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SODIUM AZIDE MUTAGENESIS IN DIPLOID AND HEXAPLOID OATS AND COMPARISON WITH ETHYL METHANESULFONATE TREATMENTS*

H. W. RINES

U.S. Department of Agriculture–Agricultural Research Service, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, U.S.A.

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RINES H. W. *Sodium azide mutagenesis in diploid and hexaploid oats and comparison with ethyl methanesulfonate treatments.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **25**, 7–16, 1985.—Sodium azide was tested as a mutagen in a diploid oat, *Avena strigosa* L. ($2n = 2x = 14$), and in hexaploid cultivated oats, *A. sativa* L. ($2n = 6x = 42$). Optimized treatments of a 1-hr exposure of diploid oat seeds to 2–10 mM azide at pH 3 or 4.5 after a 4-hr water presoak gave frequencies of chlorophyll deficient mutants in progeny populations similar to those from EMS treatments of 50–70 mM EMS for 4 hr after an 8-hr presoak. In the diploid oat, for each mutagen about 60% of the M_1 generation plants grown from mutagenized seed had mutants segregating among their M_2 generation progenies. In contrast, in the three hexaploid cultivars tested only 1–2% of M_1 plants gave mutant progeny following treatments with azide and 2–4% with EMS. On a total M_2 plant basis the frequency of chlorophyll deficient mutants was $<0.3\%$ in the hexaploid oats compared to 3–5% in the diploid oat. The lower frequency of mutants in the hexaploid vs diploid oats could be accounted for by the presence in hexaploids of duplicative loci masking the effects of mutation at individual loci. Mutants with either meiotic synapsis deficiency, shorter plant height, or possible male sterility were recovered in hexaploid oats following azide mutagenesis. Their recovery indicates that azide mutagenesis can be effective in cultivated hexaploid oats although the overall frequency of mutant recovery may be low compared to that obtained in a diploid species.

INTRODUCTION

AZIDE, through an *in vivo* metabolite, was found in the 1970s by Nilan, Kleinhofs, and coworkers to be highly effective in producing mutations in barley (*Hordeum vulgare* L.), peas (*Pisum sativum* L.), *Salmonella typhimurium*, and several other plant and microbial species.[8,9,11,15,16] The relative effectiveness of azide treatments of seeds varied with pH of the mutagen solution, azide concentration, length of soaking in water prior to azide exposure, plant species, and cultivar. Azide as a mutagen has the desired characteristic of producing primarily, if not solely, point mutations (base substitutions) with no evidence of chromosomal aberrations as are commonly found with ionizing radiation and even occasionally with alkylating agents including ethyl methanesulfonate (EMS).[10,18,20,22] This lack of gross chromosomal changes should make azide induced mutations generally more specific and more efficient to manage in a plant improvement program than some of the variants induced by other mutagens.

A mutagenesis program in cultivated oats

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(*Avena sativa* L.) was undertaken with the overall objective to recover variants for male sterility, dwarfness, earliness, and other qualitative traits of potential value in a breeding program. The specific objective of the studies described in this report was to test the effectiveness and efficiency of azide as a mutagen in oats. Mutation rates based on the frequency of chlorophyll deficient seedlings in the second (M_2) generation following azide treatments were compared to those obtained with EMS, a chemical mutagen which has been used previously in oats, wheat, and other cereals.[2,5,20,25] In initial trials, where azide mutagenic procedures effective in barley[15] were used, three oat cultivars gave an average of 1.3% M_1 plant rows with chlorophyll deficient M_2 seedlings following azide treatment and 4.7% following EMS treatment (unpublished results). In contrast, up to 64% of M_1 spike rows of barley had mutants after azide treatment.[15] The much lower mutation frequency in oats than barley was not unexpected since barley is a diploid ($2n = 2x = 14$), whereas the common cultivated oat (*A. sativa*) is an allohexaploid ($2n = 6x = 42$) with potential for duplicative loci whose expression could mask the expression of induced mutations at individual loci. Similar low mutation frequencies had been reported in hexaploid wheat (*Triticum aestivum* L.)[1,25] and oats[1,13] after EMS and radiation mutagenesis. However, not so readily explained in the initial trials was why in oats the frequency of observed mutants following azide treatment should be markedly less than that for EMS treatments while in barley azide produced mutant frequencies comparable to EMS.[16]

