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# THE YIELD OF *ARNICA MONTANA* L. PLANTLETS BY MEANS OF BIOTECHNOLOGIES IN VIEW OF REPOPULATING THE SPECIES' NATIVE HABITAT

***Diana-Elena Maftei, Loredana - Iuliana Petrea***

***Key words****: Arnica montana, in vitro, habitat preservation*

## INTRODUCTION

This present scientific study is a sequel of our previous research on this valuable medicinal species [5, 6, 7], aiming to provide as many arnica plants by means of *in vitro* cultures in view of repopulating some native habitats of this species in Eastern Romania. *Arnica montana* (Photo 1) is a well-known and valued species; its inflorescences are mainly used (*Arnicae flos*) in the pharmaceutical industry. 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 hundreds of industrial products (e.g. tinctures, ointments, gels, infusions, gargles, cataplasms)[3, 6, 11, 12].

The species bears many folk names in various countries: in England - mountain tabacco, wolfbane, leopard's bane, mountain arnica; in Denmark: gouldlomme, bjerg volverlei; in France: arnica, amique panaceea des chutes, tabac des vosges, souci des alpes, amique des montagnes; in Italy: arnica; in Germany: arnika, kraftwurz, fallkraut; in Russia: arnica gornaia, amika, barannik; in Hungary: mariafii, arnika, arnyekfii, amgylitoefii [8, 10, 11, 12]. Matthaeus Sylvaticus named the species *arnich,* a word of Arab origin. [8].

Photo 1. *Arnica montana* L. (in bloom) (http://www.google.ro)

## 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 [5]. It was intriguing and rather difficult to provide new plantlets from germinating seeds. The references mention a 80% yield for arnica germination. The source of seeds was diverse: we tested a genotype from the Botanical Garden in Oberholz (Germany), a genotype from a population in Teleorman (Romania), and one from Gârda de Sus (Gheţari village). Some of our lab tests were not successfull. The best germination yield was achieved using the populations from Gârda de Sus and from Oberholz.

A part of the new plantlets did not survive in lab conditions, as the species is an alpine one, with requirements specific for its native habitat. Seeds of all the tested genotypes were placed in Petri dishes, as well, either on moist filter paper, or on moist cotton wrapped in sterile gause. Despite the applied pre-treatments, the seeds did not start to germinate.

The pre-treatments were: a seed batch from all the tested genotypes were treated with hydrogen peroxide ($H_2O_2$) and subsequently immersed in a fertilizer (Atonik); half of the seeds were grown in soil pots afterwards, and half on Petri dishes to induce germination.

Other pre-treatments applied to the seeds consisted of their maintainance in a solution of giberellic acid for 6 hours at 20°C, subsequently placed in still water in a refrigerator for 24 hours. The seeds were inoculated on the sterile culture medium variants MS, BA1, and BGA.

The most efficient method to provide seedlings was by seed germination (previously sterilized in ethylic alcohol and HgCl2) directly on sterile culture medium (Oberholz genotype) and by seed germination directly on soil (the genotype from Gârda de Sus)(photo 6, 8).

The seeds from Gârda de Sus were characterized by a good germination on the soil pots surface (in our laboratory). The soil was an acidic one, favourable for alpine species (pH = 3).

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 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 comprising many variants of the culture medium (table 1) 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).

## RESULTS AND DISCUSSIONS

This present scientific study is a sequel of our previous research on this valuable medicinal species [5, 6, 7], thoroughly studying many references on arnica [3, 4, 9, 13], in order to provide as many arnica plants by mean of *in vitro* cultures in view of repopulating some native habitats of this species in Eastern Romania.

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 caulogentic response was very appropriate on the newly tested medium variants ZB and BK. 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.

Table 1. The culture medium variants used within the experiments

| Species | Medium variant | Basic medium | Amount of growth regulators (mg/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | IAA | IBA | BAP | KIN | 2.4-D | ZEA | NAA | GA3 |
| *Arnica montana* L. | MS | MS | - | - | - | - | - | - | - | - |
| | Half MS | MS | - | - | - | - | - | - | - | - |
| | A2 | MS | 2.0 | - | - | - | - | - | - | - |
| | BD | MS | - | - | 0.5-1.0 | - | 0.5 | - | - | - |
| | BB2 | MS | - | 1.0 | 2.0 | - | - | - | - | - |
| | BB1 | MS | - | 0.5 | 1.0 | - | - | - | - | - |
| | BA1 | MS | 0.5 | - | 1.0 | - | - | - | - | - |
| | BK | MS | - | - | 1.0 | 0.5 | - | - | - | - |
| | BN | MS | - | - | 0.5 | - | - | - | 0.5 | - |
| | ZB | MS | - | 0.5 | - | - | - | 2.0 | - | - |
| | BGA | MS | 0.5 | - | 0.5 | - | - | - | - | 0.5 |

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

## CONCLUSIONS

*Arnica montana* is a rare and vulnerable species, and it is on the Red List of IUCN [2]. The advantages of micropropagation are: a better genetic control on the plant species, several successive harvests in a shorter period of time as if in conventional cultures, regardless of the seasons. The septic and the asptic germination of the arnica seeds, as well as the in vitro seedlings' growth are time-consuming, thorough processes. The plant growth (either in soil pots, or on sterile medium culture variants) is rather slow for all the studied arnica genotypes.

