MICROPROPAGATION OF THE ENDEMIC SPECIES FOR ROMANIAN FLORA *ANDRYALA LEVITOMENTOSA* (E. NYÁR.) SELL.

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In this paper are presented the stages of micropropagation of the endemic species *Andryala levitomentosa* for the first time. This plant is considered one of the rarest and endangered species in European flora.

Key words: micropropagation, endemic species, *Andryala levitomentosa.*

INTRODUCTION

According to the flora and vegetation studies in the European flora there are only five species of *Andryala* genus: *A.agardhii, A.laxiflora, A.levitomentosa, A.integrifolia* and *A.rangusina.* Among these *A.levitomentosa* is the only one belonging to this genus in Romanian flora. It was quite recently discovered (Nyárády, 1961) and accepted as Romanian phytoendemit.

This plant is considered one of the rarest and endangered species in the European flora. It is found only on "Pietrosul Broştenilor" Mountain, on "Pietrosul Bogoloin" summit, being the only member of this genus which grows at such a high Northern latitude (47°), like the other species belonging to the Mediterranean zone (42°).

This relict plant makes botanists raise a lot of questions about its restrictive ecology and peculiarities of multiplication (merely by apomixis). Since 1961, a lot of botanists have tested the seed germination and the results show that the seeds are completely infertile. The multiplication merely on vegetative basis seems to be the main cause of the stenochory of this species.

Our purpose is to regenerate the whole plant by micropropagation and to repopulate the classic place and other areas with this endangered species.

MATERIAL AND METHODS

The samples were collected from "Pietrosul Broştenilor" Mountain, 1800m high. At the time of the collection (1st of July 2001) we appreciate that there are almost 200 plants.

The induction of callus culture was established on an MS (Murashige-Skoog) medium supplemented with 1 mg/l 2,4-D (2,4-dichlorphenoxyacetic acid) and 1 mg/l K (kinetin). Stem (rhizome and aerial stem) and leaf (petiole and lamina) 1 × 0.5 cm tissue fragments, were used as sources of explant.

The culture was transferred monthly to fresh medium in order to homogenize the growth of the callus. During the experiments the callus was grown at darkness and 18–19°C, in the culture room. We selected each inoculum from calluses with the same color and texture and aseptically weight about 0.5 g/inoculum. For regeneration each inoculum of *Andryala* callus, weighing about 0.5 g, was selected from stock callus culture (maintained on supplemented medium) and placed 3–4 per culture dish, on MS medium with no phytohormones. During the differentiation and the regeneration the inoculum was maintained at 20–23°C, with 16 hours photoperiod at 2 700 lux and transferred monthly to fresh medium.

Microphotographs were taken from Nikon Eclipse E-600 microscope with the Nikon E-950 camera.

RESULTS

Micropropagation of the endemic species *Andryala levitomentosa* was based on the inducing of regenerative process "via callus".

After the inoculation, callus developing has started after 3–4 weeks but only by foliar (lamina and petiole) explants. This was characterized by a hypertrophy of the tissues associated with a color modification from green to yellow.

After 6 weeks it becomes obvious the cell proliferation as small protuberances. In the first stage they were developed on the extremity of the explants but in 7–10 weeks morphogenetic zones comprises the whole explant. From the aspect and texture point of view the callus was heterogeneous, being formed by yellow-white and translucent nodule of undifferentiated tissue – meristemoids – from which new cells and adventitious structure arise, and yellow-brown compact islands, too. This texture reflects the functional differentiation of the callus in a meristematic and in a parenchymatouse one.

For somatic embryogenesis we inoculate callus fragments on an inductive MS medium, without hormones.

After 2 weeks from inoculation it was obvious the hypertrophy of the callus and changes in initial aspect, also. The color of the embryogenic callus becomes green. From this, by successive processes of differentiation were regenerated embryos (after 16 weeks) and in approximately 22 weeks, organs or organ-like structures (roots, shoots, etc.), so the regeneration process was complete.

