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CELL BIOLOGY & MOLECULAR GENETICS

Haploid Oat Plants Produced by Application of Maize Pollen to Emasculated Oat Florets

H. W. Rines* and L. S. Dahleen

ABSTRACT

Only six haploids in oat (*Avena sativa* L.) have been previously reported, five of spontaneous origin and one from anther culture. Our objective was to develop more efficient methods for producing oat haploids to use in selecting mutants, recovering aneuploids, and producing doubled-haploid lines for genetic and breeding studies. In a series of experiments, pollen from maize (*Zea mays* L.) was applied to previously emasculated oat florets. Twelve to 15 d later excised ovaries/caryopses, or embryos taken from them, were placed onto an amino acid-supplemented Murashige and Skoog medium containing 7% sucrose for embryo rescue. Recovered plantlets were potted in soil and grown to maturity. Root tips and meiotic tissues were sampled for cytological analyses. Overall, 14 haploid oat plants were recovered by embryo rescue following application of maize pollen to approximately 3300 emasculated oat florets. Root tip cells in each of the recovered plants had the oat haploid chromosome number of 21. Presumably these oat haploids originated from interspecies hybrid zygote formation followed by elimination of maize chromosomes during initial cell divisions, as has been described in haploid wheat (*Triticum aestivum* L.) formation in wheat × maize hybridizations. In the initial experiment, which involved combinations of various oat and maize genotypes, each of the four oat haploids recovered was from a different oat cultivar and each involved a different source of maize pollen; thus, indicating that the process is not genotype unique. Meiotic cells of the recovered haploid plants were characterized by aberrant chromosome behavior and numerous micronuclei, as expected in a haploid. Occasional seed were set on haploid plants and both euploid (2*n*) and aneuploid (2*n*-1 and 2*n*-2) progeny were obtained. The use of maize pollinations provides a new approach for obtaining haploid oat plants for genetic and breeding studies.

INTEREST IN HAPLOIDS as a genetic and breeding tool has increased during the past several years with the development of more efficient methods for their production (Snape et al., 1986). Two major techniques employed for haploid recovery have been anther/microspore culture (Dunwell, 1985) and wide hybrid crosses with subsequent elimination of the paternal genome during embryo development (Kott and Kasha, 1985). Anther culture has found application across a wide number of species (Dunwell, 1986), while crosses of the wild barley (*Hordeum bulbosum* L.) to cultivated barley (*H. vulgare* L.) and to certain genotypes of hexaploid wheat have produced high frequencies of haploids in these two cereals (Kott and Kasha, 1985). Doubled haploid-derived cultivars have been developed and released for several crops including rice (*Oryza sativa* L.), wheat, and barley (Han and Shui, 1980; DeBuyser et al., 1987; Ho and Jones, 1980, 1984). In addition, haploids have been of value for direct selection or identification of recessive mutants (Hoffman et al., 1982), the production of aneuploid stocks (Nishiyama et al., 1968), and the analysis of chromosome pairing relationships (von Bothmer and Subrahmanyam, 1988).

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In cultivated oat only six haploid ($n = 3x = 21$) or aneuhaploid ($n = 20$ and $n = 19$) plants have been previously reported. Five of these were of spontaneous origin, having been recovered either from twin embryo seeds or as progeny of crosses involving aneuploids (Leggett, 1977). The sixth was from anther culture (Rines, 1983). Efforts to derive oat haploids by anther culture involved the isolation and culture of over 65 000 oat anthers with only the single haploid plant recovered. A more efficient scheme for recovery of haploids in oat is needed to do the types of haploid-based studies described for other cereals.

Reports of early embryo development in wide hybrid crosses formed the basis for the development of an alternative method of obtaining oat haploids. Zenkteler and Nitzsche (1984) reported cytologically observable early-stage embryos in crosses between several unrelated cereals, including wheat × maize. Laurie and Bennett (1986) demonstrated the presence of both wheat and maize chromosomes in zygotes following application of maize pollen to emasculated wheat florets. They further noted that the maize chromosomes were eliminated during initial cell divisions. Embryos in early stages of development were observed in over 20% of their wheat × maize pollinations. When the maize-pollinated florets were left to develop on the maternal plant, only 1 in 2440 produced an embryo large enough to be rescued as a haploid wheat plant, presumably because there was insufficient or no accompanying endosperm development to allow continued growth of the embryo (Laurie and Bennett, 1988a). The development of methods for in vitro culture of individual spikelets that had been detached and cultured 2 d after maize pollination, however, enabled Laurie and Bennett (1988a) to recover 31 wheat haploids from 706 maize-pollinated wheat florets.