One possible explanation for the apparently higher yield of mutants following EMS treatment as compared to azide treatment in hexaploid oats is that the azide treatment regime used in this initial experiment may not have been optimal for oats. Species differences in azide sensitivity have been reported.[17] A major problem encountered in trying to identify optimal azide treatments in hexaploid oats was that the frequency of chlorophyll deficient mutants was so low that meaningful treatment comparisons could not be made with the limited size populations which were practical to grow. A diploid forage oat, *A. strigosa* cultivar 'Saia', was therefore used to determine if a diploid species of *Avena* would give the higher frequencies of mutants characteristic of barley and to determine optimal azide treatment procedures for a diploid oat species with the idea that they might also be optimal for hexaploid oats.

This report describes the influence of azide concentration, length of water soaking prior to azide treatment, and pH on the efficacy of azide mutagenesis in a diploid oat. The relative effectiveness of azide and EMS treatments on mutation induction is compared in both diploid and hexaploid oats. In addition, characteristics of some mutants recovered in progeny populations from azide and EMS treated hexaploid cultivated oats are briefly described.

MATERIALS AND METHODS

Seed sources

Three cultivars of *A. sativa*, 'Moore', 'Noble', and 'Portal', were used in this study. Although the cultivars had undergone several generations of selfing in their development, there is heterogeneity within each since the cultivars were developed as bulks of F_4 and F_5 derived lines. For this reason, 'seed' (caryopsis) populations for mutagenic treatment were derived from a single plant in each cultivar to minimize inherent variation within the materials mutagenized. The *A. strigosa* cultivar 'Saia' seed used was a bulk. The *A. sativa* seeds were all dehulled prior to mutagenic treatment, the thinner hulled *A. strigosa* seeds were not.

Mutagenesis procedures

Water presoaks, mutagen treatments, and rinses were done with seeds suspended in approximately 0.5 ml of liquid per seed in Erlenmeyer flasks. The flasks, which were never filled more than one-third full, were shaken on an orbital platform shaker at 120 cycles per minute to provide constant aeration throughout the experiment. Following presoaking of the seeds in water for the designated time intervals, the water was decanted and mutagen solutions added to initiate treatments. All azide solutions were made just prior to use. Sodium azide was dissolved in 0.1 M potassium phosphate buffers for pH 3, 4.5, 5.5, and 7.0 and in 0.1 M Tris–HCl buffer for pH 8.5. The flasks were sealed with rubber stoppers

during both azide and EMS treatments, and all treatments and rinses were conducted in a fume hood. At the end of the treatment times, which were 1 hr for azide and 4 hr for EMS treatments, the mutagen solutions were decanted and the seeds rinsed with 5–6 changes of water with the last 3 rinses extending over a 30-min period with shaking for aeration. Rinsed seeds were drained and then spread on paper in a fume hood to dry.

Growth of M_1 plants

Percent emergence and height reduction of seedlings from mutagenized seed lots were measured in the greenhouse by planting two 50-seed replicates of each sample 1 cm deep in rows 30 cm long and 4 cm apart in sand flats. Emergence percentages and plant heights were measured after 8 days growth at about 20°C. In the field, treated seeds were planted with a mechanical cone seeder at a rate of about 50 seeds per 4 m row in rows 30 cm apart. After emergence, the seedlings were counted and then thinned to a minimum of 5 cm between plants to permit distinguishing individual plants at later stages. In 1980 the first panicle of a plant to emerge from its flag leaf sheath was tagged as the panicle to be harvested. In 1979 and 1982, entire plants were harvested.

Scoring of M_2 seedlings

For the diploid, *A. strigosa*, either intact main panicles or samples of 100 seeds per M_1 plant were planted in 2 cm deep trenches spaced 5 cm apart in sand benches in the greenhouse. After 10–12 days the frequencies of chlorophyll deficient seedlings were determined. Values for percent of M_1 panicles or M_1 plants producing chlorophyll deficient M_2 seedlings and values for percent of total M_2 seedlings with chlorophyll deficiencies were used to compare the relative effectiveness of different mutagenic treatments.

