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STUDY OF THE PROPAGATION POTENTIAL OF *ARNICA MONTANA* L. USING CONVENTIONAL AND *IN VITRO* METHODS

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ABSTRACT

Arnica montana L. is a medicinal species of high importance, widely used in the pharmaceutical and cosmetic industries. This species shows moderate suitability for cultivation, preferring acidic, well-drained soils and conditions similar to natural mountain habitats. However, reduced germination capacity and slow development of young plants require the use of adapted propagation techniques. In this context, three propagation methods were evaluated at INCDCSZ Brașov: clump division, sowing in seedbeds and in vitro seed germination. Clump division proved suitable for the propagation of Arnica montana L., ensuring a high establishment rate (98 %), despite its limited multiplication potential. The percentage of emerged Arnica montana L. plants 14 days after sowing was influenced by substrate composition. The most favorable treatment was the mixture consisting of 2/3 black peat and 1/3 perlite. Assessment of germination capacity revealed a higher mean germination percentage under in vitro conditions on MS medium than in Petri dishes, indicating the favorable potential of MS medium for Arnica montana L. seed germination. This experiment demonstrates that Arnica montana L. can be propagated using combined methods and that the choice of substrate and propagation technique influences germination success and plant development.

INTRODUCTION

Arnica montana L. is a European mountain species, with Romania hosting some of the most important conservation areas within the eastern part of the species' natural range. It is characteristic of oligotrophic and mesotrophic alpine grasslands across Europe, occurring at altitudes of up to 3,000 m (Schmidt, 2023). In Romania, the species occurs in alpine habitats at elevations ranging from 800 to 1,800 m within the Carpathian mountain range (Maftei & Maftei, 2018), including the Western, Eastern, and Southern Carpathians. The Apuseni Mountains and northern Moldavia represent important centers of distribution for the species in Romania and Central and Eastern Europe. Romania is considered one of the major European countries playing an important role in the conservation of the genetic resources of this species (Wolfgang 2006, Michler et al. 2008).

Arnica montana L. is a medicinally important species, with the inflorescences being the main plant material used for therapeutic purposes and harvested at the beginning of flowering (Scarlat & Tăhăneanu, 2019). The species is widely used in the pharmaceutical and cosmetic industries due to its high content of bioactive compounds, including sesquiterpene lactones, flavonoids, and volatile oils. These compounds are responsible for its anti-inflammatory, antioxidant, antimicrobial, analgesic, and wound-healing properties. The main bioactive compounds, particularly sesquiterpene lactones such as arnicin and helenalin, contribute to reducing inflammation, relieving muscle and joint pain, and accelerating the healing of wounds and bruises.

The increasing demand for plant raw material, together with the limited capacity for natural regeneration and the degradation of specific habitats, has led to a decline in wild populations of *Arnica montana* L. in many regions of Europe. In addition, climate change in recent years represents another major risk factor for wild populations. Increasing temperatures, reduced precipitation during the growing season, and the occurrence of drought during the flowering stage may result in lower inflorescence production, thereby negatively affecting the natural regeneration of the species. Consequently, *Arnica montana* L. is subject to protection and conservation measures, while the development of sustainable cultivation and propagation systems represents a priority for biodiversity conservation.

In the context of increasing pressure on natural populations due to intensive harvesting and habitat degradation, the optimization of propagation methods represents an essential step towards the introduction and expansion of *Arnica montana* L. cultivation. *In vitro* culture represents an attractive, cost-effective, and environmentally friendly approach for large-scale plant production, the propagation and conservation of species that are difficult to regenerate using conventional methods, and the production of valuable metabolites of commercial interest (Chakarova et al. 2026, Petrova et al. 2025, Bakhtiar et al. 2026). Given the increasing demand for *Arnica montana* L., in vitro propagation is considered a viable alternative. It has been demonstrated that key factors such as explant type, culture medium composition, and plant growth regulators influence the efficiency of micropropagation in (Bakhtiar et al. 2026, Conchou et al. 1992, Petrova et al. 2021).

Both conventional methods, based on seed propagation and clump division, and *in vitro* propagation biotechnologies offer solutions for obtaining healthy, uniform plant material adapted to the requirements of commercial production and conservation programmes. Evaluating the efficiency of these methods contributes to identifying optimal strategies for the production of planting material and the sustainable utilization of this species of high medicinal value. Given its protected status, the introduction of *Arnica montana* L. into cultivation represents a sustainable approach to biodiversity conservation while providing a reliable source of raw material for the pharmaceutical and cosmetic industries.

