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INFLUENCE OF DIETHANOLAMINE SALT OF 4-NITROBENZOIC ACID IN CALLUS CULTURE AT *MOMORDICA CHARANTIA* L.

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Abstract

Medicinal plants and their products are an important solution to improve the treatment of people in the whole world. Momordica charantia L. *is a well-known species for its biological activity (antioxidant and antimicrobial activity) and contains a complex of beneficial compounds such as: vitamins, minerals and antioxidants that can be used for treating a wide range of illnesses, especially diabetes.*
Plant tissue culture is an important and facile method for the somatic variability induction and tissue lines selection inorder to obtain valuable secondary metabolites. The use of the substances that control growth and synthesis capacity of tissues allow us to produce under aseptic conditions significant quantities of plant metabolites. The controlled conditions give to the tissue culture a suitable microenvironment for the successful growth and biosynthesis. Phytohormons and other substances with the same effect can determine in tissue culture the increase of biosynthetic capacity which can lead to obtaining and selection of proliferative tissue lines producing secondary metabolites.
The present work aims to study the influence on M. charantia L. *tissue culture of a new biological active compound, diethanolamine salt of 4-nitrobenzoic acid (4-NO_2BA DEA), synthesized by the Institute of Chemistry Timisoara of Romanian Academy. In this research we used 6 hormonal balances in which we associated 4-NO_2BA DEA with cytokinin BAP and also the new compound alone on the MS culture medium, for the selection of tissue lines with high growth capacity.We note that increasing the amount of 4-NO_2BA DEA in the MS culture medium could have beneficial effects on tissue culture at M. charantia.*

Key words*: auxins, callus, Momordica charantia* L.

INTRODUCTION

Momordica charantia L. is a medicinal plant commonly known as bitter melon, balsam pear, bitter cucumber, or bitter gourd, karela (India), fukwa (China), and ampalaya (Philippines). The plant can grow on different types of soils. The flower comes into blossom about one month after planting.
Bitter gourd has a beneficial effect in the treatment of cancer, viral infections (HIV, herpes, Epstein Barr, hepatitis, influenza and measles), bacterial infections (*Staphylococcus*, *Streptococcus* and *Salmonella*), bitter digestive aid (dyspepsia and sluggish digestion), but is well known for the hypoglycemic effect (Budrat and Shotipruk, 2008; Kim et al., 2003), this plant being known as the insulin plant. The main constituents of *Momordica charantia* L. that are responsible with the antidiabetic effects are: triterpene (charantin), protein, steroid, alkaloid, inorganic, lipid and phenolic compounds (Grover Yadav, 2004). The callus culture under the hormone influence allows the selection of tissue lines with high growth capacity, which can be used in the secondary metabolites production (Simina et al., 2014).
Benzoic acids and their derivatives are important compounds involved in various physiological processes in plants. Furthermore, they regulate seed germination (Ng et al., 2003; Crisan et al., 2014;Crisan et al., 2009;Crisan et al., 2007), have been functionally associated with disease resistance and stress tolerance in plants (Dempsey et al., 1999). Ethanolamine salts of different substituted benzoic acids are new compounds synthesized by the Institute of Chemistry Timisoara of Romanian Academy, some of them revealing auxin-likeplant growth regulatory activity on *Arabidopsis thaliana* and *Cucumissativus* L. (Crisan et al., 2014). In contrast to corresponding benzoic acids, the

alkanolamine salts are water soluble, significantly influencing the plant growth activity. This research focuses on testing of new compound 4-NO_2BA DEA in association or not withcytokinin BAP in order to study the growth capacity of *Momordica charantia* L. tissue culture.

MATERIALS AND METHODS

Momordica charantia L. callus was cultivated on MS medium (Murashige and Skoog, 1962) supplemented with 6 variants of hormonal balances, representing combinations of auxins, cytokinins in which we associated auxin 4-NO_2BA DEA with cytokinin BAP and also the new tested compound alone on the MS culture medium, for the selection of tissue lines with high growth capacity.

The new compound, 4-NO_2BA DEA, was obtained by controlled method, *via* proton exchange reaction, from 1:1 molar amounts of 4-nitrobenzoic acid and diethanolamine, in acetone solvent.The different variants of hormonal balances used in our experiment are showed in Table 1.

Table 1. Phytohormonal variants

Hormonal Balance	Phytohormons (mg/l)	
	*4-NO_2BA DEA	**BAP
BH1	1.5	0.0
BH2	1.5	1.0
BH3	1.0	0.0
BH4	1.0	1.0
BH5	0.2	0.0
BH6	0.2	1.0

*4-NO_2BA DEA = 4-nitrobenzoic acid
**BAP = 6-benzylaminopurine

RESULTS AND DISCUSSIONS

Based on the results presented in Table 2, it is showed that the effect of the hormonal balances in the callus culture of *Momordica charantia* L. has a significant influence on the growth of callus at *Momordica charantia* L., instead the combined effect of the hormonal balance and the duration of culture did not show a significant effect on the growth of callus at *Momordica charantia* L.

