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Somatic Modifications Occurred at Soybean, as an Effect of the Chemical Mutagen Agents

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Abstract: The usage of chemical mutagen factors in culture media allows the emphasis on phenotypical modifications at soybean cultivated in vitro, but the effect, in the majority of cases, is not homogenous, caused by the nature of the explant, the concentration of the mutagen substances, the treatment time, and the genotype.

Keywords: somatic, macroscopic modification, in vitro mutagenesis, soybean, mutagen agent.

INTRODUCTION

The method of cultivating cells and tissues *in vitro* is at present one of the most efficient techniques for obtaining soma-clonal variations, experiments of inducing the artificial mutations were induced at *Soja hispida* (MURASHIGE, SKOOG, 1965; RAICU and collaborators, 1984, 1990).

It was demonstrated that soybean shows a good plasticity of response at mutagen stimuli, the regeneration being able to occur by forming bipolar structures and roots or by organogenesis, forming stems and roots (CORNEANU, 1989)

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

MATERIAL AND METHODS

Researches of inducing mutations were performed *in vitro* using as biologic material soybeans of Diamant and Agat type, created at SCDA Turda. As mutagen substances two alkilate agents were used, DE = diethyl sulphate and DM = dimethyl sulphate, in two concentrations introduced in an aseptic medium.

The meristematic explants, in a number of 100 for each variant, were observed under the following aspects: the ability of regeneration *in vitro*, the new 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. Because the effects of the two hormones on the general development of plants (AIA and BA) were well known and taking into account the antagonist effects of the two hormones, the culture medium of the witness, variant v_1, AIA concentration was 10 times smaller than in v_2, while BA concentration was 10 times smaller than in v_1.

Given the specific conditions offered by the *in vitro* cultures, the two concentrations of mutagen agents were introduced in the culture media used for the explants' assay: 0,2 ppm

DE in the variant of medium DE_1; 2 ppm DE in the variant of medium DE_2; 0,2 ppm DM in the variant of medium DM_1; 2 ppm DM in the variant of medium DM_2.

For each variant of medium (MS + DE_1; MS + DE_2; MS + DM_1; MS + DM_2) 100 meristems per type were assayed in three repetitions.

The meristems' assay was done in aseptic conditions, in the vapour hood with a sterile laminar flux. Meristems of 0,5 mm length were assayed, 100/type and were introduced in test tubes (12×420 mm) on approximate 5 ml of culture medium that contained the above mentioned concentrations of mutagen agent.

The treatment time was chosen according to the mutagen's ability of adsorption by the biologically treated material. After 24 hours the biological material was put on a fresh culture medium, identical with that of the witness variant M, with the following content of hormones: kinetin 10 mg/l; AIA 0,5 mg/l.

The test tubes were moved in the growing room, together with the witness variants, studying the following: the moment of initialising the cells' multiplication processes; the inoculi rhythm of growing; the initialising of the organogenesis; the plantlets' growing speed, in a close dependence with the changing of the culture layer content and modifying the conditions of the ambient medium.

Observations and biometric measurements were carried on for the biological material to be tested with mutagen agents in all the phases of the *in vitro* development, from the plantlets' assay, under the phenotypical aspect.

The vegetal material was obtained from seeds selected from the above mentioned type. 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, a 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 their nature, in order to show more clearly the possible mutagen effect.

The observations were done after 30 days at the subculture of the embryos on V1, V_2, V_3 media. The *Soja hispida* embryos, for both types, kept for 12 hours on the mutagen media, didn't show visible differences from the witness. It can be noticed that the content of the culture media didn't imply different evolutions; the neo plantlets regenerated by V_2 had a similar evolution to those on V_3. So, the phenotypical similitude to the non-treated biologic material is seemingly due to the reduced time spent with the mutagen factors.

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RESULTS AND DISCUSSION

The mutagen agents have an influence in the first generation (M0) in the conditions of the *in vitro* culture, on some quantity characters. The morphologic anomalies from M0 can affect all the organs, but more frequently the leaves and the stem.

Tab. 1

Synoptic table with the macroscopic modifications noticed at Diamant type

Macroscopic modifications	VARIANTS											
	Diethyl sulphate (DE)						Dimethyl sulphate (DM)					
	M_1			M_2			M_3			M_4		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
At the radicular level	+	-	-	+	-	-	-	-	-	+	-	-
At the foliar level	-	-	-	-	-	+	-	-	-	-	-	+
At the level of branched stems	-	-	+	-	-	+	+	-	-	+	-	+
Reddish colouring	+	-	-	+	+	-	-	-	-	-	-	-
Anomalies, necroses	-	-	-	-	-	-	-	+	+	-	+	-

The somatic, macroscopic modifications are shown according to the effect of the mutagen substances (noted with + or -).

M_1= DE 0,2ppm
M_2= DE 2,0 ppm
M_3=DM 0,2 ppm
M_4=DM 2,0 ppm
V_1=witness medium (MB)
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
ANA = alpha-naphtyl-acetic acid
Z = zeatin
AIB = indolil butyric acid

Tab. 2

Synoptic table with the macroscopic modifications noticed at Agat type

Macroscopic modifications	VARIANTS											
	Diethyl sulphate (DE)						Dimethyl sulphate (DM)					
	M_1			M_2			M_3			M_4		
	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3	V_1	V_2	V_3
At the radicular level	+	-	-	+	+	-	+	-	+	+	-	-
At the foliar level	-	-	-	-	-	-	-	-	-	+	-	-
At the level of stems – branches	-	-	+	-	-	+	+	-	-	-	-	-
Reddish colour -bale	+	-	-	-	-	-	-	-	-	-	-	-
Anomalies, necroses	-	+	-	-	+	-	-	+	-	-	+	+

The macroscopic modifications are shown according to the effect of the mutagen substances (noted with + or -).

