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SHORT COMMUNICATION

EFFECTS OF HORMONES ADDITION FOR *IN VITRO* PLANT DEVELOPMENT OF *CALENDULLA OFFICINALIS*

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Abstract: The current study presented *in vitro* plant development of *Calendula officinalis* on MS (Murashige and Skoog) medium supplemented with different formula of hormones. The morphogenetic response was evaluated by recording the number of plantlets, the plant height and weight. The results showed that small amounts of hormones can improve the plantlets growth in the case of *Calendula officinalis*.

Keywords: *Calendula officinalis, hormones, medicinal plant, plant growth, plantlets*

INTRODUCTION

Common marigold (*Calendula officinalis*) is a well-known plant widely used in traditional medicine as an important source for the production of phytomedicines and cosmetics especially ointments and extracts.
In the flowers and the leaves of marigold different classes of active compounds were identified: flavonoids, coumarines, quinones, triterpenoids, carotenoids, volatile oil, lipids and amino acids [1].
Plant pharmacological studies have suggested that *Calendula* extracts may have antioxidant, anti-inflammatory, anti-tumor properties [1 - 4]. For that reason, *in vitro* proliferation of *Calendula officinalis* can constitute a significant step in supplying the demand for such plants.
It is well-known that the technique of plant-tissue culture represents an alternative for the improvement of crops in a short period of time. The growth and morphogenesis of plant tissue cultures can be improved by small amounts of hormones. It is very important to find the appropriate hormone for developing a specie on culture media [5].
Previously, researches on plant development of *Caledula officinalis* on hormone-free medium show that low rates of plant formation are registered [6].
Our research aims is to highlight *in vitro* behavior of various explants of *Calendula officinalis*, cultured on several nutritive solutions, in view of elaborating a technology of unconventional propagation of the species and exploring the possibility to obtain economically valuable somaclonal variations.
This study evaluated the influence of hormones addition for *in vitro* plant development of *Calendula officinalis.*

MATERIALS AND METHODS

The research was conducted using as biologic material the selected seeds of *Calendula officinalis* provided by Botanical Garden from Iasi (Romania). The seeds were sterilized and inoculated *in vitro* on the hormone-free Murashige and Skoog (MS) medium [7] for obtaining sterile plantlets. Apex and nodes were used as explants source for testing the morphogenetic reaction on MS medium supplemented with three hormones: IAA (indoleacetic acid), BAP (benzylaminopurine) and IBA (indole-3-butyric acid) in different concentrations (Table 1).

Table 1. *The hormones concentration used for testing the in vitro morphogenetic reaction of explants of Calendula officinalis*

Medium	Concentration of hormones [mg·L^{-1}]		
	IAA	BAP	IBA
A_2	2	-	-
B_1	-	1	-
B_2	-	2	-
BA_1	0.1	1	-
BA_2	0.5	1	-
BB_1	-	1	0.1
BB_2	-	1	0.5

After 1 month, growth rate and biomass accumulation were evaluated for all samples and compared with the control sample (without addition of hormones).
For that, after removal of culture medium, the number of plantlets, the plant height and weight were recorded.
Each experiment was performed in duplicate.

RESULTS AND DISCUSSION

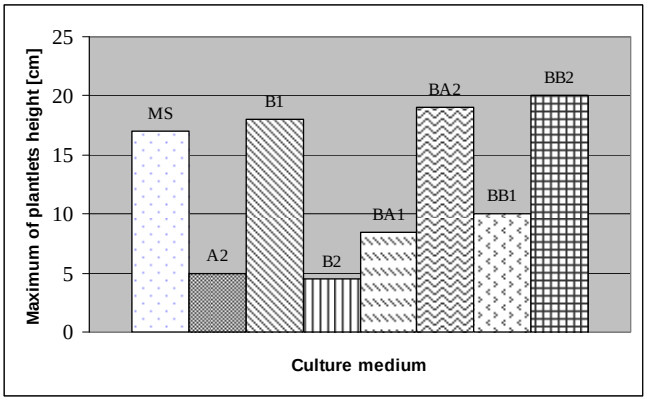
The results of morphogenetic response of *Calendula officinalis* plantlets within 30 days are presented in Table 2.

