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THE CUMULATIVE EFFECT OF KINETIN AND AIB ON SOYBEAN ORGANOGENESIS *IN VITRO*

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Abstract

In our study kinetin and AIB were used for making the culture medium more effective. The nature of the phytohormones used, and also their concentration, the differences of the hormonal balances have an important role in the organogenesis processes.

Key words: soybean, indolylbutyric acid, organogenesis, cytokinins, phytohormons, auxines,;

INTRODUCTION

Soy is one of the plants that presents a high plasticity of the response to stimuli mutagens, its regeneration can be achieved by forming the bipolar structure of cotyledons and his roots (CORNEANU, 1989).

Phyto-regulators, or chemical, organic regulators that influence plant growth and development are synthesized compounds that mimic the effects of phytohormones, with a particular practical importance in plant biotechnology (DORINA CACHIŢA-COSMA şi colab., 2004).

Phytohormones occupy a dominant position in the processes of cell multiplication and growth, in organogenesis, respectively in the differentiation and dedifferentiation of plant cells.

SKOOG şi MILLER (1957) ,have established the concept of phytohormonal control of organogenesis, demonstrating experimentally that the differentiation of rootstocks and plant stems in vitro is dependent on the auxin / cytokinin ratio present in the environment.

For cultivation of cells and tissues in vitro, auxins, cytokines, and rarely gibberellins (BOXUS şi colab., 1995).

From an organogenetic point of view, the plant can be considered to consist of two concentric coats, one superficial one having the ability to form buds and the other profound, characterized by its rhizogenic capacity.

The control of cell division, cellular stretching and differentiation processes is controlled by hormones that can have an endogenous nature,

synthesized by plants or synthesis, obtained by humans, close to or different from the chemical formula of endogenous ones, but which develop the same biological activity as the first ones.

MATERIAL AND METHODS

In the present study, soybeans were cultivated: Diamond, Pearl and Agat, introduced into the Murashige-Skoog culture medium (1962). The auxine used to induce cell divisions and rhizogenesis was indolylbutyric acid (AIB) 0.5-2.0 mg / l. The synthesis cytokinin used was chinetin (K) (6-furfuryl-aminopurine)).

Four experimental variants were performed, in terms of the germplasm used, the explant source, the culture media, and the combination of growth regulators. During the experiments, observations were made at 15, 30 and 60 days of cultivation, on the number of shoots on meristems, the height of the shoots and the rooting (number of root roots).

Tabel 1

Layout of the experiments carried out to optimize a protocol for direct organogenesis of soybean meristems

Experi-ment	*Explant source*	*Cultivars*	*Medium and plant growth regulators*
I	*Stem and crown meristems*	Diamant Perla Agat	B5 (0,2 mg/l ANA) B5 (0,2 mg/l AIB +0,2 mg/l 2iP); *at 30 days transferred to the same medium* LS (0.004 mg/l PIC+1 mg/l K *at 30 days transferred to RL*(0.2 mg/l AIB)
II	*Stem meristems*	Diamant Perla Agat	MS (*plant growth regulator free*); *at 15 days transferred to the same medium* LS (0.004 mg/l PIC+1 mg/l K *at 15 days transferred to RL*(0.2 mg/l AIB)
III	*Stem meristems*	Diamant Perla Agat	MS (0.004 mg/l PIC+1 mg/l K; *at 15 days transferred to MS (0.2 mg/l AIB)* LS (0.004 mg/l PIC+1 mg/l K) *at 15 days transferred to RL*(0.2 mg/l AIB)
IV	Meristeme tulpinale *Stem meristems*	Diamant Perla Agat	LS (0.003; 0.004; 0.005 mg/l PIC şi 0.5; 1.0 mg/l K) *at 15 days transferred to RL*(0.2 mg/l AIB

RESULTS AND DISCUSSION

It is noted that in vegetal vitrocultures, organogenesis can be regulated, within certain limits (reaction depending on endogenous factors) by changing the concentration, respectively the ratio of the two main types of phytohormones - auxin and cytokinin - present in the culture layer.

The best results on the behavior of genotypes were observed at 60 days of culture, when 25% of the LS mediums developed plants suitable for transplantation, compared to 10% on the B5 medium. It is also noted that both coronary and testicular meristems have formed normal growth plants, with the observation that coronary meristems develop a large number of explants.

