SCHEMA DE PRODUCERE *IN VITRO* A MATERIALULUI SĂDITOR PENTRU SPECIA *ARNICA MONTANA* L.

IN VITRO PROPAGATION SCHEME OF PLANTING MATERIAL FOR *ARNICA MONTANA* L. SPECIES

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Abstract

The paper reveals the *in vitro* propagation biotechnology for *Arnica montana* L. species, as part of project no. 32160/2008, regarding the chorological study of sozological categories for Arges county flora, to restore endangered phytopopulations through conventional and biotechnological methods of propagation. The established biotechnology provides the use of the next nutrient media: for initiation – base medium MS, with ½n, 0,005:2 mg/l auxins/citokinine, 20 mg/l NaFeEDTA, 20 g/l sucrose, 7g/l agar; for multiplication – base medium LF, with n concentration, 0,02:0,4 mg/l auxins/citokinine 0,02:0,4 mg/l, 32 mg/l NaFeEDTA, supplemented with 40 g/l dextrose and 7g/l agar; for rooting – the use of ½n macro and micronutrients MS, n vitamins LS, 0,1mg/l AG_3, 0,2mg/l IBA, 38 mg/l NaFeEDTA 38 mg/l, 30 g/l dextrose, 7g/l agar and 0,3 g/l activated carbon.

Keywords: *Arnica montana* L., initiation, multiplication, rooting, acclimatization
Cuvinte cheie: *Arnica montana* L., iniţiere, multiplicare, înrădăcinare, aclimatizare

1. Introduction

Arnica montana L. este o plantă erbacee perenă, cu organe vegetative hibernante, medicinală, întâlnită în tot lanţul carpatic în fâneţe şi păşuni umede, rar prin poieni şi tufărişuri, până în regiunea subalpină, rar în zona alpină. Este cuprinsă în lista taxonilor europeni ameninţaţi datorită colectării ca plantă medicinală.

2. Material and methods

Stock plants were harvested from Cheile Brusturetului area, from which were taken vegetative apexes (Alexiu, V., 1998; Pop, O., 2006).

Biological material disinfection was performed by washing with water and 2 drops of chlorine based disinfectant, followed by sterilization in 96% ethanol (C_2H_5OH) for 2 minutes and 6% calcium hypochlorite ($CaCl_2O_2$) for 4 minutes. Throughout the sampling explants, the biological material was kept in distilled water sterilized by autoclaving.

Protocol requirements for *in vitro* propagation biotechnology were followed for all culture stages.

Culture media used for vitroplants initiation, multiplication and rooting are presented in table 1.

Taking into account that *in vitro* culture biotechnology for *Arnica montana* is completed, please note that instead of presenting all the experimental variants for all 3 phases, we displayed only the final ones.

During the experiments, in the growing chamber were provided controlled conditions for: temperature (24°C ± 2^{0}C), photoperiodism (14 hours) and light intensity (3000 lux).

For vitroplants acclimatization substrate we used a mixture of peat for professional use and perlite 1:1 and perlite only.

3. Results and discussions

The results have been recorded as follows: growing percentage for the initiation phase, multiplication rate (rosettes/explant) for the multiplication phase, rooting percentage for the rooting phase and acclimatization percentage for the acclimatization phase.

For initiation phase we note that explants were represented by meristematic tissue and 2-3 leaflets, taken from active growing plants; several nutrient solid media were tested varying the phytohormones and their concentration; the use of culture medium: macronutrients MS, micronutrients MS, vitamins MS, all with ½n, 0,005:2 mg/l auxins/citokinine, 20 mg/l NaFeEDTA, 20 g/l sucrose, 7g/l agar has resulted in 90% growing plants.

For multiplication phase, using the nutrient medium: macroelements LF, microelements LF, vitamins LF, with n concentration, 0,02:0,4 mg/l auxins/citokinine 0,02:0,4 mg/l, 32 mg/l NaFeEDTA, supplemented with 40 g/l dextrose and 7 g/l agar, the multiplication rate was 5 rosettes/explant.

The culture medium: ½n macro and micronutrients MS, n vitamins LS, 0,1mg/l AG_3, 0,2mg/l IBA, 38 mg/l NaFeEDTA 38 mg/l, 30 g/l dextrose, 7g/l agar and 0,3 g/l activated carbon was used for rosettes with 97,4% rooting percentage.

The two culture substrates: peat/perlite 1:1 and only perlite assured the same vitroplants acclimatization percent - 87%.

4. Conclusions

We present the *in vitro* culture phases for *Arnica montana* L. (Fig 1.):

- 0 stage: active growing plants;
- Ist stage (20 days): transition of meristematic tissue and 2-3 leaflets on the nutrient medium;
- IInd stage (35 days): plantlets the transfer from initiation medium on the multiplication medium;
- IIIrd stage (30 days): multiplied plantlets individualization and their transfer on the rooting medium;
- IV stage (25 days): acclimatization of *in vitro* rooted plantlets (Fig 2.).

The established biotechnology will provide a large number of plants that will be fortified and used in the subsequent stages to repopulate the problem-areas.

