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PRELIMINARY RESEARCH ON *ARNICA MONTANA* L. PROVIDED *IN VITRO*

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INTRODUCTION

Arnica montana L. is a perennial species belonging to the Compositae family spread in the mountain areas of Romania; it grows merely in the whole Europe. It can be found in Asia and North America as well. The ethimology of the word appears to be the "arnikos" (lamb leather). Its folklore names are: mountain arnica, fairies' chariot, mountain podbal. It prefers the acid well-drained soil and sunny meadows. It is an endangered species in its native habitat. The vegetal product (*Arnicae flores*) consists of the its capituliform inflorescences. The flowers are harvested during June and July and should be dried immediately after harvesting. As it is protected by law in many countries, another source for the vegetal product is *Arnica chamissonis* Less ssp. *foliosa* (Nutt.) Maguire.

Arnica comprises: anti-inflammatory sesquiterpenic lactons (among which the most valuable for medicine is the helenaline), flavonoids (responsible for the tonifying action on the blood vessels, and for the local anti-inflammatory property as well), polyphenols and polyacetilens (antimicrobial and antifungical). The vegetal product contains 0.2 – 0.35% volatile oil (coloured in orange), and of a semisolid consistency. Arnica serves mainly for external use. It may be administered internally with much causion (as an infusion, tincture, oily extract) in hyperpressure, respiratory stimulant. Its most important properties are: antiseptic, wound healing, antitheumatic, analgesic, being cicatrisant, antirheumatic, analgesic; it is recommended in the treatment of sprains, dislocations, bruises, haematoma, edema associated with fractures, superficial phlebitis, inflammation caused by insect stings, inflammation of the mucous membrane. Arnica is part of more than 270 industrial products. Used as tinctures, ointments, gels, infusions, gargles, cataplasms. The internal use should be avoided or accomplished with extreme causion, because the sesquiterpenic lactons are very toxic (3, 4, 5).

The volatile oil, very pretious for therapeutical purposes, lead to the excessive harvesting of this species. This fact, together with the intensive agriculture and with the fragmentation of this species' habitat endangered *Arnica montana* (it is included in the Red List in Romania as a rare and vulnerable species)(1). The benefits of micropropagation are that they offer a better control of the genetic material; several crops are provided in vitro in a shorter period of time, whilst the conventional cultures require more time; the disadvantage of season cyclicity is surpassed.

Our research's aim was the use of the *in vitro* micropropagation to preserve this endangered species by repopulating some native habitats. In addition to that, several comparative studies on the germination rate, on the morphogenetic response, and some phytochemical tests as well will be effected on vegetal matter originating in Romania and in Germany.

MATERIAL AND METHODS

Our research started with the *in vitro* culture initiation on *Arnica montana* L. from seeds brought from the Botanical Garden in Oberholz, Germany. The most effective method to provide the seedlings was by germination of previously disinfested seeds directly on sterile solid culture medium variants.
The germination capacity for this species was previously tested (9).

The disinfestation of seeds followed some steps: 1. A former sterilization in absolute ethyl alcohol 10"; 2. Disinfestation 5-10" in HgCl2 $1^0/_{00}$, followed by 5 rinses in sterile distilled water. The *in vitro* provided regenerants will be tested phytochemically. The medium formuli used were Murashige-Skoog (1962) (tab.1) and other MS variants, as well (A2, BB2, BD), various combinations and concentrations of growth regulators (auxins and cytokinins). The charbon source was saccharose (30 g/l); the agar-agar (8,5 g/l) solidified the medium culture. After culture mediums preparation pH fixing with NaOH (5.5), they were sterilized in an autoclave at 121°C for 20 minutes, the pressure being 1 atm. The inoculated vials were incubated in a room with half-climatised conditions within the Genetics and Biotechnologies Laboratory of the "Vasile Alecsandri" University of Bacău (temperature of about 22^o C, light 2500 lux, photoperiod 16/8 hours).