Table 2. *Morphogenetic response of Calendula officinalis plantlets*

	Medium							
	MS	**A_2**	**B_1**	**B_2**	**BA_1**	**BA_2**	**BB_1**	**BB_2**
Average of plantlets height [cm]	12.8	3.7	6.6	3.5	4.7	8.8	7.7	11.3
Maximum of plantlets height [cm]	17	5	18	4.5	8.4	19	10	20
Weight of roots [g]	1.73	0.17	1.04	0.07	0.78	**1.06**	0.69	0.47
Weight of leaves [g]	1.78	0.55	1.71	0.43	1.67	**1.85**	0.92	**1.98**

As shown in Figure 1, the increasing rates of plantlets height and the leaves weight compared with the control sample (MS) proved that two medium supplemented with hormones (BB_2 and BA_2 formulas) are efficient for plantlets growth.

a)



b)

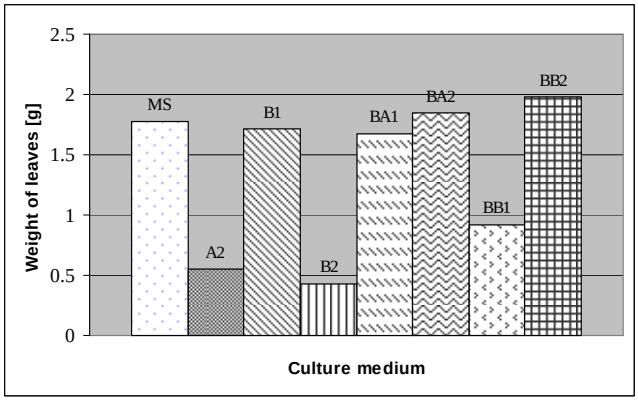


Figure 1. *Graphics of plantlets height (a) and leaves weight (b)*

The highest rate for leaves growth was obtained using BB_2 medium followed by BA_2 medium as can also be observed in Figure 2.

a) *b)* *c)*

***Figure 2**. Samples of Caledula officinalis on different growth medium a) MS, b) BA_2, c) BB_2*

Use of IAA as hormone growth (medium A_2) proves that this acts like an inhibitor.
Similar results to those of hormone-free medium (MS) concerning the leaves growth are obtained in the case of B_1 medium when the concentration in BPA is 1 $mg \cdot L^{-1}$.
Correlating the results with the concentrations of hormones, it can be easily observed that increasing the concentration of BPA to 2 $mg \cdot L^{-1}$ (B_2 medium) growth inhibition occurs at both the leaves and roots.
In the case of BA_2 which consist in adding of IAA (0.5 $mg \cdot L^{-1}$) and BPA (1 $mg \cdot L^{-1}$) in the MS medium, a small synergistic effect on leaves growth was found consisting in a 4 % increase of leaves weight.
A more pronounced synergistic effect was observed in the case of BB_2 medium in which IAA was replaced with IBA (0.5 $mg \cdot L^{-1}$), the increasing of leaves weight being 11 %.

CONCLUSIONS

The results of this research showed that small amounts of hormones can improve the plantlets growth in the case of *Calendula officinalis*.
Among the seven medium supplemented with hormones used in this study, only two (BB_2 and BA_2) stimulate plant growth especially of the leaves. The best results were registered in the case of BB_2 medium.
The most evident inhibitor medium for both growth of leaves and roots is B_2 medium.
The preliminary qualitative analysis of metabolites accumulated in plantlets by thin layer chromatography (TLC) and UV-VIS spectroscopy analysis of the alcoholic extracts is underway and will be the subject to a future publication.

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