Selection among M_2 plants

Seeds threshed from M_1 plants of *A. sativa* cultivars were planted in the field as single M_1 panicle rows or M_1 plant rows. The seeds were planted with a mechanical cone seeder at a rate of 25 seeds per 4 m row, 30 cm between rows, with every third row left blank to facilitate observations and selection of M_2 variants. M_2 plants were observed as 14-day-old seedlings and twice again as they neared maturity. Plants with reduced height were selected for potential lodging resistance. Plants with reduced or no seed set were selected as possible male steriles. Care had to be taken in scoring these traits since commonly occurring barley yellow dwarf virus infection also produced plant shortness and sterility. M_3 and subsequent generations were checked to determine if the observed variations were heritable.

RESULTS AND DISCUSSION

Diploid oats—presoak time

Authors of previous reports involving azide mutagenesis in plants agreed that presoaking seeds in water prior to azide treatment increased the frequency of mutants, but the presoaking intervals varied widely, even for the same species, concerning the optimal timing. Reported optimal presoak times in barley included 4 hr[9] and 8 or 16 hr,[15] and in rice 4 hr[19] and 48 hr.[7] In the diploid oat, a presoak also proved beneficial (Fig. 1). Presoaks of 4, 8, 12, or 16 hr all gave increased frequencies of mutants compared to no presoak (Fig. 1); however, presoaks of 8, 12, and 16 hr resulted in progressively greater reductions in M_1 plant survival while giving no consistent increases in frequencies of mutants beyond that obtained with a 4-hr presoak. Presoaks of 24 hr prior to 2 or 4 mM azide treatment resulted in $<20\%$ M_1 plant emergence and too few survivors to test for frequencies of mutants among progenies. For the 4-hr presoak the combination of a high frequency of mutants and less reduction in M_1 plant emergence resulted in the highest total number of mutant plants produced, thus making it the preferred of the presoak times tested.

Diploid oats—pH and azide concentrations

Nilan, Kleinhofs, and coworkers in their initial reports on the mutagenicity of azide found that the effects of low azide concentrations were much greater at low pH, with pH 3 being the most effective.[9,16] They postulated that the uncharged hydrazoic acid molecule, the predominant form in which azide exists at low pH, penetrates the cell more readily than the N_3^- ion.[16] Also the cell membrane may be more permeable at low pH.

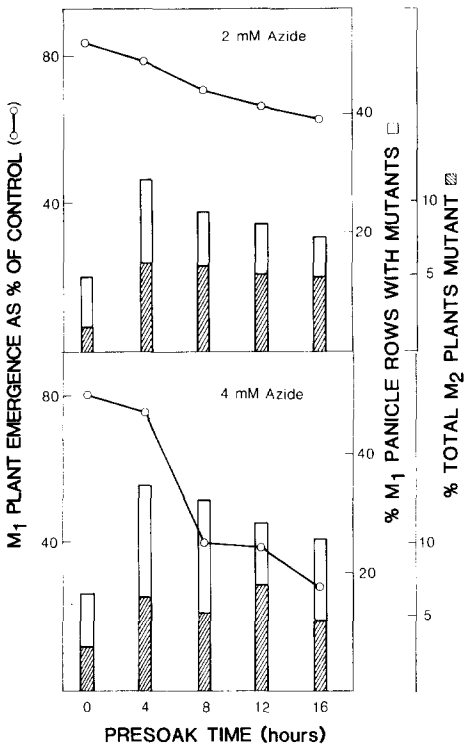

FIG. 1. M_1 plant emergence and frequencies of M_2 chlorophyll deficient mutants as a function of time seeds were presoaked in water prior to 2 and 4 mM azide treatments on a diploid oat, *A. strigosa* cv. Saia. The values are based on 400 seeds planted per treatment (320 emerged in control), 54–107 panicle rows tested per treatment, and 823–1884 total M_2 plants per treatment.

