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THE MICROPROPAGATION OF SOME ENDEMIC AND RARE TAXA FROM GILĂU – MUNTELE MARE MASSIF, APUSENI MOUNTAINS, ROMANIA

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Abstract: The biotechnology of *in vitro* vegetal cultures has led to the reconsideration of the classical concepts concerning the multiplication, amelioration, protection, conservation and even obtaining new species. The researches presented in this paper were conducted as part of the CNCSIS-ANSTI-A18, CNCSIS-B20 and CNCSIS A103/52 grant, entitled "Studiul populaţiilor unor specii vegetale endemice rare şi periclitate din M-ţii. Gilău-M-tele. Mare (M-ţii. Apuseni) în perspectiva stabilirii strategiilor optime de conservare" ["The study of some rare endemic and endangered vegetal species from Gilău-M-tele Mare Massif (Apuseni Mountains) in the perspective of achieving the optimal conservation strategies"]. The taxa proposed for study, have never been conserved or micropropagated through *in vitro* cultures. In the case of the species *Centaurea reichenbachii* DC., endemic [4] and rare [7, 15, 21, 23], being protected in the Reservation Scărița-Belioara [8, 12, 22] a micropropagation and a multiplication of 10-15 neoplantlets/inoculum were achieved, along with its acclimatization. A characteristic for this species is the appearance, in time, of the callus at the base of the explants, a phenomenon that disturbs the micropropagation. *Aquilegia nigricans* Baumg. ssp. *subscaposa* (Borb.) Soó, is an endemic taxa, having a spreading area limited to the Romanian Carpathians [25], considered by Boşcaiu *et al*. [5], Oltean *et al*. [21] and Moldovan *et al*. [18] as rare in the spontaneous flora of the country or vulnerable/rare [15]. Taking into consideration some few and irrelevant data found, at present, in the literature regarding the *in vitro* multiplication, the week germination capacity of the seeds of this genus and the fact that our attempts to initiate the *in vitro* culture for this taxa have led to the infection of the whole medium every time, we consider that, for its micropropagation, a series of preliminary experimental variants have to be done in order to eliminate the exo- and endo-infections of the seeds. For both of the taxa the best results were obtained when the sterilization was done using oxygenated water. Based on the studies conducted, it can be inferred that, in the case of the aseptic cultures initiation a great variability exists, especially linked to the species, the vegetation stage in which the sampled material was and also the habitat from where it was harvested.

Introduction

The biotechnology of *in vitro* vegetal cultures has led to the reconsideration of the classical concepts concerning the multiplication, amelioration, protection, conservation and even obtaining new species. In the case of some unfavorable pedoclimatic or ambiental conditions, the representatives of some vegetal populations of interest can be conserved and, along with that, can be multiplied with the help of aseptic cultures.

A series of taxa, from our country, present in the Red Lists elaborated for the Romanian flora, were studied in the past decade, concerning their introduction and their behaviour *in vitro* [6, 10, 11, 17, 27, 28, 31-34, etc.]. Until present, anyway, important representatives (from the botanical and phytogeographical point of view) of the C*entaurea* L. and *Aquilegia* L. genera were never studied *in vitro*. Even at an international scale the representatives of these two genera were less studied from their micropropagation point of view [14, 16, 29]. The researches presented in this paper were conducted as part of the CNCSIS-ANSTI-A18, CNCSIS-B20 and CNCSIS A103/52 grant, entitled "Studiul populaţiilor unor specii vegetale endemice rare şi periclitate din M-ţii. Gilău-M-tele. Mare (M-ţii. Apuseni) în perspectiva stabilirii strategiilor optime de conservare" ["The study of some rare endemic and endangered vegetal species from

