BIOLOGIA PLANTARUM 39 (4): 523-529, 1997

Pre-replication recovery from methyl methanesulphonate induced chromosomal damage in *Vicia faba* seeds

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Abstract

Vicia faba seeds were treated with methyl methanesulphonate (MMS) and stored at 50 % water content for 0, 14 and 28 d. This water content prolongs the period between the mutagenic treatment and the onset of DNA synthesis. Storage of seeds after mutagen treatment at the selected water content led to a significant decrease in DNA damage, manifested as a reduction in the frequency of chromosomal aberrations.

Additional key words: alkylating agents, chromosomal aberrations, faba bean, seed storage.

Introduction

The storage of barley seeds at defined water content after mutagen treatment leads to a significant decrease or increase of DNA damage, depending upon the storage conditions. This was first described by Velemínský and co-workers in a series of reports (for review see McLennan 1988), when chromosomal aberrations (CAs), single-strand breaks (SSBs), M_1 survival, M_1 seed setting and frequency of M_2 chlorophyll mutants were studied. These results were extended for *Vicia faba* by evaluating unscheduled DNA synthesis in growing roots and stored embryos (Murín 1990), sister chromatid exchanges (Murín and Mičieta 1994), double-strand breaks (Murín and Mičieta 1994) and distribution patterns of chromatid aberrations induced in mutagen-treated seeds as affected by experimental storage (Murín and Mičieta 1996).

Experiments focused on the relationship between CAs and DNA repair synthesis after maleic hydrazide-induced DNA damage (Murín 1993) showed the storage-effects in detail. As maleic hydrazide is a well known plant mutagen we were

Received 14 February 1996, *accepted* 8 August 1996.

Abbreviations: CAs - chromosomal aberrations; DES - diethylsulphate; MH - maleic hydrazide; MMS - methyl methanesulphonate; SSBs - single-strand breaks.

Acknowledgements: We are deeply indebted to Dr. J. Velemínský and Dr. T. Gichner (Prague) for their advice and encouragement. The technical assistance of L. Gallová is very much appreciated.

interested to see whether the above-mentioned storage effect is a general phenomenon by using other types of mutagens, alkylating agents particularly. This paper shows the results of series of experiments with storage of MMS-treated *Vicia faba* L. seeds.

Materials and methods

Vicia faba seeds were treated for 5 h with 4 or 6 mM methyl methanesulphonate (MMS, *Merck*, Darmstadt) in distilled water at pH 4.8. Before MMS treatment the seed-coat of dry seeds was penetrated in order to obtain a higher uniformity of the soaking and a greater synchronization of mitotic activity (Thomas and Davidson 1981). After treatment, seeds were washed for 2 h in tap water, and then re-dried to 50 % water content (2 h at 37 °C in a thermostat with a fan). For checking water content during the experiment, extra samples of ten seeds were weighed before and after drying (8 h at 105 °C) to determine their water content. Seeds were stored for 0, 14 or 28 d at 25 °C above 600 cm^3 sterile water in a closed vessel. The seeds were germinated immediately after treatment, washing and re-drying; or after 14-d or 28-d storage and the roots were fixed after 32, 48, 56, 72 and 80 h.

Roots of MMS-treated seeds were fixed in ethanol:acetic acid (3:1), squashed and stained with aceto-orcein. The frequency of chromosomal aberrations was evaluated in ana-telophase cells. On average, 200 ana-telophases (50 in control) per recovery time were evaluated. Isochromatid breaks, duplication-deletions, intercalary deletions, and chromatid translocations were evaluated according to Rieger *et al.* (1977) after 0.05 % colchicine treatment (1 h).

For testing vitality, the length of roots (from 35 seeds each time) was measured after 36-, 48-, 72-, 80- and 96-h (120-, 144-, 168- and 176 h if necessary) germination.

