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THE IMPACT OF CYTOKININES ON SOYA ORGANOGENESIS *IN VITRO*

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Abstract: *In vitro regeneration and organogenesis at some species of plants is an essential condition to accomplish vegetative multiplication. 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, hormonal balances, organogenesis, cytokinin.

INTRODUCTION

Soybeans (*Glycine max* (L) Merril), are a major source of edible protein and is one of the most important agricultural crops in the world (SMITH and HUYSER, 1987).

It was shown that soybeans shows a remarkable plasticity of response to mutagens stimuli, regeneration can be achieved by forming a bipolar structure with cotyledons and roots (Corneanu, 1989).

To complete some aspect of the `previous study we aimed to detail the role of two new cytokinine, benzilaminopurina (BAP) and 2-Isopentyl-adenine (2iP) on the development of soybean plants obtained *in vitro* from the apex node, and floral bud juvenile pods, following capacity for regeneration, organogenesis and multiplication of these explants depending on the nature, its concentration and other components of the hormonal balance.

The phyto-physiological effects of the cytokinins can be summed up, depending on the concentration and their type, as facilitating the formation of buds and stems (caulogenesis), being antagonistic, as a rule, to root formation.

MATERIAL AND METHODS

It was followed the behaviour of aseptic explants consisting of the apex node, floral bud and juvenile pods (while forming), taken from mature plants from three soybean varieties examined: Pearl, Diamond and Agate. After collecting and sterilizing tissues, they were grown on culture medium, consisting of a basic medium MS (Murashige-Skoog after 1962), macro-and micronutrients iron, nicotinic acid and pyridoxine in concentrations of 0.5

mg/ l to facilitate regenerations, and double dose 1 mg / l mesoinositol in the medium of 100 mg/ l, sucrose 30 mg / l, pH 5.7, to stimulate the plants multiplication (Table 1) .

Tabelul 1.

Composition of aseptic media in plant regeneration in soybean

Elements(mg/l)	Regeneration	Multiplication
Macroelements	MS	MS
Microelements	MS	MS
FeEDTA	MS	MS
Mezo-inozitum	100 mg	100 mg
Tiamine	0,5	1,0
Pyridoxine	0,5	1,0
Nicotinic acid	0,5	1,0
Sucrose	30 g/l	30 g/l
Agar	7 g/l	7 g/l
Adenin sulfat($AdSO_4$)	40 g/l	80 mg/l
pH	5,7	5,7

MS - basal medium after Murashige-Skoog, 1962

After aseptisation, explants were passed on a few environment variables (Table 2). Averages were abbreviated from T0 to T4. T0 as the witness was placed on basic medium without hormones-MS medium (with the components specified in Table 2). The others show a rigorous balance, each cytokinine being accompanied by a certain amount of auxin – ANA, Naphthylacetic acid in dose of 0.5 and 1.0 mg/l. The basic environment and the variants with hormones in low doses contained 40 mg/l AdSO4, while the ones with higher doses contained 80 mg/l AdSO4.

Tabelul 2.

Experimental hormonal balance for soybean meristems

Variant	*Basal medium*	*Cytokinines*	*Concentration* (mg/l)	*Auxines*	*Concentration* (mg/l)	*Addition* $AdSO_4$ (mg/l)
T_0	MS	-	-	-	-	40
T_1	MS	BAP	1,0	ANA	0,5	40
T_2	MS	BAP	2,0	ANA	1,0	80
T_3	MS	2iP	1,0	ANA	0,5	40
T_4	MS	2iP	2,0	ANA	1,0	80

RESULTS AND DISCUSSION

At about eight weeks after re-breeding on the environments mentioned in Table 2, certain macroscopic parameters were monitored as regards explants' breeding and growing: i.e. the explants' evolution in terms of percentage depending on share of regenerated explants, number of regenerated plants, the conformation of soybean plants regenerated *in vitro*, the presence of root-system.

The outcomes on the *in vitro* behaviour of the four soybean explants species as regards their apex, apex, core, floral gem, and juvenile pods, depending on the environment variants mentioned above are presented in Table 3. We were interested in the *in vitro* regenerative capacity of several soybean cultivars, depending on the explants donor, their nature and the culture medium. This aspect was analyzed on four abbreviated culture media (T1 and T4).On T1, the explants prelevated from nodes gave a 92-95% regeneration percentage, forming 1-2 neoplantlets out of each node, with an initiation of the root system. Unlike the T4 medium, the number of plants on node is lower, but with a more powerful root formation process

The initiation of *in vitro* tissue cultures requires the formation of a base culture medium with a certain mineral formula, which can stand and stimulate a rapid cellular division of the meristematic explants. The mineral formula is supplemented with auxines and cytokinins in order to initiate or to block the organogenesis of the roots and sprouts. In our experiments auxines and cytokinins were used for making the culture medium more effective.

