THE EFFECTS OF COCONUT EXTRACT ADDED TO THE CULTURE MEDIUM ON THE "IN VITRO" MULTIPLICATION OF *CHYSANTHEMUM MORIFOLIUM* RAMAT

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Abstract

Coconut milk extract experiments on Chrysanthemum morifolium Ramat. were initiated with plantules which were obtained in vitro, from which the apex was removed and then placed on different variants of medium: M-control and V_1 - V_9 with added coconut extract, either alone or mixed with auxine or cytoquinine. After two months of culturing on the above mentioned mediums, the best rate of multiplication and root system differentiation was obtained on the medium with 1 mg/l coconut extract + BA - 0.1 mg/l, cca. 20 plantlets/explant, with 5.1 cm in hight, with a mean of 30 roots/explant with cca. 2.5 cm in length each. The coconut milk extract was proven to be a good stimulator for the multiplication of Chrysanthemum morifolium Ramat., either on its own, or in combination with other phytohormones. In great doses of 5-10%, and in the presence of BA, it inhibits the formation of a root system.

Key words: *Chysanthemum morifolium* Ramat., apex, extract, coconut, benzyl adenine, βindolil butiric acid, multiplication, regeneration.

INTRODUCTION

The *Chrysanthemum morifolium* Ramat. species is one of the flower species of great economic and ornamental interest, a fact which justifies research done towards the optimization of *in vitro* multiplication in its case. (Zăpârţan, M şi Cachiţă-Cosma D.,1982a and 1982b). Depending on the hormonal or medium balance, research has studied the regenerative ability of different varieties of chrysanthemum (Zăpârţan, M., and Cachiţă-Cosma D.,1989), followed by morphogenesis concerning different types of chrysanthemum explants (Zăpârţan, M**.** and Cachiţă-Cosma D.,1983), as well as certain aspects of ultrastructure of chrysanthemum plant cells obtained *in vitro* (Zăpârţan, M., et al., 1984).

From the very beginning of the experiments on *in vitro* tissue cultures and plant cells, different extracts were added to the culture medium, such as: beer yeast extract, hydrolyzed casein, citrus extract, coconut milk, immature endosperm extract from certain seeds, etc., which were proven to have complex effects, and were used generally in small concentrations of up to 1 g/l (Cachiţă – Cosma, D. 1987). Natural extract sollution composition is complex and little known, having an experimentally proven role in the rapid *in vitro* multiplication of certain species, and in the induction of plant regeneration from callus. (Butiuc, A.L., and Zăpârţan M.,1996; Yamagishi, M., 1995).

Of the effects natural extracts have when used in the composition of culturing mediums, we would like to mention the stimulating effect on the *in vitro* differentiation of mini-bulbs in certain species which multiply via bulbs or tuber-bulbs. (Zăpârțan M., 1996,Laslo V.2006,Laslo V 2011). The use of natural extracts for *in vitro* plan regeneration via somatic embryogenesis is also well known. (Schiak, C. E., et al., 1996).

This research aims to use coconut milk in culture mediums, both for *in vitro* plant tissue regeneration and as a substitute for hormones in the medium, considering the fact that their cost is high. This study uses results obtained towards this purpose in some vegetal species of spontaneous flora as reference. (Butiuc, A. L., and Deliu C., 2000).

MATERIALS AND METHODS

The plant material used was the *apex* (cca. 0.8 cm), harvested from chrysanthemum plants from an aseptic environment, inoculated on different medium variants (Table 1), using the Murashige. T., Skoog A, 1962 medium as a base. The coconut milk extract was administered in three doses, 1g/l, 4 g/l and 8 g/l respectively. The experimental variants were devised in such a way as to allow for a control sample (M), followed by the mediums containing only coconut extract (V_1, V_2, V_3), then the mediums containing extract with an added auxine (V_4, V_5 and V_6) or cytoquinine (V_7, V_8 and V_9). The final, mixed variants were devised in such a way as to observe the combined effect of natural extract with phytohormones.

