

THE ARTIFICIAL INDUCTION OF ANEUPLOIDS IN AVENA SPECIES

BY

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ABSTRACT

In the production of a monosomic series in common oats, progress has been impeded by a lack of a highly productive source of these aneuploids. Consequently a number of methods were tested for their efficiency in inducing aneuploidy. Physical and chemical treatments were applied to whole panicles before anthesis so that the aberrations induced in the gametes would be directly incorporated into the progeny. The treatments included ultrasonic vibration; 2 chemicals, 8-ethoxycaffeine (EOC) and Myleran; and X-irradiation. The chemical and sonic treatments were not successful, inducing an average of only 0.7% and 2.4% aneuploidy respectively. X-irradiation induced a total of 65 monosomics, 15 double monosomics and 21 plants with 41+f which are potential monosomics. The average percent monosomics induced by X-rays was 12.3%.

Tetraploid and diploid species of Avena were observed after similar radiation treatment. Percent fertility, percent germination, seedling lethality, lack of vigour and other phenotypic abnormalities were observed to be inversely related to the ploidy level. The frequency of chromosomal aberrations induced was directly related to the ploidy level.

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INTRODUCTION

Common oats, Avena sativa L., ranks fourth among cereals in world production exceeded only by wheat, rice and corn. The world area devoted to oats is estimated at 130,000,000 acres. Considering its importance as a major cereal, this crop has received comparatively little attention from geneticists and cytogeneticists. This lack of effort to obtain such information is probably due primarily to the nature of the inflorescence and sensitivity of the plant to minor environmental changes. The oat panicle lacks the order and symmetry of a spike, the flowers are difficult to emasculate without injury and the pollen is highly sensitive to changes in temperature and humidity. Seed set from hybridization is usually low.

Avena sativa is an allohexaploid species similar to Triticum aestivum in that it has 42 chromosomes and is comprised of three different genomes (A, C and D). Since a monosomic series has been produced in T. aestivum, it is reasonable to expect a similar series could be produced in A. sativa. At present no monosomic series exists in A. sativa, but the production of such a series would greatly aid in cytological and genetic investigations. Linkage associations, association of genes with specific chromosomes and homoeologous relationships, which have been studied in other polyploids by the use of monosomics, could also be studied in oats. In addition monosomics may be of practical use in plant breeding particularly in a program of whole chromosome substitution.

The major difficulty in the production of a monosomic series in oats has been the lack of a suitable starting material. The chief source of monosomics in wheat was a haploid pollinated with normal pollen, while additional monosomics were derived from a nullisomic which was partially asynaptic (3B). To date no haploids have been reported in A. sativa and although asynaptic nullisomics have been reported they have been sterile. Another possible source of monosomics would be from interspecific hybridization. Such hybrids, however, are difficult to make and do not produce many seeds. Furthermore, since the A genome is common to all species of Avena, only fourteen different monosomics, those in the C and D genomes, could be produced.

A few monosomics and nullisomics have been found in natural populations of A. sativa through screening of large samples. This is a long and tedious process. It is obvious therefore, that a simple and efficient method of producing aneuploids in oats is required. The present study was conducted to determine the efficiency of chemicals, sonic vibration, and X-irradiation as agents for inducing aneuploidy.

LITERATURE REVIEW

Monosomics were reported in wheat as interesting phenomenon by Kihara in 1924 (14) and Thompson in 1928 (46), long before any practical application was made of them. Since then Sears (39,40,41) has produced all 21 possible monosomics and nullisomics in Triticum aestivum, and Clausen and his associates (3,4) have obtained all 24 monosomics in Nicotiana tabacum. Monosomics have been reported by Endrizzi and Brown in Gossypium (2,8).

The first monosomics reported in Avena were fatuoid 41 chromosome plants described by Huskins in 1927 (12) and Nishiyama in 1931 (31). In 1935 Philp found a monosomic which segregated producing 41 chromosome green plants and 40 chromosome albinos (32). In 1938 Philp reported a third monosomic which was concerned with leaf width (33). McGinnis and associates (23,24,25) have recently located genes for chlorophyll production on the short arm of chromosomes 14 and 21 (now designated chromosomes 21 and 5 respectively according to the standard karyotype of Rajhathy (34)) by means of aneuploid analysis.

The majority of monosomics in A. sativa reported to date have been found spontaneously occurring in natural populations. Riley and Kimber in 1961 reported 7 monosomics from 623 seedlings examined in the variety Sun II (37). In 1962 McGinnis analysed 4,023 seedlings from Foundation lines of the variety Garry and reported 2 nullisomics,

17 monosomics and 5 trisomics (22). In 1963 Hacker and Riley reported 6 nullisomics, 40 monosomics and 4 trisomics from 3,453 seedlings of the variety Sun II (11). Although the frequency of naturally occurring monosomics was low in these cases, both agreed that a monosomic series could be obtained in this way. McGinnis (22) pointed out that the major advantage of selecting spontaneously occurring aneuploids is that the stocks should be highly homogeneous. However, the screening method is extremely long and tedious.

Costa Rodrigues in 1954 (7) irradiated panicles of A. sativa with 300 r of X-rays. His objective was to produce a break in the two chromatids of a chromosome with consequent formation of a dicentric which would be involved in a breakage-fusion-breakage cycle. Such chromosomes would be lost and the plant would become monosomic for the affected chromosome. In 279 plants resulting from irradiated gametes, he obtained 20 monosomics (7.2%). Such a high frequency of monosomics suggests that this method may prove practicable for producing a series in polyploids where they cannot be readily obtained by other means. Sears (41) obtained 3 monosomics in T. aestivum by fertilizing a normal disomic with irradiated pollen. Except for the two latter cases, no other artificial methods have been attempted with the main purpose of obtaining monosomics.

There are very few reports of whole chromosome deficiencies resulting from chemical treatments (19,21,35). The general procedure in these cases was to treat the root tips or the flower buds, allow a

short recovery period and then examine the effect on the chromosomes. Chromatid and chromosome breakage and reunion were scored and fragmentation, stickiness and mutation as well as other aberrations were observed. These studies dealt more specifically with an analysis of the mechanism and localization of the breakage and the effect of the treatment at a biochemical level.

Auerbach (1) was the first to discover that minute doses of nitrogen mustard induced gene mutation. Subsequently Koller (18) studied its effects on the chromosomes. These investigations precipitated an extensive study of chemical mutagens. Such mutagens can be classified in the general categories of:

1. some inorganic salts
2. oxidizing agents
3. alkylating agents
4. substituted purines

The first two act mainly as gene mutators and seldom cause chromosomal aberrations. Alkylating agents and some purines, however, show direct action on the chromatin material. They possess the ability to break chromatids and chromosomes producing deletions, translocations, inversions and other aberrations ensuing from breakage (36). They therefore should have the potential of producing monosomics.

Myleran (1-4 dimethanesulphonyloxybutane) was found by von Wettstein, Gustaffson and Eherenberg (49) to have strong mutagenic activity in plants. The frequency of chromosome breaks and their local-

ization have been followed during the development of root tips in several plant species (30). Moutschen (28,29) and Moutschen-Dahmen (30,31) described the peculiar properties of this compound to break chromosomes without giving rise to much reunion. In Vicia faba the breaks induced with Myleran were strictly localized in or near the heterochromatic regions of the genome. It also produced a large number of localized breaks in acrocentric chromosomes with another maximum of breaks in the zone of the nucleolar organizer. Fahmy and Fahmy (9) studied the genetic and cytogenetic action of mesyloxyesters in *Drosophila*. Myleran was found to have a relatively low mutation rate with respect to recessive lethals (3.17%) and a high percent of major chromosome rearrangements (16.9 to 41.5%).

Kihlman (15,16) studied the activity of a purine derivative, 8-ethoxycaffeine (EOC) during various stages of the mitotic cycle. EOC produces chromatid breaks, chromatid exchanges and isochromatid breaks which result in dicentric chromatids plus an acentric fragment. In Vicia faba roots the chromatid aberrations were randomly distributed among the chromosomes (17). Kihlman states that the effect of N-methylated oxypurines like EOC is similar to radiation, and the effects are non-delayed unlike alkylating agents.

Conger (5) studied the cytogenetic effects of sonic energy applied simultaneously with X-rays. He found sonication at 9100 cycles per second increased the frequency of aberrations produced by irradiation, but there was no effect of the sonic treatment alone. Wallace

and his associates (48) treated root tips with ultra sonic vibration at 400,000 cycles per second. They reported chromosome breakage, however, only the treated tissue was affected and the plants recovered on maturing. Johnson and Obolensky (13) studied the effect of ultra-sonic vibration on the development of barley seedlings. The effects of sonication mimicked those of radiation but much less intensely. They noted inhibition of germination, lateness of maturity and in a few cases extreme luxuriance in growth. There was no cytological examination of this material, however.

MATERIALS AND METHODS

A total of five oat species were subjected to a number of treatments to induce aneuploidy. The species and type of treatments are listed in Table I.

