of marker retention and inferred maize chromosome 9 breaks. A subset of 24 radiation hybrids (7 from the 30-krad, 9 from the 40-krad, and 8 from the 50-krad groups) were found to contain unique patterns of detectable chromosome breaks that were representative of the entire panel of 55 M9RHs that were studied (Figure 7). This subpanel of M9RHs was used to dissect maize chromosome 9 into 27 distinct regions. Analysis of marker retention in these individuals revealed that M9RHs with 1–10 breaks per chromosome were produced (Figure 7). The average number of

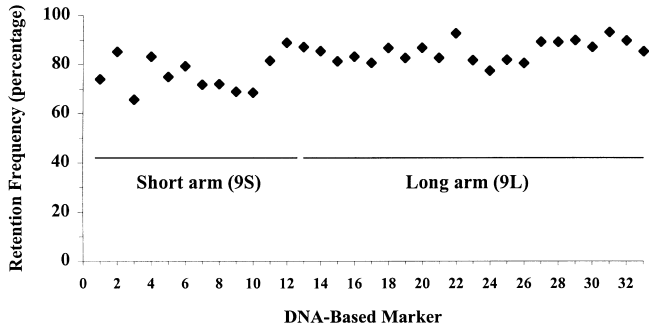

FIGURE 4.—Average retention frequencies for DNA markers for the short (9S) and long (9L) arms of maize chromosome 9. Marker retention frequencies across radiation treatments are shown. Markers 1 through 33 correspond in order to those shown in Figure 2, number 1 being umc109 and number 33 corresponding to csu54.