Gilău-M-tele Mare Massif (Apuseni Mountains) in the perspective of achieving the optimal conservation strategies"]. Gilău – M-tele Mare Massif is characterized by the presence of the calcareous rocks and a vegetation specific for the mountain belt. The altitude varies between 600 and 1300 m.s.m. The forms of relief vary a lot, from the abrupt slopes (as in Runc Gorges) to the lawns that characterize the Scărița-Belioara Reservation. From the climatic point of view the zone is characterized by a long wet period, most of the year, interrupted by short periods of dryness during the months of July and August. The soils belong, in general, to the mountainous rendzines and pseudorendzines genetic group but on the less inclined terrains forest brown soils were formed. The endemic vegetal species from this region have never been a target for the *in vitro* multiplication studies before. For all the studied species and especially when using fragments of plants for the inoculation, a small number of inocula were used in order to extract the smallest number of individuals from their natural surroundings. In a previous paper [17], the results obtained for the taxa *Dianthus petraeus* Waldst. & Kit ssp. *simonkaianus* (Péterfi) Tutin were presented, this taxa being mentioned as rare, in the Red Lists elaborated for Romanian flora [15, 21] and which is to be found in the Gilău – M-tele Mare Massif. In this paper we present the results of the studies conducted for the introduction of aseptic cultures and micropropagation for other two species included in the grant mentioned above, i.e. *Centaurea reichenbachii* DC. and *Aquilegia nigricans* Baumg. ssp. *subscaposa* (Borb.) Sóo.

Centaurea reichenbachii DC. (Syn. *Centaurea reichenbachioides* Schur; *C. reichenbachii* DC. var. *epapposa* Simk.) [24] is a species considered endemic for the first time by Borza [4]. The status of this species is: rare [7, 15, 21, 23], being protected in the Scărița-Belioara Reservation [8, 12, 22]. This species is found on calcareous rocks in the mountain belt being endemic for the Apuseni Mountains. *Aquilegia nigricans* Baumg. ssp. *subscaposa* (Borb.) Sóo is an endemic species, having a spreading area limited to the Romanian Carpathians [25]. It was considered by Boşcaiu *et al* [5], Oltean *et al* [21] and Moldovan *et al* [18] as rare in the spontaneous flora of the country or vulnerable/rare by Dihoru *et* Dihoru [15]. This taxa is found in mountainous lawns in Scărița, Vultureasa and at Poşaga in Şesul Craiului.

Material and Methods

In the case of both species, for the initiation of *in vitro* culturing fragments of plant and seeds harvested in the studied region were used. The culturing media utilized for the introduction of the vitrocultures and for the micropropagation of the two taxa are presented in table 1 and table 2.

Table 1: The base composition of the culture media used for the micropropagation in *Centaurea reichenbachii* and *Aquilegia nigricans* ssp. *subscaposa*.

	Composition		**Quantity/ 1l of medium**
Base medium	Components according to Murashige-Skoog (1962) [19]	macroelements	100 ml
		microelements	1 ml
		FeEDTA	5 ml
	Vitamines	thiamine	1 mg
		pyridoxine	1 mg
		nicotinic acid	1 mg
	myo-inozitol		100 mg
	saccharose		20 g
	agar		7 g

The phytohormones used were NAA (naftaleneacetic acid) and IBA (indolilbutiric acid) as auxins (which are known to be stimulators for the rhizogenesis) and BAP (6-benzylaminopurine), K (kinetine = 6-furfurylaminopurine), 2iP (6-dimetilaminopurine) and TDZ

(tidiazurone) as cytokinins (which are known to be stimulators for cellular multiplication and plantlets neoformation).

Table 2: Variants of culture media used for the micropropagation on *Centaurea reichenbachii* and *Aquilegia nigricans* ssp. *subscaposa* depending on the phytohormones contained

Variants	Phytohormones (mg/l)							Phytohormones balance (cytokinins/auxins)
	Auxins		Cytokinins				Giberelic acid (GA_3)	
	NAA	IBA	BAP	K	2iP	TDZ		
V1	1	-	-	1	-	-	-	1/1
V2	-	0,5	-	-	-	1	-	2/1
V3	0,5	-	-	-	2	-	-	4/1
V4	0,1	-	1	-	-	-	-	10/1
V5	-	-	-	-	-	-	1 or 100	-

Knowing the sterilization problems and the low germinative capacity of these genera, several variants and more sterilization agents were used (Tab. 3).

