MULTIPLICATION OF ARNICA MONTANA L. USING AGAR-SOLIDIFIED NUTRIENT MEDIUM AND TEMPORARY IMMERSION BIOREACTORS

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Arnica montana L. is a European endemic species used for centuries to treat various diseases due to its anti-inflammatory properties. The plant contains valuable secondary metabolites such as sesquiterpene lactones, phenols, essential oils, etc. *A. montana* is listed in the European Red List of Vascular Plants as Least Concern (LC). However, it faces threats in many European countries due to habitat loss and overharvesting from natural populations. The plant deficiency stresses the need for the development of effective propagation methods. Theoretically, optimizing *in vitro* culture conditions and techniques can produce millions of elite clones from the mother plant within a year. Micropropagation using an agar-solidified medium produces numerous true-to-type plants that are free of pathogens and microbial contaminants. A temporary immersion system is an advanced liquid-culture micropropagation method where plant tissues are periodically immersed in liquid nutrient medium. It provides maximum gas exchange, increased nutrient uptake, reduced hyperhydration of plant tissues, ensuring better *in vitro* plant growth and secondary metabolite yield.

Materials and methods

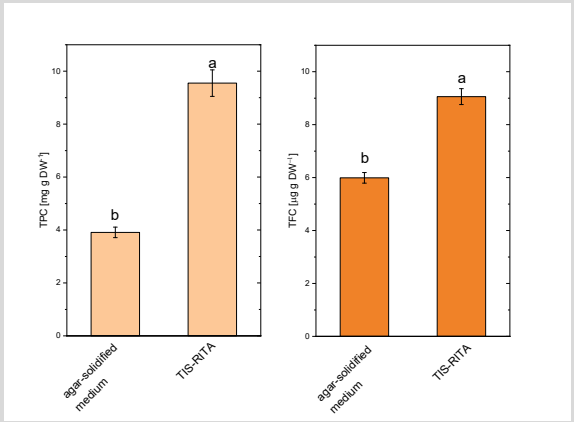
Arnica montana seeds (Poland origin) were used to initiate *in vitro* culture. Shoots (1.0 cm) were isolated from three-month old *in vitro* micropropagated *A. montana* plants and used as explants for experiments. Murashige and Skoog (MS) nutrient medium supplemented with 1 mg/L 6-benzylaminopurine and 0.1 mg/L indole-3-acetic acid was utilized. The experiments were conducted using both liquid and agar-solidified medium (0.6% agar). In the first case glass tubes (150 x 25 mm) with 6 ml of medium were used, with one explant per tube. Explants were vertically cultivated, slightly inserted into the agar-solidified medium. In the temporary immersion system, each vessel contained 10 shoots and 200 ml of liquid medium. The vessels were connected to an air supply operating at 0.25 vvm, with a schedule of 6-minute immersions occurring four times a day. Cultures were kept in a growth chamber at 25 ± 2 ºC under a 16-hour photoperiod, provided by cool-white light at an intensity of 40 µmol m^{-2} s^{-1}. Total phenolic content was determined using the method of Pfeffer et al. (1998). The method of Zhishen et al. (1999) was used to determine the total flavonoid content.

Results

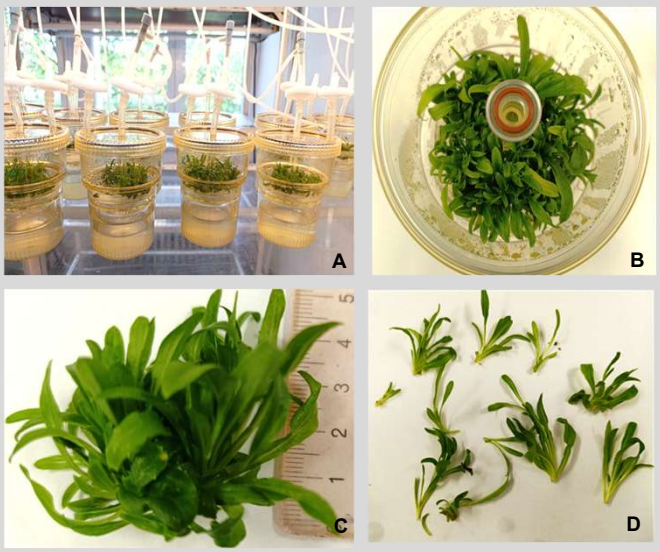
Micropropagation using agar medium A. Multiple *in vitro* plants cultured on agar solidified medium in glass tubes, B. Close-up of micropropagated plant, C. Measurement of plant height

Table 1. Morphometric parameters of *in vitro* propagated *Arnica montana* grown on agar medium and immersion culture type RITA®

Culture system	Shoots/ explant	Plant height, cm	Fresh weight, g
Agar-solidified Culture	4.6±0.33^{b}	1.81±0.07^{b}	0.45±0.03^{b}
Temporary immersion system RITA®	11.3±0.5^{a}	2.74±0.15^{a}	1.27±0.07^{a}
LSD	1.22	0.34	0.16



The content of metabolites with antioxidant power (total phenols, total flavonoids) in *Arnica montana* plantlets grown on agar solidified medium and in liquid medium using TIS RITA®



Micropropagation using an automated temporary immersion system (type RITA®) A. General view of the system, B. Micropropagated plants in the culture vessels after 4 weeks of cultivation, C. Measurement of micropropagated plants. D. Shoots divided from shoot clumps

Higher levels of phenolics and flavonoids were observed in plantlets grown in a RITA® bioreactor with liquid medium compared to the amount detected in plantlets grown on agar-solidified medium. The developed method in a temporary immersion bioreactor significantly improved the multiplication and synthesis of valuable metabolites in *A. montana* and may provide vigorous, disease-free plants, which would aid in the conservation of the species.

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