Most azide mutagenesis experiments reported since have used a pH 3 buffer, although Tomlinson[24] and de Flora[3] reported effective azide mutagenesis in *Salmonella typhimurium* at near neutral pH's. If the effect of pH is primarily one of azide penetration into the cell, then higher external concentrations of azide might offset the effect of reduced cell penetration. This possibility was tested with the hope that the use of higher pH treatment solutions might produce a more favorable ratio of mutagenesis to physiological damage, and hence result in higher frequencies of recoverable mutants.

The effects of 0.1, 1, 10, and 100 mM azide treatments at pH 3.0, 4.5, 5.5, 7.0 and 8.5 are shown in Table 1. The amount of total damage to M_1 plants is indicated by the amount of reduction in M_1 seedling height in the greenhouse and in plant emergence in the greenhouse and in the field. Frequencies of mutants are given on both an M_1 plant progeny basis and M_2 total progeny basis. The results show that azide solutions are highly mutagenic in the diploid oat even at near-neutral or alkaline pH's, particularly at high concentrations. It is also evident that low pH greatly enhances the treatment effects of a given concentration of azide; 100 mM azide was totally lethal at pH 3, and nearly so at pH 4.5, but only mildly reduced seedling emergence at pH 7 or 8.5. The need for a low pH to attain high azide effects could be partially overcome by the use of higher azide concentrations. This observation indicates that differential azide penetration may be involved in the pH effect; however, the highest frequencies of mutants were recovered with the lower pH treatments. The two treatments that show the highest percent M_2 mutant plants in Table 1, 10 mM azide at pH 3.0 and 100 mM at pH 5.5, also gave the poorest emergence of those treatments giving enough seed-producing M_1 survivors to permit determination of M_2 mutant frequencies. Commonly, in the different experiments, the highest percentage of M_2 mutants was observed when there was low plant survival. Individual plant progeny rows, particularly ones from treatments giving relatively high frequencies of mutants, often contained mixtures of albino, yellow-green, and virescent seedlings indicating that more than one mutational event had occurred in the M_1. For this reason direct comparisons between treatments giving high and low frequencies of mutants would tend to underestimate the effectiveness of the treatment producing the higher frequency of mutants.

Differences between Fig. 1 and Table 1 in terms of the frequencies of observed mutants should be noted. For the values in Fig. 1 a progeny row from only the main culm of each M_1 plant was grown. However, for the values in Table 1 the whole M_1 plant was harvested and seed from all culms of the plant bulked, and 100 seeds randomly selected for testing as a progeny row. A higher proportion of M_1 plant rows (Table 1) than M_1 panicle rows (Fig. 1) is likely to contain mutants because a much larger seed sample is represented in the

Table 1. Effect of treatment pH and azide concentration on M_1 plant emergence and frequencies of M_2 chlorophyll deficient mutants in the diploid oat Avena strigosa *cv. Saia**

		M_1 effects			M_2 chlorophyll deficient mutants			
		Greenhouse		Field	M_1 plant rows		M_2 plants	
pH	Azide dosage (mM)	Emergence† (%)	Seedling height‡ (%)	Emergence§ (%)	Total tested (No.)	Total with mutants (%)	Total tested (No.)	Total mutant (%)
3.0	0.1	92	83	81	76	31.6	7270	0.9
	1.0	86	67	64	75	57.3	7099	2.7
	10	42	32	17	36	77.8	2352	5.3
	100	0	0	0	—	—	—	—
4.5	0.1	88	86	78	78	35.9	7470	1.1
	1.0	90	70	66	82	56.1	7310	2.2
	10	70	46	42	78	56.4	7043	2.3
	100	22	15	2	—	—	—	—
5.5	0.1	90	96	93	88	15.9	8530	0.4
	1.0	92	86	98	74	23.0	7150	1.0
	10	86	74	66	72	44.3	7010	1.4
	100	61	39	31	20	40.0	1414	3.6
7.0	0.1	94	95	99	86	1.2	8330	0.01
	1.0	84	90	93	80	13.8	7640	0.4
	10	94	84	84	75	28.0	7020	1.0
	100	86	79	74	53	32.1	3646	1.4
8.5	10	88	87	81	83	18.1	7870	0.6
	100	96	94	82	80	28.8	7632	1.0

* Seeds presoaked in water for 4 hr were treated for 1 hr with sodium azide dissolved in 0.1 M potassium phosphate buffer (pH 3.0, 4.5, 5.5 and 7.0) or 0.1 M Tris–HCl buffer (pH 8.5).