CONCLUSIONS

Our studies and observations indicated different reactivity of the explant type used. In experimental conditions we used, the petiole explants presented a higher reactivity as compared with stem (rhizome and aerial stem) and lamina. This

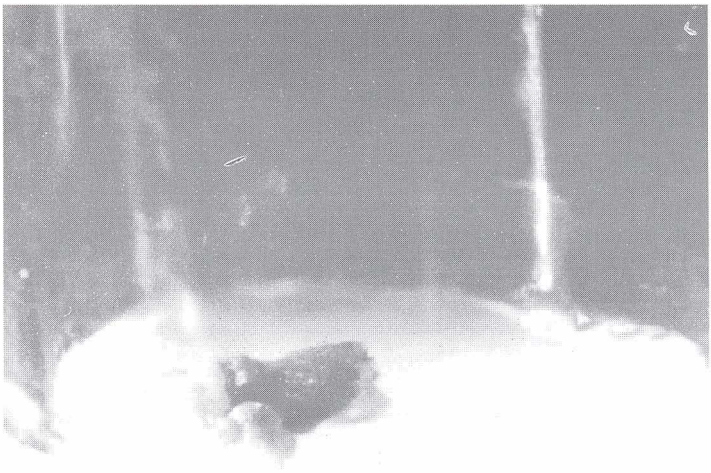


Plate I – Fig.1 – initial undifferentiated callus.

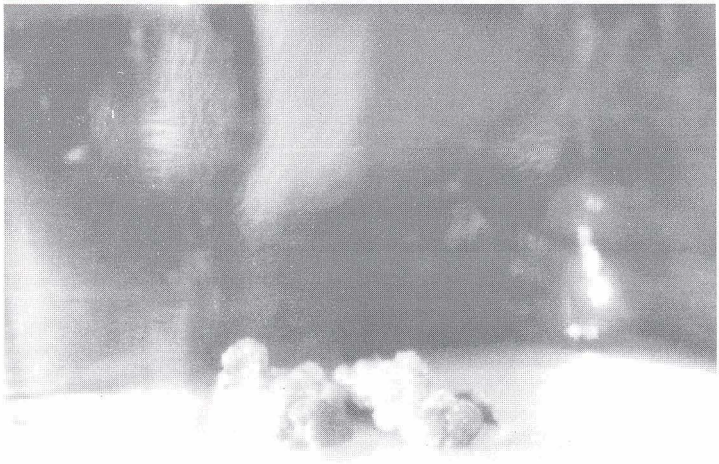


Plate I – Fig. 2 – undifferentiated callus.

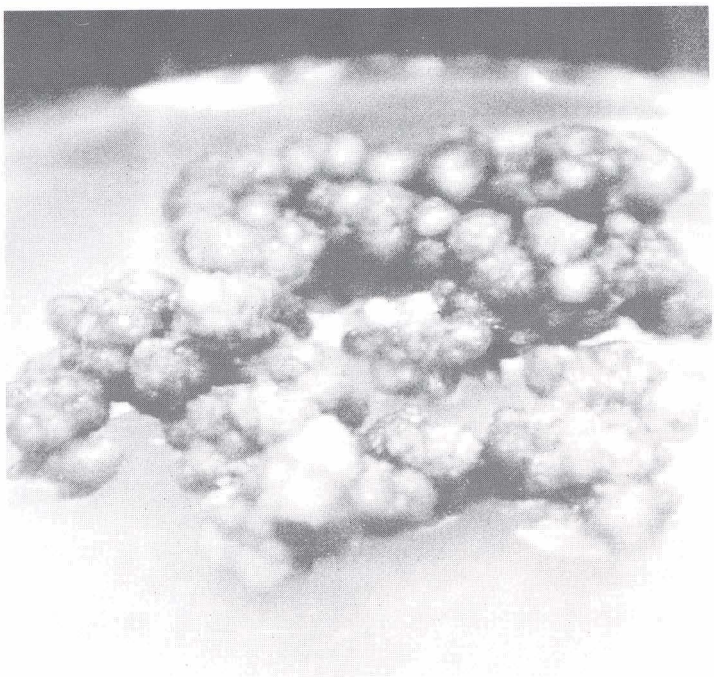


Plate I – Fig. 3 – undifferentiated proliferated callus.

