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„IN VITRO" MULTIPLICATION OF *CHRYSANTHEMUM MORIFOLIUM RAMAT.*

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Abstract: The tissue-cultured shoot primordia have distinct characteristics of high stability of chromosome number, high ability of regeneration and rapid proliferation. Rapid propagation of *Chrysanthemum morifolium Ramat*. "Prince de Monaco" and "Romica" was achieved through tissue culture technique. The procedure involved aseptic culture of shoot tips followed by rapid shoot multiplication, rooting and finally establishment of plantlets in soil. The agar solidified MS medium containing 2 mg/l BAP and 0,002 mg/l NAA was optimum for rapid mass production of plantlets. These can be rooted on MS medium in the absence of growth regulators. The indirect micropropagation of *Chrysanthemum morifolium Ramat.* was achieved through callus cultures obtained from single-node stem segments excised from other two cultivars of *Chrysanthemum morifolium Ramat.:* "Escorte" and "La Cagouille", belonging to the collection of Botanical Garden from Iasi.
Key words: *Chrysanthemum*, micropropagation, shoot tips, callus

Introduction

The modern approaches of plant propagation based on cell and tissue culture techniques are able to increase the efficiency of breeding processes [1], [2]. They offer opportunities for rapid clonal propagation of some unique, superior genotypes[3], [4]. The unconventional techniques permit the multiplication and maintenance of these genotypes.

Material and methods

Shoot apices, detached with a small portion of cotyledonary and hypocotyle tissues were sterilized with calcium hypochlorite 3 % and cultured „ in vitro" in 12 hrs light and 8 hrs darkness dayly at 24º C on MS medium with 2mg/l BAP and 0,002 mg/l NAA. Shoots of 2-3 cm, elongated „in vitro" were rooted on the MS medium both in the absence of growth regulators and by adding low concentration of BAP (2mg/l).

Explants consisting in single-node stem segments were cultured on MS medium with 1mg/l 2,4 D and 2mg/l BAP. The shoot fragments were sterilized by sinking in Na hypoclorite 3% for 7-8 minutes. The explants were rinsed several times with distilled sterile water. The capacity of shoot differentiation was observed on MS medium with 2mg/l BAP and 0,002 mg/l NAA. Shoots of 2-3 cm, elongated "in vitro" were rooted on the MS medium in the absence of growth regulators. The regenerated plants were transferred "ex vitro" to soil and the first few days were protected with a transparent cover and watered with water at room temperature.. The culture substrate that has been used consisted of a mixture of soil. This mixture has been sterilized by autoclaving.

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Table 1-Nutritive media used for micropropagation of *Chrysanthemum morifolium Ramat.*

Variant	Basal medium	Hormones	Reactions
1	MS	2mg/l BAP 1mg/l 2,4 D	callus induction
2	MS	2mg/l BAP 0,002mg/l NAA	shoot induction
3	MS	2mg/l BAP	shoot elongation
4	MS	-	root induction

Results and discussions

One of the main aims of „in vitro" cultures is to increase the rate of clonal, vegetative multiplication of important cultivars and to produce pathogen- free plants

We studied the „in vitro" response of survival and rooting of the microcuttings excised from two cultivars of *Chrysanthemum morifolium Ramat.*: „Prince de Monaco" and „Romica"

Explants consisting in apices generated 1,8-2 cm long shoots with 4-6 pairs of normal leaves, in about one month (Photo 1 and 3).

Every shoot may be considered a microcutting that may be used for studyng their behaviour on media which permite rooting. The multiple shoots displayed a rapid elongation capacity .

The multiple shoot cultures can be maintained for long periods by regular subculturing and detaching the most elongated shoots.

When the shoots growing on MS medium with 2mg/l BAP and 0,002 mg/l NAA were transferred to a medium lacking the growth regulators, differentiation of a large number of roots occurred

The autonomous regenerated plants displayed an vigurous growth and adaptative capacity to the normal environment (Photo 2 and 4). This step was performed by transferring the plants on soil and covering the aerial parts with transparent plastic for maintaining high humidity.

It were obtained and multiplicated the calli of other two cultivars of *Chrysanthemum morifolium Ramat*. :"Escorte" and "La Cagouille"which have been subjected to regeneration.