TABLE I
Treatment Used to Induce Aneuploidy in
Species of Avena

Species	Chromosome No	Treatment		
		Chemical	Sonic	X-ray
<u>Avena sativa</u> L.				
var. Garry	42	+	+	+
var. Rodney	42	+	-	+
<u>Avena barbata</u> L.	28	-	-	+
<u>Avena brevis</u> L.	14	-	-	+
<u>Avena wiestii</u> L.	14	-	-	+
<u>Avena strigosa</u> L.	14	-	-	+

I. CHEMICAL TREATMENT

The two chemicals, EOC and Myleran were chosen because of their reported effects on chromosomes in other plant species (15,29). In order to determine whether they would also effect the chromosomes of A. sativa a preliminary experiment was conducted on root tip cells. EOC is soluble in water, but Myleran is not and it was necessary to first dissolve it in 2% acetone and make dilutions to the required concentrations (15). The roots of A. sativa seedlings were treated by the following procedure:

Seeds were germinated at room temperature for 72 hours after which time the roots were 3 to 4 cm. long. The seedlings were placed in test tubes containing 5 cc. of

- 1) Myleran 10^{-3} M. pH 4.0
- 2) EOC 10^{-3} M. pH 4.0
- 3) 2% acetone
- 4) distilled water

for 2 hours, 1 seedling per tube, 10 seedlings per treatment. They were then removed, washed in running water for 30 minutes and divided into two equal groups.

In one group the root tips were excised immediately, pretreated in ice water for 24 hours at 1°C (26,47) and fixed in Farmer's fixative (3:1 alcohol-acetic acid). After 48 hours the root tips were stained with Feulgen and examined by the aceto-carmine squash technique at metaphase and anaphase.

In the second group the seedlings were allowed to recover at room temperature in the dark for 5 hours. The root tips were then excised, fixed, stained and examined as described above. PMCs from both groups were collected and examined at meiosis. Since both chemicals induced chromosome aberrations in the root tip cells (described under Results), they were subsequently used on pre-flowering panicles to determine their effectiveness in producing aneuploids.

The chemicals were applied to hexaploid plants at post meiosis but before anthesis in an attempt to induce chromosome aberrations in the gametes. In this way aberrations produced would be incorporated into

the zygotes. Solutions of EOC and Myleran were used in concentrations of 10^{-3} , 10^{-4} , 10^{-5} and 10^{-6} M. These concentrations have induced chromosome aberrations in other species (15,29). Four plants were treated at each concentration. The chemical treatments were administered as follows:

1. Injection. (a) 0.1 cc. of chemical solution was injected by means of a 0.5 cc. syringe and a number 26G1/2 needle into the floret, taking care not to injure the anthers or the ovary. (b) 0.1 cc. of chemical solution was injected into the stem of the plant above the floral node.
2. Cut-leaf method. The fifth leaf (last leaf before the flag leaf) was cut diagonally about 8 cm. from the tip. The cut end was immersed in a vial containing 3 cc. of the chemical solution which was taped to a supporting stake. Parafilm and masking tape were used to seal the mouth of the vial to prevent evaporation. The treatment was continued for 6 days, the tip of the leaf being trimmed once in this period.
3. Wick method. The method of Kreizinger (20), who studied chemical mutagens in corn, was used with some modifications due to reduced uptake in a smaller plant. Wicks were made from 10 cm. strips of 4 layers of gauze rolled and covered near one end with 4 cm. of masking tape. The long end of the wick was moistened with distilled water in the case of EOC and with .002% acetone in the case of Myleran to prevent

absorption of the solution in the vial. Vials with 2 cc. of solution were taped to stakes and the long end of the wick placed into the vial. Masking tape was used to seal the top of the vial. A small hole was punctured in the stalk of the plant just above the last node and the short end of the wick inserted. This was also covered with tape. The wicks were left in this position for 6 days.

II. ULTRA SONIC VIBRATION

Panicles of 8 plants of Avena sativa just previous to anthesis were treated in a Raytheon Sonic Oscillator, model DF 101. The plants were inverted and supported on a ring stand with the inflorescence immersed in 25 cc. of distilled water in the water cooled chamber. Plants were subjected to a sonic level of 10 kilocycles per second for periods of 5, 10, 20 and 40 minutes.

The chemical and sonication treatments were applied first to the hexaploid species and the results analysed. They were discontinued for the tetraploid and diploid species due to the low frequency of aneuploids induced in A. sativa by these methods.

III. X-IRRADIATION

A total of 72 plants of diploid, tetraploid and hexaploid oats were exposed to X-irradiation at a pre-anthesis stage when most florets had completed meiosis. The number of emerged florets at the time of treatment of A. sativa was varied from 5 to 33 to determine the effect

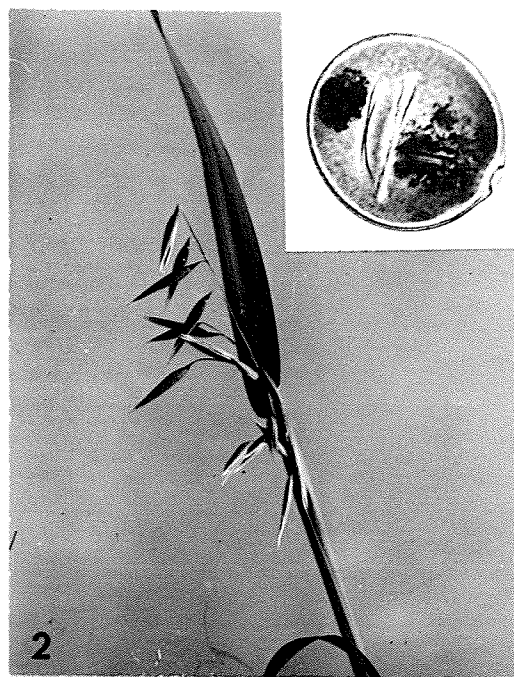
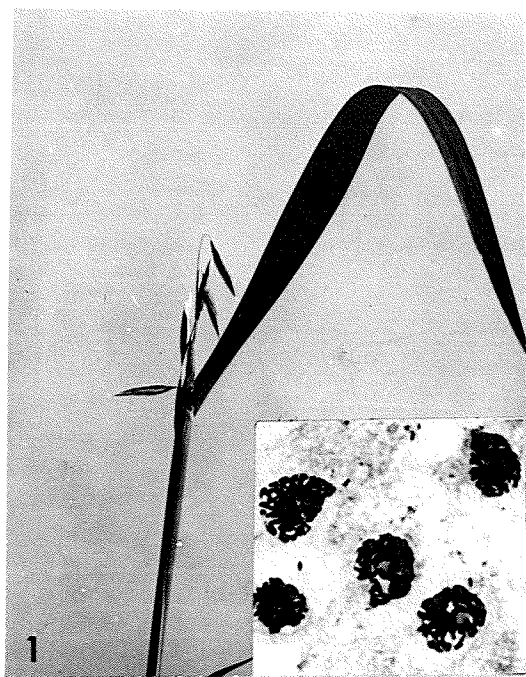
PLATE I. Number of emerged florets and the stage of development of the pollen at time of X-irradiation.

Figure 1. 5 florets emerged. Pollen is at early prophase in the youngest flower.

Figure 2. 13 florets emerged. Pollen is binucleate in the youngest flower.

Figure 3. 20 florets emerged.

Figure 4. 33 florets emerged. Pollen is trinucleate in both 3 and 4 in the youngest flower.



of a slight difference of age on the frequency of aneuploids produced (Plate I). Pollen from plants corresponding to the different ages in X-ray treatment was also examined to show corresponding differences in its development.

Radiation was administered with a therapeutic X-ray machine, 3 Ma, unfiltered at a dose rate of 180 r per minute at a distance of 30 cm. from the X-ray tube. The doses used were 75 r, 150 r, 300 r, and 600 r. Four plants of each of the hexaploid and tetraploid species and two of each diploid species were treated at each dose level.

The plants at each ploidy level were grown at the same time and under the controlled conditions of a growth cabinet. After treatment the plants were transferred back to the growth cabinet at 22° to 24°C with 18 hours of light until maturity.

The seed from these plants was treated with a fungicide and germinated at room temperature in the dark in moist vermiculite. If the seed had been freshly harvested, it was subjected to 48 hours cold shock at 4°C in moist vermiculite to break the dormancy. When the roots were 3 to 4 cm. long the root tips were excised, pretreated for 24 hours at 1°C in ice water, fixed in Farmer's solution and stained with Feulgen.

Plants with abnormal somatic chromosome counts were transplanted into 6" clay pots in growth cabinets. Immature panicles were collected from these plants to study the meiotic configurations. The panicles were fixed in Carnoy (6:3:1 alcohol-acetic acid-chloroform) for a

minimum of 24 hours. Anthers were selected with pollen mother cells undergoing meiosis, squashed in a drop of aceto-carmin and the chromosomes examined. Meiosis was studied at metaphase I, anaphase I and telophase I, and in the some cases anaphase II and telophase II as well as the diad and tetrad stages of microspore division.

RESULTS

There were wide variations in the effectiveness of the different methods attempted to induce aneuploids. The frequency and types of aneuploids resulting from these treatments are presented in Tables 2, 3 and 4.

