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Chromosome damage induced by artificial seed aging in barley

3. Behavior of chromosomal aberrations during plant growth *

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Summary. Barley (*Hordeum vulgare* L. 'Himalaya') seeds were artificially aged under two storage conditions (32 °C/12% moisture content (m.c.) and 38 °C/18% m.c.) to study the behavior of induced chromosomal aberrations during plant growth. The frequencies of aberrant anaphases at first mitosis in root tips were correlated with loss of germinability. However, after 3 and 5 weeks' growth, aberration frequency declined. In plants grown from artificially aged seeds, the frequency of aberrant anaphases appeared to be stabilized at about 1% after 5 weeks' growth, in spite of the large differences in the frequencies at first mitosis. This suggests that because of their genetic imbalance, cells with chromosomal aberrations induced by seed aging were being excluded during plant growth. Meiotic chromosome configurations at MI were normal (7 II) in all plants studied, although a few precocious separations were found. Meiotic aberrations were found at AI-TI, AII-TII and the tetrad stages in the pollen mother cells of plants grown from the control and artificially aged seeds. However, there were no clear differences among the control and the two aging treatments. It was obvious that some cells with meiotic chromosomal aberrations were lost between the AI-TI and AII-TII stages, and still more between the AII-TII and tetrad stages. The frequency of tetrads with micronuclei in plants produced from artificially aged seeds was the same as in the control. The plants grown from artificially aged seeds showed high pollen fertility (95.2 to 97.0%) and seed fertility (90.1 to 97.2%) which was comparable to the control values (97.4 and 97.9%) respectively, indicating no special effects of seed aging. Anaphase cells of the first mitosis in the next (A_2) generation were analyzed to study the transmission of chromosomal aberrations through mitotic and meiotic cell divisions in the A_1 generation. Aberrant anaphases in the progeny from the artificially aged seeds were not higher than those of the control progeny. This indicates that the chromosomal aberrations induced by seed aging are not transmitted to the next generation.

Key words: Artificial seed aging – Barley – Mitotic and meiotic aberrations – Pollen and seed fertilities – Transmission of chromosomal aberrations

Introduction

The occurrence of genetic changes such as chromosomal aberrations and gene mutations during seed storage is potentially a serious problem for the preservation of seed germplasm (Roberts 1973, 1975, 1978).

Chromosomal aberrations induced by seed aging have been studied at first mitosis in roots and/or shoots soon after germination and it has been shown that the frequency of chromosomal aberrations is correlated with loss of germinability (Harrison and McLeish 1954; Harrison 1966; Abdalla and Roberts 1968; Murata 1979; Murata et al. 1981). However, a progressive decrease or elimination of chromosomal aberrations with root or shoot elongation has been reported (Nawaschin 1933; Peto 1933; Nichols 1941; Kato 1954; Abdalla and Roberts 1969). This fact indicates the necessity of studying the behavior of chromosomal aberrations induced by seed aging during plant growth in order to evaluate the cytological damage resulting from seed aging. It is also necessary to investigate meiotic chromosomal aberrations in plants grown from aged seeds, because some structural

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changes such as inversions and translocations would not appear as aberrations in mitotic cells.

Using artificially aged barley seeds, we systematically analyzed the fate of chromosomal aberrations during plant growth from first mitosis in root tip cells to meiosis in the pollen mother cells, as well as the transmission of the chromosomal aberrations to the next generation. Pollen and seed fertility data are also presented to show the effects of seed aging on meiotic aberrations and gene mutation.

Materials and methods

Materials (seeds of barley, *Hordeum vulgare* 'Himalaya') and the methods for artificial seed aging and cytological analysis at first mitosis have been reported previously (Murata et al. 1981). After collecting primary roots for the analysis at first mitosis, the seedlings from three sampling times of each of two storage conditions: 32 °C/12% moisture content (m.c.) and 38 °C/18% m.c. were saved and allowed to grow in order to trace the fate of chromosomal aberrations during plant growth. Twenty seedlings per aging treatment were grown in peat pots for 3 weeks in a growth chamber under short-day conditions (8 h light at 18 °C and 16 h dark at 10 °C) to increase the number of tillers. After 3 weeks' growth, three roots per plant were collected individually for cytological studies. Normal appearing plants were transplanted into 4-inch pots in a greenhouse under long-day conditions (16 h light at 20° to 25 °C and 8 h dark at 10° to 15 °C). Two weeks after transplanting (5 weeks' growth), three roots per plant were again cytologically investigated. The 5-week-old plants, the roots of which were already collected, were again transplanted into 5-inch pots and allowed to grow for the analyses of meiotic aberrations and pollen and seed fertilities.

To learn the frequency of chromosomal aberrations at meiosis, ten plants per aging treatment and three tillers per plant were sampled. Meiotic divisions were investigated at metaphase I (MI), anaphase I to telophase I (AI-TI), anaphase II to telophase II (AII-TII), and tetrad stages in the pollen mother cells. One anther out of three in the same flower was used to determine the stage of development. If the appropriate stage was found the other two anthers were fixed in a 3:1 mixture of ethanol and glacial acetic acid. Squash preparations were made in 0.8% acetocarmine staining solution after fixation for 2 to 3 days.