MATERIAL AND METHODS

The low germination rate and slow development of *Arnica montana* L. plants require the use of adapted propagation techniques. In this context, at the National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Brașov, three propagation methods were evaluated during the 2024–2026 period: clump division, seed sowing in nursery beds, and in vitro seed germination.

1. Vegetative propagation by clump division

Arnica montana L. develops rhizomes and lateral rosettes, which allow vegetative propagation through the division of mature clumps (Sugier et al., 2013). Clump division was performed by separating the mother plants into fragments containing rhizomes and early-stage buds, with each fragment having the potential for regeneration (Figure 1). The divided plant material was planted in pots at the end of March, and establishment and initial development were carried out under protected conditions in a greenhouse. The plants obtained were subsequently transplanted into the experimental field at the end of April, after developing a minimum of six leaves in the rosette, thus ensuring good acclimatization to outdoor environmental conditions.



Figure 1. Division of mother plant clumps and planting of rhizomes in pots (Original photograph by Nina Bărăscu)

2. Seed propagation

Seed propagation represents the natural reproductive method of the species and is an important means of maintaining the genetic variability of populations (Sugier et al., 2013). Seeds were collected in 2025 from plants obtained through clump division and cultivated in the experimental field in 2024. The seeds were stored over winter at a temperature of 16–18 °C and were used in 2026 for propagation, with their germination capacity being assessed first. Thus, at the beginning of April 2026, 30 seeds were placed for germination in each of three covered Petri dishes on a moist substrate consisting of filter paper (Figure 2).

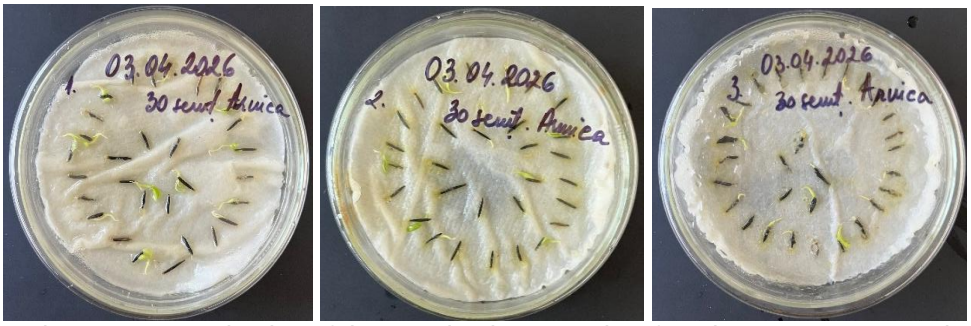


Figure 2. Determination of the germination capacity of *Arnica montana* L. seeds (Original photograph by Nina Bărăscu)

Numerous authors recommend producing seedlings under protected conditions and subsequently transplanting them into the field, as this method ensures a higher establishment rate than direct sowing (Sugier et al., 2013). Therefore, seedling production in nursery beds was selected for seed propagation, using several substrate formulations. To maintain constant moisture in the substrate and ensure adequate drainage and aeration, thereby preventing substrate compaction and promoting uniform germination and emergence, vermiculite, zeolite, and perlite were used in mixtures with black peat at ratios of 1:2 and 1:3, while black peat alone was used as the control substrate. The influence of the substrate on seedling emergence was tested. Thus, six substrate mixture treatments and one control treatment were established, with each treatment consisting of four replicates. Fourteen seeds were used for each replicate: V1 – mixture of 2/3 black peat and 1/3 vermiculite; V2 – mixture of 2/3 black peat and 1/3 zeolite; V3 – mixture of 2/3 black peat and 1/3 perlite; V4 – mixture of 3/4 black peat and 1/4 vermiculite; V5 – mixture of 3/4 black peat and 1/4 zeolite; V6 – mixture of 3/4 black peat and 1/4 perlite; V7 – black peat – control. The percentage of emerged plants was determined 14 days after sowing.

3. In vitro propagation

In parallel with the germination capacity assessment in Petri dishes, seed germination was also evaluated under *in vitro* conditions on Murashige and Skoog (MS) culture medium. Since 2024, research on *Arnica montana* L. has been conducted at the NIRDPSB Brașov, focusing on seed sterilization, culture media for seed germination, and nutrient media for plantlet micropropagation. For seed disinfection, 70 % ethyl alcohol was used as the sterilizing agent. In the literature, in addition to ethanol, other sterilizing agents have been reported, including commercial bleach based on sodium hypochlorite (Chakarova et al., 2026; Bakhtiar et al., 2026; Petrova et al., 2025), detergents, antibiotics, fungicides, and chlorine (Nieto-Trujillo et al., 2021), sodium hypochlorite alone (Kolesnyk et al., 2025), or mercuric chloride (Panciu et al., 2014).