Table 3: Disinfection variants used for the *in vitro* culture initiation in *Centaurea reichenbachii* and *Aquilegia nigricans* ssp. *subscaposa.*

Variants	H_2O jet washing	Presterilization (min.)		Sterilization (min.)		Sterile H_2O cleansing
		H_2O_2 4%	C_2H_5OH 96%	Domestos (<5% NaOHCl)	H_2O_2 10%	
D1	40 min.	-	-	90%, 17 min.	-	4 times
D2	40 min.	-	-	100%, 25 min.	-	4 times
D3	30 min.	-	40 sec.	-	15 min.	4 times
D4	120 min.	-	60 sec.	100%, 20 min.	-	4 times
D5	120 min.	12 h	60 sec.	-	18 min.	4 times

In the case of the species ***Centaurea reichenbachii*** fragments of plants (nodes) were sterilized using method D1. For the initiation of the aseptic cultures, starting from seeds, vegetal material originating from 3 localities (Cheile Runcului, Cheile Poşegii and Poşaga) was used. For the disinfection of the seeds other two methods of sterilization were tested: D2 – with a higher concentration of Domestos (from 90% to 100%) and the prolonging of the disinfection time from 17 to 25 minutes in respect to the previous method – and D3 variant of disinfection, using a presterilization with ethanol (C_2H_5OH) 96% and as a disinfection agent, instead of Domestos, oxygenated water (H_2O_2) was used, in order to achieve a more efficient disinfection and to get the seeds out of dormancy. They were inoculated on a base medium, diluted 50%, without phytohormones (Tab. 1) and kept in light and in dark. The generated vitroplants were transferred on cultural media containing the cytokinins tidiazuron – V2 variant of medium, and 2iP – V3 variant of medium, for stimulating the multiplication, besides the added auxins, as rhysogenetic phytohormones (Tab. 2). Also for stimulating the rhysogenesis some of the plants were transferred on a medium with active coal, 3 g/l, without phytohormones.

In the case of the experiments for introducing aseptic cultures of ***Aquilegia nigricans* ssp. *subscaposa***, starting from fragments of plants sampled in the field, fragments of stem and petiole were used. The disinfection of the vegetal material was done using the D1 method. In the case of introducing *in vitro* cultures starting from seeds, knowing the low germination capacity of the *Aquilegia* genus, and the sterilization difficulties of the seeds, a new variant of disinfection was carried out (D4), prolonging the duration of washing the seeds under a continuous jet of water – up to 2 hours and using a presterilization with absolute ethanol, for 1 minute. After that the disinfection of the seeds was done using Domestos 100%, for 20 minutes. Later one other disinfection method based on H_2O_2 was testes (D3). A final disinfection variant (D5), and the inducing of the germination *in vitro* for the seeds of this species, was done, besides the

sterilization with H_2O_2 10 %, using a double presterilization with H_2O_2 4 % for 12 hours and ethanol for 1 minute. In this latter case, the seeds were inoculated on a base medium, with 100 mg/l GA_3 (V5), some of them being exposed to light and the rest to dark, in the refrigerator. We compared the disinfection results for this taxon, with the results obtained for other taxa: *Aquilegia nigricans* Baumg. ssp. *nigricans*, *Dianthus callizonus* Schott & Kotschy, *Dianthus spiculifolius* Schur, *Dianthus petraeus* Waldst. & Kit ssp. *simonkaianus* (Péterfi) Tutin, *Dianthus glacialis* Haenke subsp. *gelidus* (Schott, Nyman & Kotschy) Tutin [11].

For both of the species the cultures were kept at a temperature of 25 ± 2^0C, a light intensity of 87 μmol/m^2/s and a phoperiod of 16 h light/8 h dark.

Results and Discussions

In the case of the species *Centaurea reichenbachii*, for the initiation of *in vitro* cultures starting from fragment of plants, the explants used were nodes from the principal stems or from the branches. For this species, taking into consideration the characteristics of the surface tissues, the method D1 of disinfection was inefficient, the infection rate being of 100%. It might be also possible that, the plants that were already in the state of vegetative rest, presented an infection degree, with phytopatogens, much more higher than the young plants, before blooming.

The results obtained in disinfecting the seeds are shown in table 4.

Table 4: Infection and germination rates in *Centaurea reichenbachii*

Inoculum type	Disinfection variant	Days from the inoculation	Light/dark	Infection rate	Germination rate (after 56 days)
Plant fragment	D1	14	light	100%	-
Seeds	D2	9	light	61%	-
			dark	58%	5-8%
	D3	9	light	65%	18%
			dark	50%	

As seen in the table, using the method D3, was obtained the best rate of sterilization. Using this method of disinfection, based on H_2O_2 action, looking to achieve disinfection more efficient and also to get the seeds out of dormancy, the infection rate at 9 days after the inoculation was of 65% in light and of 50% in dark. Using H_2O_2 has indeed allowed a better germination rate, of 18%. This germination rate can be considered sufficient for the introduction of an aseptic culture.