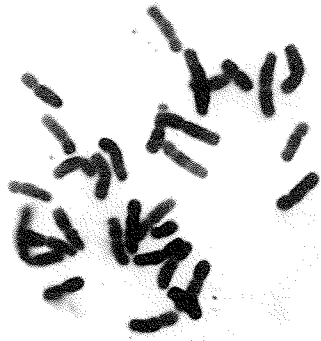
I. CHEMICAL TREATMENTS

The initial investigation of the effects of Myleron and EOC on root tip chromosomes indicated that these compounds were capable of inducing chromosomal aberrations in those cells with which they come in contact. The types of abnormalities observed were: polyploidy, aneuploidy (chromosome numbers from 27 to 64), stickiness in the case of Myleron, fragmentation, chromatid breaks, interchanges, interference with centromere division and retardation of mitosis (Plate II, Figs. 1 to 6). Three plants of the EOC and Myleran treatments, and of each of the controls were examined at metaphase I of meiosis. In all cases there appeared to be complete recovery from the chemicals, all PMCs having the normal 21 bivalents.

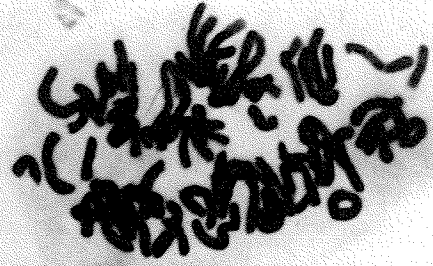
As can be seen in Table 2, the chemical treatment of preflowering panicles did not appear to give a significantly higher yield of aneuploids than the control. Due to the structure of the oat inflorescence, direct application of the chemical solutions was difficult. Attempts to get the plant to take up the chemical systemically also apparently failed. In all cases of chemical treatment, the tissue immediately

PLATE II. Root tip chromosomes of Avena sativa after chemical treatment.

- Figure 1. Metaphase cell with 30^I (Myleran).
- Figure 2. Early anaphase with 62 daughter chromosomes (Myleran).
- Figure 3. Anaphase with 41^I and 1 fragment at each pole (EOC).
- Figure 4. Part of a metaphase cell with a fragment and showing early division of the centromere (EOC).
- Figure 5. Part of a metaphase cell with stickiness (far left) and chromatid breaks (left and top centre) (Myleran).
- Figure 6. Portion of figure 5 magnified 3 times.



1



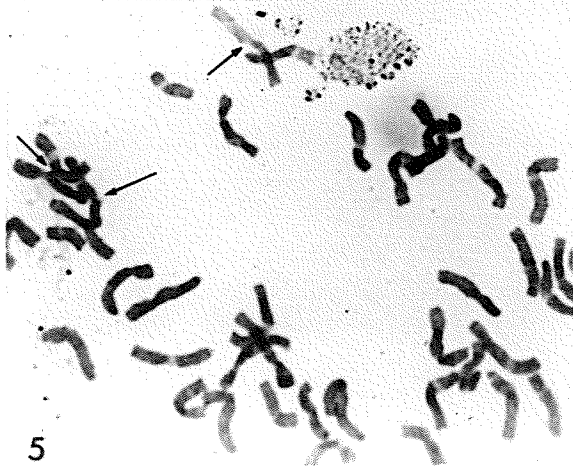
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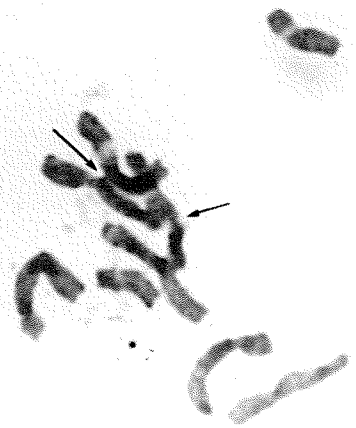
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4



5



6

TABLE 2. Aneuploids and their frequencies resulting from treatment of two varieties of hexaploid oats.

Treatment	Root Tips Counted	Aneuploid										Total	%
		36-38	39	39+f	40	40+f	40+2f	41	41+f	42+f	43		
Garry													
Control	500							3				3	0.60
Sonic Vibration	5 min.										)		
	10 min.										)		
	20 min.							1			)	4	2.39
	40 min.				1			2			)		
Control-2% acetone	115											-	0.00
Myleran	10-6 M.										)		
	10-5 M.										)		
	10-4 M.										)	1	0.55
	10-3 M.							1			)		
Control-distilled water	23											-	0.00
EOC	10-6 M.										)		
	10-5 M.										)		
	10-4 M.										)	1	0.74
	10-3 M.										)		
X-irradiation	75 r											1	1.37
	150 r							3	1			4	5.55
	300 r				1	1		15	2			19	7.66
	600 r				6	2		12	6			29	9.90
		1(38)	1									1	

TABLE 2 CONTINUED

Treatment	Root Tips Counted	Aneuploid										Total	%
		36-38	39	39+f	40	40+f	40+2f	41	41+f	42+f	43		
Rodney													
Control - 2% acetone	19											-	0.00
Myleran 10 ⁻³ M.	90											-	0.00
Control-distilled water	25											-	0.00
EOC 10 ⁻³ M.	126							1				1	0.79
X-irradiation 75 r	108							2	1			3	2.78
150 r	158							5	1		1	7	4.43
300 r	173			1*	1	2		12	5#	1		23	13.29
600 r	160	1(37)	1	2+	7	4**	1	14	5	2		37	23.13
Total	2666	3	2	3	16	9	1	73	21	3	2	133	4.99

+ 39+2 fragments
 * 38+ dicentric + fragment
 ** one had 40 + centric fragment
 # one had 41 + centric fragment

around the point of application died and there was no indication that the compound reached the reproductive cells.

II. SONIC TREATMENT

Ultrasonic vibration increased the frequency of aneuploids only slightly above the spontaneous frequency. Three monosomics and one 40 chromosome plant were obtained from 167 seedlings resulting from this treatment. It is most probable that the length of treatment and/or the intensity of the sonication was not sufficient to induce a high rate of aberrations.

III. X-IRRADIATION TREATMENT

X-irradiation produced a high frequency of aneuploids in the hexaploid and tetraploid species, the numbers increasing with the dose (Tables 2 and 3). There appeared to be a varietal difference in the frequency of aneuploids in the hexaploid oats, a higher frequency occurring in the variety Rodney than in Garry (Plate III). The cause of this difference was not determined and may have been fortuitous. As is shown in Plate I, the pollen ranged from pre-meiotic prophase in the youngest floret on the youngest panicle treated, to fully developed trinucleate pollen in the youngest florets on the two oldest panicles treated. The data indicates that a slight variation in the age of the panicle at time of treatment does not result in a significant difference in aneuploid induction (Plate IV).

