THE USE OF *IN VITRO* SELECTION, FOR THE EMPHASIS OF TOLERANT SOY MUTANTS OF THE ALUMINUM IONS

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Abstract: *Using in vitro selection in order to point out several genotypes of soybean that are tolerant to aluminum ions (Al^{+}); regarding the creation of genetic variability in soybean by using chemical mutagenic factors and the use of in vitro selection in order to identify the lines tolerant to aluminum ions ($Al^{+)}$.*

Key words: *soybean, aluminum ions, possibly mutant.*

INTRODUCTION

The application of a selection in order to obtain a resistant line to various stress factors, has the effect of temporary inhibition of increase of culture, in the division being included only the cells resistant to the newly created conditions.

At the important species of culture studies referring to the genetic determinism of resistance to aluminum were performed. It was observed that, in general, the resistance has a dominant multigenical character, which may be controlled by one or several major genes and a few minor ones (ANIOL and GUSTAFSON, 1984; MARTEN, 1991; PHAN THI BETU and colab., 2003).

Of special importance for the agricultural practice is the creation of resistance genotypes to various stress factors, and especially to soy, that towards Al^{+} ions. To this end, we have to mention that in the traditional soy culture areas, especially in Transylvania, we can find large areas where the acidification progress is high, also determined by the excess aluminum ions, causing relatively low productions. In the conditions of a slightly acid or neutral pH, the aluminum is also found as oxides or aluminum silicates. As the pH value is decreasing, in the soil solution are issued the phyto-toxic forms of aluminum at levels affecting the increase of roots and, implicitly, of plants.

In the sense of those mentioned, in order to extend the soy culture on fields less adequate for the respective culture, with favorable effects, of this plant on soils, it would not be without interest to track down genotypes with a high resistance to Al^{+} ions.

The *in vitro* selection may represent an interesting solution in order to obtain soy mutants resistant to aluminum.

MATERIAL AND METHODS

In our work we present the results of research done in order to obtain cell lines tolerant to Al^+ ions from the embryogene and non-embryogene calluses, in order to verify if the potential mutants signaled by us may manifest a certain tolerance to Al^+. Our studies aimed not treated biological material, as well as biological material treated with chemical mutants (DMS and EMS).

The vegetal material used was made up of the three soy versions, Diamond (Diamant), Pearl (Perla) and Agate (Agat).

The introduction and subculture of callus was achieved after the method described by Mihaela CIALÂCU and VARGA (1994) and by Gabriela VICAŞ (2009), using the basic culture B5, GAMBORG and colab., (1976).

For the *in vitro* selection, the B5 environment with supplemented with 2,0 mg/l naphtylacetic acid (NAA) and Al^+ ions in concentration of 0,5% (86 mM), respectively 1,0% (171 mM) (Blum ,1993).

The development of callus on the selective environment was achieved with the help of an indirect method presented by DIXON (1985) and which is represented by the determination of surface of viable callus transferred each passage.

The biological material (callused) was differentiated on culture environments on which it was acted with different concentrations of aluminum ions, in order to divide, with a high accuracy, their resistance capacity.

In order not to amplify the volume of experiences, we used only mutant biological material achieved after treatments with maximum concentration of mutant agents (2,0 ppm). In relation to the origin of callus, from assumed mutants we achieved a differentiation from cell lines which were tested, in which concerns the resistance to aluminum ions. 10 sources for each mutant were separated and tested in the first phase in which concerns the reaction of callus cultures towards the aluminum ions (chart 1). The differentiated cell lines come from the three soy cultures available.

We mention the fact that the plants resulted from the cell lines which manifested a certain tolerance to the aluminium ions were not tested *in vivo*, the information obtained being reduced to the cell resistance towards the acid stress. Tolerant cell lines point out a variability earned under the aspect of tolerance to the aluminium ions, and plant regeneration out of these lines may be an interesting material to study to elucidate the genetic and molecular mechanism of tolerance to the aluminium ions and the selection of several potential genotypes adaptable to crops on acid soils.

RESULTS AND DISCUSSION

In case of soy, we noticed that the culture on acid environment generated by the presence of aluminum ions (Al^{+}) influences the development of callus by slowing the decrease and appearance of necrosis of more or less enlarged areas. From the dates of chart 1 we notice response differences *in vitro*, determined by genotype and from the Al^{+} concentration.

By analyzing the answer of callus cultures coming from the non-treated biological material with mutant factors, at the level of the three cultivation versions, Diamant (Diamond), Perla (Pearl) and Agat (Agate) obvious phenotype differences is notices (chart 1). We notice that a concentration of 0,5% Al^{+} the callus increased percentage achieves only 18,8% (Agate), 22,5% (Pearl) and 25,3% (Diamond), towards the witness version, without basic culture addition of aluminum ions. The phenomenon is decisively emphasized in the case of increase of aluminum concentration in the culture (1,0%), when an obvious depression of increase of callus is achieved, of only 3,1-6,6% comparison to the non-treated witness (Mt 0). The data mentioned show lack of tolerance of soy genotypes towards the Al^{+} ions, toxic elements, of acidification of culture environments.