Tabel 2

Comparison of media MS (plant growth regulators free) and LS (0.004 mg/l PIC+ 1,0 mg/l K, for 15 days, RL+0.2 mg/l IBA afterward) after 60 days of culture (Experiment II)

Cultivar	*Number of shoots/meristem*	*Number of roots/meristem*	*Height (mm)*
Diamant	5,6	1,7	2,6
Perla	5,0	1,1	1,8
Agat	5,8	2,3	2,6
Average MS	5,5	1,7	2,3
Average LS	6,6	3,7	3,2
General average	6,0	2,7	2,5
Signification			
Cultivar	*	*	Ns
Medium	*	ns	Ns
Cult.×Medium	ns	ns	Ns
LSD 5%			
Cultivar	0,82	1,00	-
Medium	0,53	-	-

Table 3 shows the influence of indole acetic acid (AIB) in mg / l on calusogenesis and rhizogenesis in the three soybean cultivars. It is noted that at both phenomena the most favorable AIB dose is 1.5 mg / l in the environment culture, aspect mentioned, phenomenon mentioned in the literature (CHIRILEI et al., 1970; BANDICI, 2001). In this respect, it is found that if at the level of the three cultivars used in the experiment the rhizogenesis manifests at a level of 27.1%, one observes a behavioral differentiation between the genotypes in the sense that if between Diamant and Pearl varieties the differences in reaction to rhizogenesis are small, in the case of the Agat variety, the differences are marked, 8-10% higher than

the first two varieties mentioned. The same can not be said regarding the process of calusogenesis, the differences between genotypes are less marked. Analyzing the results obtained, there seems to be a negative correlation between rhizogenesis and calusogenesis at the level of in vitro cultures, at least in soybean, on the one hand, and the caulogenetic process on the other.

Tabel 3

Cumulative effect of K and AIB on organogenesis

Cultivar	K+AIB (mg/l)	Evolution of organogenesis %			
		No development	Calusogenesis	Risogenesis	Caulogenesis
Diamant	0,0	100,0	0	0	0
	0,5	60	35	20	48
	1,0	63	40	23	50
	1,5	62	46	28	56
	2,0	56	43	33	52
	3,0	70	21	19	32
	%	62,2	37,0	24,6	47,6
Perla	0,0	100,0	100,0	0	0
	0,5	56	32	18	46
	1,0	53	42	19	54
	1,5	58	46	29	54
	2,0	60	38	36	50
	3,0	66	30	19	33
	%	58,6	37,6	24,2	47,4
Agat	0,0	100,0	100,0	100,0	0
	0,5	43	46	28	55
	1,0	40	46	36	63
	1,5	38	56	40	66
	2,0	45	52	32	50
	3,0	58	32	26	32
	%	44,8	46,4	32,4	54,4
$\overline{X}$ **/genotip**		**55,2**	**40,3**	**27,1**	**49,8**

These are illustrated by the mean percentages of genotypes, highlighting the fact that the hormonal balance achieved in the KIB combination is a very balanced one. Of the three cultivars, the Agat variety had good results for the K AIB combination: caulogeneza (54.4%), calusogenesis (48%) and rhizogenesis (32.4%).

Tabel 4

Cumulative effect of phytohormones on soy organogenesis

Cultivar	Variantă	Fără diferenţiere	Calusogenesis	Risogenesis	Caulogenesis
Diamant	K+AIB	62,2	37,0	24,6	47,6
Perla	K+AIB	58,6	37,6	24,2	47,4
Agat	K+AIB	44,8	46,4	32,4	54,4

CONCLUSIONS

The formation of different hormonal balances and differences in organogenesis (caulogenetic-rhizogenesis) make us consider that one of the most economical and efficient formula is the KIB combination. With regard to the recommended concentrations, it is found, in all variants, that doses of 1.0-1.5 mg / l of stimulators are most favorable in triggering the organogenesis of soybeans.

Analyzing the influence of auxins and cytokinins in the soybean organogenesis process, the necessity of their presence in the nutritional environment is indisputably present. In all cases it was found that the passage of the explant into the basic culture medium without the participation of the growth hormone organogenesis did not trigger.

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