The highest dose tested (600 r) produced more translocations and

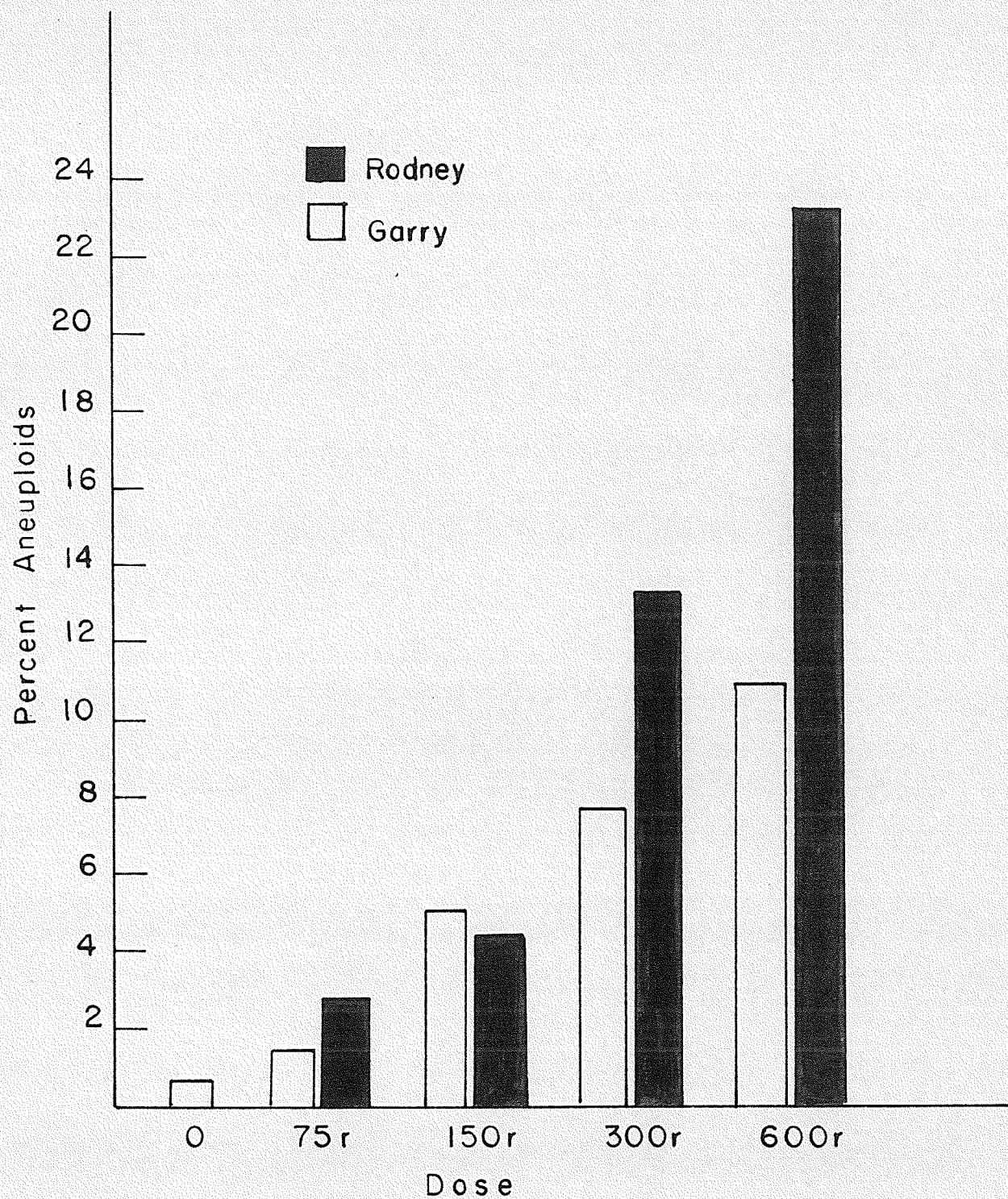


PLATE III. Percent aneuploids produced in Rodney and Garry at 4 levels of radiation.

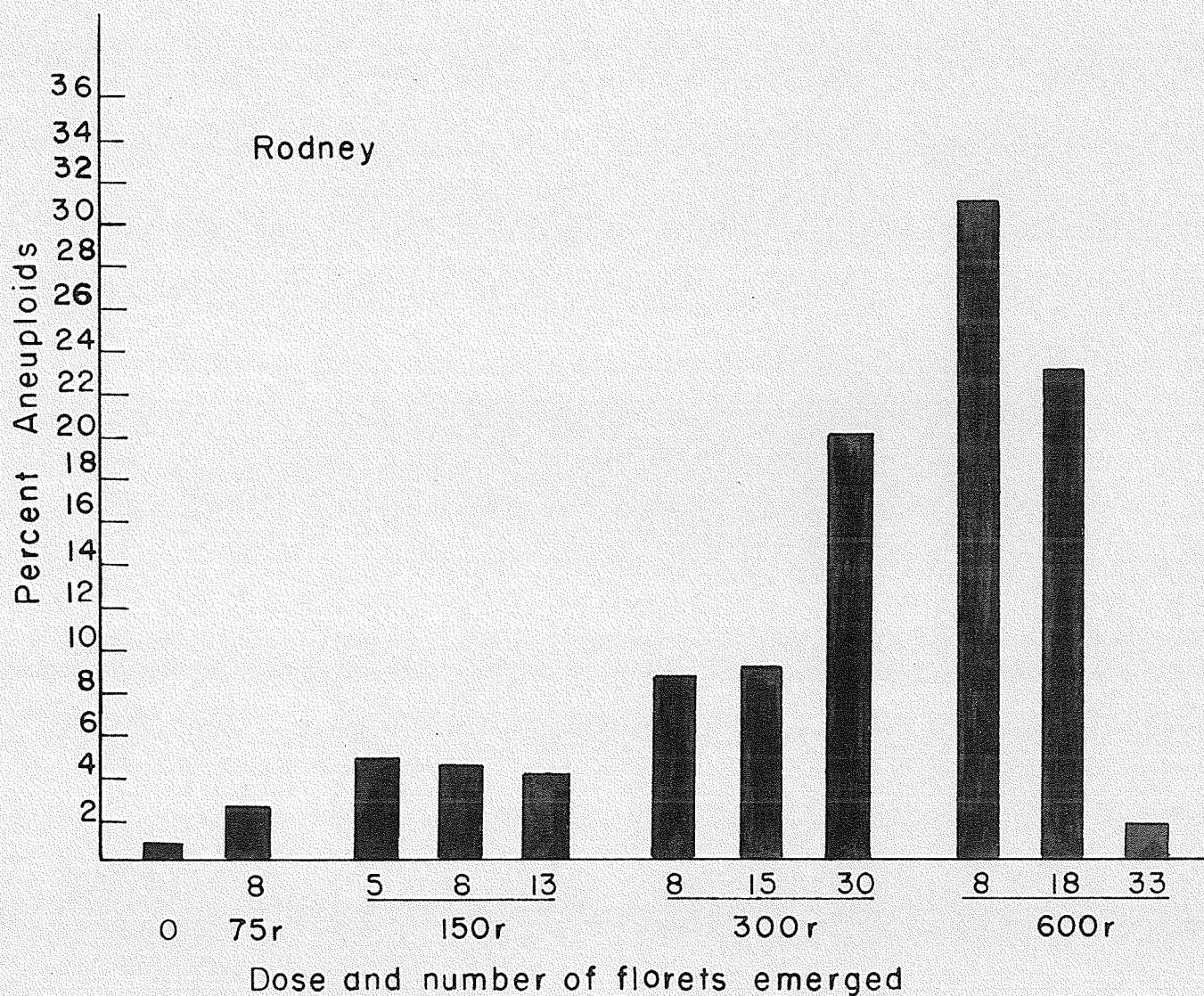


PLATE IV. Percent aneuploids produced in Rodney in relation to the number of florets exposed at time of radiation.

TABLE 3. Aneuploids and their frequencies resulting from X-irradiation of Avena barbata; $2n=28$

Treatment	Root Tips Counted	Aneuploids					Total	%
		27	27+f	27+Cf	28+Cf	Chimera		
Control	21						0	0.0
<u>X-irradiation</u>								
75 r	41						0	0.0
150 r	45	2					2	4.4
300 r	12		1	1			2	16.7
600 r	14	1	2	1*	1**	2	7	50.0
Total	133	3	3	2	1	2	11	8.27

* 27 + dicentric

** 28 + very small satellite chromosome

TABLE 4. Aneuploids and their frequencies from bulked data from X-irradiation of the three diploid species Avena brevis, Avena weistii and Avena strigosa; $2n=14$

Treatment	Root Tips Counted	Aneuploids				Total	%
		13	13+Cf	14+f	fragmented 14		
Control	75					0	0.00
<u>X-irradiation</u>							
75 r	126					0	0.00
150 r	64			1	1	2	3.13
300 r	113		1		1	2	1.77
600 r	106	1				1	0.94
	484	1	1	1	2	5	1.03

TABLE 5. Phenotypic abnormalities resulting from X-irradiation of Avena species.

Treatment	% Fertility	% Germination	Root Tips Counted	Number		Weak Plants	Dwarfs	Albinos	Twins	% Abnormal
				Seedling Lethality	Seedling					
Hexaploid 2n=42										
Control	80.9	97.5	500	0	0	0	0	0	0	0.0
75 r	74.0	97.3	73	0	0	0	0	0	0	0.0
150 r	84.8	96.0	72	0	0	0	0	0	0	0.0
300 r	79.3	96.1	248	1	0	0	0	0	0	0.4
600 r	74.9	97.3	293	7	0	0	0	0	0	2.8
Total			1186	8	0	0	0	0	0	
%				0.67	0.00	0.00	0.00	0.00	0.00	0.67
Tetraploid 2n=28										
Control	47.8	84.0	21	0	0	0	0	0	0	0.0
75 r	47.5	88.0	44	2	1	0	0	0	0	6.8
150 r	47.7	90.0	45	1	2	0	0	0	0	4.4
300 r	13.9	100.0	12	0	2	0	0	0	0	16.7
600 r	11.8	78.5	14	0	2	1	0	0	0	21.4
Total			133	3	7	1	0	0	0	
%				2.25	5.26	0.75	0.00	0.00	0.00	8.3
Diploid 2n=14										
Control	47.5	89.3	75	0	0	0	0	0	0	0.0
75 r	46.3	61.1	126	4	6	0	0	0	0	7.9
150 r	33.6	71.8	64	4	1	0	0	0	0	7.8
300 r	38.2	65.4	113	8	9	1	0	0	0	15.9
600 r	42.1	60.3	106	18	5	2	1	1	1	25.5
Total			484	34	21	3	1	1	1	
%				7.23	4.35	0.65	0.21	0.21	0.21	12.4

other aberrations than the lower doses, but did not produce more monosomics than the 300 r level of irradiation. For this reason the 600 r level is not too efficient for the production of immediately useable monosomics, since many would require back-crossing to reconstitute the original karyotype. The majority of monosomics produced by 300 r appeared meiotically normal.

Table 5 indicates the phenotypic abnormalities resulting from X-irradiation of Avena species. In Avena sativa there was some seedling lethality at the higher levels of radiation. The fertility varied closely around the normal (80%). Out of 133 seedlings of A. barbata three died and seven were very weak. There was a great reduction in fertility at the 300 r and 600 r levels of radiation. This reduction was probably caused by a period of 8 hours, shortly after treatment, during which the temperature in the growth cabinet rose to 55°C. The high temperature may also have had the effect of increasing chromosome aberrations resulting from radiation. In the diploid material there was a relatively high frequency of seedling lethality and weak plants. It is interesting to note that the percentage of aneuploids produced is inversely proportional to seedling lethality. The fertility in the diploids, although low, varied little from the control.

