EFFECTS INDUCED BY DIETHYL SULPHATE ON SOME CYTOGENETICAL PARAMETERS AND LENGTH GROWTH OF HEMP PLANTLETS

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Abstract: The hemp seeds were treated with diethyl sulphate, in four concentrations (0.1%, 0.25%, 0.5%, and 1%) and in two variants of alkylant exposure (3 and 6 hours). The length growth of plantlets, mitotic index and frequency of chromosomal aberrations were the analyzed parameters. Significant modifications were obtained at the level of parameters in variants treated with DES, comparatively with control.

INTRODUCTION

The chemical mutagenesis is a useful tool to study the physiological processes in plants and to improve the crops. By this way is possible to induce some mutation types in plant genome. The alkylants are largely used, especially because of their mutagen potential and to possibilities to obtain mutants with optimized bio productive characteristics (GILLE et al., 1986). In the most studies, the primary objective is the increase of genetic variability, not the direct investigation of mutagenic processes. The mutation induction at the level of quantitative traits is of particular interest in variability enlargement, necessary in selection and melioration (TOTH et al., 1984).

MATERIAL AND METHOD

The biologic material was represented by hemp seeds (*Cannabis sativa* L.). The experiences were carried out in more variants, one of control, in distilled water, the others in mutagen solutions, for different exposition times (3 h, 6 h). Four concentrations were tested: 0.1%, 0.25%, 0.5%, and 1.0%. The microscopic preparations were obtained by squash method. As staining reactive, the modified carbol fuchsine was used. The diethyl sulphate $[SO_2(OC_2H_5)_2]$ is a monofunctional ethyl alkylant, colourless or slightly coloured, with 154.18 molecular weight. Is an oily liquid, of moderate viscosity, with an experimental half time of 1.7 h, at neutral pH. The reactive contains 99% active ingredient.

RESULTS AND DISCUSSION

The alkylant induced dysfunctions are registered at the level of physiological, genetical and biochemical processes: increase of chromosomal restructurations frequency, mitosis inhibition, decrease of seeds germination faculty, reducing of plant growth rate, a greater sterility degree and a reduced survival, a great number of chlorophyllian and morphological mutations etc. (VERZEA et al. 1981).

DES was studied especially for its clastogenic effects. Although considered as an active mutagen, DES induces relatively few chromosomal aberrations, comparatively with the great capacity to induce chlorophyllian mutations. Many mutagenic alkylating agents react not only with nitrogen containing bases, but also with phosphate groups, resulting in phosphate triesthers. DES is more effective than DMS in alkylating of phosphate groups. The breaking of DNA double helix, as result of the hydrolysis of alkylated phosphate groups, may cause chromosome breakings and lethality.

The chromosome lesions which are not lethal, namely non affects vital functions, may lead to structural chromosome mutations, with condition that the karyokinesis mechanisms to be not affected. In cell cycles following mutagen treatment, the chromosomal aberrations are subjected

to a repair mechanism. The cell selection gradually eliminates the cells strongly affected by treatment. The response of vegetal organisms is conditioned by a lot of other biotic or abiotic factors.

DES influence on length growth of plantlets. The treatments with diethyl sulphate inhibited the growth length of hemp plantlets, indifferent to concentration and duration of exposure time on biological material to mutagen agent. The inhibitory effects are not markedly different in comparison with control values. The variant with the smallest average values of plantlets growth length was 1% DES, 6h (value with 25% smaller than C=100) (Table 1, Fig. 1).

Table 1. The average values of length growth of hemp plantlets, after DES treatment

Variant	**DES treatment duration**			
	3 hours		**6 hours**	
Control	56.77%	C=100	58.00	C=100
0.1% DES	47.06	82.89	45.98	79.27
0.25 DES	50.41	88.79	48.81	84.15
0.5% DES	52.96	93.28	47.02	81.06
1.0% DES	47.58	85.45	43.07	74.25

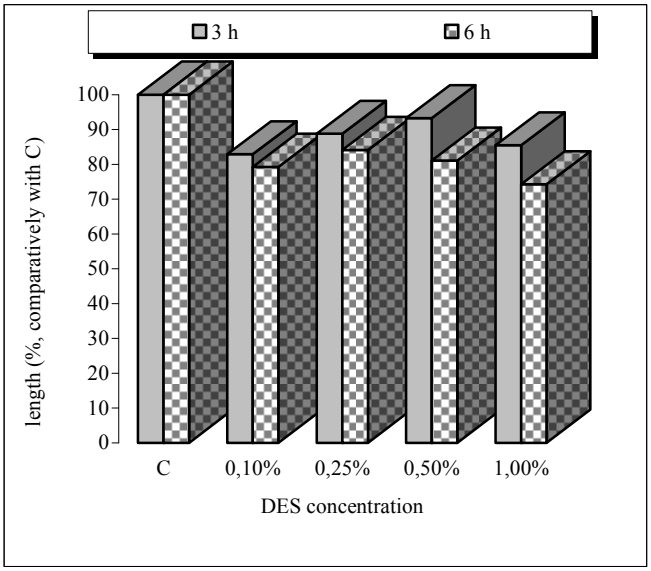

Figure 1. Influence of DES on plantlets length growth, depending on concentration and treatment duration

Evolution of mitotic index under DES treatment. The values of mitotic index, as indicator of cell division intensity, interesting variations and differences registered, depending on DES concentration and treatment duration. All DES treated variants have values of mitotic index smaller than those of control. Not even control has been a great level of cell division (55.17%). In the case of 3h treatments, the most important diminishing of mitotic index was registered for 0.5% DES (a 15% decrease, in comparison with control value) and for 1.0% DES (at which mitotic index is with 13% smaller than control). For the variants 6h-treated with alkylant, the number of cells in division was significantly reduced for 0.5% DES (with 12%) and 1% (with

17%), comparatively with mitotic index of control (Fig. 2). The 1% concentration of DES (6h) strongly inhibited cell division; the most cells were, also, blocked in prophase (36.06% from 38.18%) (Table 2). It is possible that this fact is due to the direct action of alkylant on genetic material, in the stage of synthesis and replication of this.