Based on the success in haploid production from wheat × maize hybridization, we attempted the recovery of oat haploids using a similar approach. This report describes the recovery and characterization of haploid oat plants following the application of maize pollen to emasculated oat florets.

MATERIALS AND METHODS

The four spring oat genotypes 'Stout', 'Starter', 'Steele', and 'Black Mesdag' used in the initial experiment of this study were chosen to reflect a diversity of genetic back-

grounds. Stout and Starter were used extensively in later experiments because their early maturity, short stature, and stiff stems made them easier to handle for emasculations and pollinations. The four field-grown maize genotypes used initially as pollen sources were selected for diversity and included inbreds 'A188' and 'B73', hybrid 'A619 × W64A', and 'Honeycomb', a hybrid sweetcorn from Sun Seeds, Inc. (Hollister, CA).[1] Greenhouse-grown A188 was used as the pollen source for later experiments.

Oat plants were grown in growth chambers except in a later study when plants grown in the greenhouse in the spring were compared to growth chamber-grown plants. Oat plants in the growth chamber received 12 h d^{-1} of about 400 $\mu mol\ m^{-2}\ s^{-1}$ light from a mixture of incandescent and cool white fluorescent lamps with a temperature regime of 20/15 °C (day/night), with a switch to 14 h of light 4 wk after planting. Greenhouse-grown oat plants received 14 h d^{-1} of about 100 $\mu mol\ m^{-2}\ s^{-1}$ supplemental light from cool white fluorescent lamps and were in a 21/18 °C (day/night) temperature regime. The oat plants were grown in soil, two per 12-cm diam. clay pot, with macronutrient fertilizer added biweekly from 4 wk after planting to anthesis. Greenhouse-grown maize plants received 16 h d^{-1} of about 600 $\mu mol\ m^{-2}\ s^{-1}$ supplemental light from high pressure Na lamps with the greenhouse maintained at about 24 °C. The plants were grown in 30-cm diam. plastic tubs of soil with macronutrient fertilizer added at 2-wk intervals.

Oat florets were prepared for applications of maize pollen by emasculating them 1 to 3 d before anthesis by procedures described by McDaniel et al. (1987) for the approach method of crossing oats. Individual spikelets were emasculated by removing the secondary floret and the anthers of the primary floret with fine forceps. Two to 3 d later and just before applying maize pollen, the glumes, lemma, and palea of the spikelet were clipped to just above the stigmas. Maize pollen was collected either directly from greenhouse plants or from maize tassels that had been cut from plants in the field in early morning and placed in flasks with their cut stems in water. The pollen, which was gathered by gently tapping tassels about to anthese over a sheet of paper, was either sprinkled over the trimmed emasculated oat florets or transferred to the florets using a camel-hair brush. Application of maize pollen was usually repeated 2 d after the initial application to increase frequency of fertilizations. Panicles were covered with glassine bags from the time of emasculation until ovaries/developing caryopses were harvested 12 to 15 d after the initial application of maize pollen. Oat × oat crosses for use as checks of normal oat caryopsis development were made by gently crushing individual mature anthers directly onto previously emasculated florets.

For embryo rescue from maize-pollinated oat florets, the ovaries/developing caryopses were isolated from the florets and surface sterilized by placing them in 950 mL L^{-1} ethanol for 15 s and in 25 g L^{-1} NaOCl solution for 5 min, followed by three rinses in sterile H_2O. On the rare occasion when an embryo was larger than 1 mm, it was dissected from the caryopsis and placed onto rescue medium. Otherwise, the ovaries/caryopses were placed either intact on the medium or the tips containing any developing embryos were cut off and placed on the medium. The rescue medium was MS medium (Murashige and Skoog, 1962) supplemented with the amino acids used by Norstog (1973) for barley embryo culture, 70 g L^{-1} sucrose, and 6 g L^{-1} agar. Fifty milliliters of the rescue medium was added to each 25 by 100-mm plastic petri dish. Media modifications tested included either the omission of the amino acid supplement or the substitution of 100 mL L^{-1} fresh filter-sterilized coconut milk in place of the amino acid supplement.