Tabelul 3.

In vitro morphogenesis of soybean explants, cultivated on media with benzylaminopurine (BAP) and 2 izopentyl-adenine (2iP)

Variant	*Explant*	% *Regene-ration percentage*	*No of plants*	*Height* (cm)	*No of roots*	*Roots length*	*Other types of tissues*
T_0		80	1	**1,5**	1-2	2,5	-
T_1		85	2	1,0	3	2,5	-
T_2	***Apex***	90	**5**	1,1	5	2,0	-
T_3		90	2	1,3	3	2,5	-
T_4		**94**	3	1,1	**8**	2,0	-
T_0	***Node***	70	2	1,0	2	1,8	-
T_1		80	**8**	0,7	4	1,0	-
T_2		70	6	0,8	-	-	calus (50%)
T_3		82	9	1,0	5	1,5	-

T_4		**88**	6	0,9	-	-	calus (30%)
T_0	***Bud***	57	3	0,8	2	1,0	-
T_1		**60**	8	**1,8**	5	1,0	-
T_2		50	14	0,8	2	0,4	calus
T_3		**61**	10	1,5	5	0,6	-
T_4		50	**16**	0,5	5	0,5	calus
T_0	***Juvenile pods***		6	0,9	3	1,0	-
T_1			12	1,2	4	1,0	calus
T_2			14	**1,5**	3	0,5	-
T_3		93	10	1,6	5	0,5	-
T_4		**97**	**18**	0,8	5	0,5	-

T_0 – mediul de bază după Murashige-Skoog + 40 mg/l $AdSO_4$
T_1 – MS + 1 mg/l BAP + 0,5 mg/l ANA + 40 mg/l $AdSO_4$
T_2 – MS + 2 mg/l BAP + 1,0 mg/l ANA + 80 mg/l $AdSO_4$
T_3 – MS + 1 mg/l 2iP + 0,5 mg/l ANA + 40 mg/l $AdSO_4$
T_4 – MS + 2 mg/l 2iP + 1,0 mg/l ANA + 80 mg/l $AdSO_4$

Phyto-physiological effects of cytokinine, can be summarized in general, as follows: depending on their concentration and types, *in vitro* breeding they stimulate the forming of adventitious shoots and small stems (caulogenesis), being, in general, antagonistic to risogenesis; the cytokinines serve to maintain cell viability, by supporting new plantelets capacity to survive and fostering the cells dedifferentiation and multiplication, and prevents senescence.

It must be emphasized the physiological effect of auxins and cytokinines which is the so called hormonal balance.

The organogenesis may be adjusted within certain by changing the concentration, i.e. the ratio between the two phytohormones – auxininc and cytochemical - present in the layer of culture. Thus, the presence, in breeding environment, of an enhanced amount of auxine, along with cytokinines, both stimulates the formation of adventitious shoots and growth of small stems. It is worth mentioning that in the presence of high but balanced concentration levels of the components mentioned above, one may generate callus formation and growing along with the morphogenesis (see Table 3).

CONCLUSIONS

By summarising the data collected throughout our experiment *in vitro* on behaviour of certain explants, apex, core, floral gem, and dolphin pods, sampled from soya plants on breeding environment with two cytokinines (BAP and 2iP) in various auxine (ANA) concentrations and additions, one can draw the following conclusions:

The apex regenerated completely formed plants by multiplying and rooting on all the environment variants (T0-T4) which proved to be the most favourable. The core has also generated, on almost all environments variants, new plantelets, but in a lower number than the apex. On the environments with 2iP the regeneration share reached 82-88%, but the occurrence of callus at the basis of new plantelets inhibits the radicle system formation. The floral gem regenerate a lower share – 50-60%- but it generates the largest number of new planteles (roughly 14-16/explant) with an appropriate radicle system. This explant generates the forming of callus on environments with larges doses of cytokinines. The juvenile pods, along with the meristems originating in apex, seem to be the most favourable as regard the regenerating shares, but superior as regard the number of plants formed.

The outcomes we have reached at make us to recommend a moderate use of cytokinines and auxines as ell as of the adenine sulphate in the basic environment.

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