After inoculation, explants were maintained in growth chamber condition, at a temperature of cca. 29°C, illuminated by flourescent tubes (87 μmol/m^2/s) for 16 hours out of 24. The culture was evaluated after cca. 2 months (8 weeks), observing the multiplication of the apex (the number of plantlets/apex and their length) and the differentiation of the root system (root number and length).

Table 1

Coconut milk content culture mediums
(MS = T. Murashige., Skoog, *A*.,1962 medium; AIB = βindolil butyric acid; BA = benzyl amino purine)

Var.	MB	Coconut extract(Ec) (g/l)	Auxine (mg/l)	Cytoquinine (mg/l)
M	MS	Proba martor	-	-
V_1	MS	1.0 g/l	-	-
V_2	MS	4.0 g/l	-	-
V_3	MS	8.0 g/l	-	-
V_4	MS	1.0 g/l	0.1 mg/l AIB	-
V_5	MS	4.0 g/l	0.1 mg/l AIB	-
V_6	MS	8.0 g/l	0.1 mg/l AIB	-
V_7	MS	1.0 g/l	-	0.1mg/l BA
V_8	MS	4.0 g/l	-	0.1mg/l BA
V_9	MS	8.0 g/l	-	0.1mg/l BA

RESULTS AND DISCUSSION

After the *in vitro* cultivation period, variants were analyzed, observed and measured, and the mean result of the observed parameters was recorded in Table 2. *Plan regeneration* was the parameter which excelled even on surprising variants. The mean regeneration rate of plantlets was good, ranging between 5 plantlets on the control sample up to 20 plantlets on some variants with coconut extract. The best results were observed on mediums with a high dose of extract (V_3, V_6 and V_9), but the highest number of plantlets was obtained in the case of the mixture between 1g/l of extract + 0.1 mg/l BA (V_7). In this particular case, plant height exceeded the 5 cm mean, which means the combination of extract with cytoquinine was beneficial.

Table 2

In vitro multiplication and root formation in *Chrysanthemum* morifolium Ramat. on coconut extract culture mediums

Variante	No. plantlets/explant	Length of plantlets(cm)	No. roots/explant	Length of roots(cm)
M	5.2 ± 0.80	1.20 ± 0.20	9.2 ± 0.83	1.50 ± 0.12
V_1	8.5 ± 0.60	**5.05 ± 0.20**	**15.0** ± 1.60	1.75 ± 0.20
V_2	11.6 ± 1.15	3.50 ± 0.10	10.4 ± 0.90	1.85 ± 0.05
V_3	13.5 ± 1.82	4.50 ± 0.10	**13.1** ± 1.30	**1.95** ± 0.05
V_4	13.6 ± 1.20	4.45 ± 0.10	11.0 ± 1.20	2.06 ± 0.10
V_5	10.0 ± 0.80	2.30 ± 0.17	**15.0** ± 1.10	**2.25** ± 0. 20
V_6	3.40 ± 0.90	**3.00** ± 0.10	4.5 ± 0.60	1.80 ± 0.10
V_7	**20.0** ± 1.00	5.10 ± 0.10	**30**.0 ± 1.30	2.40 ± 0.10
V_8	4.0 ± 0.5	2.30 ± 0.15	-	-
V_9	13.0 ± 1.00	4.40 ± 0.10	-	-

Chrysanthemum plantlet evolution, their number and length are also represented in fig. 1, which evidently shows the stimulating effect of coconut extract in its highest concentration (8 g/l), but not only that, also illustrating the combination between 1 g/l extract + auxine (AIB), the V_4 variant, on which the mean plantule regeneration rate is over 13 plantules/apex, reaching almost 5 cm in height. We'd also like to point out a well known fact - multiplication is registered on the control sample (M) as well, albeit at a decreased rate and height. Generally the plantlet height is almost level (between 2 and 5 cm), reaching slightly higher values on variants with combined natural extract and phytohormones.