Table 1. The culture mediums used during the experiments

Species	Medium variant	Basal medium	Amount of growth regulators (mg/l)					
			IAA	IBA	BAP	KIN	2.4-D	GA3
Arnica montana L.	MS	MS	-	-	-	-	-	-
	A2	MS	2.0	-	-	-	-	-
	BD	MS	-	-	1.0	-	0.5	-
	BB2	MS	-	1.0	2.0	-	-	-

IAA = indole acetic acid; NAA = naphtyl acetic acid; BAP = benzylaminopurine;
KIN = kinetine; IBA = indole butiric acid; 2.4-D = diclorfenoxiacetic acid

RESULTS AND DISCUSSIONS

There were some previous tests regarding the morphogenetic reaction of *Arnica montana* in the scientific journals (2,6). Our preliminary studies reavealed the following : the morphogenetic response on the A2 medium variant was extremely favourable, the multiple shoots grew intensely on this variant. This medium stimulated the root growth (the root length was of about 3 cm, the merystematic root tips could be well observed). On the basal hormone free MS, the shoots were characterised by a better length growth compared to the previous culture medium (3-7 cm in height). The multiple shooting was absent. The roots are very long, extremely thin, without secondary ramifications.

The BD medium variant provided friable average proliferative callus, cream greenish colour. 50 % of the explants provided callus as well, and shoots via callus (indirect caulogenesis). The shoots had small thick dark green leaves. It was observed that the rhyzogenesis was absent on this medium variant. The best morphogenetic response was evinced on the BB2 medium variant, consisting of: multiple vigorous shoots, and a rapid shoot growth. The rhyzogenesis was well represented on this medium variant (the roots were more developed and greener compared to the rest of the tested variants). The main root's length reaches 10 cm, and it is endowed with secondary ramifications.

CONCLUSIONS

The plantlets grown from seeds germinated on sterile medium variants (Figure 1) are an important explant source in view of micropropagation. Regarding the morphogenetic response, there was ascertained that: the best caulogenetic response was provided on the BB2 medium variant, followed by: A2, MS, BD. The best rhyzogenesis was obtained on BB2 medium variant, as well; the rhyzogenesis was absent on the BD variant, that provided callus, and shoots via callus (Figures 2-6).

The *in vitro* plants were acclimatized, and they will be further tested phytochemically.

The shoots provided by means of micropropagation are meant to repopulate some of this species' native habitats.

ABSTRACT

Arnica montana L. is a rare and valuable medicinal species. Arnica (mountain arnica) is a plant in the family Compositae, about 30-50 cm tall, with yellow flowers that bloom in June-July. The inflorescences are used (Arnicae flos) and they contain volatile oil (0.05-0.15%). Used externally as an anti-inflammatory, and in external bruises, sprains, arthritis, phlebitis and thrombophlebitis, venotonic, varicose veins, etc. Used internally, it causes gastrointestinal disorders and hypertension. The study aims at the *in vitro* micropropagation of this species, in view of repopulating some native habitats of this species in Eastern Romania. Further phytochemical and biometrical comparative studies will be accomplished on two genotypes of *Arnica montana* L.

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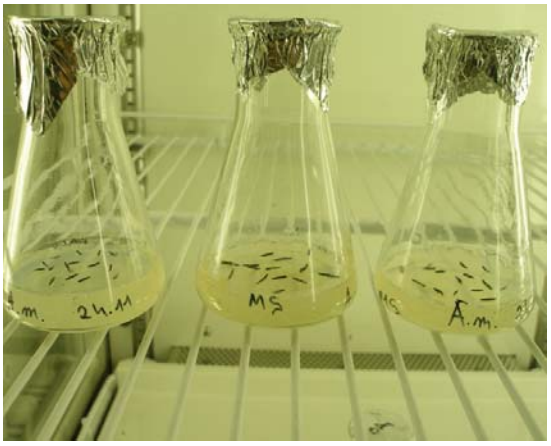

Figure 1. Arnica sterile seeds on solid medium culture in the growth chamber

Figure 2. Multiple shoots of *Arnica montana* on the BB2 medium variant

Figure 3. Arnica shoots on A2 medium

Figure 4. Cream greenish friable callus on BD medium variant

Figure 5. *In vitro* culture vials in the half-climatised growth chamber

Figure 6. *Arnica montana in vitro* (the morphogenetic response on different medium variants)