Pollen grains were collected from mature anthers in the middle of the spikes. Three spikes per plant and ten plants per treatment were sampled to determine pollen fertility. Pollen grains were stained with 0.4% acetocarmine staining solution. About 500 pollen grains per preparation were analyzed according to Kihara's (1937) classification.

Seed fertility was determined in each spike previously checked for pollen fertility. Seed fertility as used in this study implies the percentage of seed set in a mature spike excluding two sets of three flowers at each of the top and bottom parts of the spike where seed set is usually poor. Mature seeds of each spike were harvested, placed in a paper envelope, and stored at 10 °C for 3 weeks.

The stored seeds (A_2 generation) were germinated and chromosomal aberrations were determined in root tips of ten seeds from each of two spikes per plant. The roots collected were 2 to 4 mm long, presumably in the first mitotic cycle.

Results

Mitotic chromosomal aberrations

Chromosomal aberrations in the root tips of plants grown from the control seeds and the six aging treatments were studied at three growth stages; first mitosis, and after 3 and 5 weeks' growth (Table 1).

Germination of the control seeds was 100%, while seeds stored 100, 120, and 140 days at 32 °C/12% m.c. germinated 85, 59, and 25%, respectively. Germination of seeds stored at 38 °C/18% m.c. for 8, 12, and 14 days was 85, 45, and 33%, respectively.

The frequency of aberrant anaphases at first mitosis increased as the time of storage increased and the percentage germination decreased. The control showed 0.76% aberrant anaphases. In the 32 °C/12% m.c. condition, the aberrant anaphases increased from 2.55 to 4.30% as the storage time increased from 100 to 140 days and the germination decreased from 85 to 25%. Increased time in storage from 8 to 14 days under the 38 °C/18% m.c. condition induced a similar loss in germination from 85 to 33%, and at the same time increased the frequency of aberrant anaphases from 2.30 to 3.78%. In both storage conditions, evident differences in the frequency of aberrant anaphases were found at first mitosis, resulting in a correlation between loss of germinability and frequency of aberrant anaphases (Murata et al. 1981).

After 3 weeks' growth, however, aberration frequency was reduced in all treatments. In the control plants, the decrease from the first mitosis to 3 weeks was 0.11%. The differences in the frequency of aberrant anaphases between the first mitosis and 3 weeks were very similar in both storage conditions. The differences were 1.13 to 2.61% (1.60% on the average) and 0.94 to 2.14% (1.71% on the average) at 32 °C/12% m.c. and 38 °C/18% m.c., respectively. However, the amount of decline was different in plants with different germination percentages. The frequency of aberrant anaphases was reduced by about 1% in plants raised from seeds with 85 and 59% germination, and about 2% in those with 45 and 33% germination. In plants grown from seeds with 25% germination, the decrease from the first mitosis to 3 weeks' growth was 2.61%. The higher the frequency of aberrant anaphases at first mitosis, the greater the reduction in aberrant anaphases after 3 weeks' growth.

Further reduction in the frequency of aberrant anaphases was evident after 5 weeks of growth. The control plants had 0.42% aberrant anaphases at this stage. In the plants grown from artificially aged seeds, the frequency of aberrant anaphases appeared to stabilize at 1.13 to 1.40% (1.25% on the average) and 0.75 to 0.90% (0.81% on the average) in seeds stored under

Table 1. Frequencies of aberrant anaphases at three different growth stages – first mitosis, 3 and 5 weeks' growth – in plants grown from control and seeds artificially aged at 32 °C/12% and 38 °C/18% m.c.*

Storage condition (°C/% m.c.)	Storage time (days)	Germination (%)	No. of seedlings observed	No. of anaphases observed	Aberrant anaphases (%)	Difference from the former stage (%)
First mitosis						
Control	0	100	53	1,848	0.76	–
32/12	100	85	50	1,528	2.55	–
32/12	120	59	50	1,740	3.10	–
32/12	140	25	49	1,327	4.30	–
38/18	8	85	52	1,086	2.30	–
38/18	12	45	54	1,252	3.19	–
38/18	14	33	54	1,799	3.78	–
3 weeks						
Control	0	100	20	1,250	0.65	−0.11
32/12	100	85	20	986	1.42	−1.13
32/12	120	59	20	1,239	1.94	−1.16
32/12	140	25	20	888	1.69	−2.61
38/18	8	85	19	2,639	1.36	−0.94
38/18	12	45	14	1,509	1.13	−2.06
38/18	14	33	17	917	1.64	−2.14
5 weeks						
Control	0	100	20	2,372	0.42	−0.22
32/12	100	85	20	2,785	1.22	−0.20
32/12	120	59	20	2,121	1.13	−0.81
32/12	140	25	20	1,994	1.40	−0.40
38/18	8	85	19	3,225	0.75	−0.62
38/18	12	45	14	1,226	0.90	−0.43
38/18	14	33	16	1,246	0.80	−0.84

* Data for first mitosis from Murata et al. 1981, 1982

the 32 °C/12% m.c. and 38 °C/18% m.c. conditions respectively, in spite of a large difference in the frequency at first mitosis.