† Average of % emergence of two 50-seed samples planted in trays of sand.

‡ Mean seedling height of 11.2 cm for untreated control assumed to equal 100%. Heights measured on 10 plants selected at random in each of two samples planted with 50 seeds each in trays of sand.

§ 400 seeds planted for each treatment. Emergence expressed as % of control (340 plants emerged).

plant row. However, the proportion of total M_2 plants expressing mutations was generally higher in panicle rows (Fig. 1). This latter observation indicates that the main culms were more likely to contain mutations than were the tillers. Both procedures give valid comparisons within themselves, but specific values are not comparable from one procedure to the other.

To analyze the azide concentration effect in more detail, seed lots were treated with 2, 4, 8, and 16 mM azide at pH 4.5. Increasing M_1 damage and M_2 mutant frequencies were observed with increasing concentrations of azide (Table 2). At 16 mM azide at pH 4.5 the M_2 mutant frequency of 5.2% was near that attained with 10 mM azide at pH 3.0 (Table 1) and was coupled with less reduction in M_1 greenhouse emergence, seedling growth, and field emergence than the pH 3 treatment. More detailed tests at both pH's are needed, however, to determine if pH 4.5 with slightly higher azide concentrations consistently performs as effectively and with less damage to plant survival than pH 3 treatments in azide plant mutagenesis.

Another approach used to try to increase the ratio of mutagenesis to physiological damage was

Table 2. Dosage effects of azide and EMS on M_1 *greenhouse emergence, seedling growth, and field emergence and on frequencies of* M_2 *chlorophyll deficient mutants in a diploid oat,* A. strigosa *cv. Saia*

	Treatment		M_1 effects			M_2 chlorophyll deficient mutants			
			Greenhouse		Field	M_1 plant rows		M_2 plants	
Mutagen	Dosage (mM)	Presoak time (Hours)	Germination (%)	Seedling height (%)	Emergence (%)	Total (No.)	Total with mutants (%)	Total (No.)	Total mutant (%)
Azide*	0	4	100	100†	100‡	86	0	8904	0
	2	4	86	80	70	80	54	7044	2.3
	4	4	82	68	58	83	63	7302	2.7
	8	4	72	58	39	95	60	7342	2.6
	16	4	50	49	35	84	61	5621	5.2
Azide*	2	4, 8, 12§	62	71	45	84	74	5669	3.8
	4	4, 8, 12	60	44	21	55	75	3138	4.6
EMS¶	50	8	84	70	79	99	58	7660	3.5
	70	8	80	54	68	82	48	3104	4.3

* Seeds presoaked in water were treated for 1 hr with sodium azide dissolved in 0.1 M potassium phosphate buffer pH 4.5.
† Mean seedling height of 11.2 cm for untreated controls assumed to equal 100%.
‡ Emergence of 170 seedlings from 200 untreated seeds assumed to equal 100%.
§ Treatments were pulsed by treating with fresh azide solutions for 1 hr at 4, 8, and 12 hr after seeds first placed in water.
¶ Seeds presoaked in water were treated for 4 hr with EMS dissolved in 0.1 M potassium phosphate buffer pH 7.

to administer the low pH azide treatments in 1-hr pulses, with 3-hr intervals of neutral pH and no azide between treatments to allow respiratory (metabolic) recovery in the plant tissues (Table 2). Pulse treatments at 4, 8, and 12 hr with either 2 or 4 mM azide gave mutant frequencies at least comparable to the most effective single azide treatments.