Petri dishes with the ovaries/caryopses were incubated in the dark at 20 °C and checked daily. If developing shoots appeared, the dishes were transferred to a growth cabinet providing 12 h d^{-1} of about 40 $\mu mol\ m^{-2}\ s^{-1}$ light at a constant 20 °C. After 3 to 5 d, the plantlets were transferred to 250 mL flasks containing 50 mL MS medium with 20 g L^{-1} sucrose and 6 g L^{-1} agar and placed back in the same growth cabinet. When the plants were about 10 cm tall, they were transferred to pots of sterilized soil and placed in growth chambers set to provide 12 h d^{-1} of about 400 $\mu mol\ m^{-2}\ s^{-1}$ light with 20/15 °C (day/night) temperature regime. Root tips were collected for chromosome counts when the plants were first put into soil, or a few days thereafter. At about 6 wk after transfer to soil, plants were subcloned by breaking tillers away from the crown region of each primary plant and placing these 3 to 5 subclones per plant into fresh pots of soil. Two weeks later the photoperiod was adjusted to 14 h of light d^{-1} to promote floral development. As panicles developed, some of them were collected for meiotic analysis of pollen mother cells (PMCs) and others left to mature. Seed produced on these haploid plants were germinated on moistened filter paper in petri dishes for 40 h in the dark at 20 °C and root tips collected for cytological analysis. The seedlings were then potted in soil and placed in the greenhouse. PMCs were collected and analyzed to confirm the root tip analyses.

Collected root tips of haploid plants and progeny seedlings were incubated in 0.5 g L^{-1} colchicine for 5 h at 20 °C and then fixed in ethanol: glacial acetic acid (3:1). Fixed root tips were heated in 1 *M* HCl at 60 °C for 12 min and squashed in proprionocarmine stain. Root tip chromosome counts were based on at least three cells per root tip. Meiotic analyses in PMCs from developing panicles were performed using the 3:1 fixative and gentle squashing in acetocarmine stain. Percent seed set on mature panicles was calculated by dividing number of seeds produced per panicle by the total number of primary and secondary florets on the panicle and multiplying by 100.

The initial experiment to determine if haploid oats could be recovered was conducted in late summer and involved combinations of maize pollen collected from various maize genotypes in a field nursery with four oat genotypes grown in a growth chamber. In follow-up attempts in the fall, Stout and Starter oat were grown in a growth chamber and the A188 maize pollen donor plants in a greenhouse. Both main culms and tillers of the oat plants were used. In the spring, oat plants were grown in both the greenhouse and the growth chamber and only main culms were used. A portion of the emasculated oat florets had no maize pollen applied prior to their being excised and cultured 12 d post emasculation. Also, an embryo rescue medium with the amino acid supplement omitted and one with coconut milk added were compared to the standard medium using portions of the maize-pollinated ovaries. No statistical analyses were applied because the low frequencies of plantlets recovered in each factor combination and the limited number of florets tested precluded valid comparisons of treatments.

RESULTS

Fourteen haploid oat plants, verified by chromosome counts of root tip cells, were obtained in a series of experiments involving application of maize pollen to over 3300 emasculated oat florets. In our initial experiment, pollen from various field-grown maize genotypes was applied to emasculated florets on eight plants of each of four oat cultivars. At the same time emasculated florets on a ninth plant were pollinated

[1] Mention of a trademark, vendor, or proprietary product does not constitute a guarantee or warranty of the product by the Univ. of Minnesota or the USDA, and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

with oat pollen. Among 600 ovaries excised from the maize-pollinated florets 12 to 15 d post pollination, 32 were enlarged and had green color, but they were thinner than caryopses of the same age from the oat × oat pollinations (Fig. 1). White, embryo-like structures up to 1.2 mm in length were visible in the tips of a few of these ovaries/caryopses; however, they were poorly formed compared to the 2.5 to 3.0 mm embryos present in caryopses from the oat × oat crosses, and endosperm was essentially lacking (Fig. 2). These green elongated ovaries, or embryos isolated from them, when placed on embryo rescue medium were the source of 3 of 4 haploid plants obtained in the initial experiment and 8 of 14 haploid plants recovered in all experiments (Fig. 3a and 3b). Plant development from these structures usually occurred within a few days after they were placed on the medium. Overall, 143 of the 3300 total ovaries isolated from maize-pollinated oat florets in this study were green and elongated; however, no consistent effects on their frequency were noted among different genotypes and growth conditions of oat and maize plants eventually tested.

The other six haploid plants recovered, including one in the initial study, came from the over 3150 ovaries that were indistinguishable in appearance from unfertilized ovaries at the time they were excised 12

Fig. 1. Comparison of nonpollinated oat ovaries 12 d post-emasculation (left), a caryopsis 12 d post-application of maize pollen (middle), and a caryopsis 12 d post-application of oat pollen (right).

Fig. 2. An oat caryopsis 15 d post-application of maize pollen with possible haploid embryo and little or no endosperm.