5. References

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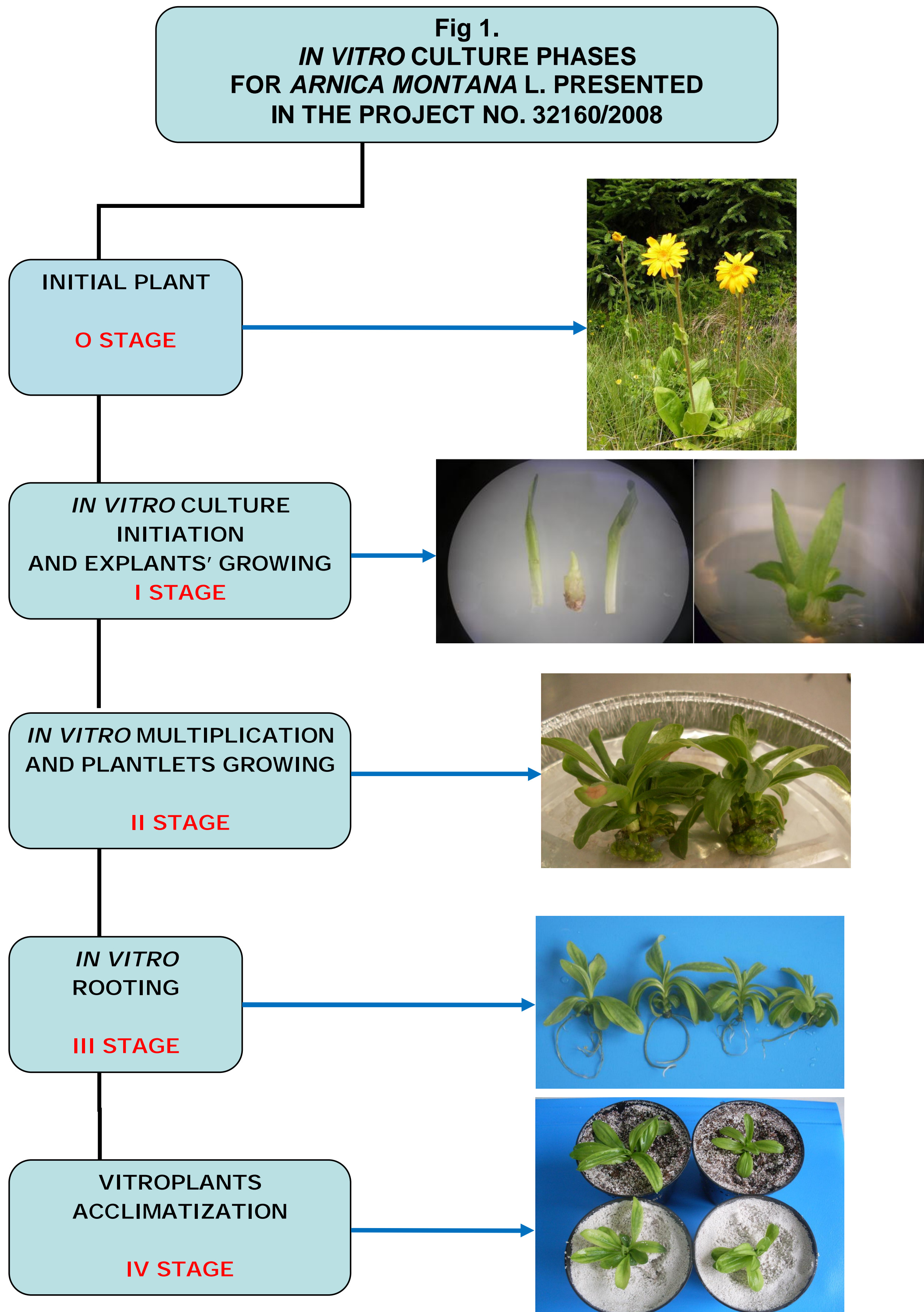
Tables and Figures

Table 1. Nutrient media composition for biotechnology phases

Composition	Initiation	Multiplication	Rooting
Macro MS	½ n	-	-
Micro MS	½ n	-	-
Vitamins MS	½ n	-	-
Sucrose (g/l)	20	-	-
Agar (g/l)	7	-	-
BAP (mg/l)	2	-	-
NAA (mg/l)	0,005	-	-
NaFeEDTA (mg/l)	20	-	-
Macro LF	-	n	-
Micro LF	-	n	-
Vitamins LF	-	n	-
Dextrose (g/l)	-	40	-
Agar (g/l)	-	7	-
BAP (mg/l)	-	0,4	-
IAA (mg/l)	-	0,2	-
NaFeEDTA (mg/l)	-	32	-
Macro MS	-	-	½ n
Micro MS	-	-	½ n
Vitamins LS	-	-	n
Dextrose (g/l)	-	-	30
Agar (g/l)	-	-	7
G_3A(mg/l)	-	-	0,1
IBA (mg/l)	-	-	0,2
NaFeEDTA (mg/l)	-	-	38
Activated carbon(g/l)			0,3

Legend: MS = Murashige-Skoog (1962), LF = Lee-Fossard (1977), LS = Linsmaier-Skoog (1965).



Plant	Biotechnology phases (Weeks)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arnica montana L.	**Initiation phase (20 days)**															
				Multiplication phase (35 days)												
									Rooting phase (30 days)							
													Acclimatization phase (25 days)			

Fig 2. Implementation period of each biotechnology phase