The plantlets grown from seeds germinated on sterile medium variants (photo 8) 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: BK, ZB, 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 (photos 9-13). 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.


Photo 2. Seeds of *Arnica montana* L.( Gârda de Sus population)

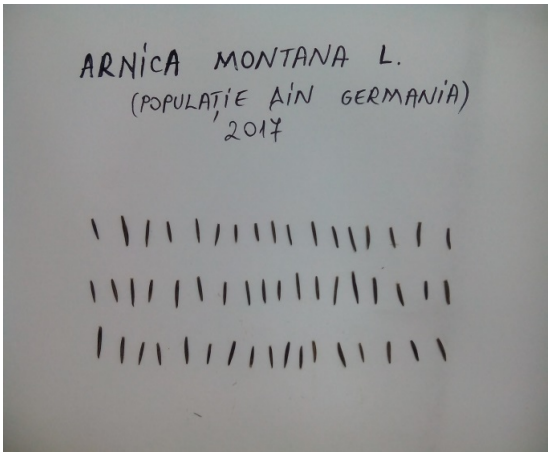


Photo 3. Seeds of *Arnica montana* L. (Oberholz, Germany)


Photo 4. Germinating seeds on moist filter paper in Petri dishes.


Photo 5. Germinating seeds on moist cotton in Petri dishes.

Photo 6. Seedlings provided by germinated seeds on soil pots (Gârda de Sus population)

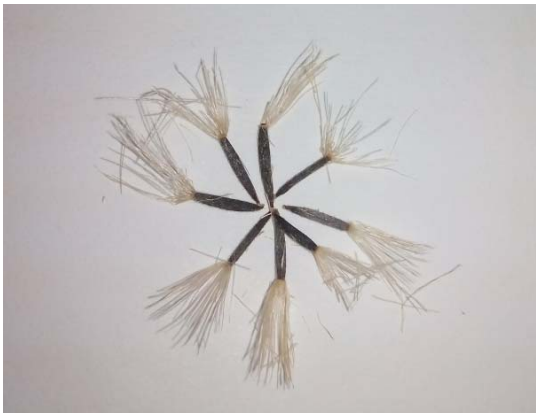

Photo 7. *Arnica montana* L. seeds with pappus.

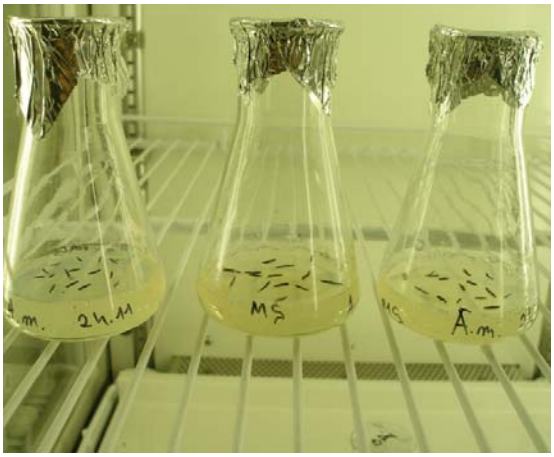

Photo 8. Sterile arnica seeds on solid culture medium in the *in vitro* growth chamber

Photo 9. *Arnica montana* shoots on the BB2 medium

Photo 10. Arnica seedlings on the A2 medium variant

Photo 11. Friable cream and green callus BD

Photo 12. Vials with arnica shoots in the *in vitro* growth chamber

Photo 13. *Arnica montana in vitro* (several madium culture variants)


## ABSTRACT

*Arnica montana* L. is one of the most rare and valuable medicinal species, one of the best-known homeopathic remedies. It is an alpine plant, growing in nutrient-poor soil. It can potentially reach a height of 60 cm, but this is unusual given the harsh conditions at high altitudes. It grows in meadows up to 3,000 metres above sea level, where it is exposed to strong sunlight. The higher the altitude, the more aromatic the plant will become. It is found throughout Europe.

Arnica (mountain arnica) is included in the Compositae family, about 30cm tall, with yellow flowers that bloom in June-July. The inflorescences are used (*Arnicae flos*) for treatment and they comprise 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 to provide seedlings and plantlets of mountain arnica by conventional culture and by means of plant biotechnologies, in view of repopulating some native habitats of this species in Eastern Romania. Further phytochemical and biometrical comparative studies will be accomplished on several genotypes of *Arnica montana* L.


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**AUTHORS' ADDRESS**

MAFTEI DIANA ELENA – „Vasile Alecsandri" University of Bacău, Faculty of Science, e-mail: diana.maftei@ub.ro;

PETREA LOREDANA – IULIANA – „Vasile Alecsandri" University of Bacău, Faculty of Science, e-mail lollyana.petrea@yahoo.com.