Most plants which had chromosomal aberrations at first mitosis showed a decrease or disappearance of aberrations during plant growth. However, a few of the plants without aberrations at first mitosis showed some chromosomal aberrations after 3 weeks' or 5 weeks' growth. It should be noted that the chromosomal aberrations appeared in one or two out of three roots collected from the same plant after 3 and 5 weeks' growth. There were no plants in which all three roots had chromosomal aberrations.

The types of chromosomal aberrations after 3 and 5 weeks' growth were analyzed in the same way as was used at first mitosis (Murata et al. 1982). Proportions of each type of aberration per total chromosomal aberrations observed are shown in Table 2. Single bridge (1B), single fragment (1F) and double bridges (2B) comprised about 80% of the total aberrations at both the 3 and 5 week stages. The large proportion of these three types was very similar to that at first mitosis. After 5 weeks' growth, however, the proportion of 1F decreased to 9.22% compared to 21.21% at first mitosis and 22.31% at 3 weeks, while the 1B increased to about 60% vs. about 43 and 45% at first mitosis and after 3 weeks' growth. Chromosome stickiness was again frequently observed at the 3 and 5 week stages and accounted for a high frequency of the bridge aberrations.

The combinations of fragments and bridges were less frequent after 3 and 5 weeks' growth than at first mitosis. In the chromosomal aberrations observed at first mitosis, some 1F+1B, 1F+2B, 2F+1B and 2F+2B types appeared; however, only one 2F+1B type was found after 3 weeks and five 1F+1B type aberrations after 5 weeks of growth.

Meiotic chromosomal aberrations

Meiotic chromosomal aberrations were investigated at MI, AI-TI, AII-TII, and the tetrad stage in the pollen mother cells of plants grown from the control and artificially aged seeds. All plants studied showed the

Table 2. Types and proportions of chromosomal aberrations at first mitosis and after 3 and 5 weeks' growth in roots of plants grown from artificially aged seeds

Type of aberrations	No. of aberrations (%) at		
	First mitosis	3 weeks growth	5 weeks growth
1. Single bridge (1B)	128 (43.10%)	59 (45.38%)	84 (59.57%)
2. Single fragment (1F)	63 (21.21%)	29 (22.31%)	13 (9.22%)
3. Double bridges (2B)	43 (14.48%)	16 (12.31%)	18 (12.77%)
4. Double fragments (2F)	20 (6.73%)	8 (6.15%)	7 (4.96%)
5. Single fragment and single bridge (1F+1B)	11 (3.70%)	0 (0%)	5 (3.55%)
6. Multiple bridges (mB)	7 (2.36%)	9 (6.92%)	6 (4.26%)
7. Multiple fragments (mF)	6 (2.02%)	6 (4.62%)	4 (2.84%)
8. Double fragments and single bridge (2F+1B)	4 (1.35%)	1 (0.77%)	0 (0%)
9. Single fragment and double bridges (1F+2B)	2 (0.67%)	0 (0%)	0 (0%)
10. Double fragments and double bridges (2F+2B)	2 (0.67%)	0 (0%)	0 (0%)
11. Others	11 (3.70%)	2 (1.54%)	4 (2.84%)
Total	297 (100.00%)	130 (100.00%)	141 (100.00%)

Table 3. Frequencies of meiotic aberrations at AI-TI, AII-TII and tetrad stages in the pollen mother cells of plants grown from control and artificially aged seeds under 32 °C/12% and 38 °C/18% m.c

Storage condition (°C/% m.c.)	Storage time (days)	Germination (%)	AI-TI		AII-TII		Tetrad	
			No. of cells (spikes) observed	Aberrant anaphases (%)	No. of cells (spikes) observed	Aberrant anaphases (%)	No. of cells (spikes) observed	Aberrant tetrads (%)
Control	0	100	2,017 (18)	0.89	1,408 (16)	0.27	2,044 (17)	0.10
32/12	100	85	2,471 (18)	1.73	1,418 (17)	0.35	3,705 (25)	0.13
32/12	120	59	2,089 (22)	1.68	2,215 (20)	0.81	3,299 (24)	0.18
32/12	140	25	2,641 (20)	1.89	2,359 (21)	0.93	3,956 (25)	0.14
38/18	8	85	3,109 (21)	0.95	2,846 (22)	0.53	3,816 (26)	0.18
38/18	12	45	1,076 (14)	1.12	1,209 (14)	0.58	1,848 (15)	0.16
38/18	14	33	1,146 (13)	1.22	1,100 (14)	0.64	1,666 (14)	0.18

normal chromosome configuration with seven bivalents at MI (Fig. 1a) although a few cases of precocious separation were observed (Fig. 1b).

In contrast, meiotic chromosomal aberrations were found at the AI-TI stage (Fig. 1c–i), and at the AII-TII and tetrad stages (Fig. 2a–h). The frequencies of aberrations at these stages are shown in Table 3. At AI-TI, the control plants had 0.89% aberrations. In plants grown from the seeds stored at 32 °C/12% m.c., the frequency of aberrant anaphases was 1.68 to 1.89%. The frequency of aberrant anaphases from the 38 °C/18% m.c. treatment was 0.95 to 1.22%, which was less than in the 32 °C/12% m.c. condition. However, within a storage condition, there was no difference among the three storage times.