There was a wide range of aneuploids produced by the various treatments. These are described below:

Avena sativa (Table 2)

36^I+f, 37^I, 38^I. One plant of each of these chromosome numbers was produced, but only the one with 38 chromosomes survived past the

seedling stage. This plant produced only one panicle which was collected for analysis at the PMC stage. No counts could be obtained since the anthers of all flowers were rudimentary and empty and the pistils also appeared poorly developed.

39^I , 39^I+f , 39^I+2f . Five plants were obtained in this category. Only the two plants with 39^I+2 fragments survived beyond the seedling stage. Counts of $19^{II} + 1^I + 2f$ were obtained on both at metaphase I. The chromosomes of one plant showed stickiness producing bridge-like structures at anaphase I and 0 to 1 micronuclei per tetrad. The second plant had 2 to 7 lagging univalents and fragments at anaphase I and II with 5 to 8 micronuclei in the tetrad stage (Plate V, Figs 1 and 2). Both were induced by 600 r X-irradiation.

40^I . Sixteen plants with 40 chromosomes were found. One was discovered in the progeny of a plant treated with ultra sonic vibration, two resulted from 300 r treatment and thirteen from the 600 r treatment. The majority of these showed metaphase I configurations of $19^{II} + 2^I$ with a few cells with 20^{II} . There was some stickiness and the occasional laggard. In the tetrad stage there were 0 to 1 micronuclei with a preponderance of tetrads with none.

$40^I + f$. Eight plants with $40^I + 1$ acentric fragment and one with $40^I + 1$ centric fragment were induced at 300 r and 600 r levels of radiation. Another, with $40^I + 2$ fragments was induced in Rodney at 600 r, died as a seedling. Of the former nine plants, one showed multivalent associations at metaphase but exact counts could not be obtained

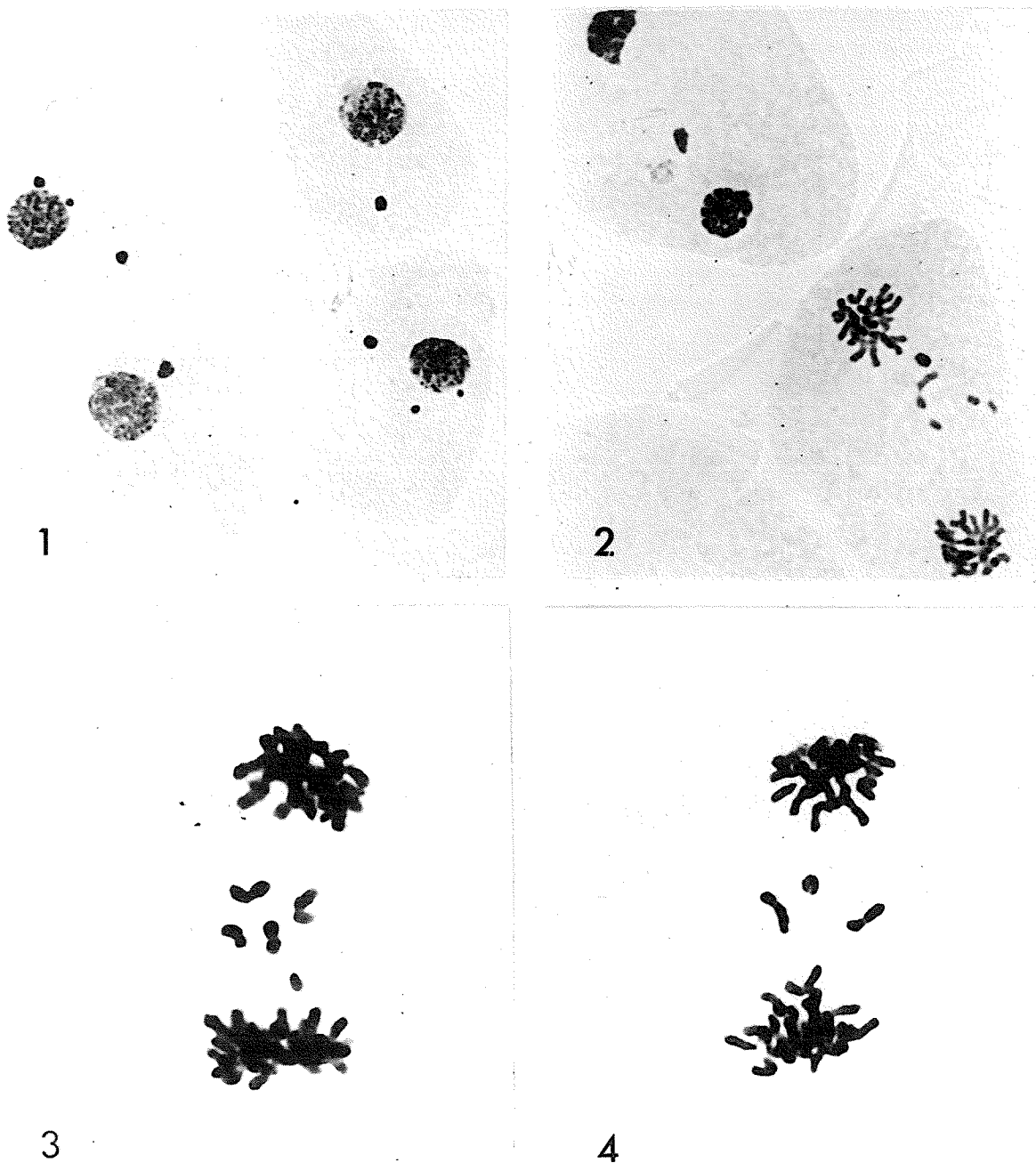


PLATE V. Pollen mother cell analysis of aneuploid plants.

- Figure 1. Tetrad stage of a plant with $39^I + 2f$ showing 8 micronuclei.
- Figure 2. Anaphase II of a plant with $39^I + 2f$ showing laggard univalents and fragments.
- Figure 3. Late anaphase I of a plant with $40^I + f$ showing 4 laggard univalents and a laggard fragment.
- Figure 4. Late anaphase I of a plant with $40^I + f$ showing 2 laggard univalents and a laggard fragment.

because of the stickiness of the chromosomes (Plate VI, Fig.4). This plant had 0 to 2 micronuclei per tetrad. Two others could not be counted at metaphase I due to the chromosome stickiness but 1 to 3 univalents and possibly a chain of three could be determined. At anaphase I, two univalents and a fragment were laggards (Plate V, Fig.4). A fourth plant in this group showed fragments and laggards (Plate V, Fig.3) and stickiness (Plate VI, Fig.2), at anaphase and tetraphase I with 0 to 4 micronuclei per tetrad. The remaining five plants had metaphase counts of 20^{II} and $20^{II} + f$ with no, or very few, small micronuclei. The plant with 40^I and a centric fragment had the same metaphase configuration and had a single large bridge at anaphase I (Plate VI, Fig.1).

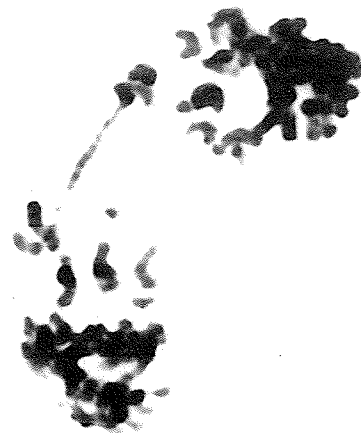
41^I . A total of seventy-three plants had 41 chromosomes in their root tip cells (Plate VII, Fig.2). Three of these plants were derived from controls, three from sonic and three from chemical treatments. At metaphase I these had the expected $20^{II} + 1^I$ (Plate VII, Fig.1). The three monosomics produced at 75 r X-irradiation, and the eight at 150 r also had normal meiosis. Of the 27 monosomics from 421 seedlings induced at the 300 r level only four showed meiotic aberrations. All others had the normal $20^{II} + 1^I$ (Plate VII, Fig.1). The aberrant plants were as follows: one $20^{II} + 1$ long dicentric (Plate VIII, Fig.1) and a few cells with $20^{II} + 1^I$; $19^{II} + 3^I$, $19^{II} + C^{III}$ (Plate VIII, Fig.3) and $20^{II} + 1^I$; $20^{II} + 1^I$ with a few cells with $19^{II} + R^{III}$ (Plate VIII, Fig.2) and $19^{II} + C^{III}$; $20^{II} + 1^I$ in which the univalent chromosome

PLATE VI. Pollen mother cell analysis of aneuploid plants.