Results

Storage at 50 % water content of *V. faba* seeds treated with 4 mM MMS led to a significant decrease in the frequency of chromosomal aberrations both in ana-telophases (from 39.4 ± 4.6 % without storage to 16 ± 2.24 and 14.4 ± 3.45 % after 14 and 28 d of storage, respectively, Fig. 1) and metaphases (from 40.12 ± 1.88 to 16.4 ± 3.36 and 16.88 ± 4.05 %, Table 1). Storage of seeds treated with 6 mM MMS also led to a significant decrease of frequency of chromosomal aberrations both in ana-telophases (from 64.5 ± 3.79 to 24 ± 2.88 and 18.8 ± 5.04 %, Fig. 1) and metaphases (from 70.77 ± 3.61 to 22.02 ± 1.89 and 17.46 ± 3.2 %, Table 2). Differences in frequencies (*i.e.* effectiveness of DNA repair) were much higher in the first 14 d than in the second two weeks. The decrease in repair capacity was caused by decreasing water content of seeds. The first two weeks of storage of seeds treated with 4 mM MMS reduced the yield of chromosomal aberrations in ana-telophases by

about 1.7 % (max. 2 %, min. 0.6 % according to particular recovery times) chromosomal aberrations perday. The next two weeks of storage under identical conditions resulted only in a 0.045 % (max. 1.2 %, min. -1.1 %) reduction of aberrations per day (see Fig.1). In the 6 mM dose, reduction dropped from 3.01 % per day (max. 3.4 %, min. 2.7 %) to 0.47 % (max. 1.5 %, min. -0.65 %) per day on average for all recovery times evaluated in ana-telophases (see Fig. 1). These data

Table 1. Influence of seed storage on chromosomal aberrations induced by 4 mM methyl methanesulphonate for metaphase cells.

Storage [d]	Recovery time [h]	Metaphases scored	Damaged metaphases	Damaged metaphases [%]	Isochro-matid break	Chromatid translo-cation	Dupl. deletion	Intercalary deletion
0	32	199	71	35.7	69	2	4	0
	48	175	71	40.6	57	15	5	2
	56	104	46	44.2	45	6	4	1
	72	131	47	35.9	38	2	3	0
	80	147	65	44.2	62	12	0	1
14	32	137	20	14.6	45	0	1	1
	48	162	39	24.0	35	4	1	1
	56	-	-	-	-	-	-	-
	72	99	8	8.1	4	2	1	0
	80	58	11	18.9	8	1	0	1
28	32	157	20	12.7	22	0	1	0
	48	110	7	6.4	6	0	0	1
	56	110	14	12.7	17	0	1	0
	72	68	19	27.9	17	2	0	0
	80	85	21	24.7	22	0	0	0

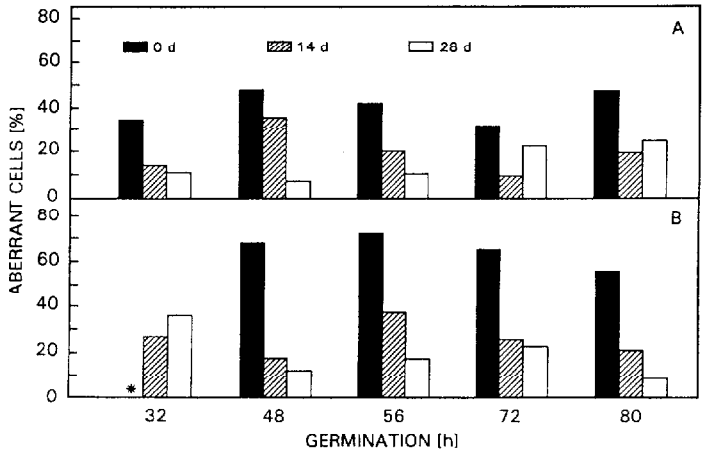

Fig. 1. Frequency of aberrant ana-telophases induced by 4 mM (A) or 6 mM (B) MMS in dependence on duration of storage (0, 14 or 28 d). The frequency was measured 32, 48, 56, 72 or 80 h after treatment. * - not evaluated.

show greater differences between the first and second two weeks of storage than in the case of 0.6 mM maleic hydrazide (from 2.72 % to 1.58 %) used in our previous experiments (Murín 1990). However this tendency is confirmed by the level of vitality showed on growing roots of treated seeds (Fig. 2).