A reduction of meiotic chromosomal aberrations was seen at the AII-TII stage. In the control plants, the frequency of meiotic aberrations at AII-TII stage was 0.27%. This value was much lower than the 0.89% at AI-TI stage. The meiotic aberrations were halved from AI-TI to AII-TII stages in the plants grown from the artificially aged seeds, except the plants from the seeds stored 100 days under the 32 °C/12% m.c. condition, which showed a reduction of about 80% (1.73 to 0.35%).

The occurrence of micronuclei at the tetrad stage (Fig. 2g–h) probably resulted from the chromosomal aberrations observed at AII-TII. The frequency of aberrant tetrads with micronuclei was 0.10% in the control plants and 0.13 to 0.18% in plants grown from artificially aged seeds (Table 3). A small difference in the frequency was seen between the control and aged seeds. There was no correlation between the frequency of aberrant tetrads and degree of seed aging.

From these results, a clear decrease in the frequency of meiotic aberrations was evident. The control plants had 0.89% aberrations at AI-TI. These aberrations de-

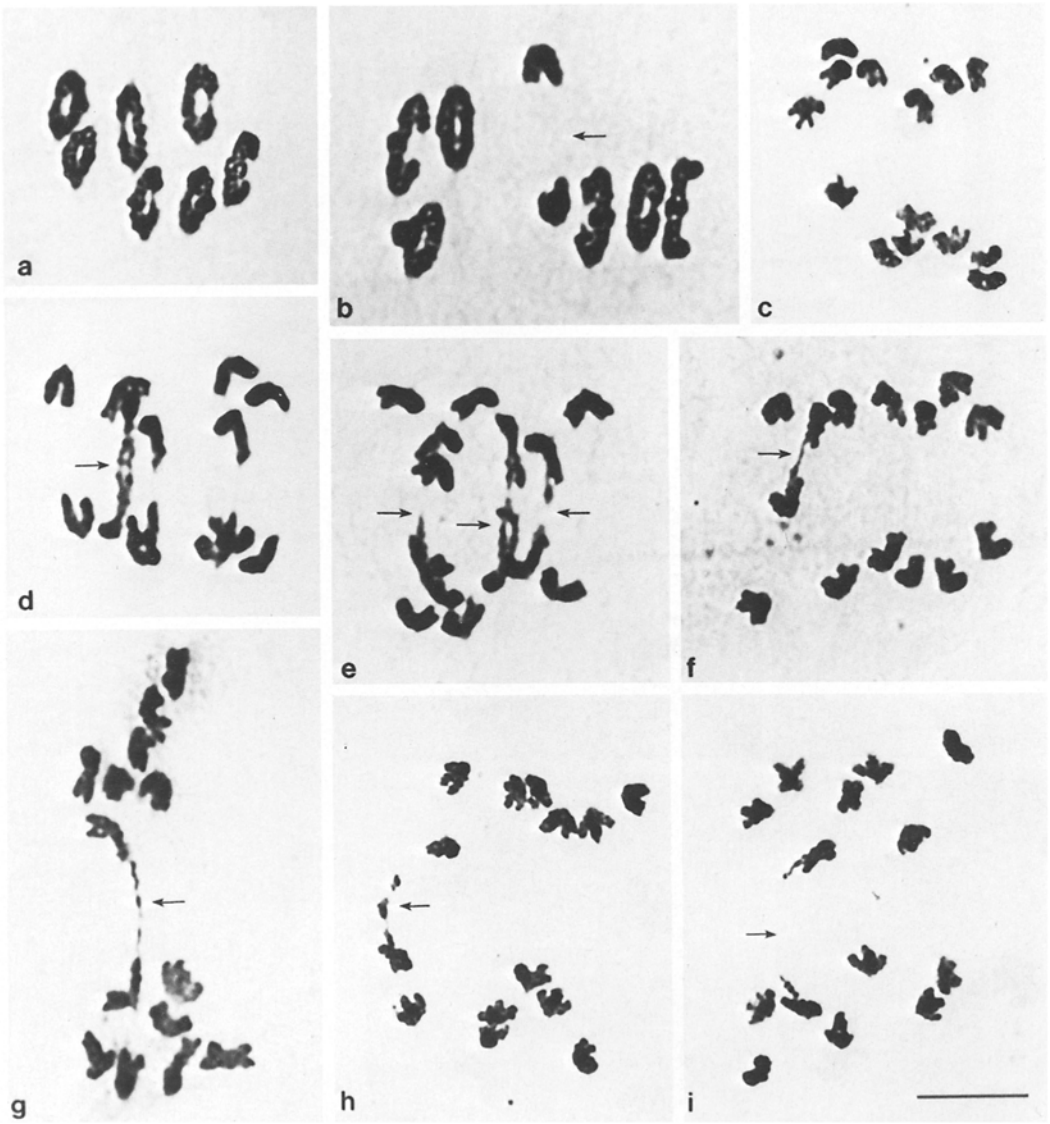

Fig. 1 a–i. Meiotic chromosomal aberrations at MI and AI-TI stages in plants grown from artificially aged seeds. **a** normal chromosome configuration with 7II at MI; **b** precocious separation in one pair of chromosomes (*arrow*); **c** normal AI cell; **d** single bridge due to chromosome stickiness (*arrow*); **e** triple bridges due to chromosome stickiness (*arrows*); **f, g** single bridge (*arrows*); **h, i** single broken bridge (*arrows*). Bar represents 10 μm

