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THE CUMULATIVE EFFECT OF KINETIN AND ANA ON SOYBEAN ORGANOGENESIS

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Abstract

In our study kinetin and ANA 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,, organogenesis, cytokinins, phytohormons,auxines,hormonal balance ;

INTRODUCTION

The cumulative physiological effect of auxins and cytokines, called hormonal balance, is highlighted in the literature. (CACHIŢA-COSMA and colab., 2004; CACHIŢA-COSMA and Camelia SAND, 2000; RAICU and colab., 2000) .

Analyzing the effects of artificial auxins on soybean ontogenesis, it is found that its reaction differs in terms of percentage depending on the genotype analyzed. This leads us to believe that in the process of in vitro multiplication of soybeans, the individualization of the process must be deepened, in the sense that each cultivar has a specific profile of the reaction to hormonal activity and as such there is a need for each genotype to individualize the structure. culture media.

SKOOG and MILLER (1957), they founded the concept of phytohormonal control of organogenesis, demonstrating experimentally that the differentiation of the roots and stems of seedlings in vitro, is dependent on the ratio auxin / cytokinin, present in the environment.

Auxins are involved in many physiological processes and interact with various endogenous substances, especially other phytohormones, especially cytokinins, gibberellins and ethylene.

Auxins, cytokines and less often gibberellins are used in vitro for cell and tissue culture (BOXUS and colab., 1995).

MATERIAL AND METHODS

In the present study, soybean cultivars were used: Diamond, Pearl and Agate, introduced into the Murashige-Skoog culture medium (1962). The auxin used was alpha-naphthylacetic acid (ANA) at a concentration of 0.5-2.0 mg / l.

The physiological action of auxins on plants, although very complex, can be reduced to the fact that they act on the growth of cells in length; permeabilizes plasma membranes for water and certain ions; along with cytokinins, auxins stimulate cell division; net rhizogenic action (CACHIŢA-COSMA and colab., 2004).

The synthetic cytokinin used was kinetin (K) (6-furfuryl-aminopurine). MILLER et all, identified the first cytokinin - kinetin (6-furfurylaminopurine), which has been shown to stimulate caulogenesis and bud formation in the inocula, from which stems are generated.

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 culture, regarding the number of shoots on meristems, the height of the shoots and the rooting (number of main roots).

Table 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 +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 ANA)
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 ANA)
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 ANA)* LS (0.004 mg/l PIC+1 mg/l K) *at 15 days transferred to RL*(0.2 mg/l ANA)
IV	*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 ANA

RESULTS AND DISCUSSION

The best results on the behavior of genotypes were observed at 60 days of culture, when 25% of the meristems grown on the LS medium developed plants suitable for transplantation, compared to 10% on the B5 medium. It is also found that both the coronary and stem meristems formed normally growing seedlings, with the observation that the coronary meristems develop a large number of explants.

Observations on the reaction of soybean genotypes to the culture media and growth regulators used showed that callus (variant I) formed on the B5 medium supplemented with ANA as a source of auxin. The explanation of this phenomenon consists in the presence of naphthylacetic acid (ANA) in the culture medium, knowing the influence of this auxin on the generation of callus.

Table 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 IAA 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	-	-

The reported differences are entirely due to the surplus of growth regulators that benefited the LS culture medium, referring to the presence of kinetin (K) and indolylacetic acid (ANA).

From the presented data, the behavioral differentiations of the genotypes under the influence of ANA introduced in the culture environment are highlighted. If in all three cultivars the phenomenon of caulogenesis does not show any evolution, in the case of rhizogenesis and callusogenesis some notable aspects appear. it is found in the case of naphthylacetic acid (ANA) that it has a positive influence especially on

callusogenesis. Under the influence of this auxin there is also a process, quite modest, of differentiation of caulogenesis at the level of the three cultivations (table 3).

In the case of the Agat cultivar, at a concentration of 1.5 mg / l of ANA, a percentage of 9% caulogenesis is achieved, obviously superior to the other two cultures. Under the influence of this phytohormone, callusogenesis and rhizogenesis are fully favored, a fact found, in fact, in the case of AIA and AIB (CHIRILEI et al., 1970; BANDICI, 2001).

Analyzing the obtained results, it seems that there is a negative correlation between in vitro cultures, at least in soybeans, between rhizogenesis and callusogenesis, on the one hand, and the process of caulogenesis, on the other hand..

Table 3

Cumulative effect of K and NAA on organogenesis

Cultivar	K+ANA *K+NAA* (mg/l)	*Evolution of organogenesis %*			
		No development	*Calusogenesis*	*Risogenesis*	*Caulogenesis*
Diamant	0,0	100,0	0	0	0
	0,5	53	32	27	46
	1,0	50	36	33	50
	1,5	48	46	38	54
	2,0	60	38	28	50
	3,0	63	16	14	29
	%	54,8	32,4	28,0	45,2
Perla	0,0	100,0	0	0	0
	0,5	49	38	31	39
	1,0	45	38	38	46
	1,5	43	46	38	56
	2,0	56	32	26	42
	3,0	60	19	18	30
	%	50,6	34,6	30,2	42,6
Agat	0,0	100,0	0	0	0
	0,5	46	39	36	51
	1,0	43	43	39	58
	1,5	40	48	46	63
	2,0	46	40	42	48
	3,0	58	26	19	26
	%	46,6	39,2	36,4	49,2
$\overline{X}$ /genotip		**50,7**	**35,4**	**31,5**	**45,9**

The mentioned ones are illustrated by the values of the average percentage of the genotypes, highlighting the fact that the hormonal balance achieved at the K + ANA combination, is a very balanced one. Of the three

cultivars, the Agat variety had the best results in the case of the K + ANA combination: in the case of caulogenesis (49.2%) and rhizogenesis (36.4%). In terms of recommended concentrations, it is found in all variants that the doses of 1.0-1.5 mg / l of stimulants are the most favorable in triggering soybean organogenesis.

It is found that in plant vitro crops, organogenesis can be regulated, within certain limits (the reaction depending on endogenous factors) by changing the concentration, respectively the ratio of the two main types of phytohormones - auxins and cytokinins - present in the culture layer.

The hormonal balance response highlights the existence of a negative correlation between caulogenesis and rhizogenesis if we look at the percentage ratios highlighted by the K + ANA combination.

CONCLUSIONS

The presented data show the favorable effect of the combinations between kinetins and auxins, in the sense of favoring the process of organogenesis.

However, the fact of the appearance of some differentiations of answer according to the genotype is highlighted, the Agat variety having the most favorable answer to the combinations mentioned in the hormonal balance.

Analyzing the influence of auxins and cytokinins in the process of organogenesis in soybeans, there is an indisputable need for their presence in the Murashige-Skoog nutrient medium. In all cases it was found that the passage of the explant on the basic culture medium without the participation of growth hormones organogenesis is not triggered.

It can be concluded that the presence in culture media of an increased concentration of auxins, together with cytokinin, stimulates the processes of rhizogenesis, while an increase in cytokinin content stimulates the formation of buds, their growth and the generation of stems. It is also observed that the existence in the culture environment of equal concentrations of compounds with auxin and cytokinin action, can imply - along with the processes of morphogenesis - both the generation of calusm and its increase.

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