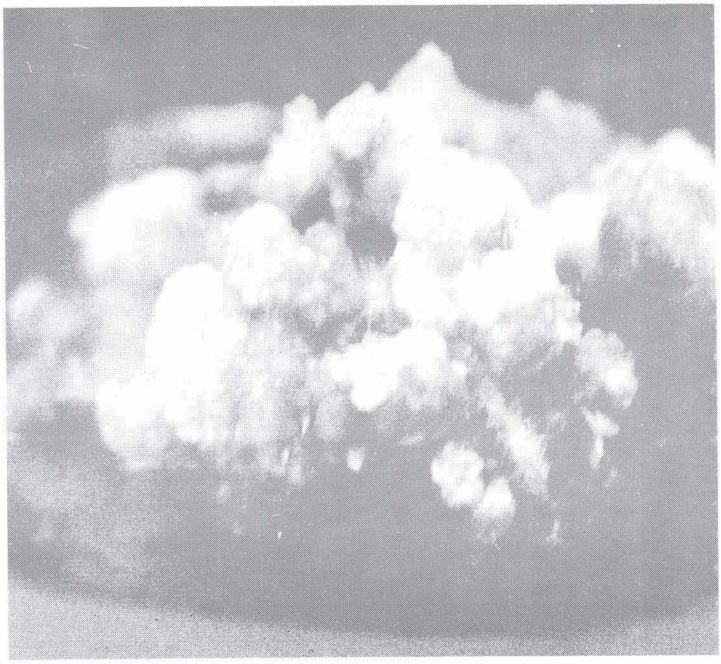
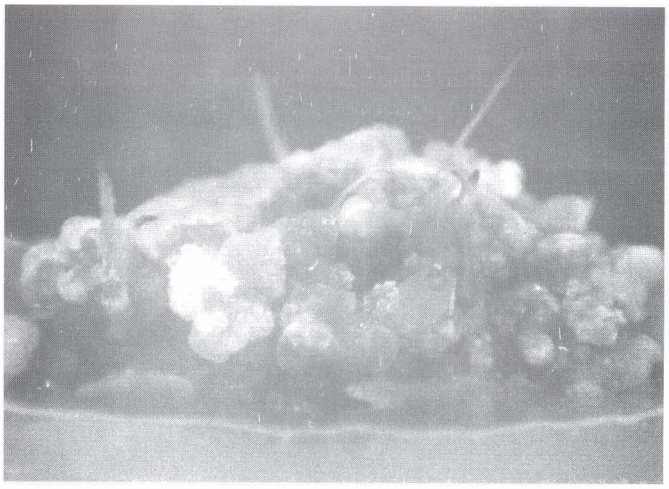
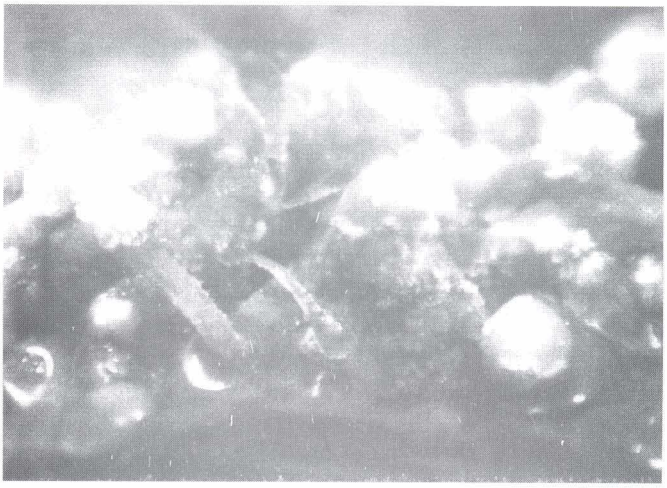


Plate I – Fig. 4 – embryogenic callus.