Tabel 1

The soybean genotypes callus development in the control medium and with different concentrations of Al^{+}

Genotype	*No callus*	*Control (without Al^{+})*		*Al^{+} concentration*			
				0,5%		1,0%	
		Callus area (mm^2)	%	*Callus area* (mm^2)	%	*Callus area* (mm^2)	%
Diamant	95	4136	100	2100	53,2	798	19,3
Perla	95	3210	100	1396	43,5	659	17,4
Agat	98	3024	100	942	23,4	130	4,3

In order to test the possibilities of emphasis of a soy biological material, previously treated with mutagens (DMS and EMS, 2,0%), in which concerns the resistance and tolerance to aluminum, on the basis of previous observations (morphological and cytological), 10 cloning lines were followed in which concerns their answer to the presence of aluminum ions in the culture environments (chart 2).

Tabelul 2.

Dynamic of callus growth in genotypes of soybean probably from mutant seedlings on culture medium with different concentrations of Al^+

Variant	*Origin*	*Callus number*	*Al^+ concentration*					
			Control no Al^+		0,5%		1,0%	
			mm^2	%	mm^2	%	mm^2	%
LC_1DMS	Diamant	60	3310	100,0	3330	100,6	3290	99,4
LC_2DMS	Perla	60	3460	100,0	3480	100,6	3390	98,0
LC_3DMS	Perla	60	3432	100,0	3396	99,0	3000	87,4
LC_4DMS	Agat	60	3120	100,0	2990	95,8	3120	100,0
LC_5DMS	Agat	60	2990	100,0	2990	100,0	3100	103,7
LC_1EMS	Diamant	60	3336	100,0	3300	98,9	3225	96,7
LC_2EMS	Diamant	60	3415	100,0	3384	99,1	3300	96,6
LC_3EMS	Perla	60	3390	100,0	3380	99,7	3250	95,9
LC_4EMS	Agat	60	2876	100,0	3000	104,6	2915	101,4
LC_5EMS	Agat	60	3030	100,0	3040	100,3	3000	99,0
LC_6EMS	Agat	60	3114	100,0	3100	99,6	2998	96,3
LC_7EMS	Agat	60	2956	100,0	3000	101,5	2889	97,7
LC_8EMS	Agat	60	2970	100,0	2950	99,3	2950	99,3
LC_9EMS	Agat	60	2868	100,0	2900	100,4	2796	97,5
$LC_{10}EMS$	Agat	60	3013	100,0	2987	99,1	3000	99,6

The results introduced in chart 2 emphasize an interesting fact in which concerns a positive reaction in relation to the tolerance to aluminum ions, of those 10 cloning lines tested. This fact seems to be revealed by the increase or stagnation of surfaces of callus cultures, towards the witness (controlled version), as well as at the level of concentrations of aluminum ions of 0,5%, respectively 1,0%.

The data presented makes us to believe that the possibly mutant lines present a tolerance and not a resistance to aluminum ions, because in case of resistance, the processes of increase of callus surfaces should continue to have a dynamics.

From the cloning lines considered tolerant to aluminum ions were harvested cells which were sent at callus and rhyzogene culture (with AIA growth hormones and cytokinine). After the initiation, the somatic embryos were treated with Al^+ ions with concentration of 2,0%.

Tabelul 3.

The effect of medium with 1,0% aluminium ions on embryos
from probalbly soybean mutants

Variant		Somatic embryos			
		pure culture		*tolerant*	
		nr.	%	nr.	%
LC_1DMS	2,0%	75	100,0	69	92,0
LC_2DMS	2,0%	60	100,0	60	100,
LC_3DMS	2,0%	60	100,0	58	96,7
LC_4DMS	2,0%	75	100,0	70	93,3
LC_5DMS	2,0%	90	100,0	88	97,8
LC_1EMS	2,0%	60	100,0	60	100,0
LC_2EMS	2,0%	60	100,0	60	100,0
LC_3EMS	2,0%	75	100,0	72	96,0
LC_4EMS	2,0%	75	100,0	70	93,3
LC_5EMS	2,0%	75	100,0	68	90,7
LC_6EMS	2,0%	75	100,0	75	100,0
LC_7EMS	2,0%	60	100,0	60	100,0
LC_8EMS	2,0%	60	100,0	60	100,0
LC_9EMS	2,0%	75	100,0	79	97,3
$LC_{10}EMS$	2,0%	90	100,0	88	97,8

From the data of chart 3, we emphasize the fact that between the treated and not treated versions with aluminum ions, 2,0%, the average differences are not marked. Despite that, the data indicates an increased rate of tolerant mutants at aluminum ions, at the level of LC_2DMS, LC_1EMS, LC_2DMS, LC_6EMS, LC_7EMS and LC_8EMS, which did not suffer any differentiation in comparison to the witness versions not affected by Al^+.

CONCLUSIONS

The use of this working method with somatic embryos coming from plants considered potentially mutant, following the treatments with chemically mutant agents, might represent an experimental version for the identification of soy genotypes tolerant to Al^{+}.

The tolerant cell lines, as well as their regenerated plants may represent an interesting study material for the clarification of genetic and molecular mechanisms of the tolerance to aluminum ions, process which was unclear until nowadays.

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