creased to 0.27% at AII-TII and to 0.10% at the tetrad stage. A similar reduction of meiotic aberrations was found in the plants grown from the artificially aged seeds. In the 32 °C/12% m.c. condition, 1.68 to 1.89% aberrations were present at AI-TI. These frequencies were about 1% higher than in the control plants. However, they decreased to 0.35 to 0.93% at AII-TII and finally to 0.13 to 0.18% at the tetrad stage. The plants grown from seeds stored 8, 12, and 14 days at 38 °C/18% m.c. showed 0.95 to 1.22% aberrations at AI-TI, and 0.53 to 0.64% aberrations at AII-TII. The frequency of abnormal tetrads with micronuclei was only 0.16 to 0.18%.

The types of meiotic aberrations at AI-TI and AII-TII are summarized in Table 4. The bridge type aberrations were more frequent than fragment or other types at AI-TI. The bridges appeared more frequently at early AI, while the fragment types were observed mostly at late AI to early TI. A high proportion of bridges at early AI was due to chromosome stickiness (Fig. 1 d–f). At later stages of AI, these aberrations were more clearly seen as bridges (Fig. 1 g). Some bridges were broken by chromosome separation (Fig. 1 h, i). The highest frequency among the types of aberrations was 1B. The frequency of 2B was much lower than that of 1B and only one cell with 3B was found (Fig. 1 e).