Table 2. Influence of seed storage on chromosomal aberrations induced by 6 mM methyl methanesulphonate.

Storage [d]	Recovery time [h]	Metaphases scored	Damaged metaphases	Damaged metaphases [%]	Isochro-matid break	Chromatid translo-cation	Dupl. deletion	Intercalary deletion
0	32	-	-	-	-	-	-	-
	48	300	186	62.0	192	21	5	4
	56	140	111	79.3	75	31	6	9
	72	100	69	69.0	53	15	7	4
	80	367	267	72.8	294	42	10	11
14	32	169	43	25.4	47	2	0	1
	48	84	15	17.9	12	0	1	2
	56	154	41	26.6	46	1	0	3
	72	92	16	17.4	18	3	0	1
	80	92	21	22.8	40	2	0	1
28	32	56	15	26.8	17	0	0	0
	48	98	11	11.9	13	0	0	0
	56	115	20	17.4	24	0	0	0
	72	105	23	21.9	26	0	0	0
	80	75	7	9.3	8	1	0	0

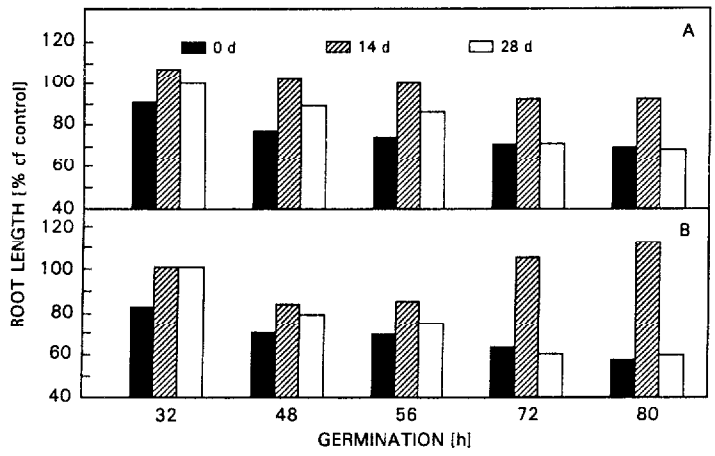

Fig. 2. Root length after 32-, 48-, 56-, 72- and 80-h germination of seeds treated by 4 mM (*A*) or 6 mM (*B*) MMS and stored for 0, 14 or 28 d.

Evaluation of types of aberrations in metaphase cells showed a higher yield of breaks than of chromatid translocations (Tables 1, 2). The most important are the changes in the yield of different types of chromatid aberrations in relation to the storage effect. Pronounced reductions in the frequency of chromatid translocations during storage showed significant changes in isochromatid break ratio for 4 mM dose (0:14:28 d = 7.32:13.14:42.00). A similar pattern was observed with 6 mM MMS (0:14:28 d = 5.60:20.37:176.00). It seems that with a longer time of storage greater differences between concurrent lesions of individual chromosomes occur per cell.

The number of aberrations per cell during storage in comparison with unstored samples showed both for 4 mM MMS dose (0:14:28 d = 1.12:1.35:1.10) and 6 mM dose (0:14:28 d = 1.21:1.36:1.16) an interesting decrease of the ratio between 14 and 28 d to a similar level as it was without storage. Thus more complex chromosomal arrangements are present in the remaining damaged cells (approx. 16.4 % of cells for 4 mM MMS and 22.02 % for 6 mM MMS after two weeks of storage). After four weeks of storage it represents again 16.9 % of cells for 4 mM MMS and 17.46 % for 6 mM MMS dose.