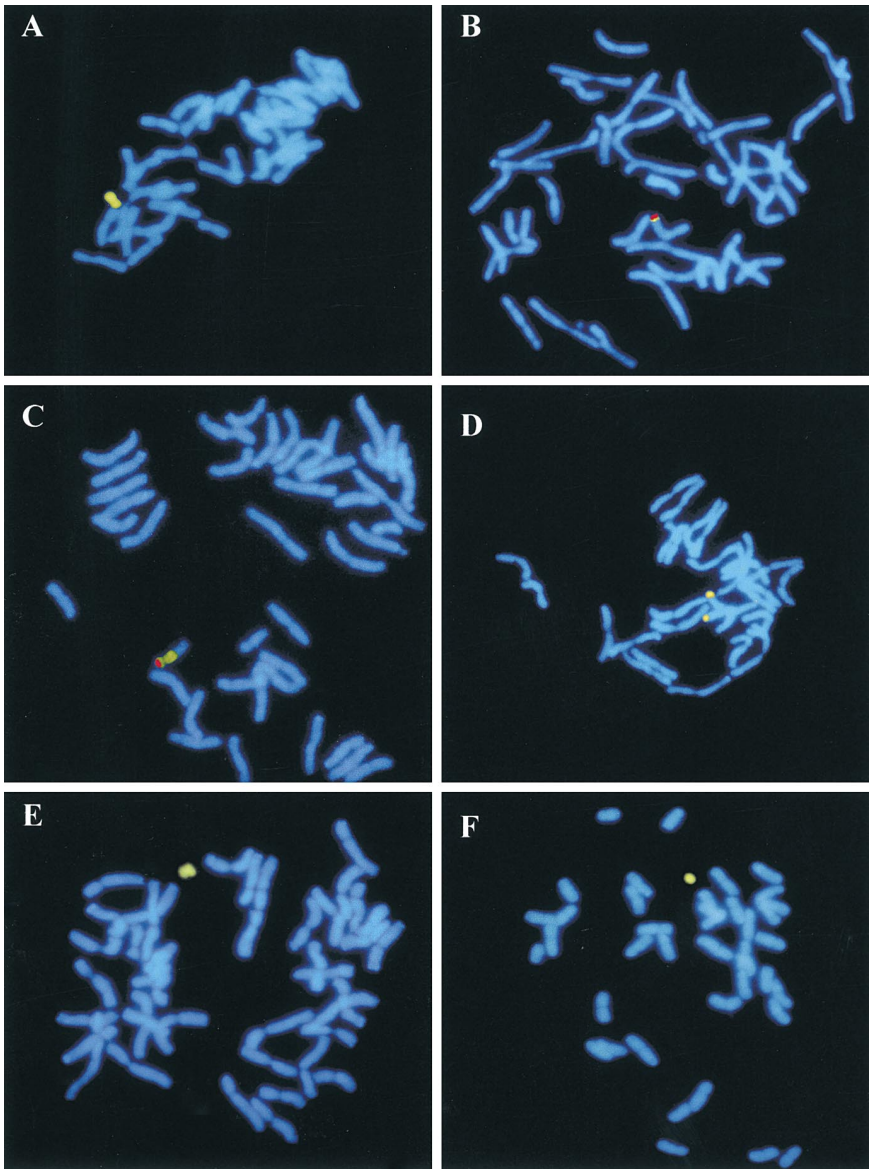

FIGURE 5.—Fluorescence *in situ* hybridization of a maize chromosome 9 monosomic addition line (A) and 11 radiation hybrid lines (M9RH0355, B; M9RH0901, C; M9RH0872, D; M9RH0393, E; M9RH0413, F; M9RH0481, G; M9RH0964, H; M9RH0362, I; M9RH0688, J; M9RH0968, K; M9RH0681, L). Unlabeled blue oat chromosomes were counterstained with DAPI. Fluorescein-labeled maize genomic DNA was used as the probe for genomic *in situ* hybridization in A, D, E, F, I, and L. Yellow-green fluorescence marks maize chromatin. Multicolor fluorescence *in situ* hybridization using two maize-specific repetitive probes (180-bp knob-specific sequence and the maize-specific multiprobe) were used in B, C, G, H, J, and K. Red fluorescence marks the 180-bp knob homologous sequences and yellow fluorescence marks sequences homologous to the maize-specific multiprobe.

breaks per chromosome for these subpanels of M9RHs from the 30-, 40-, and 50-krad treatment groups was 3.6, 3.1, and 2.5, respectively (Figure 7).

Stocks with discrete parts of maize chromosome 9: We identified a number of individuals that apparently contained discrete regions of maize chromosome 9. M9RH0481 (Figure 5G), for example, possesses an intergenomic translocation involving the short arm of maize chromosome 9 and a small piece of the long arm. M9RH0355 (Figure 5B) possesses an intergenomic translocation involving the distal end of the short arm of maize chromosome 9. M9RH0864 and M9RH1105 possess intergenomic translocations involving the long arm of maize chromosome 9. Finally, M9RH0872 (Figure 5D) possesses an intergenomic translocation involving the distal end of the long arm of maize chromosome 9. These lines may be used to isolate DNA clones that are specific for a chromosome region.

DISCUSSION

Irradiation of maize chromosome 9 monosomic addition line seeds resulted in the production of novel oat lines possessing various rearrangements of maize chromosome 9. Of rearrangements that involved maize chromosome 9, simple intergenomic translocations were less frequent than rearrangements with single or multiple deletions with or without a translocation (Table 3). The preponderance of intrachromosomal aberrations (single and multiple interstitial deletions) over interchromosomal aberrations (simple intergenomic translocations without a deletion) is consistent with chromosome aberration yield studies in human cells following ionizing radiation treatments. In humans, a bias for intrachromosomal aberrations over interchromosomal aberrations has been attributed to "proximity effects," *i.e.*, the preferential interaction or reattachment of double-strand breaks initially formed closely in space and/or

