

IN VITRO CULTIVATION OF THE ENDEMIC SPECIES *ANDRYALA LEVITOMENTOSA*

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Keywords: *Andryala levitomentosa*, callus, embryogenesis

Abstract: In vitro cultivation of the endemic and threatened species *Andryala levitomentosa* represents an unconventional strategy and action plan for the biological diversity conservation. This plant is considered one of the rarest species in the European flora and in Romania it is founded only on „Pietrosul Brostenilor” mountain. The micropropagation of *Andryala levitomentosa* implies measures which should allow to conserve and perpetuate this species. The plants of *Andryala levitomentosa* have been regenerated from callus cultures. The callus cultures were established from stem and leaves explants on MS medium, supplemented with indolylacetic acid and benzylaminopurine.

INTRODUCTION

In vitro cultivation of the endemic species *Andryala levitomentosa* represents an alternative to conserve and multiply plant resources (Dihoru and Pârnu, 1987), (Dihoru and Dihoru, 1994).

Somatic embryogenesis and subsequent plants of *Andryala levitomentosa* have been obtained from callus cultures (Păunescu Aiftimie and Vântu, 2002). The micropropagation was achieved through tissue culture technique and involved callus induction followed by somatic embryos development, rooting and establishment of plantlets in soil.

MATERIAL AND METHODS

The callus cultures were established from stem and leaves explants on MS medium, supplemented with IAA and BAP (Table 1). The different variants of MS medium (I, III, IV) were used for callus induction (Vântu et Sărăcuțu, 2005), (Vântu, 2006) The stem and leaves explants were sterilized for 10-15 minutes in sodium hypochlorite solution 3 % and washed with sterilized distilled water

The regeneration of *Andryala levitomentosa* were obtained through somatic embryogenesis on callus cultures derived from leaves explants. The embryogenic structures were induced on callus cultivated on MS medium, supplemented with 2mg/l BAP and 0,2 mg/l IAA. They were isolated and cultivated for maturation on the same basal medium without growth regulators.

Table 1-The variants of MS medium

The variants of basal MS medium	Auxins- IAA mg/l	Cytokinins- BAP mg/l
I	0,2	0,2
II	0,2	2
III	2	0,2
IV	2	2
V	-	-

IAA-indolylacetic acid; BAP- benzylaminopurine

RESULTS AND DISCUSSION

The primary callus cultures were obtained after 4 weeks from the in vitro cultivation, when the entire explant dedifferentiated. The callus cultures obtained on agar medium were multiplied.

Considerable variation in the frequency of callus formation were observed on different type of explants. Some variation in callus growth rate and regeneration potential was noted among leave and stem explants.

This callus derived from leaves explants was generally friable, green-yellow in colour (Photo 1). After six weeks of incubation, one of these callus masses formed organized structures.

Embryogenic lines were formed and maintained upon transfer to MS medium, supplemented with growth regulators.

The use a combination of a synthetic cytokinin such as BAP in excess and an auxine led to a increase in the frequency of somatic embryos differentiation in callus cultures.

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

The embryogenic structures were induced on callus cultivated on MS medium, supplemented with BAP and IAA. They were isolated and cultivated for maturation on the same basal medium without growth regulators. Early stages of somatic embryogenesis were observed after a week from the initiation of cultures (Photo 2, 3, 4, 5).

It were encountered the specific steps of embryo development: globular, cardo, torpedo and cotyledonary stages

The main factors for somatic embryogenesis induction were: the selection of cells, the use of growth regulators in some concentrations and combinations, the photoperiode.

Indirect regeneration of plantlets through somatic embryogenesis is a three step process: callus induction, embryo development, followed by plants acclimatization. Fully grown plantlets were finally transferred to the pots containing a mixture of soil (Photo 6, 7, 8).The pots were maintained for 2 weeks in a growth chamber, before they were transferred to field conditions

The acclimatization of regenerated plants was concerned in transfer to nonsterile conditions with humidity control and temperature control.

The regenerated plants were transferred in soil and grown in a controlled environment chamber with 16 hours photoperiod at 24°C.

CONCLUSIONS

The capacity for callus formation depends on type of explants.

Callus cultures obtained from leaves had a greater proliferation capacity than those obtained from stems.

The morphogenetical potential has been established to be greater in primary callus cultures derived from leave explants.

The embryogenic callus was obtained on MS medium containing 2mg/l BAP and 0,2 mg/l IAA.

The use a combination of a synthetic cytokinins such as BAP in excess and a auxine led to a increase in the frequency of somatic embryos differentiation in callus cultures.

The maturation of somatic embryos was achieved in MS medium without growth regulators.

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Photo 1- Initiation of callus cultures



Photo 2- Initiation of somatic embryogenesis

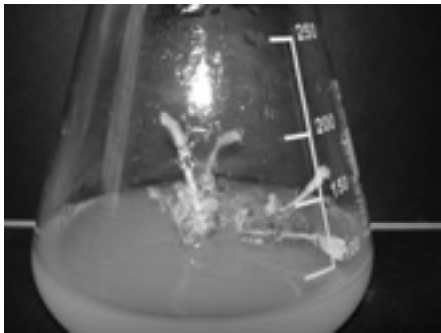


Photo 3- Somatic embryos development

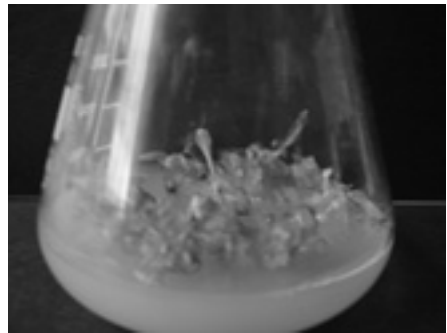


Photo 4- Stages of somatic embryogenesis



Photo 5- Stages of somatic embryogenesis



Photo 6- Regenerated plants

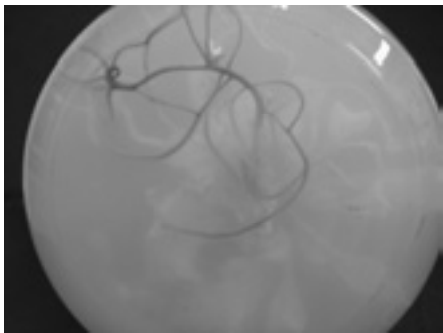


Photo 7- Roots development



Photo 8- Plants acclimatization