Diploid oats—comparison of azide and EMS effects

Data on M_1-plant effects and M_2 mutagenic frequencies produced by treatments with the commonly used mutagen EMS are presented in the lower portion of Table 2. Comparison of the more effective of the azide and EMS treatments indicates that the two mutagens are similarly effective in the diploid oat. The percentages of the chlorophyll deficient mutants that were of the albino type following azide and EMS treatments were 60.4 and 53.1%, respectively, indicating a similar spectrum of mutants. Nilan *et al.*[15] reported that in barley the proportions of albinos induced by azide and an alkylating agent diethyl sulfonate (DES) were similar to each other, but this proportion was unlike that obtained with gamma radiation. They postulated that the differences might be attributable to the much higher frequencies of chromosome breaks induced by the radiation.

Hexaploid oats—comparison of azide and EMS effects

In contrast to the high frequencies of chlorophyll deficient mutants induced in the diploid oat species by azide and EMS treatments, few such mutants were recovered in hexaploid oats. On an M_1 plant row basis the frequency was 3.6% overall for the hexaploids (Table 3) compared to as high as 70% for the diploid oat (Table 2), and on an M_2-plant basis $<0.3\%$ for the hexaploids compared to 3–5% for the diploids. This low incidence of mutants in the hexaploids occurred even though mutagen treatment levels were sufficient to reduce seedling emergence to $<50\%$ of controls in all cases. The frequency of mutants remained low over a number of treatment modifications tried over several years on several oat cultivars (Table 3). In spite of the low frequencies of mutants recovered, there did appear to be some consistent differences among cultivars for sensitivity to the mutagens. Azide produced about one-third as many mutants overall as did EMS.

Table 3. Frequencies of M_2 *chlorophyll deficient mutant frequency expressed on an* M_1 *plant row basis following azide and EMS mutagenesis of 3 cultivars over* 3 yr *in hexaploid oats,* A. sativa

		M_1 plant rows with M_2 mutants/total M_1 plant rows (%)			
		Individual treatments			Total
Mutagen	Cultivar	1979*	1981†	1982‡	over years
Azide	Noble	3/200	4/361	0/110	7/671 (1.0%)
	Moore	5/400	0/114	3/152	8/666 (1.2%)
	Portal	—	—	2/96	2/96 (2.1%)
	Total	8/600 (1.3%)	4/475 (0.8%)	5/358 (1.4%)	17/1433 (1.2%)
EMS	Noble	8/100	10/350	6/118	24/568 (4.2%)
	Moore	1/100	2/120	4/121	7/341 (2.1%)
	Portal	5/100	—	4/106	9/206 (4.4%)
	Total	14/300 (4.7%)	12/470 (2.6%)	14/345 (4.1%)	40/1115 (3.6%)

* In 1978 dehulled seeds were treated with 2 mM azide at pH 3 for 2 hr after a 4-hr presoak or with 50 mM EMS at pH 7 for 4 hr after a 4-hr presoak.

† In 1980 dehulled seeds were treated with 4 mM azide at pH 4.5 for 1 hr after an 8-hr presoak or with 50 mM EMS at pH 7 for 4 hr after an 8-hr presoak.

‡ In 1981 dehulled seeds were treated with 2 mM azide at pH 4.5 for 1-hr intervals at 4, 8, and 12 hr after seeds first placed into water or with 60 mM EMS at pH 7 for 3 hr after an 8-hr presoak.

The low incidence of chlorophyll deficient mutants in the hexaploid vs diploid oats is in agreement with previous studies in hexaploid oats and wheat where chlorophyll deficient mutants were rare.[1,12,13,23,25] Such results can be readily explained by assuming that in a hexaploid there are duplicative loci with the same function able to compensate for, and phenotypically mask, the functional loss of a mutated locus. Among the chlorophyll deficient mutants observed in the hexaploid oats, no albinos were found, whereas albinos comprised more than half the chlorophyll deficient mutants observed in the diploid oat. This result indicates that in these hexaploids some types of mutations are more likely to be phenotypically expressed either through diploidization or dominance effects than are others.