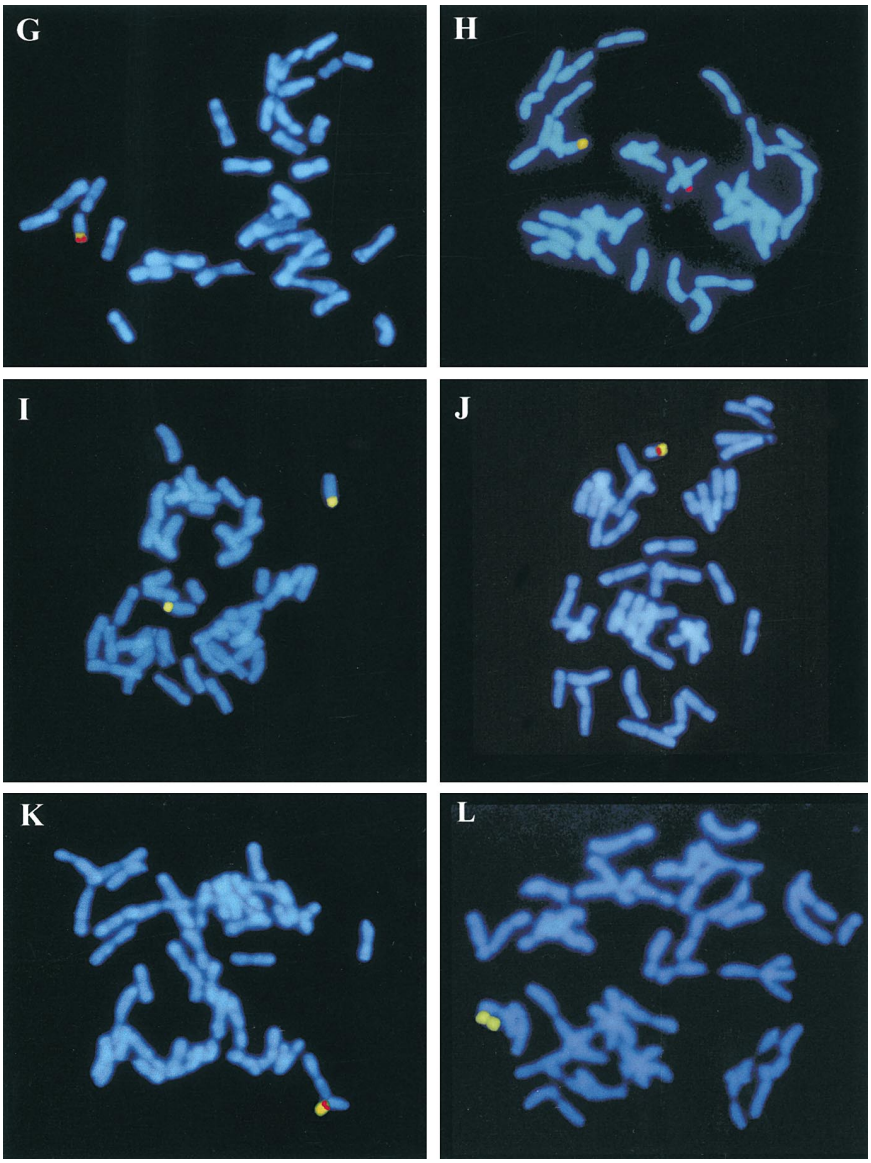

FIGURE 5.—*Continued.*

time (SACHS *et al.* 1997). Because chromosomes occupy localized subdomains within a cell during interphase (CREMER *et al.* 1993; YOKOTA *et al.* 1995), double-strand breaks on the same chromosome are, on the average, formed closer together than double-strand breaks on two different chromosomes (SACHS *et al.* 1997). The localization of chromosomes to subdomains in interphase cells has also been observed in plants (SCHWARZACHER *et al.* 1992). Similarly, the localization of maize chromosomes to discrete domains in interphase nuclei has also been observed in maize chromosome addition lines of oat (BASS *et al.* 2000). Thus, intrachromosomal aberrations induced by radiation were also expected to be more frequent than interchromosomal aberrations in our study. About 77% of all the aberrations observed involved at least one interstitial deletion of maize chromosome 9. On the other hand, 23% of aberrations involved a translocation without a deletion. These observations may indicate a bias for intrachromosomal aberrations in our study. It is important to point out that the chromosome rearrangements involving maize chromosome 9 in each radiation hybrid have passed through one meiotic event before being studied. Thus, intrachromosomal aberrations such as centric ring and dicentric chromosomes are, in general, eliminated before analysis.

The basic strategy in RH mapping is to use radiation-induced breakage for mapping. In addition, radiation hybrid mapping panels with incrementally higher levels of resolution may be produced by inducing incrementally higher levels of chromosome breakage (STEWART *et al.* 1997). In animal systems, RH mapping panels with substantial levels of chromosome breakage are possible due to the strategy that is used in their development. First, a donor cell culture line, with a functional thimidine kinase (TK+) or hypoxanthine phosphoribosyl transferase (HPRT+), is lethally irradiated. Second, lethally irradiated cells with highly fragmented chromo-