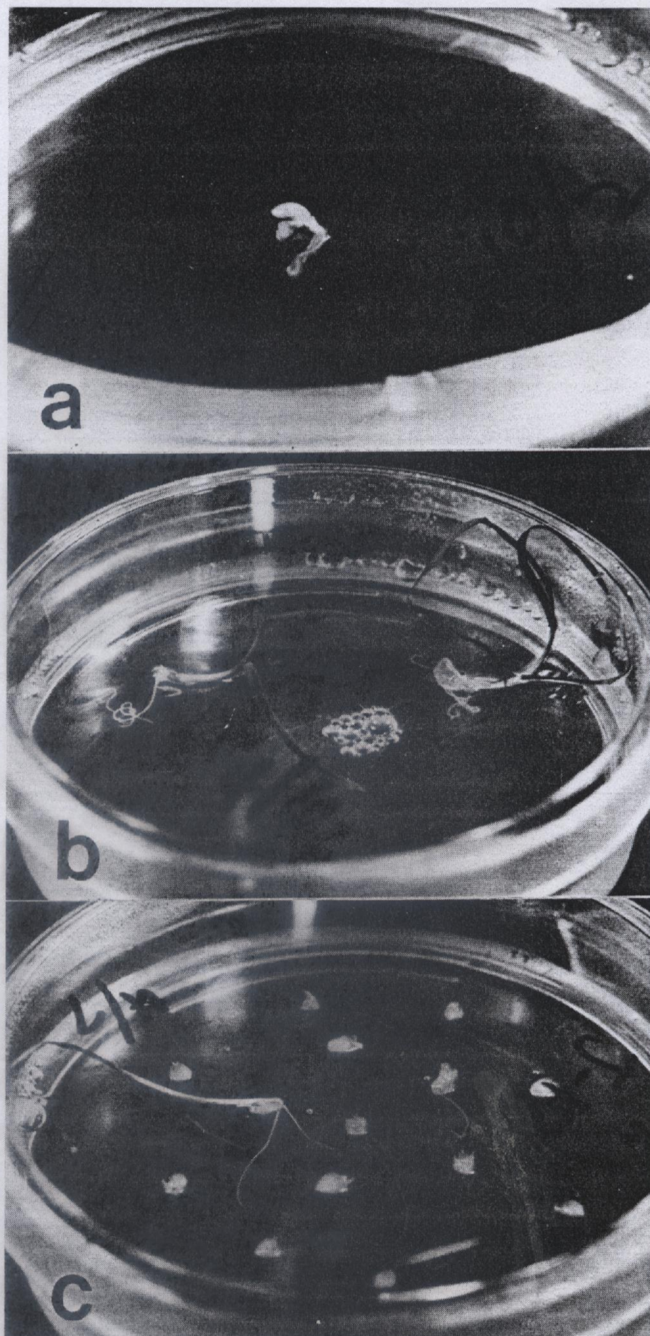

Fig. 3. Plant development following oat × maize pollinations: (a) germinating embryo 2 d after being isolated from a 12-d post-pollination caryopsis, (b) two plantlets 10 d after embryos were isolated from 12 d post-pollination caryopses, and (c) one plantlet and one root-only structure 18 d after ovaries were placed on culture medium.

Table 1. Oat × maize combinations from which single oat haploid plants were recovered by embryo rescue following application of pollen from field-grown maize to emasculated florets of growth chamber-grown oats.

Oat cultivar†	Maize pollen source‡
Stout	A188
Starter	B73
Steele	Honeycomb
Black Mesdag	A619 × W64A

† From 105 to 187 emasculated florets of each genotype were pollinated.
‡ Pollen sources were selected at random from a maize genetics nursery.

to 15 d post-pollination (Fig. 3c). Plantlets arising from these cultures did not appear until 7 to 14 d after the ovaries had been placed on the culture medium. Why certain ovaries became green and elongated following maize pollinations and whether there was a relationship between ovary greening and the occurrence or developmental state of embryo or endosperm was not determined.

In addition to the 14 haploid plants that were recovered from ovaries of maize-pollinated florets, there were four plants that were diploid in all root tips and PMCs examined and two plantlets that grew only a few centimeters tall and never had roots vigorous enough to collect for root tip chromosome counts. Also, small root-only growths (Fig. 3c) were produced from 37 of the maize-pollinated ovaries or their isolated embryos and small coleoptile sheaths without shoots were produced from seven others.