In vitro propagation involved the inoculation of *Arnica montana* L. seeds (Figure 3) onto (MS) culture medium (Murashige and Skoog, 1962), without the addition of plant growth regulators. A total of 35 seeds were inoculated per replicate, with three replicates established.



Figure 3. *Arnica montana* L. seeds and their in vitro germination (Original photograph by Mihaela Cioloca)

The *Arnica montana* L. plantlets obtained from seed germination were transferred to the multiplication medium, which had the following composition: MS

basal medium, 4.4 g/L; sucrose, 30 g/L; agar, 8.8 g/L; 6-benzylaminopurine (BAP), 1 mg/L; kinetin (KIN), 1 mg/L; α-naphthaleneacetic acid (NAA), 0.2 mg/L; gibberellic acid (GA_3), 0.25 mg/L; vitamin C, 20 mg/L; and activated charcoal, 0.5 g/L.

The culture medium was adjusted to pH 5.8. Sterilization of the medium was performed by autoclaving for 20 min at 121 °C. After sterilization, the medium was dispensed into polypropylene vessels (100 mL per vessel), which were sealed with lids equipped with a gas-exchange filter. The culture vessels were incubated in a growth chamber (Figure 4) under controlled light conditions (16/8 h photoperiod) and temperature (22 ± 2 °C). The plantlets were periodically subcultured onto fresh medium.



Figure 4. In vitro micropropagation of *Arnica montana* L. (Original photograph by Mihaela Cioloca)

The experimental data were analyzed using descriptive statistics, including the mean, minimum and maximum values, standard deviation, median, percentiles, and coefficient of variation. Differences among treatments were assessed using analysis of variance (ANOVA), while mean comparisons were performed based on least significant differences (LSD) and the corresponding statistical significance levels. The results were graphically represented using Microsoft Excel, with Chart-type graphs. The relationships among the experimental treatments were analyzed and visualized using Matrix plots in PAST 5.

RESULTS AND DISCUSSIONS

1. Vegetative propagation by clump division

Clump division showed a limited multiplication potential, as only a small number of viable propagules could be obtained from each mature clump. Nevertheless, the method resulted in a high establishment rate (98 %), confirming the good viability and adaptability of the obtained plants. Potted plants were transplanted into the experimental field one month after clump division. (Figure 5).



Figure 5. Growth and development of plants transplanted from pots to the experimental field (Original photograph by Nina Bărăscu)

2. Seed propagation

The substrate factor had a differential effect on the emergence of *Arnica montana* L. seedlings 14 days after sowing. The most favorable treatment was V3, consisting of 2/3 black peat and 1/3 perlite, which resulted in a significant 29.2% increase compared with the control. In contrast, substrates containing zeolite resulted in the lowest emergence values, with a highly significant reduction of 41.7% compared with the control.