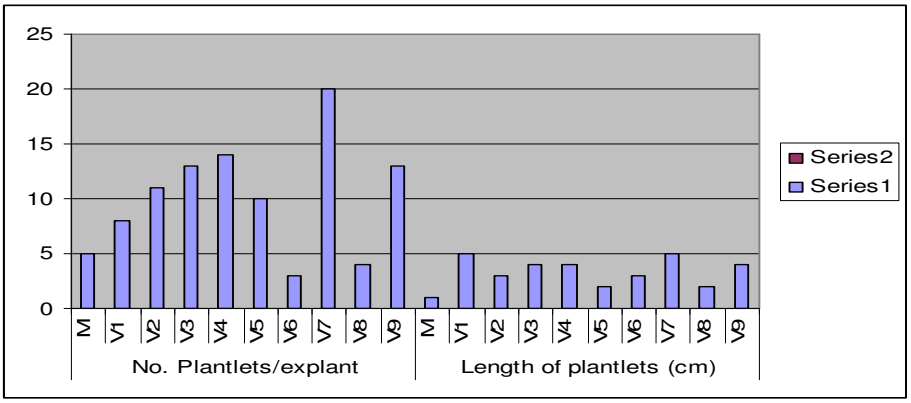

Fig. 1. Chrysanthemum plantlet formation and length on medium with coconut extract

The *root system* shows good evolution, but we ecounter a phenomenon observed for the very first time with this species, namely that in the presence of large concentrations of extract (4-8%) + BA, roots do not differentiate. The phenomenon was ardently observed, and the fact that even after a further two months of cultivation the root system did not differentiate was taken note of. It appears that coconut extract in high concentrations, combined with a cytoquinine, inhibits root formation. Adversely, in the case of small extract concentration with added BA (V_7), the highest number of roots is generated - cca. 30/plantlet, of cca. 2.4 cms in length (see Fig. 2). Other variants yiealded a balanced root system, both in terms of root number and length.

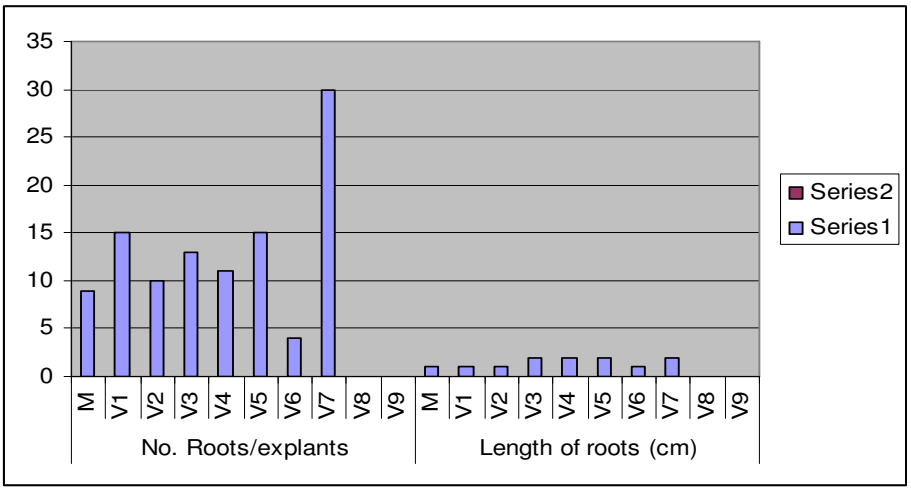

Fig. 2. Root system differentiation from chrysanthemum apex cultivated on mediums with added coconut extract

CONCLUSIONS

1. *Chrysanthemum morifolium* Ramat. apex yiealds multiplication even hormone-free medium (cca. 5 plantlets/explant), but in a smaller ratio than on the other variants
2. On coconut milk extract exclusive mediums (V_1, V_2, V_3), multiplication is good, as well as the formation of superior root systems.
3. The combination of an auxine (AIB) and the natural extract has a beneficial effect in smaller extract concentrations (1% and 4% respectively), resulting in cca. 10-13 plantlets, with a root system of 11-15 roots/plantlet.
4. Coconut extract mixed with BA favors massive multiplication in low concentrations (1%) amd the complete organization of unformed chrysanthemum plants.
5. High concentrations of extract (4% and 8% respectively) when mixed with BA (V_8, V_9), inhibit the formation of a root system (V_8, V_9), a phenomenon not previously encountered in other species and other types of extract.

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