In the initial experiment, which involved field-produced maize pollen and growth chamber-grown oats, each of the four recovered plants was from a different oat cultivar and each involved a different source of maize pollen (Table 1); thus, indicating that the process was not unique to a particular genotype. Root tips taken when the four plants were small revealed the haploid number (21) of chromosomes (Fig. 4) in each recovered plant. In developing panicles in these plants, behavior at meiosis and pollen development was as expected for a haploid (Nishiyama and Tabata, 1964) with either little pairing or only loose nonsynaptic associations at Meiosis I (Fig. 5a), abnormal chromosome distribution to poles in Anaphase I (Fig. 5b), numerous micronuclei and nuclear sizes in quartet microspores (Fig. 5c), and multi-size aberrant pollen (Fig. 5d). This meiotic behavior was observed in gametophytic cells in all 4 to 7 panicles examined from each of the four haploids initially recovered. Other tillers on these plants were left to develop and mature. In these panicles, seed set ranged from none on less vigorous tillers up to 23% on more vigorous tillers in three of the four plants. The Stout plant produced no seed. Samples of seed from these plants were germinated and root tips collected for chromosome counts. Among the progeny seedlings, 11 had 42 chromosomes, 5 had 41, and 1 had 40 chromosomes. Both 42 and 41 chromosome seedlings were recovered from the three haploids that produced seed. The occurrence of both euploids and aneuploids among the progeny of each of the three haploid plants was verified by PMC analysis based on the presence or absence of an unpaired chromosome in early meiotic stages and high micronuclei frequencies in quartet microspores.

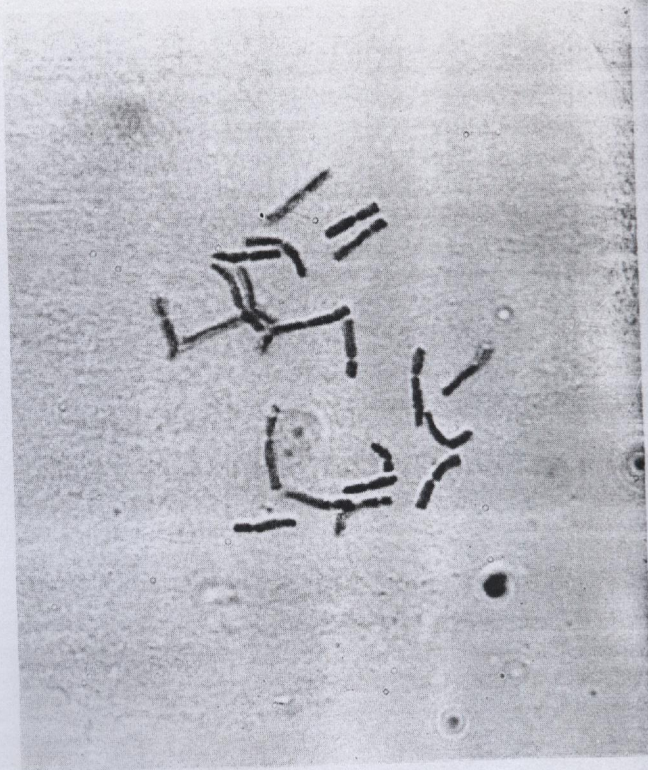

Fig. 4. Root tip cell with the haploid number (21) of oat chromosomes from a plant produced by oat × maize pollination.

Ten additional haploid plants were recovered from about 2700 cultured maize-pollinated ovaries during the subsequent experiments involving variations in plant growth conditions and embryo culture media. In these tests, the frequency of plant production remained too low (<1%) and inconsistent to draw definitive conclusions on treatment effects with the limited number of maize-pollinated oat florets tested. In an experiment involving plants grown in growth chambers in the fall, however, four plants were recovered from 635 maize-pollinated florets from main culms whereas no plantlets were obtained from 33[illegible] florets on later tillers of these plants. This result indicates that the vigor of the maternal oat tiller ma[y] influence haploid embryo development. Also, maternal oat plants grown in the greenhouse in the sprin[g] produced a higher frequency of haploids (6 from 94[illegible] florets) than did growth chamber-grown materna[l] plants (1 from 780 florets). In this comparison [of] greenhouse and growth-chamber growth condition[s] the plants were pollinated from the same sources [of] maize and only main culms were used.

Application of maize pollen appeared to be essenti[al] for plantlet development from cultured ovaries of t[he] emasculated oat florets. In a comparison study usi[ng] only main culms and both greenhouse and grow[th] chamber-grown plants, no plantlets or root structu[res] were produced from cultured ovaries of 650 nonpo[l]linated emasculated oat florets, whereas two pla[nts] and seven root structures were produced in the pa[r]allel set of cultured ovaries from 650 maize-pollinat[ed] florets.