Table 2. Variance analysis regarding the effect of hormonal balances and *in vitro* culture duration on the growth of *Momordica charantia* L. callus

Source of variation	SP	GL	S^2	Test F
Total variation	14854.5	95		
Hormonal balance	4072.5	5	814.5	5.67**
Culture duration	223.5	3	74.5	0.52
Balance x Duration	224.5	15	15.0	0.10
Error	10334.0	72	143.5	

Regarding the effect of *in vitro* cultivation period on callus growth at *Momordica charantia* L. (Table 2) we can see that in the first 14 days of culture, the growth of *Momordica charantia* L. callus culture recorded an amplitude variation of 1.4%, with values between 114.8 % after seven days and 116.2% after 14 days of culture. After 21 days of culture,a decrease in the amplitude variation to 3.1% with the average of 113.1% is observed. The decrease in the amplitude measurements continue until reaching 0.8%, with an average value of 112.3% at the end of the determinations.

Table 3.The effect of *in vitro* culture duration on the growth of *Momordica charantia* L. callus

Culture duration (days)	Callus growth (%) compared to baseline		Relative values (%)	Difference/ Significance
14 - 7	116.2	114.8	101.22	1.4
21 - 7	113.1	114.8	98.52	-1.7
28 - 7	112.3	114.8	97.82	-2.5
21 - 14	113.1	116.2	97.33	-3.1
28 - 14	112.3	116.2	96.64	-3.9
28 - 21	112.3	113.1	99.29	-0.8

$DL_{5\%}$=6.9 $DL_{1\%}$=9.1 $DL_{0.1\%}$=11.9

Based on the results obtained (Table 4), we can say that the duration of *in vitro* culture does not have an influence on callus growth of *M. charantia* L. throughout the period of our determinations. It also notes that in the first 7 days of culture, the effect of hormonal balance on callus growth does not occur. But after 14 days of culture, significant differences between hormonal balances BH1 (4-NO_2BA DEA 1.5mg/l), BH3 (4-NO_2BA DEA 1.0 mg/l) and

BH4 (4-NO_2BA DEA 1.0mg/landBAP 1.0mg/l)can be observed. After 21 days of culture we can note that there is a differentiating effect of hormonal variations, recording significant differences between BH1 (4-NO_2BA DEA 1.5mg/l), BH2 (4-NO_2BA DEA 1.5mg/l and BAP 1.0mg/l) and BH3 (4-NO_2BA DEA 1.0 mg/l), which last until the end of measurements (28 days).

Table 4.The effect of hormonal balance and *in vitro* culture duration on the growth of *Momordica charantia* L. callus

Hormonal balance	Culture duration (days)					
	7	**14**	**21**	**28**	$\bar{x} \pm s_{\bar{x}}$	$S_{\%}$
BH1	x118.0a	x119.0ab	x115.0ab	x114.5ab	116.6±1.3	4.41
BH2	x122.5a	x128.0a	x127.0a	x125.0a	125.6±4.2	13.28
BH3	x115.0a	x116.0ab	x110.0b	x108.0b	112.2±1.8	6.44
BH4	x109.5a	x109.5b	x106.5b	x108.0b	108.4±2.5	9.17
BH5	x108.5a	x107.5b	x103.0b	x103.0b	105.5±1.4	5.38
BH6	x115.5a	x117.5ab	x117.0ab	x115.5ab	116.4±3.8	13.05
$\bar{x} \pm s_{\bar{x}}$	114.8±2.1	116.2±2.6	113.1±2.8	112.3±2.7	114.1±1.3	
$S_{\%}$	9.19	11.08	12.12	11.64	10.96	

$DL_{5\%}$=16.9 $DL_{1\%}$=22.4 $DL_{0,1\%}$=29.1

Under the aspect of callus growth variation (Figure 2) in case of using the hormonal balance 4-NO_2BA DEA 1.5mg/l and BAP 1.0 mg/l it is noted a proportional increase during the duration of the callus culture until 19 days, when it reaches a maximum gain of 28 % compared to the original value. Subsequently in the last nine days of culture a decreasing trend is observed, so in the end a 25% growth increase is achieved.When using the hormonal balance 4-NO_2BA DEA 1.5 mg/l, there is a regressive evolution of callus growth from the fourth day of culture, when it is recorded the maximum size equivalent to 118.5% compared to the original value and finally ending after 28 days of culture to 114.5% compared to originalvalue.

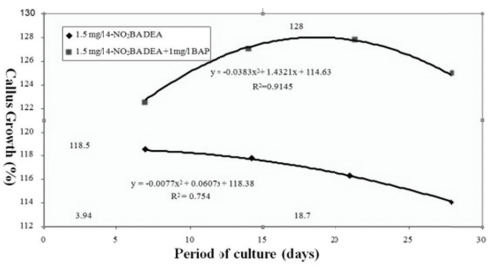

Figure 1. The growth rate of *M. charantia* L. callus under BH1 (4-NO_2BA DEA 1.5 mg /l) orBH2 (4-NO_2BA DEA 1.5 mg /l and BAP 1.0 mg /l)hormonal balances

Decreasing the amount at 1mg/l 4-NO_2BA DEA has led to the regression of callus growth from the fourth day of culture, when it is recorded a maximum size equivalent to 115.6% from baseline and finally ending after 28 days of culture at 107 5% compared to baseline (Figure 2). When 4-NO_2BA DEA (1.0 mg/l) and BAP (1.0 mg/l) is combined, there is again a regressive evolution of callus growth from the fourth day of culture, when it is recorded a maximum size equivalent to 109.6% compared with the original value, reaching 108%. Then a period of stagnation isfollowed which will be recorded until the end of measurements.