Azide treatments have been reported to produce no detectable chromosomal aberrations in barley root and shoot tip cells whereas EMS produces a low level of chromosomal breakage.[14,18,20,22] If the chromosomal breaks lead to losses of large segments of chromatin, and hence, loss of multiple loci, then such mutations would be more likely to show phenotypic expression than simple point mutations. In the case of hexaploid wheat, and probably also hexaploid oats, the simple loss of chromosomes or chromosome segments is not expected to produce visible chlorophyll deficiency since none of the complete set of 21 nullisomics in 'Chinese Spring' hexaploid wheat shows chlorophyll deficiency[21] nor do any of the 15 nullisomics identified in hexaploid oats.[6] Thus, the production of an apparent 3-fold higher frequency of chlorophyll deficient mutants by EMS than by azide in at least certain cultivars of hexaploid oats (Table 3) probably cannot be accounted for simply by assuming EMS produces more chromosomal aberrations than azide. Many nullisomics do, however, show reduced vigor, fertility, and other developmental–morphological effects. The occurrence of induced losses of whole or portions of chromosomes and their effects may be one of the reasons why morphological variations are more common in mutagenized hexaploid wheat than are chlorophyll deficiencies.[12,21]

Varietal differences in relative frequencies of chlorophyll deficient mutants from EMS and azide were observed among the three hexaploid oat cultivers (Table 2) and may reflect differences in uptake or metabolism of the mutagens, cellular repair mechanisms, or the site specificity and mode of action of the two mutagens. Varietal differences in frequencies of detectable mutants may also be reflective of different degrees of diploidization among varieties.

Hexaploid oats—mutants recovered

In spite of the low frequency of azide-induced chlorophyll deficient mutants recovered in hexaploid oats, azide mutagenesis may have value because of the type of mutations it produces. Two meiotic synapsis deficient mutants were recovered from azide mutagenized populations of hexaploid cultivated oats. These mutants, which will be described in detail elsewhere (Rines, in preparation), are unique from all other synapsis deficient mutants described in oats in that they are inherited as simple recessives. Other synaptic variants in hexaploid oats have involved loss of whole chromosomes or chromosome arms.[6] Both of the synapsis deficient mutants recovered here were sterile and were recovered by growing out seed of fertile heterozygous siblings. If the seed of M_1 plants had been planted as one large bulk rather than as M_1 plant progeny rows, the mutants probably would not have been recovered as the plants heterozygous for the synapsis mutations are phenotypically normal.

Mutants for shorter plant stature, possible male sterility, and other traits of potential value in a breeding program were also recovered from azide and EMS treated populations of cultivated oats. One shorter line from an azide treated population performed well in preliminary trials and has been advanced into multi-location trials. Five azide derived and three EMS derived shorter stature variants gave unacceptable levels of yield reduction (20–40%) when tested directly in preliminary yield traits, but may have value for crossing into very tall lines. About 15 independently derived M_2 plants showing partial or total sterility (lack of seed-set), while retaining high plant vigor, were selected from mutagenized populations as potential male steriles. The completeness and stability of their male sterility and female fertility as well as their inheritance patterns are being checked in subsequent generations. In hexaploid wheat following EMS muta-

genesis,[5] the recovery of nine male sterile mutants, including one that is inherited as a dominant, indicates that recovery of male sterility by mutagenesis also should be possible in hexaploid oats.

The potent mutagenicity of azide in both diploid and hexaploid species of oats, as demonstrated in this report, also reveals complications in proposed direct uses of azide for agronomic purposes. As an example, based on the ability of azide to stimulate breakage of seed dormancy of the wild oat *A. fatua*, Fay and Gorecki[4] proposed that more effective herbicide or tillage control of this noxious weed could be achieved if infested fields were first treated with 11.2 kg/hectare azide to promote uniform germination of the wild oat seed. One possible complication of the azide treatment would be that it might induce mutations for resistance to the herbicides used subsequently for wild oat control. The same possibility would exist when azide is used directly as a herbicide. Dangers from other toxicity, carcinogenicity, and mutagenicity potentials[8] should also rule out azide use in this way.

In spite of the dangers of large scale use of azide, the results in this report demonstrate that azide can be an effective, highly specific mutagen for use in controlled conditions for generating potential useful variants in diploid and hexaploid oats. A reduced rate of mutants recovered with azide as compared to other mutagens may be compensated for by the specificity of azide and by a lack of the chromosomal aberrations that may accompany the use of other mutagens.

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