Discussion

The time between the start of seed imbibition and the first wave of semiconservative DNA replication offers a "window" (Osborne *et al.* 1984) for pre-replicative DNA repair. This time could be prolonged from hours to days by means of a simple physiological tool and the effect then occurred according to the level of water content in the seeds. Murata *et al.* (1981, 1982, 1984) suggested 12 or 18 % water content for barley, when germination of seeds was delayed and reduced. In parallel, the frequency of aberrant anaphases increased. Gichner and Gaul (1971) observed a drastic decrease of the height of barley seedlings that survived storage at 13 or 20 % water content. On the contrary, higher water content (30 % for barley) leads to significant recovery and repair of SSBs induced by alkylating agents; in plant cells this was described for the first time by Velemínský *et al.* (1972). We should expect the same for *Vicia faba* as we know that approximately 73 - 96 % of *Vicia faba* dormant radicle cells are in G-1 phase as a result of decreasing water content in the developing embryo (Jakob and Bovey 1969). Decrease in water content under 75 % arrested DNA synthesis and under 65 % the mitotic cycle is stopped (Brunori 1967). Re-drying of the imbibed *V. faba* seeds to 50 % water content caused prolongation of the G-1 phase of cells. Storage of seeds at this condition consequently could allow recovery from the mutagen induced chromosomal damage.

Our previous results with storage effects were based mainly on experiments with maleic hydrazide (MH treatment (Murín 1990, 1993). Therefore our aim was to use another type of mutagen, MMS, as an alkylating agent. Alkylating agents are known as a strong mutagens and one of the strongest carcinogens in our environment (Velemínský and Gichner 1982). They have often been used in storage experiments with barley (McLennan 1988). Although more than 100 mutagens have been tested on *Vicia faba* seeds (Sýkorová 1984), only few experiments were made with MMS.

The storage effect observed in our experiments was described for the first time by Sýkorová (1984) in *Vicia faba*. She applied both MH and MMS treatments and observed similar differences between the first and the second 14-d storage. In all cases (both doses, 0.2 and 0.4 mM MH, and both types of aberrations, ana-telophases and metaphases) MH-treatment led to non-significant differences between results of 14 and 28 d of storage although there was a clear and significant repair in comparison with unstored samples. This ment that the first two weeks of storage of seeds treated with 0.4 mM MH reduced the yield of chromosomal aberrations in metaphases by about 1.47 % chromosomal aberrations per day. The next two weeks of storage under identical conditions resulted only in the 0.64 % reduction of number of aberrations per day. This may indicate that both for alkylating agents, like MMS, and non-alkylating agents, like MH, DNA repair proceeds during storage after treatment, and that after some days (*e.g.* first two weeks) it could reach a peak of storage effect.

The observed higher yield of breaks than of chromatid translocations is in accordance with the observations of Michaelis and Rieger (1963) with 0.5 mM MH treatment of *Vicia faba* seeds. Gichner and Velemínský (1977) described changes of chromatid- to chromosome-type aberrations during storage after treatment with diethyl sulphate (DES). They proposed an explanation that the changes in aberration types are connected with the conversion of induced single- to double-strand DNA breaks. Nevertheless, another set of experiments made by Gichner and Velemínský (1979) showed that whereas the number of DES-induced chromatid-type aberrations decreased in the course of seed storage, the number of chromosome-type aberrations increased. However, this was valid only after DES treatment, whereas treatment by MMS and the subsequent seed storage led to reduction of both chromatid and chromosomal aberrations. The authors proposed two alternative explanations: *1*) the lesions leading to chromatid aberrations are repaired to a greater extent than lesions giving rise to chromosomal aberrations; and *2*) in the course of seed storage, the lesions in chromatid-type aberrations are either repaired and/or converted to lesions giving rise to chromosome-type aberrations.

Heindorff *et al.* (1987) have reported that there exist in plants at least two independent inducible DNA repair systems - one for non-alkylating and another for alkylating agents. Regarding our previous results with treatment by a non-alkylating agent, MH, and the results of the same method of storage with an alkylating agent, MMS, shown above, we could conclude that DNA damage from both these S-dependent mutagens was repaired during the storage of damaged seeds at 50 % water content.

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