The basal part of the plantlets generated *in vitro* from seeds disinfected using D2 and D3 methods was transferred to varied culture media. The best results were obtained, in a first stage, on V1 medium, containing NAA 1mg/l and K 1 mg/l, also (Fig. 1).

The vitroplants generate, from their basal region, up to 4 neoplantlets/inoculum. On V1 medium (Fig. 2), after several transfers, the callusal volume generated at the base of the plant grew, affecting the plant itself. On the medium containing NAA 0,1 mg/l and BAP 1 mg/l (V4) the best multiplication is achieved and the generated amount of callus is lower. This result can be explained by the fact that, in the case of this medium, the hormonal balance is 10/1 (cytokinins/auxins) and, as it is known, the cytokinins favour the multiplication. But the radicular system was not very well developed. Through successive transfers a multiplication rate of 5-10 neoplantlets/inoculum was obtained (Fig. 3).

Later on, the vitroplants were transferred on culture media containing tidiazuron (V2) and 2iP (V3), besides the added auxins – as rhisogenetic phytohormones, in order to stimulate the multiplication. We used these two cytokinins for studying their effect on the vitrocultures of this species. These tow phytohormones led to no better results, in the case of *Centaurea reichenbachii*, than the combination of auxins and cytokinins, presented above.

Some of the vitroplants were transferred on a medium containing active coal, 3 g/l, lacking in phytohormones, in order to stimulate the rhizogenesis and the effect was satisfactory. The rooted vitroplants using this medium were acclimatized after a stage of *in vitro* photoautotrophy.

Fig. 1: *Centaurea reichenbachii* vitroplant generated from explants originating from sterilized seeds, inoculated *in vitro*, on V1 medium (NAA 1 mg/l, K 1 mg/l), after 25 days from transfer

Fig. 2: *Centaurea reichenbachii* vitroplants, on V1 medium (NAA 1 mg/l, K 1 mg/l), 36 days from transfer

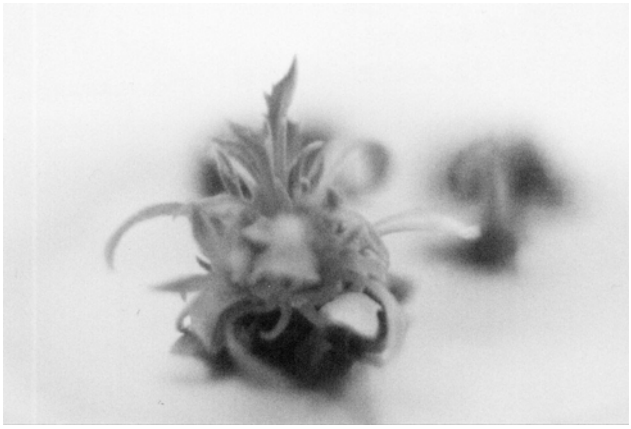

Fig. 3: *Centaurea reichenbachii* vitroplant with neoplantlets generated at the base

Centaurea genus, as like as *Aquilegia* genus, was relatively little studied from *in vitro* cultures point of view. From the species of this genus, *in vitro* cultivated, we can cite *C. junoniana* Svent. [16], C. *cyanus* L. [29] şi *C. spachii* Sch. Bip. ex Willk. [14]. The medium culture that was used by this authors was, mainly, Murashige-Skoog [19], solid or liquid. The growth regulators that were used, were BAP 0,5-5 mg/l, ANA 0,2-2 mg/l or IBA 0,01 mg/l. The inocula used were cotyledons, leaves and apexes of plantlets, nodes or inflorescences. Following the introduction of the *in vitro* cultures, the growth and multiplication of the vitroplants were achieved, or, in the case of the cultures on liquid media, cellular suspensions were obtained. Our collective has studied another representative of this genus aiming to introduce *in vitro* cultures for *Centaurea kotschyana* Heuff. – originating from Piatra Craiului Mountains – from nodal fragments and foliar fragments. The results from the preliminarily studies were not satisfactory because of the same very high infection index. Another difficulty in the multiplication of this species is due to the relatively low germination capacity of the seeds.