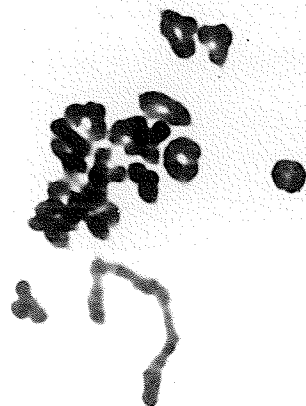
- Figure 1. Anaphase I of a plant with $40^I +$ centric fragment showing a large bridge.
- Figure 2. Anaphase I of a plant with $40^I + f$ showing stickiness producing bridge-like structure.
- Figure 3. Metaphase I of a plant with $18^{II} + C^V$.
- Figure 4. Metaphase I of a plant with $40^I + f$ showing multivalent associations, fragments and stickiness.



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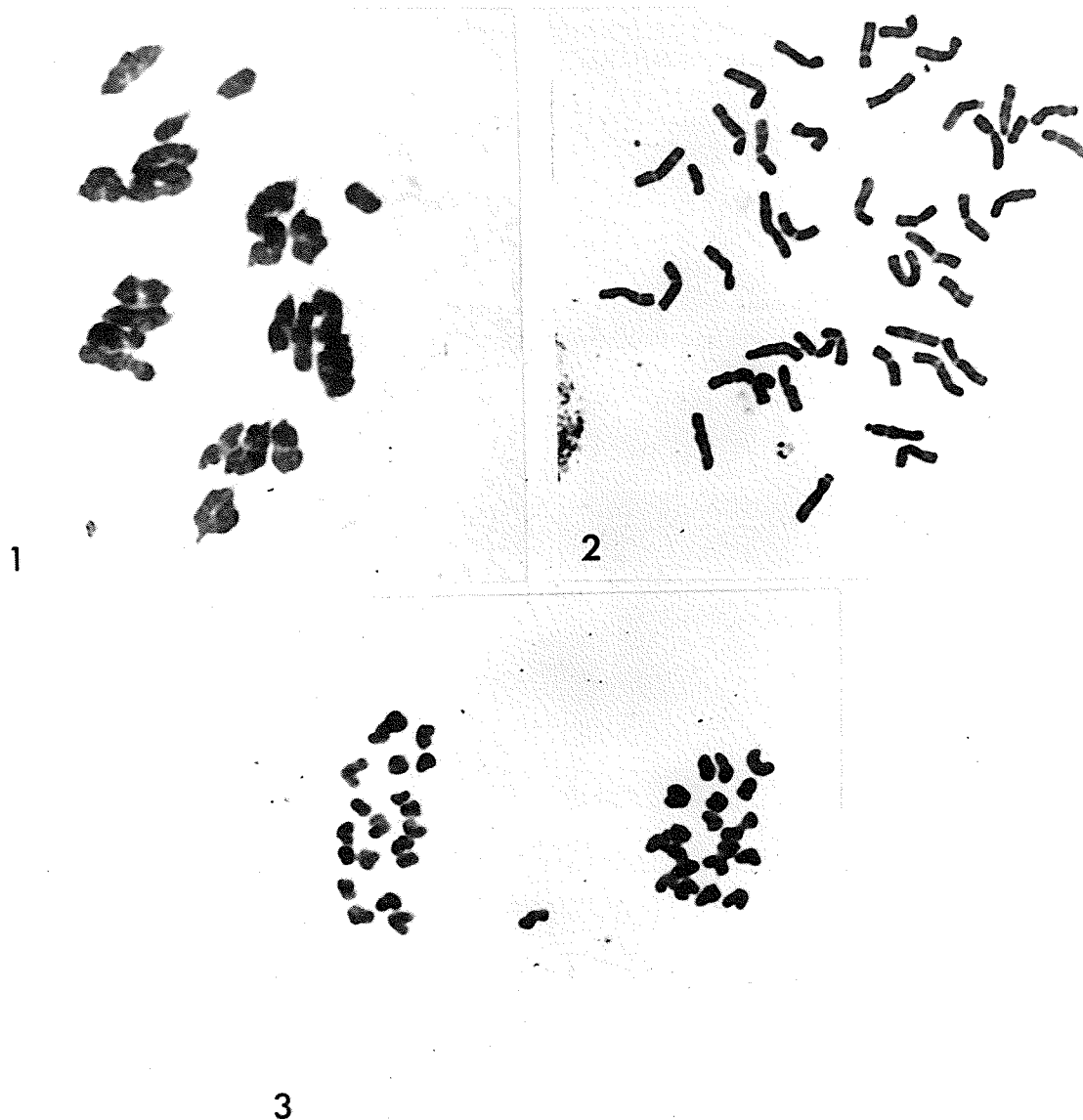


PLATE VII. Pollen mother cell analysis of aneuploid plants.

Figure 1. Metaphase I of a plant with $20\text{II} + 1\text{I}$.

Figure 2. Root tip chromosomes of a monosomic, 41I .

Figure 3. Anaphase I of a monosomic showing lagging univalent.

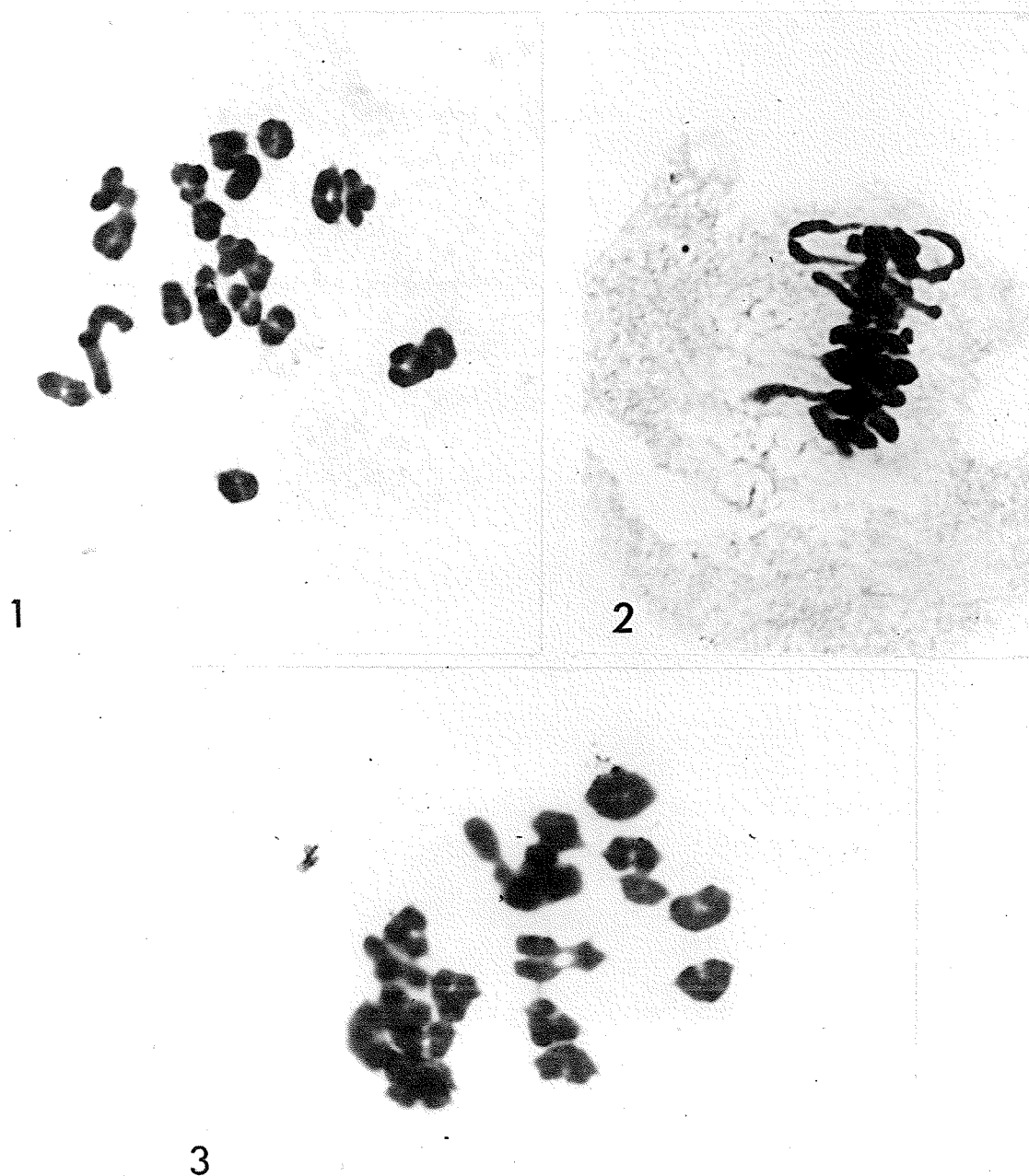


PLATE VIII. Pollen mother cell analysis of aneuploid plants.

Figure 1. Metaphase I, $20^{II} + \text{Dicentric}$.

Figure 2. Metaphase I, showing a R^{IV} .

Figure 3. Metaphase I, $19^{II} + C^{III}$.

varied in size from one PMC to another. The chromosomes of aneuploids induced at 300 r did not show any stickiness at anaphase I but there were laggards and occasional bridges with 0 to 4 micronuclei per tetrad. One plant also had a few tetrads with microcytes.

