THE EFFECT OF CHEMICAL MUTAGEN AGENTS ON SOYBEAN (TYPE AGAT), IN CULTURE *IN VITRO*

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Abstract: *In vitro mutation induction on soybean, using chemical mutagen agents leads to genetic variability, which can be used on the improvement species.*

Key words: genetic variability, *in vitro* mutagenesis, mutagen generator, soybean.

INTRODUCTION

The i*n vitro* method for cells and tissues cultures is one of the most efficient techniques for achieving soma-clonal variations. It was demonstrated that soybean shows a good response to mutagen stimuli, the regeneration being made by forming bipolar structures and roots or by organ-genesis, trunks and roots forming (CORNEANU, 1989)

The efficiency of the treatment with chemical mutagen agents can be established according to several parameters: the mutagen agent, its concentration and the treatment used, establishing the new economic potential achieved after the mutagen treatment applied (SAVATTI and collaborators, 2004).

MATERIAL AND METHODS

The research of inducing and selecting mutations *in vitro* was carried on using as biological material the Agat type soybeans, created at SCDA Turda, and as mutagen factors two chemical alkilant agents were used: DE= diethyl sulphate şi DM = dimethyl sulphate, in two concentrations introduced in an aseptic medium.

100 drawings were carried on for each variant. Each experimented variant was placed in three repetitions for the ulterior statistic data processing.

The vegetal material was obtained from seeds selected from the above mentioned type, previously disinfected with calcium hypochlorite 7% for 30 minutes and then washed 5-6 times with distilled water. The inoculation of seeds on medium for germination MS ½ for two days allowed the development of the embryo for about 0.3 cm. The embryo was then placed on M_1, M_2, M_3, M_4 mutagen media and M, control medium. The

embryos were kept on these media for 12, respectively 48 hours in the conditions of the growing room, after which they were removed and subcultivated on media abbreviated V_1, V_2, V_3, media with a balanced hormonal balance, both as the rate of hormones concentration and its nature, in order to show more clearly the possible mutagen effect (table1).

Table 1

Aseptic media used in inducing mutations *in vitro* at soybean

Contents of media	Type of media / concentration		
	For germination (MS 1/2)	Mutagen (M_1, M_2, M_3, M_4)	Culture (V_1, V_2, V_3)
Macro elements	MS 1/2	MS	LS
Microelements	MS 1/2	MS	LS
FeEDTA	MS 1/2	MS	MS
Mezo-inozitol	50 mg/l	100 mg/l	252 mg/l
Vitamins:			
Thiamine HCl	0,1 mg/l	1 mg/l	
Pyridoxine HCl	10,1 mg/l	1 mg/l	
Nicotinic Acid	0,1 mg/l	1 mg/l	
Saccharose	20 g/l	20 g/l	
Agar	6 g/l	6 g/l	
pH	5,7	5,8 (MB)	5,6 (MB)
Diethyl sulphate (DE)	-	M_1=MB+DE–2 ppm M_2=MB+DE–0,2 ppm	-
Dimethyl sulphate (DM)	-	M_3=MB+DM–2 ppm M_4=MB+DM–0,2 ppm	-
Hormones:			
Bentiladenine (BA)	-	-	V_2=MB+BA–0,5 mg/l
Naphtilacetic acid (ANA)	-	-	ANA–0,5 mg/l
Zeatine (Z)	-	-	V_3=MB+Z–0,5 mg/l
Indolil butyric acid (AIB)	-	-	AIB–0,5 mg/l

MS – media after Murashige-Skoog – 1962
LS = media after Linsmaier-Skoog – 1965
MB = basic media

The meristematic explants were observed under the following aspects: the ability of regeneration *in vitro*, neo-formation of plantlets completely conformed (number of neo-plantlets, branching, the length of neo-plantlets) and neo-formation of roots (number, length, thickness, nodules), as well as some macroscopic somatic modifications, signalled after the mutagen treatment.