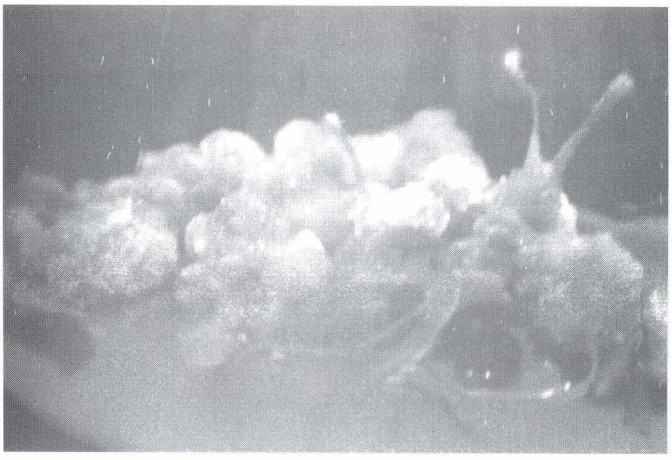


Plate II – Fig. 5–7 – embryogenic callus in different stages of differentiation.

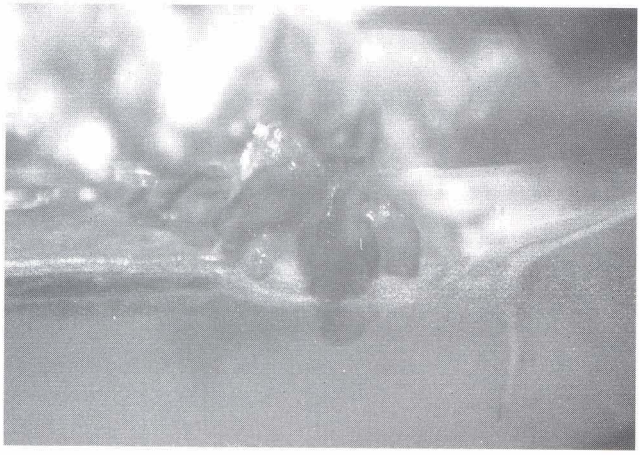


Plate II – Fig. 8–9 – embryogenic callus in different stages of differentiation.

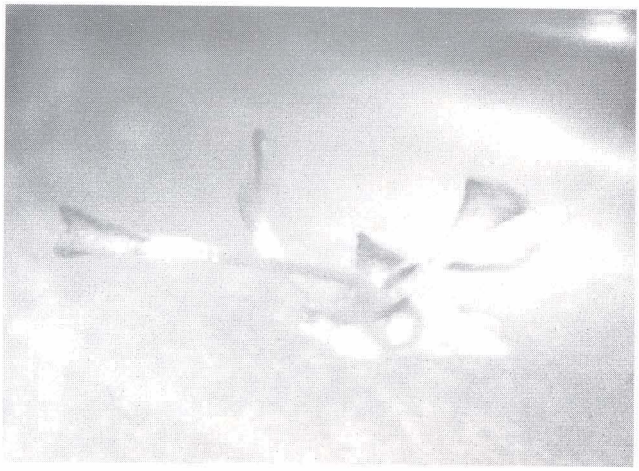


Plate II – Fig. 10 – plantlet.

recommended it as a source of explant which could be successfully used in micropropagation experiments of *Andryala levitomentosa.*

The start of callus developing process was up to 6 weeks after inoculation.

Callus has morphological and functional differentiation.

Somatic embryogenesis begins 3 weeks after the inoculation on inductive medium.

The differentiation is complete 22 weeks after the transfer on inductive medium.

We can conclude that micropropagation is a valuable method for multiplication of this rare endemic plant. *In vitro* culture also, offers the possibility to preserve this unique and endangered species in the Romanian flora.

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