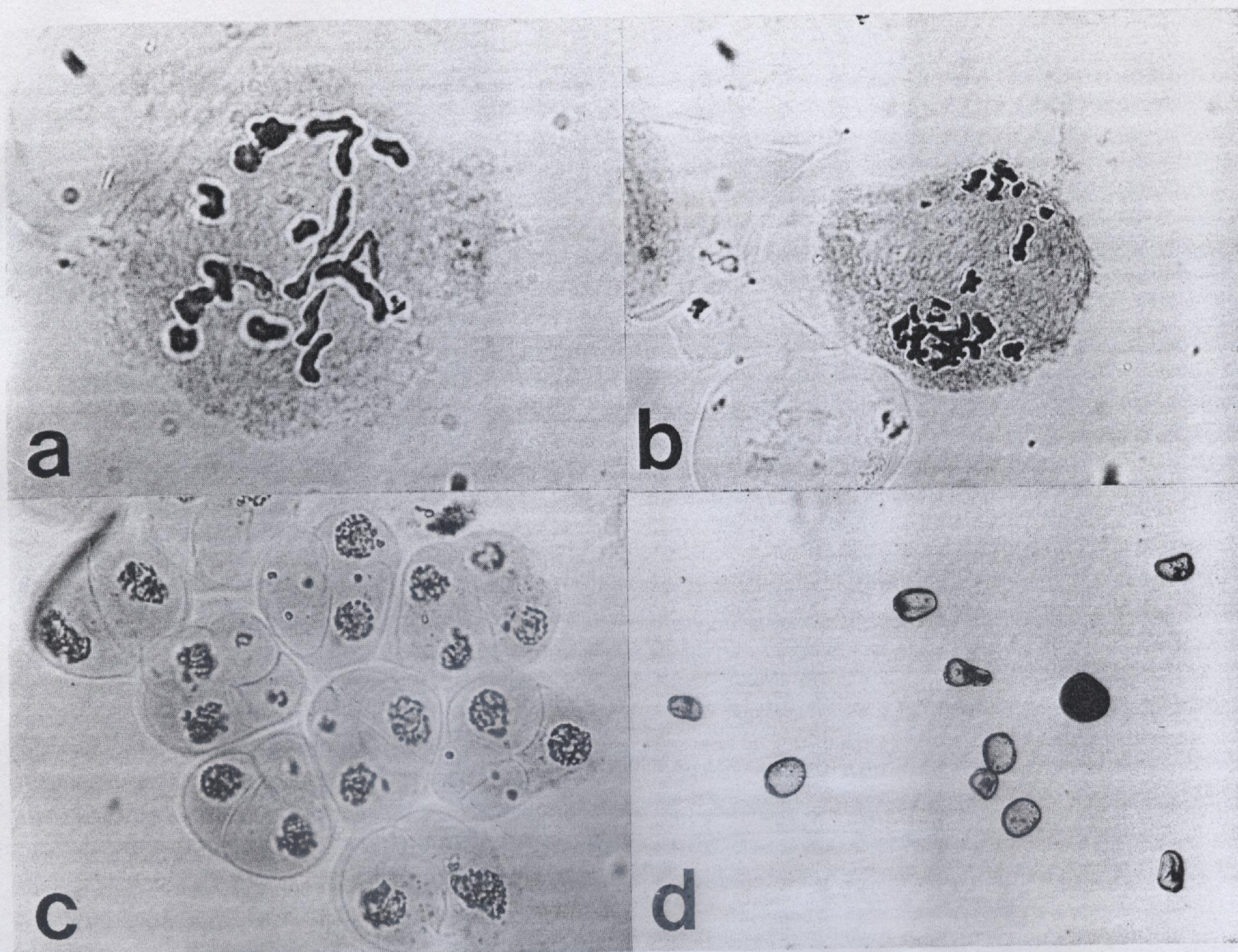

Fig. 5. Metaphase I (a), Anaphase I (b), quartet stage (c), and pollen (d) in a haploid oat plant from oat × maize pollinations.

DISCUSSION

Although the recovery of haploid oat plants by embryo rescue following application of maize pollen to emasculated oat florets was at a low frequency in this research, the 14 oat plants recovered is more than double those previously reported from spontaneous origin (five plants; Leggett, 1977) and from anther culture (one plant; Rines, 1983). Also, the maize pollination technique appears to be applicable over a wide range of oat genotypes based on the positive response with the limited number of genotypes tried (Table 1). Laurie and Bennett (1987) have reported a similar apparent lack of genotype restriction in embryo formation for wheat × maize hybridizations in that wheat × maize hybridizations were found to be relatively unaffected by the crossability loci *Kr 1* and *Kr 2*. Dominant alleles at these *Kr* loci restrict crossability of hexaploid wheat with many alien species, including rye (*Secale cereale* L.) and *H. bulbosum*.