The process of callus formation was favoured by relatively high citokinine (2mg/l BAP) concentrations in the MS medium and a photoperiode of 12/12. The calli developed after two weeks of culture were compact in texture and brownish in colour Following the subculturing of the 30 day-old callus resulted in the development at the initial culture of some callus islands having a green- yellowish colour and a disperse structure. At the level of these calli, organogenic structures occurred sporadically. (Photo 5 and 9).This callus was generally friable, green-yellow in colour. After six weeks of incubation, one of these callus masses formed adventitious shoots buds from cells close to the callus surface .


Photo 1 and 2- Caulogenesis on explants consisting in shoot apices and regenerated plants of *Chrysanthemum morifolium Ramat*. (Prince de Monaco)

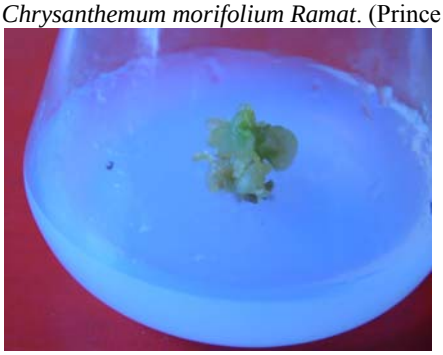


Photo 3 and 4.- Caulogenesis on explants consisting in shoot apices and regenerated plants of *Chrysanthemum morifolium Ramat*. (Romica)

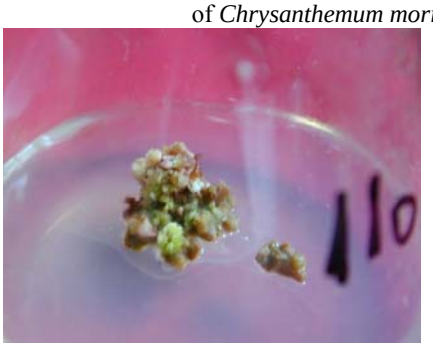


Photo 5 and 6- Callus induction from single node stem explants and stages of caulogenesis at *Chrysanthemum morifolium Ramat (La Cagouille* **)**


Photo 7 and 8- Shoots differentiation from callus and regenerated plants at *Chrysanthemum morifolium Ramat (La Cagouille)*

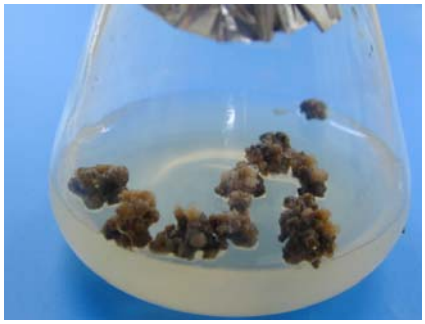


Photo 9 and 10- Callus induction from single node stem explants and shoots differentiation from callus *at Chrysanthemum morifolium Ramat.(Escorte)*

Photo 11- Regenerated plants at Chrysanthemum morifolium Ramat.(Escorte)

The regeneration of plants from callus was influenced by several factors. BAP alone did not induce shoot formation, although it did in cultures of the stem segments of the same species.This would imply that organogenetic reactions are considerably influenced by the source of explant and the hormonal balance. BAP in conjunction with NAA triggered bud initiation.

The regenerative potential of callus was observed to be greater in primary calli cultures than in calli from older cultures.

The capacity of shoot differentiation was observed on MS medium with 2mg/l BAP and 0,002 mg/l NAA (Photo 6 and 10).

Shoots of 2-3 cm, elongated "in vitro" were rooted on the MS medium in the absence of growth regulators (Photo 11). The both cultivars responded in a similar way.

Plantlets developed on this medium were transferred to soil and gave rise to normal plants (Photo 8 and 11).

Conclusions

1.The investigations led to the establishing of an efficient technique for clonal plant production at four cultivars of *Chrysanthemum morifolium Ramat*.:"Prince de Monaco", „Romica", „La Cagouille" and „Escorte".

2.The basal medium MS supplemented with 2mg/l BAP and 0,02 mg/l NAA for shoot tip cultures is mostly beneficial to regenerate large numbers of shoots suitable for large-scale plant regeneration.

3.The complete plantlets were obtained in about 2 month.

4.The capacity for shoot differentiation depended on concentration of cytokinin in the shoot-induction medium and age of the callus cultures.

5.For root induction, shoots were excised and transferred to MS medium lacking growth regulators.

6.The four cultivars of *Chrysanthemum morifolium Ramat*. regenerated "in vitro"displayed an vigorous growth capacity to the natural environment.

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