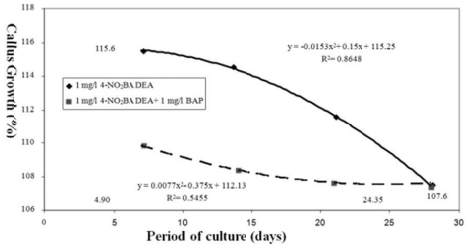

Figure 2. The growth rate of *Momordica charantia* L. callus BH3 (4-NO_2BA DEA 1.0 mg /l) or BH4 (4-NO_2BA DEA 1.0 mg /l and BAP 1.0 mg /l)hormonal balances

The callus growth variation is showed in Figure 3.The use of a concentration like 0.2 mg/l of 4-

NO_2BA DEA leads to a proportional increase in the duration of culture until about 16 days of culture and then it is followed by a period of stagnation for 3 days.After 19 days of culture it is observed a downward trend since the beginning of determinations. The use of the hormonal balance 4-NO_2BA DEA 0.2 mg/l and BAP 1.0 mg/l has led to the regression of callus throughout the period of the measurements leading to a lower value (103.5%) than that obtained in the first determination (109.5%).

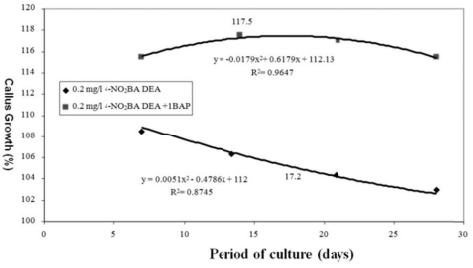

Figure 3. The growth rate of *Momordica charantia* L. callus under BH5 (4-NO_2BA DEA 0.2 mg/l)or BH6 (4-NO_2BA DEA 0.2 mg/l and 1.0 mg/l BAP) hormonal balances

CONCLUSIONS

Based on results carried out during our research we can conclude thatthe use of different concentrations of 4-NO_2BA DEA with or without BAP has a significant effect on the callus growth of *Momordica charantia* L.

Each of the hormonal balance used recorded a percentage increase of callus growth in the first 14 days of culture, followed by a slowing of the callus growth.

This preliminary study shows that the growth capacity of *Momordica charantia* L. tissue culture increases proportionally with the increasing concentrations of 4-NO_2BA DEA used.

REFERENCES

Budrat P., Shotipruk A., 2008. Extraction of Phenolic Compounds from Fruits of Bitter Melon (Momordica charantia) with Subcritical Water Extraction and Antioxidant Activities of These Extracts. Chiang Mai Journal of Science, 35(1):123-130.

Crisan M.E., Bourosh P., Maffei M.E., Forni A., Pieraccini S., Sironi M., Chumakov Y.M., 2014. Synthesis, Crystal Structure and Biological Activity of 2-Hydroxyethyl-ammonium Salt of *p*-Aminobenzoic Acid.Plos One, 9(7):e101892.

Crisan M, Grozav M, Bertea C., 2009. *Arabidopsis thaliana* seed germination and early seedling growth are inhibited by monoethanolamine salts of *para*-halogenated benzoic acids. Journal of Plant Interaction, 4(4)271DOI: 10.1080/17429140903063072.

Crisan M, Grozav M, Kurunczi L, Ilia G, Bertea C., 2007. Inhibitory Effects of some Synthetic Monoethanolamine Salts of *para*-Substituted Benzoic Acids and Corresponding Benzoic Acids on Cucumber Seed Germination. Journal of Plant Interaction, 2(1) 53–61.

Dempsey D.M.A., Shah J., KlessigD.F., 1999.Salicylic acid and disease resistance in plants.Critical reviews in plant sciences, 18:547-575.

Grover J.K., Yadav S.P., 2004. Pharmacological actions and potential uses of Momordica charantia: A Review. Journal of Ethnopharmacology, 93(1):123-132.

Kim D.O., Seung W.J., Lee C.Y.,2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry, 81(3):321-326.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3):473-497.

Ng P.L.L., Ferrarese M.L.L., Huber D.A., Ravagnani A.L.S., Ferrarese-Filho O., 2003. Canola (*Brassica napus* L.) seed germination influenced by cinnamic and benzoic acids and derivatives: Effects on peroxidase, Seed Science and Technology, 31(1):39–46.

Simina A., Botau D., Popescu S., 2014. The evaluation of somatic variability in the callus of bitter melon (*Momordica charantia* L.) using molecular methods. Journal of Horticulture, Forestry and Biotechnology,18(3):87-91.