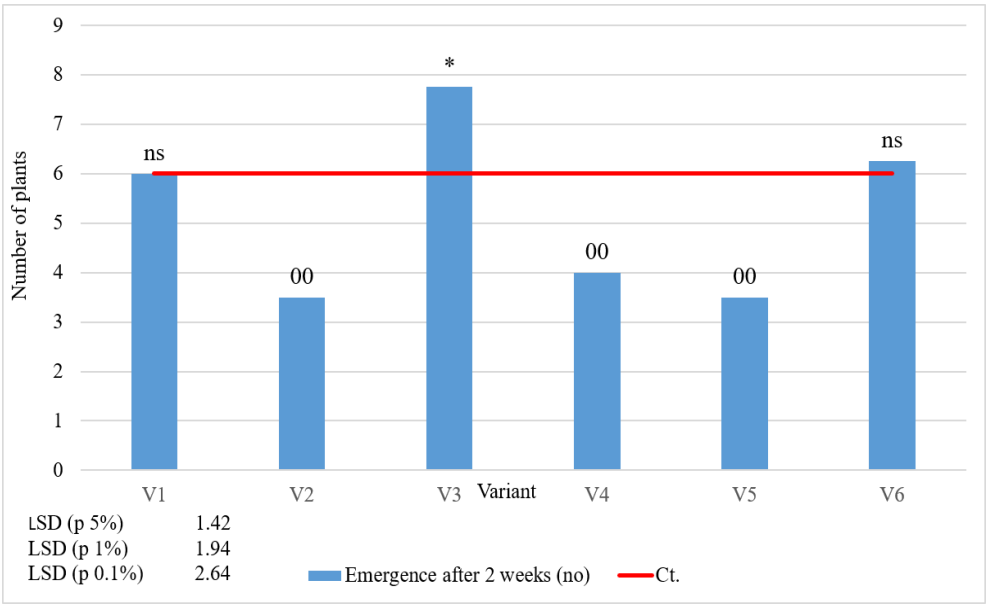


Figure 6. Effect of substrate type on the number of emerged *Arnica montana* L. plants 14 days after sowing.

Analysis of the variation series for the percentage of *Arnica montana* L. plants emerged 14 days after sowing revealed relatively high variability among the seven substrate treatments evaluated. Emergence percentages ranged from 25.00 % to 55.36 %, with a variation amplitude of 30.36 percentage points.

The mean emergence percentage was 37.25 %, while the standard deviation was 11.50 percentage points, indicating appreciable dispersion of the values around the mean. The median, at 39.29 %, was slightly higher than the mean, suggesting a slight asymmetry in the distribution of the observed values.

Analysis of the percentile values showed that 25 % of the treatments recorded emergence percentages of up to 25.00 %, whereas 75 % of the treatments had values of up to 44.64 %. The interquartile range of 19.64 percentage points further confirmed the substantial variability among the experimental treatments. The coefficient of variation (CV) was 30.88 %, indicating moderate to high variability in the percentage of emerged plants. Overall, the results demonstrate that both the type and proportion of substrate components used significantly influenced the emergence capacity of *Arnica montana* L.during the first 14 days after sowing.

	%
N	7
Min	25.00
Max	55.36
Mean	37.25
Stand. dev	11.50
Median	39.29
25 prcntil	25.00
75 prcntil	44.64
Coeff. var	30.88

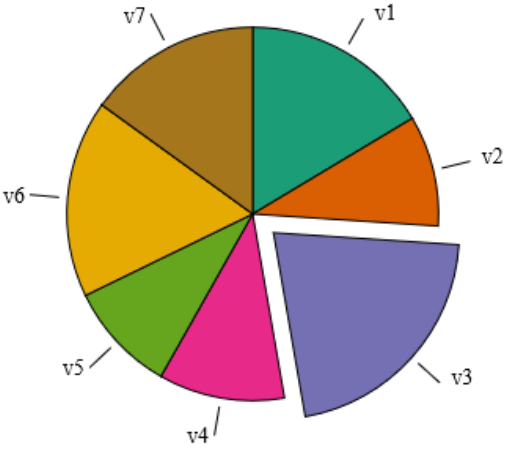


Figure 7. Descriptive analysis of the percentage of emerged *Arnica montana* L. plants according to substrate type

3. In vitro propagation

Arnica montana L. seeds inoculated onto MS culture medium without the addition of phytohormones began to germinate seven days after inoculation.

Assessment of the germination capacity of *Arnica montana* L. seeds under different conditions revealed differences between germination in Petri dishes and *in vitro* germination on MS culture medium. In Petri dishes, the percentage of germinated seeds ranged from 30.00 % to 53.33 %, whereas on MS medium, the values ranged from 42.86 % to 57.14%. These values fall within the lower range of those reported in the literature (Romero et al., 2018; Aiello et al., 2014) for Arnica montana, for which germination percentages ranging from 30 % to 90 % have been reported under optimal conditions.

In Petri dish 1, the germination capacity was 40.00 %, compared with 57.14 % on MS medium, while in Petri dish 2, the corresponding values were 30.00 % and 54.29 %, respectively. Thus, in these two determinations, the in vitro conditions promoted germination, with differences of 17.14 and 24.29 percentage points, respectively. In Petri dish 3, the results were different, with a higher germination percentage recorded in the Petri dish (53.33 %) than on MS medium (42.86 %).

Across the three determinations, the mean germination capacity was approximately 41.11 % in Petri dishes and 51.43 % on MS medium. Thus, under the experimental conditions evaluated, the use of MS culture medium resulted, on average, in a 10.32 percentage-point higher germination capacity compared with germination in Petri dishes.

However, the results revealed a variable response of *Arnica montana* L. seeds to the two germination conditions, as the beneficial effect of MS medium was observed in two of the three determinations, whereas in the third determination, germination was higher in Petri dishes.