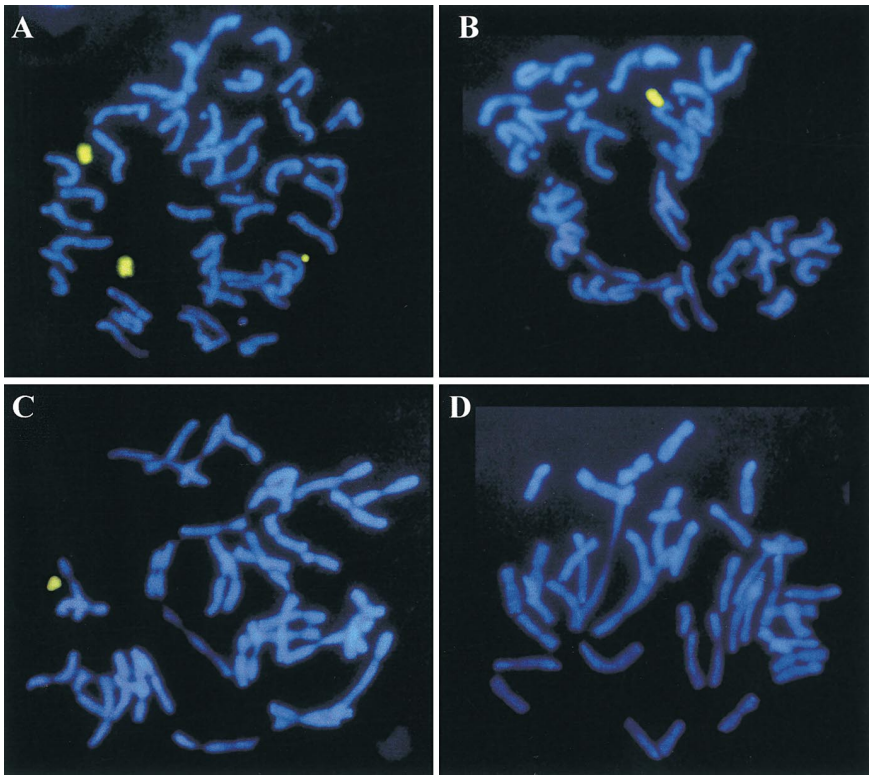

FIGURE 6.—Genomic *in situ* hybridization of four cells from a single root tip of M9RH0687. Unlabeled blue oat chromosomes were counterstained with DAPI. Fluorescein-labeled maize genomic DNA was used as the probe. Yellow-green fluorescence marks sequences homologous to the maize genomic DNA.

somes are rescued by fusion with a recipient nonirradiated cell line deficient for thimidine kinase (TK−) or hypoxanthine phosphoribosyl transferase (HPRT−). Third, viable fusion products are isolated by selecting for TK or HPRT activity. The final products are viable somatic (RH) hybrids with various subchromosome fragments from the donor species. These radiation hybrids are kept in culture for a limited period of time to prevent the loss of acentric chromosome fragments (STEWART *et al.* 1997). In this system, radiation hybrids with various chromosome fragments only experience selection for the selectable marker and their ability to proliferate *in vitro* for a brief period of time. In our case, irradiated cells with highly fragmented chromosomes were not recovered because we regenerated live and fertile plants after seed irradiation. Highly fragmented nuclei are not likely to survive the selection for morphogenetic capacity and mitotic divisions during plant development. During sporogenesis, chromosome rearrangements that have a deleterious effect on gametophyte development will also be eliminated. The fate of a particular cell in an irradiated meristem is uncertain because its fate depends on its position in the meristem at the time of differentiation (POETHIG *et al.* 1990). In our study, the number of cells in the meristem that were competent to develop was probably reduced by irradiation. Thus, cell lineages in an inflorescence probably traced back to a limited number of cells (with or without a maize chromosome 9 rearrangement) that had retained morphogenetic capacity.

A benefit of the regeneration of live and fertile plants is the availability of material for future use. The chromosomes of each M9RH have passed through one meiotic generation before being analyzed. Any maize chromosome 9 rearrangement recovered, therefore, is known to be sexually transmissible, at least through the female parent. Because a particular rearrangement may be reisolated from seed produced by self-pollination of a given M9RH, an M9RH can be considered immortal. The transmission rates of various maize chromosome 9 rearrangements by self-pollination are currently being evaluated.

Our chromosome breakage data also indicate that there is a limit to the amount of chromosome breakage that is possible when live and fertile plants are involved. The proportion of plants with maize chromosome 9 rearrangements increased from 42% in progenies of materials treated with 30 krad to 72 and 78% in progenies of material treated at 40 and 50 krad, respectively. However, the level of chromosome breakage was fairly constant among informative M9RHs (~3 breaks per chromosome) from all radiation dose groups. Chi-square tests of homogeneity of marker retention in the long and short arms of maize chromosome 9 indicated that radiation-induced breakage was homogeneous for regions covered with the markers used in this study. Thus, the level of chromosome breakage was constant but there was no indication of preferential breakage or lack of breakage for a particular chromosome region. The chromosome rearrangements that we have recovered in this study probably represent a highly selected but random subset of the rearrangements that were initially induced.