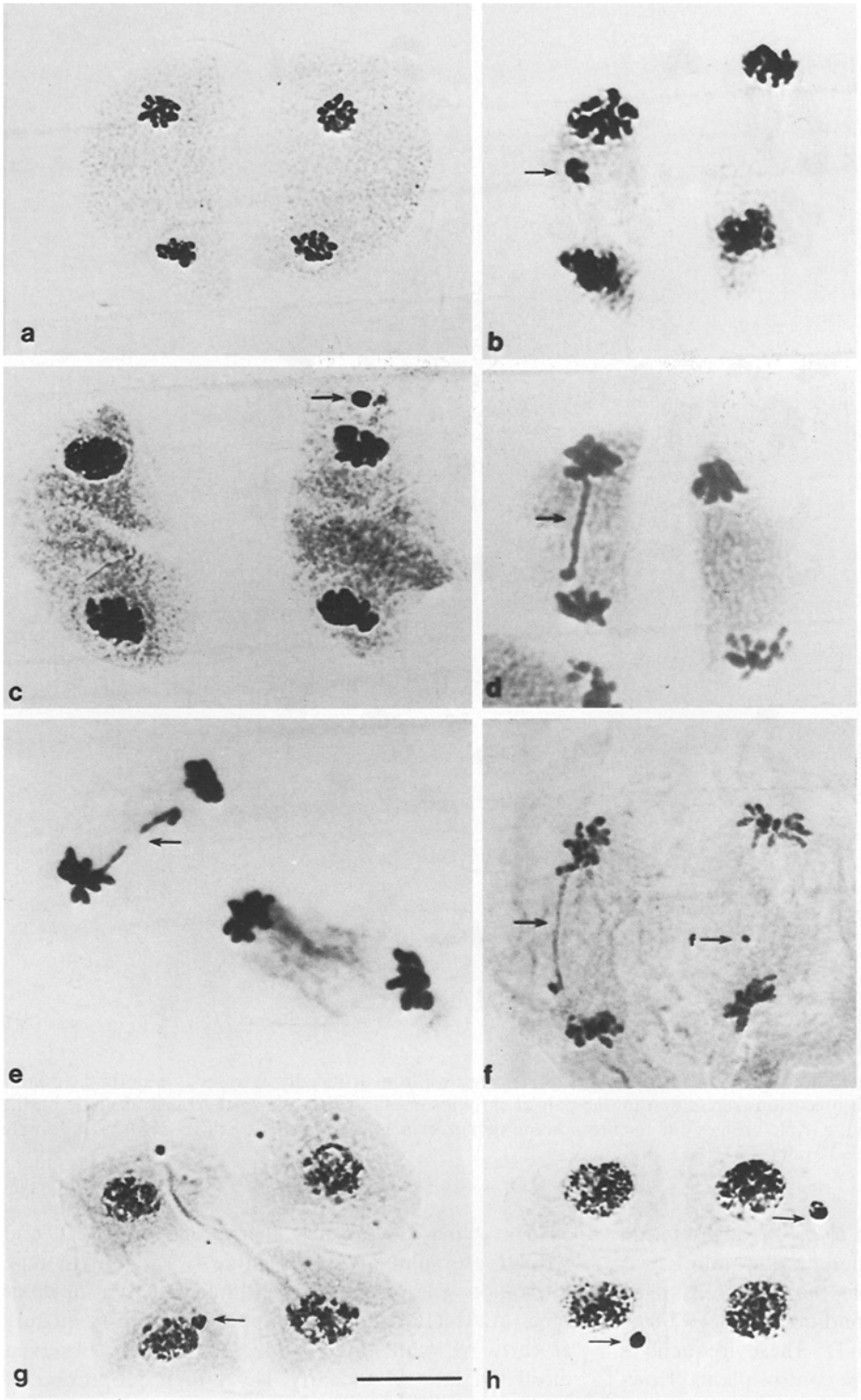

Fig. 2 a–h. Meiotic chromosomal aberrations at TII and tetrad stages in plants grown from artificially aged seeds. **a** normal TII cell; **b, c** single (micronucleus-like) fragment (*arrows*); **d, e** single bridge (*arrows*); **f** single fragment (*arrow*-f) and single bridge (*arrow*); **g** tetrad cell with one micronucleus (*arrow*); **h** tetrad cell with two micronuclei (*arrows*). Bar represents 10 μm

Table 4. Types and proportions of meiotic aberrations observed at anaphase I to telophase I (AI-TI) and anaphase II to telophase II (AII-TII) in the pollen mother cells of plants grown from artificially aged seeds

Types of aberrations	No. of aberrations (%) at	
	AI-TI	AII-TII
1. Single bridge	99 (50.77%)	21 (26.92%)
2. Single fragment	58 (29.74%)	43 (55.13%)
3. Double bridges	20 (10.26%)	0 (0%)
4. Double fragments	11 (5.64%)	11 (14.10%)
5. Single fragment and single bridge	3 (1.54%)	2 (2.56%)
6. Others	4 (2.05%)	1 (1.28%)
Total	195 (100.00%)	78 (100.00%)

Multiple fragments were not observed, and 2F was much less frequent than 1F. Combinations of bridges and fragments were observed at low frequency. More bridges appeared at early AI and more fragments at late AI or early TI indicating that breakage of bridges along with chromosome separation produced some fragments.

The meiotic aberrations at AII-TII consisted of 1F, 2F, 1B, and 1F+1B. The frequency of fragments was higher than that of bridges at the AII-TII stage, although at AI-TI the bridges were more frequent than fragments. This resulted from the rapid decrease in the frequency of 1B and 2B. The frequency of fragments remained about the same. The frequency of 1F+1B was very low at AII-TII as well as at AI-TI.

At the AI-TI stages, the bridge type (1B and 2B) aberrations comprised 80% and the fragment type (1F and 2F) aberrations comprised 16% of the aberrations whereas at AII-TII only 1B types were observed, with a frequency of 27%, and the 1F and 2F types comprised 69%. A high proportion of the fragments at AII-TII can be explained by the following two observations. Fragments observed at AII-TII formed micronuclei outside as granddaughter nuclei (Fig. 2 b, c) which suggests that they were produced late in telophase I. Furthermore, most observations of meiotic chromosomal aberrations were made at late AII and early TII when the bridges induced by chromosome stickiness might already have broken and thus were seldom observed.

Pollen and seed fertility

Pollen fertility was studied in the spikes of the plants grown from control and aged seeds (Table 5). The control plants showed 97.4% pollen fertility. In the plants grown from seeds stored at 32 °C/12% m.c. and 38 °C/18% m.c., the pollen fertility was 95.2 to 97.0%. Compared with the control, a decrease of a few percent was found in some plants, especially in those from seeds stored 12 days at 38 °C/18% m.c. However, it was difficult to say that the decrease in pollen fertility resulted from seed aging, because there was no linear decrease in pollen fertility with increased storage time and decreased germination, and because a few percent difference in pollen fertility may have been caused by other factors such as temperature and humidity in the greenhouse.

Similar results were obtained for seed fertility (Table 5). In the control plants, the seed fertility was 97.5%. The plants grown from artificially aged seeds showed no significant reduction in seed fertility from the controls, except the plants raised from the seeds stored 140 days at 32 °C/12% m.c. Seed fertility of these plants was 90.1%, although the pollen fertility was 96.7%.

Pollen and seed fertility may be related to the low frequency of micronuclei at the tetrad stage; however, no correlation was found between the frequency of chromosomal aberrations at meiosis and pollen and seed fertility. This suggests that decreases in pollen and seed fertility were not related to seed aging treatments.

Table 5. Pollen and seed fertilities (%) in plants grown from control and artificially aged seeds stored at 32 °C/12% and 38 °C/18% m.c.

