

IN VITRO MICROPROPAGATION OF *COLEUS BLUMEI* BENTH SPECIES

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Abstract

A quick and efficient methods has been tested for micro propagation of *Coleus Blumei* Benth species from binodal mini-top grafting cuttings inoculated on a MS medium with several phytohormons: BA, K and 2IP in 0.5, 1.0 and 1.5 mgL⁻¹ concentrations, for multiplication and NAA and IAA in 0.5, 1.0 and 1.5 mgL⁻¹ concentrations for rhizogenesis induction. According to our results, the greatest percent of multiplication is obtained in the case of BAP 1.5 mgL⁻¹ medium and the rhizogenesis process is the most intens in the case of MS medium without any phytohormons.

Keywords: *Coleus Blumei* Benth, cytokinin, tissue culture, multiple shoots.

INTRODUCTION

Coleus Blumei Benth (sin. *Solenostemon scutellarioides*) is an annual plant, well known and appreciated amongst the horticulturists for the colours of its leaves (Fig.1). Their shape is oval having gnawed margins, lively coloured, with incarnadin red middle and green border. The flowers are very small along a central scape, having from purple to violet colour. *Coleus blumei* Benth originates from South Asia and the Phillipines, from where it arrived to tropical areas of Mexico and became well known amongst the Mazatec indiens, mainly for its psiho-active qualities (Schultes et al, 2001).

Very few aspects are known at the present regarding the alcaloids from the ctive component of *Coleus Blumei* Benth species. What is certainly known is the fact that the plant contains alcaloids (diterpene). At prezent there is formulated an hypothesis that when diterpens are dry and exposed to high temperatures suffer structural changes that make them look like the active alcaloids which can be found in *Salvia divinorum* (Ratsch, 2005).

Traditionally, *Coleus Blumei* has been used to cure several common diseases. Most frequently the Mazatec indiens have used this plant against stomach aches, digestive problems, dysentery and even elephantiasis. In other parts of the world this plant has been used to cure headaches, ulcers and even as a contraceptive, to prevent pregnancy. Conservation of vegetal resources as vitrocultures is one of the most important directions to apply

the tissue-culture and cell-culture. At the moment it is outlined the utility of the new biotechnologies as they are of great quality through diversification of genetic component of vegetal cells that are preserved and also from quantity point of view, through multiplication of the preserved species, used in different purposes. Vitroculture preservation implies the temporary reduction or even stopping of vital processes, at the level of inoculum cells, with the option of the possibility of bringing back the cultures to active, normal life by regenerating cells, tissue. Organs, the preserved genotype, without losing the morphofiziological and biochemical capacities of the cormophitoinoculums that are preserved, for a longer or shorter period of time (Cachiță et al, 2004).

Micropropagation is one of the most used instruments for preservation of certain rare elites and of species in extinction danger. It is an efficient mean for quick multiplication of species and for ex-situ preservation (Pratibha et al, 2011).

Previous researches regarding the establishment of quick protocols for this species multiplication include experiments using different kinds of inoculums: apical meristems on IAA medium (Smith and Murashige, 1982), leaves excerpts and knot segments on NAA și BA, (Gaurav et al, 2010), medium and knot segments (Rani et al, 2006). Other studies focused on obtaining rosmarin acid in callus of *Coleus* sp. (Razzaque and Ellis, 1997; Zenk et al, 1977, Verpoorte et al, 2002).

Other studies were targetted to see the resistance of the species to the induced stress of several salts (Collins et al, 1990). The presents study has been initiated in order to develop a protocol for quick multiplication of this species.



Fig. 1 *Coleus blumei* Benth

MATERIAL AND METHODS

Preparation of the inoculum

The plants that we used in our study have been provided by S.C. Sanplant Serv Agro SRL. For this experiment we used explants consisting of binodal apical mini-slips or subapical slips with two axial leaves.

The explants have been washed using tap water jet in order to remove the dust. Sterilization has been made by first immersion of the explants into 0.5 % Chlorox 0.1 % solution for 8 minutes and then by immersion in 0.1 % mercuric chloride for 5 minutes. In order to remove these solutions from the explants we washed them in distilled water for 3 minutes, 4 times.

The explant sections have been refreshed under the hood using sterile laminar flux and then they were inoculated on Murashige – Skoog (MS) medium with 24 g /l sucrose. The cultures have been kept at $23 \pm 2^\circ \text{C}$, with a photoperiod of 16 h light (the intensity of the light was $80 \text{ micromol m}^{-2} \text{s}^{-1}$) and 8 h dark. The shoots that we obtained have been individually used for following experiments.