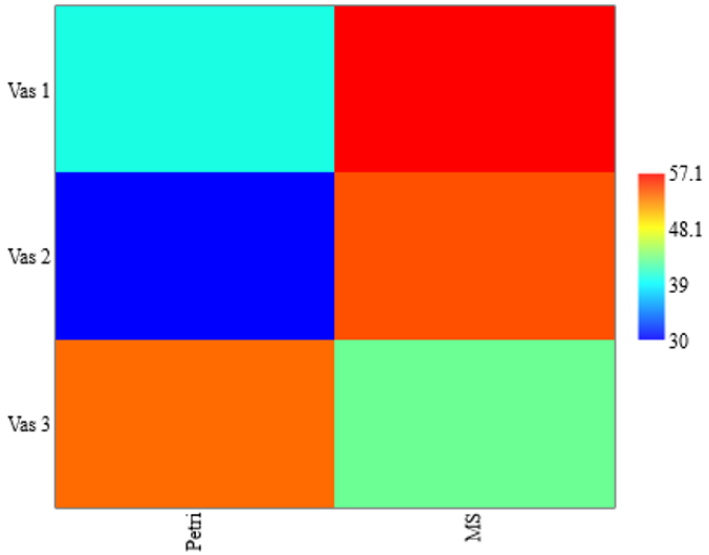


Figure 8. Comparison of the germination capacity of *Arnica montana* L. seeds in Petri dishes and *in vitro* on MS culture medium

The *Arnica montana* L. plantlets regenerated from seed germination showed a 100 % survival rate. Plantlet development and rooting continued *in vitro* on MS nutrient medium supplemented with activated charcoal and a combination of plant growth regulators, including a cytokinin, an auxin, and a gibberellin. Explants cultured on MS medium supplemented with cytokinins, auxins and gibberellic acid (GA_3) showed a maximum regeneration and in vitro rooting capacity of 100 %.

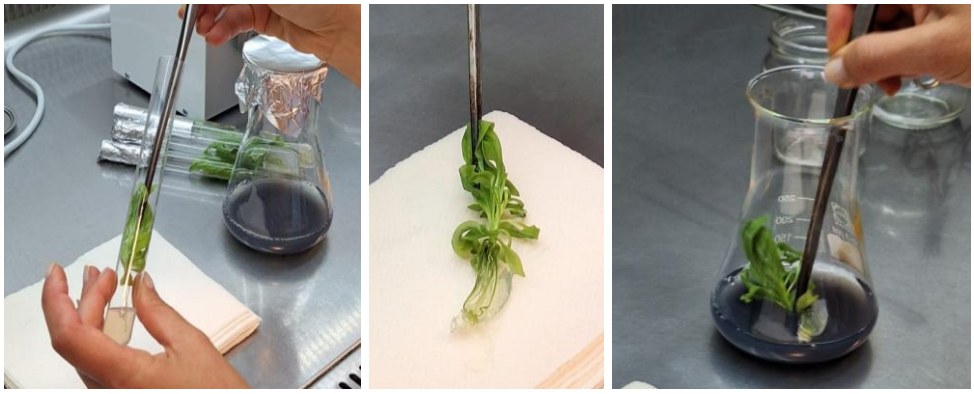


Figure 9. Cultivation of *Arnica montana* L. explants on MS growth medium (Original photograph by Mihaela Cioloca)

CONCLUSIONS

- Clump division is a reliable method for *Arnica montana* L. propagation, providing a high establishment rate (98 %) particularly recommended for the conservation of valuable genotypes; however, its multiplication potential is limited.

- The results demonstrate that *Arnica montana* L. can be propagated using combined methods, while the choice of substrate and propagation technique influences the success of seed germination and plant development.
- The results obtained showed that the percentage of emerged *Arnica montana* L. plants 14 days after sowing was influenced by substrate composition. The most favorable treatment was the mixture consisting of 2/3 black peat and 1/3 perlite, which resulted in the highest emergence percentage and a significant positive difference compared with the control. In contrast, substrates containing zeolite resulted in the lowest emergence percentages.
- Assessment of germination capacity also revealed differences between germination in Petri dishes and *in vitro* germination on MS medium. The mean germination percentage was higher under *in vitro* conditions, suggesting the favorable potential of MS medium for *Arnica montana* L. seed germination.
- Overall, the results highlight the importance of substrate selection and germination conditions for improving the efficiency of *Arnica montana* L. plant production.
- The study contributes to the optimization of cultivation technologies and supports the sustainable utilization of this valuable medicinal species.

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