Storage condition (°C/% m.c.)	Storage time (days)	Germination (%)	No. of plants (spikes) analyzed	Pollen fertility (%)		Seed fertility (%)	
				Mean ± SE	(range)	Mean ± SE	(range)
Control	0	100	10 (28)	97.4 ± 0.4	(96.8–98.0)	97.9 ± 1.9	(95.2–100.0)
32/12	100	85	9 (27)	95.9 ± 2.0	(92.7–98.6)	94.9 ± 5.1	(83.3– 98.9)
32/12	120	59	10 (29)	96.6 ± 1.3	(94.3–98.2)	97.2 ± 1.3	(90.8– 99.0)
32/12	140	25	10 (30)	96.7 ± 0.9	(94.9–97.6)	90.1 ± 4.9	(81.7– 97.9)
38/18	8	85	10 (27)	97.0 ± 0.9	(95.3–98.0)	97.1 ± 1.7	(95.1–100.0)
38/18	12	45	8 (19)	95.2 ± 1.0	(93.8–96.4)	94.9 ± 3.4	(91.7–100.0)
38/18	14	33	9 (23)	95.7 ± 1.1	(94.0–97.1)	96.2 ± 3.2	(90.5–100.0)

Table 6. Frequency of mitotic chromosomal aberrations (%) in roots of the A_2 progeny seeds from control and artificially aged seeds

Storage condition in A_1 (°C/% m.c.)	Storage time in A_1 (days)	Germination (%) in A_1	No. of A_2 seeds analyzed	No. of A_2 anaphases scored	Aberrant anaphases (%) in A_2
Control	0	100	30	1,702	0.35
32/12	100	85	30	1,534	0.33
32/12	120	59	29	1,402	0.43
32/12	140	25	26	1,686	0.36
38/18	8	85	29	1,621	0.37
38/18	12	45	27	1,498	0.33
38/18	14	33	27	1,575	0.38

Chromosomal aberrations in the A_2 generation

Progeny seeds of the plants grown from the control and artificially aged seeds were cytologically analyzed at first mitosis in order to determine if the chromosomal aberrations induced by seed aging were transmitted to the next (A_2) generation. No difference was apparent between the control and artificially aged seeds (Table 6). The A_2 seeds from artificially aged seeds showed 0.33 to 0.43% aberrant anaphases, while 0.35% aberrant anaphases were found in the A_2 seeds from control plants.

Discussion

Mitotic chromosomal aberrations

The effects of seed aging on the induction of chromosomal aberrations were most evident at first mitosis. The frequency of aberrations depended upon the germination percentage and was not affected much by root lengths from 1.5 to 7.0 mm (Murata et al. 1981). This result agreed with results in X-rayed seeds of barley in that roots between 4 and 10 mm gave no difference in aberration frequencies (Avanzi 1960). However, fewer aberrations were observed in roots varying in lengths from 4 to 20 mm (Gaul 1957). Also in aged seeds a reduction in the frequency of chromosomal aberrations with root elongation has been observed (Nawaschin 1933; Nichols 1941; D'Amato 1951). Nichols (1941) showed that chromosomal aberrations decreased from 10.4 to 1.7% with root growth from 2 to 100 mm in aged onion seeds. No aberrations were found in roots more than 20 to 30 mm long of 4-year-old pea seeds (D'Amato 1951).

We observed a decrease in chromosomal aberrations in roots after 3 and 5 weeks' growth. Similar results were obtained after treatment of barley seeds at 95 °C for 25 min (Peto 1933). He reported that the frequency of abnormal cells was reduced from 27 to 9%, 40 days after seeding. The fact that the frequency of chromosomal aberrations is reduced with root or plant growth suggests that the cells with aberrations are inferior to the normal cells in mitotic activity. The abnormal cells, not being able to maintain their cell divisions, would be replaced by normal cells, because fragments and bridges produce deficiencies and duplication of chromosomes or genes, which disturb the genetic balance of the cell (Sax 1941). In contrast, chromosomal aberrations were occasionally observed in roots from 3 or 5-week-old plants which showed no aberrations at first mitosis. The probable explanation for this would be that chromosomal aberrations were involved in the lateral roots but not in the primary root used for cytological analysis at first mitosis.

Meiotic chromosomal aberrations

No abnormal meiotic chromosomal configurations (except for a few precocious separations) were found at metaphase I (MI) in this study. However, Cartledge and his colleagues found translocations, deficiencies and other meiotic aberrations in plants grown from aged *Datura* seeds (Cartledge and Blakeslee 1934; Cartledge et al. 1936). The ring-of-four aberrations were also observed in the pollen mother cells of plants from 2 to 3-year-old hexaploid wheat, 32-year-old tetraploid wheat and diploid barley seeds (Gunthardt et al. 1953). However, their frequency was very low, but the frequency increased as the polyploidy level increased from diploid barley to hexaploid wheat. This suggests that the observed ring-of-four aberrations were caused by homoeologous chromosome pairing rather than by translocations.

In the present study, some meiotic chromosomal aberrations were observed at AI-TI, AII-TII and at the tetrad stage. The frequency of meiotic aberrations at AI-TI declined at AII-TII stage with further loss at the tetrad stage. This shows that the chromosomal aberrations are also being eliminated in the meiotic divisions. A very low frequency of micronuclei at the tetrad stage is related to the consistently high pollen fertility observed, regardless of the seed aging treatment. From this it is concluded that the slight reduction in pollen fertility is not due to the genetical effects of seed aging.

In contrast with the present result, pollen abortion mutants caused by gene and chromosomal mutations have been reported previously (Cartledge and Blakeslee 1934; Cartledge et al. 1936; Avery and Blakeslee 1936, 1943). Reduced fertility was also reported in aged seeds of wheat (Schkwarnikow 1937) and of tobacco (Gisquet et al. 1951).