The average marker retention frequencies in our hy-

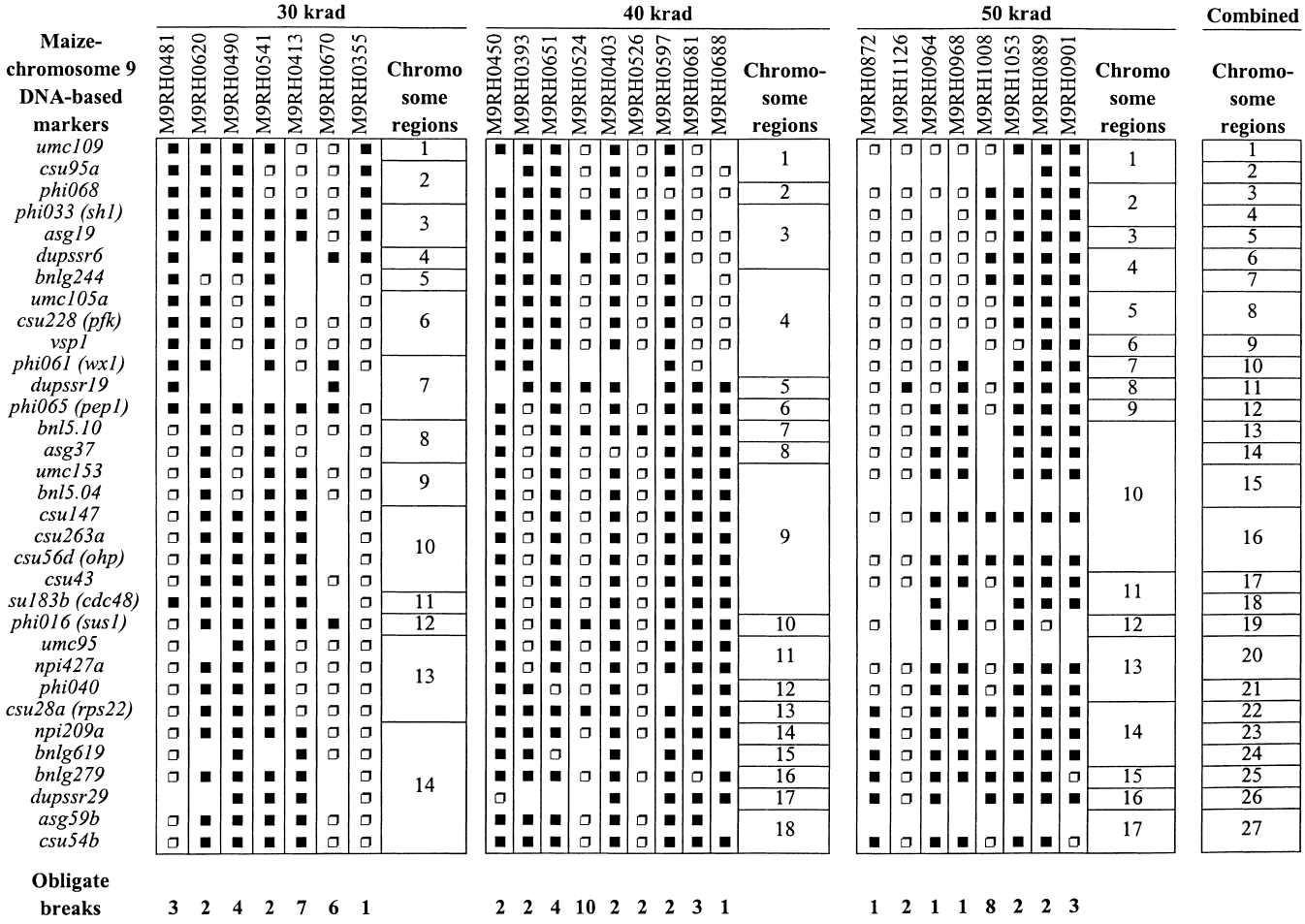

FIGURE 7.—DNA marker-based dissection of maize chromosome 9 using M9RH's. Maize chromosome 9 dissection is based on the pattern of DNA marker retention or loss in selected M9RH's from different radiation treatment groups. Maize chromosome 9 dissection based on the combined data from all of the selected M9RH's is also presented. The order of DNA-based markers is based on the 1998 UMC maize bin map (Maize DB http://www.agron.missouri.edu). The gamma-ray dose corresponds to the treatments applied to the maize chromosome 9 monosomic addition line seed that gave rise to the M9RH's. Solid squares indicate that the marker was present in a given M9RH. Open squares indicate that the marker was absent in a given M9RH. Empty spaces indicate missing data.