At 600 r 26 monosomics were found from 453 seedlings analysed. Only five of these had the normal metaphase pairing of $20^{II} + 1^I$. The remaining 21 showed abnormal divisions. These consisted of metaphase I configurations of $19^{II} + C^{III}$ and $19^{II} + R^{III}$; $20^{II} + \text{heteromorphic}^{II}$; $20^{II} + \text{univalent of unusual size}$; and one plant with $18^{II} + C^V$ (Plate VI, Fig.3) and $18^{II} + 5^I$. The amount of stickiness was greatly increased over the 300 r treatment and all 21 showed lagging, bridges and bridge-like formations due to stickiness at anaphase and telophase I and II. Micronuclei were present in both the diad and tetrad stages.

41 + f. Twenty plants with $41^I + \text{an acentric fragment}$ were induced by all ranges of radiation dosage. Again, those induced by the low doses (75 r, 150 r and 300 r) behaved as would be expected meiotically, i.e., $20^{II} + 1^I + f$ at metaphase I with the normal accompanying laggards and micronuclei. In the 600 r group the metaphase I plates also had $20^{II} + 1^I + f$ or $20^{II} + 1^I$. These displayed stickiness, laggards, bridges and micronuclei. One plant had pollen mother cells with $20^{II} + 2^I + f$, $20^{II} + 2^I$, $19^{II} + C^{III} + f$ and $19^{II} + C^{III} + 2f$ and with a number of micronuclei (2 to 6) per tetrad. The plant with $41^I + Cf$ had a metaphase I configuration of $20^{II} + 1^I + \text{isochromosome}$. At anaphase I there was a $19^I : 20^I$ chromosome segregation with a large bridge.

42^I + f. Three plants were produced with 42^I + an acentric fragment. The two induced at 600 r X-irradiation died as seedlings. The third one had 21^{II} + a small fragment which was usually associated with another bivalent. There was lagging at anaphase I and II and the tetrads had 0 to 4 micronuclei each.

43^I. Two trisomics were obtained, one at 150 r and one at 600 r. Both trisomics show a meiotic configuration of 21^{II} + 1^I at metaphase I.

42^I. Plants which had 42 chromosomes on root tip analysis were examined at meiosis to determine if abnormalities were present. Three plants at each level of radiation were studied. Those disomics at 75, 150 and 300 r all showed normal meiosis with 21^{II}, no laggards, little stickiness and no micronuclei. One plant from 600 r also displayed the same pattern. The remaining two however had univalents, laggards at anaphase I, stickiness at telophase I and a few micronuclei at the tetrad stage.

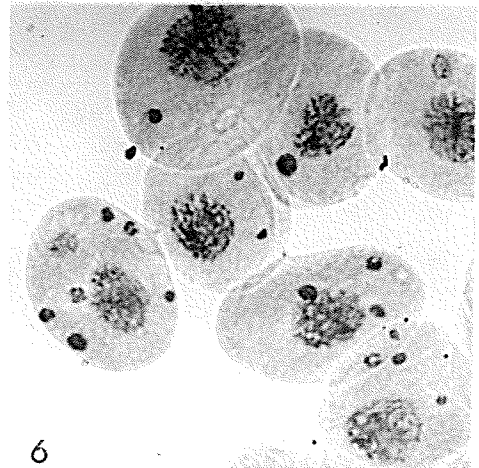
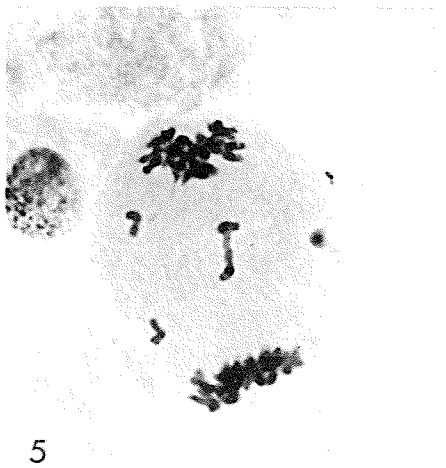
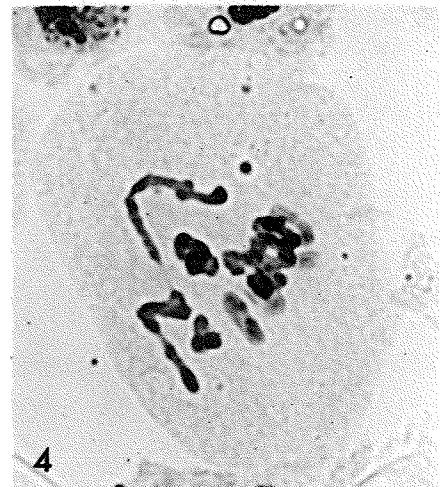
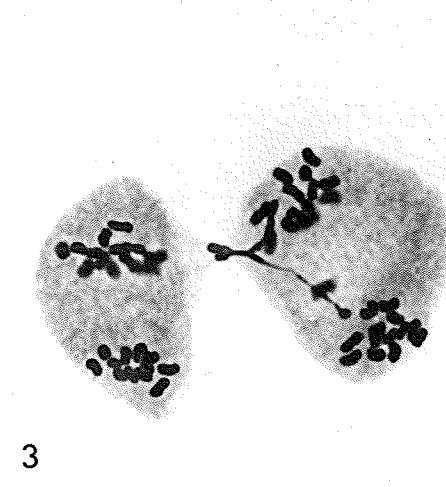
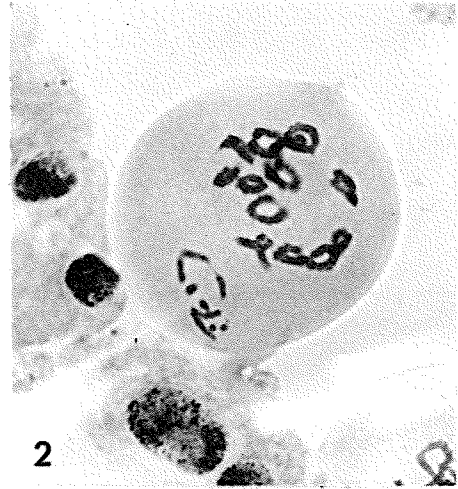
Avena barbata (Table 3).

Eleven aneuploids were obtained from X-irradiation of Avena barbata panicles. Of these, three had 27 chromosomes at mitotic metaphase (Plate IX, Fig.1). One of these plants (that from 600 r) had multivalent associations at diakinesis and metaphase I (Plate IX, Fig.2), lagging, bridges, and stickiness (Plate IX, Fig.5) at anaphase and telophase I and II, and 0 to 8 micronuclei per tetrad (Plate IX, Fig.6)(the majority of tetrads had 2,3 or 4).

Three plants had root tip counts of 27^I + an acentric fragment

PLATE IX. Aneuploids in Avena Species, Avena barbata.

- Figure 1. Root tip metaphase with 27^{I} .
Figure 2. Diakinesis with 12^{II} + a fragmented C^{IV} .
Figure 3. Anaphase II with stickiness.
Figure 4. Metaphase I with 11^{II} + C^{VI} .
Figure 5. Anaphase I with late division of 2 univalents.
Figure 6. Tetrad stage showing micronuclei.



(Plate X, Fig.5). One was induced at 300 r and two at 600 r. These plants had meiotic configurations of $11^{II} + C^{VI}$, (Plate IX, Fig.4), $12^{II} + 0^{IV}$ and $13^{II} +$ a heteromorphic bivalent. There were laggards at anaphase I and the univalent disjunction was 13:15 in the majority of cases. Laggards and bridges were present at anaphase II, and there were micronuclei present in both diads and tetrads.

One plant with $27^I +$ a centric fragment (Plate X, Fig.4) was induced by 300 r X-irradiation. It had PMCs with 14^{II} , $13^{II} + 2^I$ and $12^{II} + C^{IV}$ at metaphase I. In some metaphase plates there appeared to be a few very small univalents or fragments present. There were also micronuclei in the tetrads.

600 r gave rise to one plant with $27^I +$ a dicentric chromosome (Plate X, Fig.6). At metaphase I it had cells with $13^{II} + C^{III}$, $13^{II} + 1^I + 1$ extremely long I. It had a bridge at anaphase I and micronuclei in tetrads. The remaining three plants with abnormal mitotic chromosome counts consisted of two chimeras; $27^I +$ dicentric satellite chromosome - $27^I - 28^I$, and $26^I + f's - 27^I - 28^I$ and one plant with $28^I +$ a very small satellite chromosome.

Diploid (Table 4)

Plants of Avena brevis, Avena weistii and Avena strigosa yielded a total of five aneuploids from X-ray treatment. The results from all three of these species were bulked because of the small numbers of seed set in each species and a lack of a significant difference between the species with respect to the data collected. The aneuploids consisted

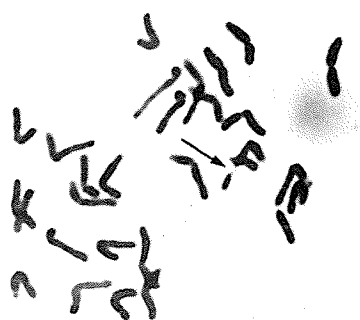
PLATE X. Root tip chromosomes of Avena species.