The origin of haploid oat embryos in our experiments is unknown but likely involves fertilization of the oat egg cell by a maize sperm nucleus followed by elimination of maize chromosomes during initial embryonic cell divisions, as occurs in wheat × maize hybrids (Laurie and Bennett, 1986). Preliminary cytological analysis (not shown) of nuclear division in 48-h postpollination endosperm revealed chromosomes not incorporated in mitotic figures and numerous micronuclei, both indicative of chromosome elimination.

The frequent lack of plant development when small embryos appeared to be present in the ovaries 12 d after pollination or when root-only or coleoptile-only structures were produced from cultured ovaries indicates that inability to recover initiated embryos is a limiting factor to oat haploid production by the attempted techniques. The oat × maize system may be analogous to wheat × maize hybridizations where chromosome elimination occurs very early in embryo development, endosperm development ceases early or never occurs, and embryos usually fail to develop to a size that can be readily rescued when maize-pollinated florets are left to develop on untreated maternal plants (Laurie and Bennett, 1986). The use of an alternative rescue method involving culture of wheat spikelets 2 d post-maize pollination, however, resulted in the recovery of 31 haploid wheat plants from 706 maize-pollinated florets (Laurie and Bennett, 1988a). Even greater efficiencies of haploid wheat recovery following maize pollination have been reported recently by Suenaga and Nakajima (1989) from the injection of 2,4-D solutions into the uppermost internodes of wheat stems immediately following and again 1 d after pollination. The 2,4-D treatments apparently allowed

embryo development to continue on the intact wheat plants until embryos were isolated for culture 10 to 12 d postpollination. Also, Laurie and Bennett (1988b) have reported that sorghum (*Sorghum bicolor* L. Moench) pollen may give even higher frequencies of fertilization with wheat than does maize pollen. Such technique modifications plus the use of a male-sterilizing agent, if an effective one becomes available in oat (Johnson and Brown, 1976), could greatly improve efficiencies of haploid oat production involving wide crosses.

Several genetic studies and applications become feasible with the capability to recover haploids in oat. The number of monosomics is limited in oat with a complete monosomic series reported in only a single genetic background (Morikawa, 1985), and the completeness of the series has not been genetically verified. Nine oat monosomic lines were obtained by Nishiyama et al. (1968) in the progeny of a haploid from a twin seedling. Initial analysis of plants grown from seed produced on the haploids recovered in the present study indicate that this seed is a rich source of aneuploids; thus, these oat haploids may allow the recovery of monosomics not available in current stocks and also of monosomics in new genetic backgrounds. Also, we have recently established cell culture lines from haploid plants produced by this oat × maize pollination technique. Such haploid cultures will permit direct in vitro selection of recessive traits, such as victorin toxin resistance (Rines and Luke, 1985). Finally, development of a highly efficient haploid scheme will allow the production of doubled haploids as instant pure lines for germplasm development and breeding procedures. However, evaluation of doubled haploids derived by this technique will be needed to determine if variability is introduced as happens in doubled haploids of wheat produced from either anther culture (Baenziger et al., 1989) or the *H. bulbosum* method (Snape et al., 1988).

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Isolation and Analysis of Genomic DNA from Single Seeds

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ABSTRACT

Our objective was to adapt a plant DNA minipreparation procedure for the rapid isolation of total nucleic acid from single legume seeds. Single-seed extractions from cultivars of soybean (*Glycine max* L. Merr.) and frenchbean (*Phaseolus vulgaris* L.) were characterized for total nucleic acid yield, DNA content, and restriction fragment length polymorphisms (RFLPs). This procedure can be employed in the analysis of a large number of individual seeds by genomic blotting and molecular hybridization. While probing seed lots with the same complementary DNA (cDNA) fragment, we found that ostensibly pure soybean seed lines contained significant levels of identical RFLPs while frenchbean seed lines from plants with dramatic morphological differences had no detectable RFLPs for the same sequence. When isolated genomic DNAs were used to detect restriction fragment length polymorphisms (RFLPs), it was possible to make limited distinctions among cultivars of both soybean and frenchbean seed lots. This technique met the criteria of simplicity, rapidity, and low cost, and yielded DNA fragments of sufficient purity and length for RFLP analysis.

Molecular mapping of the soybean genome via RFLP has recently been initiated by several research groups (Apuya et al., 1988; Keim et al., 1989; Tingey et al., 1989). Widespread application of RFLP analysis to seed science and crop genetics depends on the development of techniques to reliably and rapidly isolate genomic DNA for use as a substrate for restriction enzymes. We have adapted a procedure, originally developed as a DNA minipreparation protocol from corn (*Zea mays* L.) leaf tissue (Dellaporta et al., 1983), for use in obtaining genomic DNA fragments from individual dry seeds. DNA preparations from seeds of several cultivars of soybean and frenchbean were characterized and used as the starting material for RFLP analysis. We demonstrate here that the DNA minipreparation procedure, modified for use with dry seeds, can be employed in the analysis of a large number of seeds by genomic blotting and molecular hybridization.