brids from the 30-, 40-, and 50-krad treatments were 85, 83, and 75%, respectively (Table 2). These average marker retention frequencies are higher than 10 to 50% of donor genome retention that are considered to be best suited for RH mapping (GYAPAY *et al.* 1996; STEWART and COX 1997; STEWART *et al.* 1997). In our study, ~21% of the radiation hybrids from all gamma-ray treatment categories retained 60% or less of the markers tested (Figure 3). Although marker retention frequencies in our study do reflect lower breakage frequencies than those needed for high-resolution RH mapping, the overall concept of using chromosome breakage for mapping is, in general, applicable. The mapping resolution of a panel with a constant level of chromosome breakage depends on the number of informative hybrids that are isolated. Assuming an average of 3 chromosome breaks per informative M9RH, the potential number of chromosome breaks in a population of 100 and 200 M9RHs would be 300 (100 chromosomes × 3 breaks/chromosome) and 600, respectively. Since maize chromosome 9 is about 191 Mb in size, the average distance between random breaks would be 0.6 Mb (191 Mb/300 breaks) for 100 radiation hybrids and 0.3 Mb for 200 individuals. We estimate that ~400 evenly distributed markers would be necessary to map at a 0.5- to 1.0-Mb level of resolution. Our inability to recover a larger number of chromosome fragments will limit the mapping resolution that may be achieved in our system but it may be possible to obtain mapping panels with higher resolution by enriching the panel with hybrids with the greatest amount of chromosome breakage and mapping information (JONES 1996).

Addition lines allow the localization of a marker to a given maize chromosome and radiation hybrid derivatives will permit their placement to a chromosome region. Because mapping with maize chromosome addition lines and radiation hybrid derivatives involves assays for the presence or absence of a given marker, mono-

morphic markers such as STSs and expressed sequence tags (ESTs) can be quickly and efficiently mapped. This system is particularly amenable to automation and high-throughput formats. At present, there is no comparable system in maize to map monomorphic markers. Thus, we believe that radiation hybrid derivatives of maize chromosome addition lines of oat will play an important role in the difficult task of mapping an ever-increasing number of maize ESTs (~56,000; dbEST: database of expressed sequence tags; http://www.ncbi.nlm.nih.gov/dbEST).

Besides the use of M9RHs to map DNA markers, these materials will also permit the isolation of chromosome-segment DNA libraries for targeted cloning. The efficiency of this targeted cloning approach has already been demonstrated by the isolation of maize-specific cosmid clones from a maize chromosome 9 addition line library (Ananiev *et al.* 1997). The use of maize-specific DNA sequences to study the structure and composition of specific maize chromosome regions such as heterochromatic knobs (Ananiev *et al.* 1998a,c) and centromeres has also been demonstrated (Ananiev *et al.* 1998b). Before M9RHs are used for targeted cloning, additional DNA markers should be used to fully evaluate the integrity of maize chromosome 9 fragments in question. The radiation doses used to produce radiation hybrids in this study were intended to induce as much chromosome breakage as possible. Materials produced with lower radiation doses may be more desirable.

In summary, irradiation (gamma rays at 30, 40, and 50 krad) of a monosomic maize chromosome 9 addition line resulted in the production of M9RHs possessing different maize chromosome 9 rearrangements. The level of chromosome breakage in M9RHs that are useful for mapping was fairly constant (~3 breaks per chromosome) irrespective of radiation dose used in their production. It is estimated that 100 informative M9RHs would allow mapping at the 0.5- to 1.0-Mb level of resolution. Because mapping with maize chromosome addition lines and radiation hybrid derivatives involves assays for the presence or absence of a given marker, monomorphic markers may be quickly and efficiently mapped to a chromosome region. Radiation hybrid derivatives also represent sources of region-specific DNA for cloning of genes or DNA markers. The development of subchromosome fragment stocks for other maize chromosomes present in various maize chromosome addition lines of oat is currently underway.

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