- Figure 1. Metaphase of a diploid species with 14^I + acentric fragment.
- Figure 2. Metaphase of a diploid species with 14 fragmented chromosomes.
- Figure 3. Anaphase of a diploid species with a chromatid break.
- Figure 4. Metaphase of A. barbata, 27^I + centric fragment.
- Figure 5. Metaphase of A. barbata, 27^I + acentric fragment.
- Figure 6. Portion of a metaphase plate of A. barbata with 27^I + a dicentric chromosome.

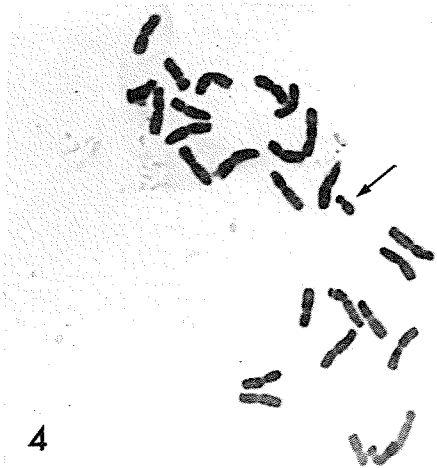


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of one 13^I derived from 600 r and which died at the seedling stage, one 13^I + a centric fragment from 300 r, one with 14^I + an acentric fragment (Plate X, Fig.1), and two with 14 fragmented chromosomes (Plate X, Fig.2). These are presented in Table 4 with their frequencies. The 14^I + f and both fragmented 14^I s died as seedlings. The plant with 13^I + Cf had PMC's with $5^{II} + C^{IV}$, $5^{II} + C^{III} + 1^I$, $5^{II} + R^{IV}$ and a few with $6^{II} + 2^I$ and 7^{II} at metaphase I. Occasionally bridge-like structures were present at anaphase I and micronuclei were present at both the diad and tetrad stages. One plant on root tip examination had a chromatid break (Plate X, Fig.3). However, at metaphase I in PMC's it had 7^{II} .

DISCUSSION AND CONCLUSIONS

The value of monosomics in genetic and cytogenetic analysis of polyploid species has been well established particularly in common wheat and tobacco where complete series exist. It is obvious that similar studies could be conducted on oats when such a series has been completed. Consequently, the 21 different monosomics must be produced as soon as possible. The results of the present study indicate that large numbers of monosomics can be induced artificially. Although chemical treatment and sonication did not prove to be efficient inducers of aneuploidy, low doses (300 r) of X-irradiation were highly productive. The effects of each of the treatments applied will be discussed below.

I. CHEMICAL TREATMENT

The attempt to produce aneuploids, specifically monosomics, by the use of chemical mutagens was not successful. Many theories have been postulated concerning the mode of action of chemical mutagens. It has been generally accepted that chromosome aberrations induced by chemical agents are due to chromatid breakage and to inactivation of the centromere or 'dynamic centres of the chromosome'. Revell (36) reported in vitro studies with DNA, RNA and polynucleotides. He states that in some cases the chemical would affect all three components. Other mutagens however affected only one or two of the three.

The most widely accepted theory concerning alkylating agents is that the reagent reacts by cross-linkage between different DNA molecules

and their phosphate groups thereby inactivating them (10,44). Experiments have indicated that compounds containing energy-rich phosphate bonds are involved in the production of chromosome aberrations by 8-ethoxycaffeine (16,17). The exact mode of action of either Myleran, an alkylating agent, or EOC is not known.

The results of the preliminary experiments on somatic cells gave good reason to expect that chromosomal aberrations could be induced in the reproductive cells. Treatment of seeds or seedlings presents certain difficulties. There would probably be a chimera effect of mutated sectors, a rapid loss of cytogenetic changes during subsequent mitosis of the treated plant and the overgrowth of mutated cells by nonmutated cells (36). It is possible that methods of application other than those attempted, such as an aerosol spray or a vacuum method would be more successful. The yield of aneuploids in such a case would have to justify the labour involved in such a treatment.

II. ULTRA SONIC VIBRATION

Sonic vibration is a known and efficient method used to disrupt the cell walls of microorganisms. This treatment requires only short periods at a fairly low frequency. It was considered that sonic treatment of preflowering panicles would weaken the chemical bonds in the chromosomes producing breakage such as was reported by Wallace (49). This may in fact have been the case in the few monosomics found from this treatment. Sonication increased the frequency of aneuploids only slightly above the control.

III. X-IRRADIATION

X-irradiation of preflowering panicles of A.sativa is an efficient method for the induction of monosomics. As was mentioned before the treatment of seeds or seedlings would be impractical. Greater efficiency would be obtained by irradiation of gametes or zygotes. In 1930 Stadler (45) explored the advantages of using zygotes or pro-embryos for inducing mutation. He found several difficulties inherent in these materials and no further studies were conducted. The gametes were considered to be the best cells to treat in this study as a greater range of aneuploidy could result here than from treated zygotes. Treatment of the pollen or egg as separate units presents a problem. This would involve artificial pollination and securing a large number of seeds from this method is extremely difficult. It seemed necessary therefore to treat the whole flower. The results obtained would be a combination of egg and pollen treatments.

It is commonly accepted that radiation produces its effect on the interphase nucleus or at early prophase (38,43). At this time the chromosomes are long, very fine threads, and therefore more susceptible to breakage by a radiation hit than in the more condensed stages. Costa Rodrigues (7) suggests that monosomics are produced by radiation when a break occurs in the two chromatids of a chromosome with consequent formation of a dicentric and an acentric fragment. The fragment will be lost and the dicentric becomes involved in a breakage-fusion-breakage cycle during the early divisions of the embryo and

is lost too. A second mechanism concerns the production of double monosomics. A chromatid of each of two non-homologous chromosomes in a microspore or a megaspore are broken by radiation with subsequent rejoining forming a dicentric. This would undergo the same fate as the dicentric described above forming an $n-2$ daughter cell. Upon union with a normal gamete, 40 chromosome zygotes are formed deficient for the two nonhomologous chromosomes. A third mechanism whereby a monosomic is produced could be by radiation inactivation or disturbance of the centromere. A chromosome thus affected would suffer non-disjunction or divide late so that $n-1$ and $n+1$ gametes could be formed. Such action could account for the low frequency of trisomics observed. There is a fourth possibility as well, that of extreme fragmentation due to radiation which might cause the loss of a chromosome.

From Table II we see the frequency of low chromosome number plants is very low and that all of these resulted from the higher doses of radiation. In these cases the amount of chromosome damage was probably very high and all four of the above had occurred. The actual frequency of such aberrations is probably higher than observed due to the self-elimination of highly aberrant gametes involved in zygote formation.

On the basis of chance, the production of nullisomics is highly improbable since this would require the union of two $n-1$ gametes both deficient for the same chromosome. The fifteen 40 chromosome plants found in the progeny of radiated parents and the one produced by sonic

treatment were most likely double monosomics. Meiotic examination did in fact indicate this. The few cases of complete pairing could be due to homoeology between the univalent chromosomes.

The fragments in the somatic cells of a number of plants resulted from fragmentation induced by radiation and their subsequent persistence through mitosis.

Although polyploids are phenotypically more resistant to radiation effects than the lower levels of ploidy, this is an expression of gene duplication rather than one of actual resistance. The sensitivity of individual chromosomes to radiation does not change in polyploids. Conger and Johnston (6) found in diploid microspores of Tradescantia (twice the target size) twice the number of chromosomal aberrations than are induced in haploid cells. Thus although irradiated polyploids show fewer mutations than diploids, the polyploids show higher frequencies of chromosome aberrations (42). This inverse relationship is evident from the data on phenotypic abnormalities presented in Table 5.

The percentage of aneuploids produced is not proportional to the level of ploidy, however. Avena barbata yielded a high frequency of chromosome aberrations. This may have been due to the temperature shock the plants received as was pointed out earlier. The numbers of aneuploids found in the hexaploid and tetraploid species increased with the dose. In the diploids there was an inverse ratio. This effect is probably due to the replication of genetic material in the higher ploidy



levels thereby relieving part of the lethal effect the aberrations may have. The diploids however would suffer the full consequences of any radiation damage.

The aneuploids induced by radiation in the tetraploid and diploid species of Avena, arose through the same mechanisms proposed earlier. The monosomic plants indicate the formation of $n-1$ gametes. Those with centric fragments could have arisen through chromosome fragmentation and persistence of the centric fragment and those with an acentric fragment through continuation of the fragment during cell division.

In two cases a dicentric chromosome was found in root tip cells. This chromosome, instead of becoming involved in a breakage-fusion-breakage cycle, probably divided longitudinally and persisted through subsequent divisions.

It can be concluded from these studies that chemical and ultrasonic treatment of preflowering panicles of Avena sativa do not appear to be efficient methods for the induction of monosomics in that species. On the other hand, X-irradiation of the panicle proved to be a highly efficient and simple method. The monosomics induced at the 300 r level of X-irradiation are for the most part meiotically normal and therefore this treatment can be recommended for producing the complete series in oats.

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