An interesting aspect is the cell blocking in prophase at 1% concentration, 6h. It is possible that DES, at this concentration and for this treatment duration to affect the chromosome replication and the forming process of division spindle components.

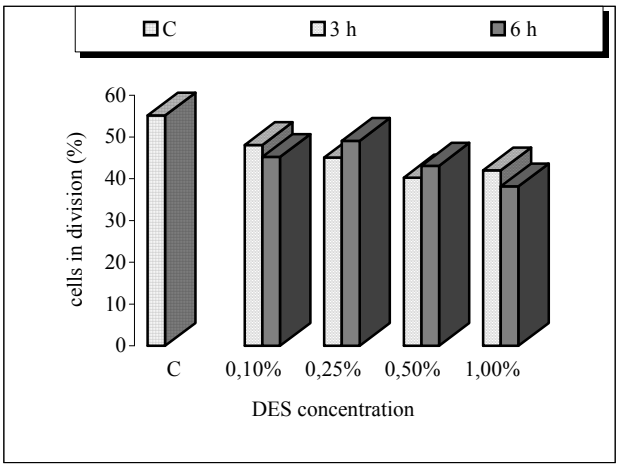

Figure 2. Mitotic index evolution in hemp, under the effect of DES

Frequency of chromosomal aberrations in root meristems of hemp, induced by DES. DES is known as inducing a large spectrum of chromosomal aberrations. For 0.25% DES, 6h, 1.01% metaphase aberrations, respectively 1.05% ana-telophase aberrations were scored, while at 0.5%, applied for 6h, DES induced 1.07% metaphase aberrations and 0.83% ana-telophase aberrations, in comparison with control distilled water treated, in which the percentage value were 0.35%, for the first category, respectively 0.44%, for the second. The cell blockage in prophase at 1% DES (6h) results in a decrease of metaphase and ana-telophase frequency. The chromosomal aberrations are also fewer in these stages of cell division.

The types of DES induced chromosomal aberrations were simple, double or multiple bridges in ana-telophases of dividing cells, lagging chromosomes, chromosome fragments, tri- or tetrapolar ana-telophases, metaphases with agglutinated chromosomes or with variable numbers of chromosomes out of equatorial plate. We observed too abnormally organized metaphases or anaphases or cells with increased ploidy levels. Aberrations were singular or in different combinations. The presence of fragments may be a proof that DES induces deletions, while the fragments may appear because DES exerts a preferential action on primary or secondary constrictions, disturbing chromosome kinetics. The chromosome dispersion and expulsion probably are due to the destroying effect of DES on components of equatorial plate or division spindle.

In most cases, the mutagenic effect of each chemical substance is confirmed by its capacity to induce chromosomal restructurations in mitotic dividing cells of root meristems or in meiosis. Some restructurations, such as isochromatidic fragments, translocations, inversions, duplications require, for their analysis, a deeper research (FLORIA, 1980).

CONCLUSIONS

DES inhibited the length growth of hemp plantlets, for all concentrations and duration variants of treatment, but these inhibitory effects were not so marked, comparatively with control. The variant 1% DES, 6 h, registered the smallest average values of growth length.

The concentration of 0.25% DES, 6h, induced 1.01% metaphase aberrations and 1.05% A-T aberrations, while 0.5% DES, 6h, determined 1.07% metaphase aberrations, respectively 0.83% aberrations in ana-telophase, all compared with control, for which 0.35% metaphase aberrations and 0.44% ana-telophase aberrations were registered.

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APPENDIX

Table 2. Effects induced by DES on cytogenetical parameters in root tips of hemp.

Variant	Total analysed cells	Mitotic cells	Mitotic index	Interphases		Prophases		Metaphases		Ana-telophases		Chromosomal aberrations			
												in metaphase		in ana-telophase	
				Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%*	Nr.	%*
C	5425	2993	55.17	2432	44.83	2480	45.71	225	4.14	288	5.30	19	0.35	24	0.44
0.1% DES 3h	4712	2266	48.08	2446	51.92	2000	42.44	100	2.12	166	3.52	23	0.48	25	0.53
0.25% DES 3h	4090	1844	45.08	2246	54.92	1532	37.45	138	3.37	174	4.25	19	0.46	10	0.24
0.5% DES 3 h	3795	1529	40.29	2266	59.71	1190	31.35	150	3.95	189	4.98	17	0.44	14	0.36
1% DES 3h	5512	2315	41.99	3197	58.01	2105	38.18	80	1.45	130	2.35	20	0.36	11	0.19
0.1% DES 6h	6024	2724	45.21	3300	54.79	2420	40.17	145	2.40	159	2.63	31	0.51	22	0.36
0.25% DES 6h	4928	2420	49.10	2508	50.90	2210	44.84	60	1.21	150	3.04	50	1.01	52	1.05
0.5% DES 6h	5123	2209	43.11	2914	56.89	1999	39.02	52	1.01	158	3.08	55	1.07	43	0.83
1% DES 6h	4520	1726	38.18	2794	61.82	1630	36.06	40	0.88	56	1.23	7	0.15	8	0.17

* The calculus of chromosomal aberrations was made by report to whole analysed cells