M_1= DE 0,2ppm
M_2= DE 2,0 ppm
M_3=DM 0,2 ppm
M_4=DM 2,0 ppm
V_1= witness medium
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
ANA = alpha-naphtyl-acetic acid
Z = zeatin
AIB = indolil butyric acid

In the case of the mutagen treatment at Diamant type, it can be noticed a normal evolution of the embryo, with a generation, at all variants, of some neo-plantlets with variable height, according to the hormonal balance, with a thick and long root of about 2.5 cm, with secondary ramifications and even with some nodes. On the other hand, at the embryos moved from mutagen media on culture media, some somatic macroscopic modifications can be noticed:

- *at the foliar level,* in case of small concentrations of DE and DM (0,2 ppm) on V_3 medium (with 0,5 mg/l Z and AIB) some modifications occurred by appearing leaves with only one lobe, or strongly segmented leaves;
- *at the radicular level,* DE (for both concentrations) implies in the variant without hormones (V_1) the occurrence of some thick and strongly branched roots, with nodes and the occurrence of a reddish colouring of the bale;
- *at the stem level* there can be noticed a ramification on DE media (for both concentrations), on medium V_3 (with 0,5 mg/l Z+AIB), DM, in both concentrations implied a ramification of the stem on the witness medium (V_1);
- *the reddish colouring at the level of the bale* appears only on media with DM in the variant without hormones (V_1) and rarely at the level of variant V_2;
- *anomalies and necroses* are produced on media with DM in variants V_2 and V_3, where in the benzyl adenine medium (BA) there are alpha-naphtyl-acetic acid (ANA), respectively zeatin (Z) and indolil butyric acid (AIB), by forming torsion roots and by appearing necroses on the plantlets.

In the case of the mutagen treatment at Agat type, the results being shown in table 2, it can be noticed a similar situation to that presented at Diamant type, the witness does not record macroscopic modifications in comparison to the variants with mutagen substances where somatic modifications occur.

There can be noticed that *at the foliar level* there are modifications only in variants that contain dimethyl sulphate mutagen (in both quantities);
-*at the stem level* there are ramifications of the bale in the variants with diethyl sulphate (DE) on V_3 medium, with a content of zeatin +AIB 0,5 mg/l, and for the dimethyl sulphate (DM), only on V_1 and at a low concentration;
- *the bale's colouring* appears in V_1, where the embryo was immersed in DE in a low concentration;
- *anomalies and necroses* are numerous at this type, producing especially at the level of variant V_2, in case of both mutagen substances and in both concentrations, phenomena probably conditioned by the incompatibility between the mutagen substances and the hormonal balance in the medium.

The neo-plantlets obtained *in vitro*, both those which show in M_0 mutant phenotypes, and those from the witness variant were observed *ex vitro*, in the greenhouse, in order to notice their ability to adapt to new conditions and to make biologic material for a new multiplication *in vitro* of M_1 material.

It was found that the mutagen agents influence in the first generation (M_0), in the conditions of the *in vitro* culture some quantity characters (NICOLAE, 1969; SAVATTI and collaborators, 2004).

The morphological anomalies from M_0 can affect, as noticed from the data previously presented, all the organs, but more frequently the leaves and stem. Their phenotypical exteriorization gets different shapes according to the genotype and the quantity of the mutagen concentration that was administrated.

The importance given to these anomalies comes from the fact that they help, in some cases, to the application of the selection from M_0, in order to obtain a bigger frequency of mutants in M_1.

The bonification shows that modifications up to 50%, or over, are recorded under the influence of the variants with DM mutagen (dimethyl sulphate), in both concentrations, at both soybean types that were tested. It can be noticed that the incidence of the possible mutants grows proportionally to the growth of the DM concentration.

The differentiated reaction of genotypes to the mutagen agent is obvious; the percentage of the possible mutants is higher at Agat type.

The observations regarding the similitude of morphological characters and the supposed mutations are due to some macroscopic modifications obtained during the experimental process. At the foliar level, leaves with a single folio appear, or leaves with a small size, especially under the influence of dimethyl sulphate (DM).

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.

Using chemical mutagen factors in the culture media allows the emphasis on some mutants that will be multiplied and observed under the aspect of the behavior shown by advanced mutant generations, obtained during moving on several culture media, *in vitro*, under the aspect of their resistance to biotic and a-biotic factors.

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. As regards the effect of the mutagen agents on the evolution of meristems in the *in vitro* culture, the effect of diethyl sulphate (DE) is noticed as regards the ability to neo-form plants from meristem, at both types; it can be notice the positive response of Diamant type as regards the ability to multiply *in vitro*, as compared to Agat type.

At this stage, the dimethyl sulphate (DM) has an inhibiting effect on the plants' ability to regenerate from meristems, indifferent of the applied dose, the differences being significantly negative compared to the witness, at both types.

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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