RESULTS AND DISCUSSION

The observations were done after 30 days for the embryos subculture on V1,V2,V3 media. The soybean embryos, kept for 12 hours on mutagen media did not show visible differences as compared to the witness. The phenotypical similitude to the non-treated biological material is due to the reduced period with mutagen factors.

The mutagen agents influence a few quantitative characters in the first generation (M0) in the conditions of the *in vitro* culture. The

morphological anomalies from M0 can affect all the organs, but most frequently the leaves and trunk.

The results of treatment with mutagen factors for 48 hours are shown in table 2.

Table 2

The effect of mutagen factors on Agat type, in culture *in vitro*, at a 48 hours treatment

Mutagen Media	Concentration	Variant	Neo-formation				Observations: phenotypical modifications
			Plantlets		Roots		
			no	height (cm)	no	length (cm)	
M	Witness	V_1	4	6,0	1	1,2	Normal evolution, developed roots
		V_2	3	2	1	1,1	Normal evolution
		V_3	3	2,5	1	1,0	Normal evolution
M_1	DE/0,2 ppm	V_1	4	1,0	1	2,4	Thick roots, secondary branches,
		V_2	-	-	1	0,3	Non-uniform evolution
		V_3	5	0,8	1	2,0	Neo-plantlets branches Non-uniform colouring
M_2	DE/2,0 ppm	V_1	2	11,0	5	3,5	No branches, plants with a reddish colouring
		V_2	-	-	-	-	Tissue mass, no differentiate neo-plantlets
		V_3	1	1,0	2	2,4	Thick roots, nodules, branches at the basis, non-uniform evolution
M_3	DM/0,2 ppm	V_1	3	2,0	1	4,1	Interesting evolution, long roots, real, reddish leaves
		V_2	-	-	-	-	No evolution
		V_3	3	0,5	-	-	Slow evolution, the explants hardly raise
M_4	DM/2,0 ppm	V_1	3	7,0	1	4,5	Long, pubescent roots
		V_2	-	-	-	-	No evolution
		V_3	1	0,8	6	2,0	Several branched roots, real leaves

M = MB – witness (after Murashige-Skoog 1962)
M_1= MB+DE 0,2ppm
M_2= MB+DE 2,0 ppm
M_3=MB+DM 0,2 ppm
M_4=MB+DM 2,0 ppm
V_1=MS1/2 (after Murashige-Skoog 1962)
V_2=MB+BA-0,5 mg/l+ANA-0,5 mg/l
V_3= MB+Z-0,5 mg/l+AIB-0,5 mg/l
BA= benzyl adenine
Z = zeatyne
AIB = indolil butyric acid
ANA = alpha- naphtil- acetic acid

The percentage of similar phenotypic plants with the initial material and the percentage of plants supposed mutant is shown in table 3.

Table 3

The percentage of similar phenotypic plants with the initial material and the percentage of plants supposed mutant

Agat type			
Variant	% of plants similar phenotypic	Supposed mutant plants %	Bonus
Testifier	100,0	-	-
DES_1 (0,2 ppm)	75,0	25,0	××
DES_2 (2,0 ppm)	70,0	30,0	××
DMS_1 (0,2 ppm)	38,0	62,0	×××
DMS_2 (2,0 ppm)	16,0	84,0	×××

Legend – without modifications
× easy phenotypic modifications, under 20%
×× modifications almost 25%
××× modifications more than 50%

CONCLUSIONS

The analysis of the treatment with chemical mutagen factors such as diethyl sulphate (DE) and dimethyl sulphate (DM) on the Diamant type, cultivated *in vitro*, was done taking into account its effect on the *in vitro* culture and on the morphological variation of M_0 and M_1 offspring.

Diethyl sulphate and dimethyl sulphate induce phenotypical modification on the soybean cultivated *in vitro*, but the effect, in most cases, is not homogenous, caused by the type of the explants, the concentration of mutagen substances, the period of treatment, genotype, and the mutants' individualization being performed in the ulterior generations of multiplication.

The occurrence of some morphological modifications under the influence of chemical mutagen agents, possibly mutant, opens favourable perspectives for selecting and fixing some quantity and quality characters and fulfilling some improvement objectives.

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