Organogenesis of the explants

In order to test the effect of the growth controller substances on the shoot multiplication, we used 3 kind of cytokinins: 6-benzylaminopurine (BA), 6-furfurilaminopurine (K), 6-(2-izopentenil)adenine(2iP) with the following concentrations: 0.5, 1.0 and 1.5 mgL^{-1} .

The intensity of caulogenesis process was in terms of number of shoots regenerated on the explant, percent of the shoots towards the mark experiment (without phytohormones version) and also in terms of length of the shoots (cm).

Testing the capacity of auxines to induce formation of roots on shoots regenerated in vitro has been made by using MS medium with auxines improvement NAA and IAA in 0.5, 1.0 and 1.5 mgL^{-1} concentrations. In order to quantify the intensity of rhizogenesis process we established as indicator the number of days from inoculation to the appearance of the first root, the number of roots on the explant and the length of the roots. In this case also the mark experiment sample was the one on MS medium without phytohormones. Each experimental version had 3 repetitions. The results have been calculated as \pm SD average. For the interpretation of the results we used the analysis of the variance.

RESULTS AND DISCUSSION

The study referring to vitro seedling regeneration at binodal mini slips and their growth according to the nature and concentration of growth

regulators from the culture medium, has shown that regeneration of *Coleus blumei* Blenth can be obtained on MS media without phytohormons (58.5% from the inoculums have been regenerated, forming an average of 2.2 springs on the explants). The presence in the culture medium of BA 1.5 mg/L (Table 1) ensures a regeneration percent of the inoculums which is with 150.8% higher than the one from the medium without phytohormons.

In terms of regenerated explant spring number and height also, the medium variant with 1.5 mg BA /L is superior to the others.



Fig. 2 Highlight of micromultiplication process on MS medium with 1.5 mg BA /L (a) and with 1.5 mg /L 2IP (b)

In vitro *Coleus* seedling regeneration on media without growth controllers reduces the costs regarding the production of material for planting due to elimination of certain compounds from the culture media that usually have a high price.

Table 1.

The effect of cytokinines in the culture medium on the regenerating capacity of the shoots at 35 days from the inoculation moment

Conc.mg/L ⁻¹	Regeneration %	Height (cm)	Number of shoots regenerated on the explant
BAP			
0.5	74.0 ± 6.0	3.9 ± 0.3	3.4 ± 0.2
1.0	82.6 ± 3.8	4.2 ± 0.2	4.0 ± 0.4
1.5	92.5 ± 5.8	5.8 ± 0.4	4.5 ± 0.6
K			
0.5	61.3 ± 4.8	3.8 ± 0.2	3.1 ± 0.4
1.0	70.5 ± 9.6	4.1 ± 0.3	3.6 ± 0.5
1.5	74.4 ± 4.7	4.6 ± 0.2	4.1 ± 0.7
2IP			
0.5	66.2 ± 4.7	3.9 ± 0.3	2.2 ± 0.3
1.0	75.3 ± 5.3	4.0 ± 0.4	3.1 ± 0.5
1.5	80.1 ± 5.8	4.7 ± 0.3	3.4 ± 0.6
Control			
0.0	58.5 ± 6.2	3.2 ± 0.4	2.2 ± 0.3

The rhizogenesis process has been more intense on the medium without phytohormones. On this medium the roots have shown up earlier than on the other media variants (Table 2).

Table 2

The effect of auxines from the culture medium on the intensity of rhizogenesis process, after 25 days from the inoculation moment:

Conc.mg/L ⁻¹	Number of days till the first root appears	Number of roots /shoot	Average length of the roots (cm)
NAA			
0.1	8	6.2 ± 0.4	5.2±0.4
0.5	10	4.8±0.3	4.2±0.3
1.0	12	3.6±0.4	3.3±0.4
IAA			
0.1	7	6.8±0.2	3.8±0.5
0.5	9	5.7±0.4	3.3±0.2
1.0	11	4.6±0.3	2.8±0.1
Control			
0.0	8	7.3±0.4	5.8±0.3

In the case of using NAA as well as IAA, the lower concentrations have stimulated in a greater measure formation of roots than in the case of using higher concentrations.



Foto. 3



Foto .4

Fig. 3 High light of rhizogenesis process on MS medium with NAA mg/L⁻¹ (Foto.3) and on MS without auxines. (Foto.4)

This result can be explained by the high endogene auxine contained available in the vitro seedling.

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