MATERIALS AND METHODS

Seed Description and Tissue Treatment

Soybean seeds included samples from the 1958 crop of cultivars Henry and Ross, which were sixth-generation inbred progeny stored by the breeder prior to the public release (Smith, 1962). The other soybean cultivars were a gift from the USDA Soybean Germplasm Facility at Urbana, IL. Seeds from frenchbean plant introduction PI 169783 (National Seed Storage Lab., Ft. Collins, CO; from Turkey in 1963) included 4 of 11 distinct sublines derived from this original collection based on seed-coat color (Roos, 1983). These samples were the result of two generations of regrowth after selection for seed-coat color (Generation C of McDonald and Kamalay, 1989). Seeds were transported at room temperature and then were stored at 4 °C (or at −20 °C for 1958 seed) upon arrival.

Isolation of Nucleic Acid

Dry seeds were split with a razor blade, and weighed after seed coats were peeled off. Tissues were ground to a fine powder in liquid N_2 using a mortar and pestle, defatted by three extractions with 5 mL of chilled chloroform/methanol (2:1, v/v) pre-chilled to 4 °C, and the resulting sediments dried in a vacuum desiccator. Cells were disrupted in 5 mL of extraction buffer [0.1 *M* TRIS-HCl (2-amino-2-hydroxymethyl-1,3-propanediol), pH 8.0; 50 m*M* disodium ethylenediaminetetraacetic acid (EDTA); 0.5 *M* NaCl; 10 m*M* β-mercaptoethanol] in a dounce homogenizer. Homogenates were brought to 1.3% (w/v) sodium dodecyl sulfate (SDS) and incubated at 65 °C for 30 min to promote cell lysis. Potassium acetate (KOAc) was added to 1.5 *M* final concentration, and the samples were left on ice for 20 min. Proteins were removed as SDS/KOAc precipitates by centrifugation of the homogenates at 25 000 *g* for 20 min at 0 °C. Supernatants were cleared of nonsedimenting debris by filtration through cheesecloth and total nucleic acids precipitated by adding 0.6 volumes of chilled (−20 °C for 30 min) isopropanol.

Precipitated nucleic acids were collected at 7000 *g* for 10 min at 0 °C. Pellets were dried in a vacuum desiccator and resuspended in 1 mL of buffer: 50 m*M* TRIS-HCl, pH 8.0; 10 m*M* EDTA. Insoluble debris was removed by sedimentation at 11 000 *g* for 10 min in a cold-water-jacketed Savant microfuge (Farmingdale, NY). Supernatants were adjusted to 0.3 *M* NaOAc and nucleic acids selectively precipitated by the addition of 0.17 volumes of 1% (w/v) cetyltrimethylammonium bromide (CTAB) (Sigma Chemicals, St. Louis, MO) (30 min at room temperature followed by −20 °C for 60 min). After CTAB precipitates were collected by sedimentation, pellets were washed with 70% w/v ethanol (3 times), dried under vacuum, and resuspended in 0.4 mL of TE buffer (10 m*M* TRIS-HCl, pH 8.0; 1 m*M* EDTA). The resuspensions were adjusted to 0.3 *M* NaOAc, and nucleic acid precipitated by the addition of 2.5 volumes of ethanol pre-chilled to −20 °C and kept at −20 °C 2 h. The TE resuspension and ethanol precipitation was repeated, and the final nucleic acid pellets were resuspended in 1 mL TE g^{-1} starting tissue and stored at −20 °C.

Ultraviolet Spectrophotometry and Fluorometry

Nucleic acid yield was calculated based on the observation that 1 mg RNA in low salt absorbs 25 optical density (OD) units at 260 nm. The OD_{260}/OD_{280} and OD_{260}/OD_{230} absorbance ratios of 1.8 or greater indicated levels of purity which correlated well with subsequent restriction endonuclease digestibility of the DNA in the extract. Relative purity of the isolated preparations generally did not vary widely, but the occasional occurrence of an aberrant absorbance profile preceded problems with restriction and subsequent analysis.

The DNA content in each nucleic-acid mixture was quantitatively assayed via ultraviolet (UV) fluorometry after staining with bisbenzimide (2 mg/L) (Hoechst no. 33258), essentially as described by Labarca and Paigen (1980). A fluorescence standard curve (Fig. 1) was constructed from measurements made on a Perkin-Elmer LS-5 fluorometer. We are currently using the Hoefer TKO-100 Mini-Fluoro-

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