In barley, broad bean, and pea seeds stored at 45 °C/12% m.c. and 25 °C/18% m.c., Abdalla and Roberts (1969) observed a few percent loss in average pollen fertility compared with the control seeds. In some barley plants grown from aged seeds with 40% germination pollen fertility was less than 80%. They mentioned that the reduction of pollen fertility was caused by pollen abortion mutation. However, more than 95% pollen fertility was observed in the majority of plants (97%, 362 out of 375 plants). Pollen fertility in the progeny of plants with low pollen fertility was not studied. Therefore, it is difficult to conclude that seed aging reduced pollen fertility in their experiment.

Transmission of chromosomal aberrations

The very low frequency of chromosomal aberrations in the roots of the A_2 generation indicates that most chromosomal aberrations induced by seed aging are not transmitted to the next generation. Frequencies of 0.3 to 0.4% aberrant anaphases in the A_2 seeds are not considered to be related to seed aging effects at the A_1 generation. This frequency in the A_2 seeds should be explained by some other factors. 'Himalaya' barley seeds are known to exhibit low levels of aberrations as shown by Caldecott and Smith (1952) and Adams and Nilan (1958). Although the frequency of chromosomal aberrations may be increased by seed storage in the A_2 generation, they will again be eliminated during the mitotic and meiotic cell cycles, and not transmitted to the subsequent generations.

This result agrees with that by Harrison and McLeish (1954) and Harrison (1966), who pointed out that chromosomal aberrations observed at first mitosis in aged lettuce seeds were not transmitted to the next generation. However, Gerassimova (1935) and Nawaschin and Gerassimowa (1936a, b) reported that the chromosomal aberrations caused by seed aging of *Crepis* could be transmitted to the next generation and their progeny both through female and male gametes. The transmission of chromosomal aberrations through pollen is usually very limited. The gametophytic stage is extremely sensitive to small genetic losses, and only spores that receive the complete haploid complement survive (Khush 1973). It is known that reciprocal translocations and inversions can be transmitted from generation to generation. However, these types of aberrations were not found in this study. Based on the present results, it is concluded that chromosomal aberrations induced during seed aging do not change the genetic information in the species or variety, even though the seed germinability is markedly reduced.

Germplasm preservation

In two previous papers in this series (Murata et al. 1981, 1982) we have examined in some detail the induction of chromosomal aberrations as a result of seed aging and the types of aberrations induced. In this paper we have monitored their fate during plant growth and reproduction. To evaluate potential genetic changes which my occur in seed germplasm stocks held in long-term storage, the question of accumulation of point mutations should also be addressed. Roberts (1973, 1975, 1978) emphasized the relationship between loss in seed viability and induced genetic damage including both chromosomal aberrations and point mutations. Based upon the induction of chlorophyll deficiency mutations, Abdalla and Roberts (1969) argued that any storage treatment which leads to a loss of viability of about 50% induces chlorophyll mutations in about 1 to 4% of the surviving seeds and would be equivalent to that produced by a treatment of fresh seeds with 10,000 r of X-rays. However, in all of the studies of induced genetic changes during seed aging, no evidence has been presented to show the persistence of mutations beyond the first or second seed increase. Evolutionary pressures will eliminate unfavorable traits. Also, induction of mutations, be they favorable or unfavorable, has the net effect of adding to the total genetic variability.

The history of agriculture is the history of selecting new and better mutations, which occurred spontaneously, by careful farmers. Also in recent years a large number of spontaneous and induced mutations have been used for breeding programs directly or indirectly. Development of many European barley varieties involved induced mutations either directly or indirectly. Sigurbjornsson and Micke (1969) listed 77 new varieties developed from induced mutations in more than 15 crop species including wheat, rice, barley, and soybeans. Of these, 67 varieties resulted from direct use of mutants. In the last 10 years, an additional 300 new varieties derived from induced mutants were listed in the Mutation Breeding Newsletter. This means a total of 400 or more varieties or lines of cultivated plants are direct or indirect products of induced mutations. These facts show that mutations, either induced or spontaneous, should not be considered simply deleterious. Some mutations are deleterious, but others are useful depending on their nature. It should be remembered that induced mutation research was initiated to broaden genetic variability.

Of far greater concern to seed germplasm banks is the potential loss in genetic variability which may result from genetic selection during seed storage and regeneration. The International Board for Plant Genetic Resources (1976) recommended that for long-term storage of germplasm no more than 5 to 10% deterioration should be allowed before regenerating a sample. However, allowing only a 5 to 10% loss in viability will result in more frequent regeneration which adds to the costs and space needed for germplasm maintenance. Furthermore, more frequent regeneration of seeds is

associated with problems such as genetic drift, when small populations are used; outcrossing with other germplasm accessions; and selection during rejuvenation (Allard 1970). Other factors such as number of components in an accession, presence or absence of diseases and pests, adverse weather and human error (mixing and mislabeling) can affect the composition of a population (Roos 1982).

Seed germplasm curators must weigh the consequences of potential genetic damage which is associated with seed deterioration vs. the genetic shifts which may occur from more frequent regenerations. Improved seed storage is one way to avoid both dilemmas.

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