In the case of the endemic and rare species ***Aquilegia nigricans* ssp. *subscaposa*,** the culture induction was started from fragments of plants, harvested in the studied region. The stem and petiole fragments were sterilized with the help of Domestos 90%, using D1 method. From the inocula with potential meristematic tissue, represented by fragments of a stem, all were infected after 17 days from the inoculation. As it is known, the sterilization of the subterranean vegetal organs, with the object of introducing the aseptic cultures, is always very difficult. From the extant petiole inocula, just 16% got infected, proving that the used disinfection method for the aerial parts of the plant was more efficient. But the regeneration did not occurred because the meristematic tissue is missing in this phase of development.

The results obtained when trying to introduce the *in vitro* cultures starting from seeds, are presented in table 5.

Table 5: The infection rate in *Aquilegia nigricans* ssp. *subscaposa* and *Aquilegia nigricans* ssp. *nigricans*

Harvesting year	Seeds inoculation	Disinfection variant	Days from inoculation/ Observations	Light/Dark	Infection rate %
Aquilegia nigricans* ssp. *subscaposa					
2000	Nov. 2000	D1	23	light	100
				dark	100
2000	June 2001	D4	23	light	85
				dark	73
2001	Dec. 2001	D3	21/on GA_3 medium	light	80
2002	Nov. 2002	D5	21/on GA_3 medium	light	65
				dark	55
Aquilegia nigricans* ssp. *nigricans					
2002	Nov. 2002	D5	21/on GA_3 medium	light	26
				dark	23

It can be seen that, knowing the week germination capacity in the case of the *Aquilegia* genus, along with the sterilization difficulties linked to the seeds, a prolonging of the washing phase was tested using a continuous jet of water for 2 hours and a presterilization with absolute ethanol, for 1 minute. Later on, the disinfection continued with Domestos 100% (D4 method). In a second experiment, after 23 days from the inoculation, in dark, 73% of the seeds were infected and none had germinated while in light, 85% of the seeds were infected and also, none had germinated. No seed germinated in the following months either.

It was established that the high rate of infection is due to the presence of a fungus (*Alternaria alternata*), probably an epiphytic one on the vegetal tissues and its spores could not be destroyed by currently used methods (i.e. disinfectants based on chlorine like Domestos). We mention that using Domestos as a disinfectant in the case of the species of *Dianthus caryophyllus*

L., *D. callizonus* Schott & Kotschy, *D. spiculifolius* Schur, *D. petraeus* Waldst. & Kit ssp. *simonkaianus* (Péterfi) Tutin, *D. glacialis* Haenke subsp. *gelidus* (Schott, Nyman & Kotschy) Tutin, etc. led to very good results [11]. Later on, a new disinfection method was used (D3), based on oxygenated water (H_2O_2). It is known to be an efficient disinfectant in situations where other disinfection agents fail and has an effect of getting out of dormancy the seeds, also. In this case the infection index was also very high, 80% for the seeds and 100% for the plant fragments. Wishing to stimulate the germination of the uninfected seeds, they were transferred on a medium culture containing GA_3 (giberelic acid), 1 mg/l. This phytohormone is utilized in normal conditions or *in vitro* culturing to get the seeds out of dormancy. The seeds did not germinate in this case, also. A last method of inducing the germination of the seeds *in vitro*, for this species, was presterelizing and stimulating the germination with H_2O_2 4%, for 12 hours followed by sterilization with H_2O_2 10%, for 18 minutes (D5 method). The seeds were inoculated on a basal culture medium, with 100 mg/l GA_3, some of them being placed in light and some in dark and in the refrigerator. Regarding this case of sterilization, after 21 days from the inoculation, the infection index of the seeds was lower (65% - in light and 55% - in dark). It must be mentioned that in the case of the same species but another subspecies (*Aquilegia nigricans* Baumg. subsp. *nigricans*) the infection index was lower, using the same sterilization, being 26% in light and 23% in dark. The seeds' disinfection of *Dianthus glacialis* ssp. *gelidus* and *Dianthus callizonus* using the same method of presterilization and sterilization with H_2O_2 led to no infection after 7 days after the inoculation.

Table 6: Infection rate for different taxa in similar disinfection conditions

Taxa	Disinfection variant	Days from inoculation	Light/ Dark	Infection rate %
Aquilegia nigricans* ssp. *subscaposa	D5	7	light	57
			dark	47
Aquilegia nigricans* ssp. *nigricans	D5	7	light	18
			dark	15
Dianthus glacialis* ssp. *gelidus	D5	7	light	0
Dianthus callizonus	D5	7	light	0

In this last case of disinfection (D5 method), experimented for *Aquilegia nigricans* ssp. *subscaposa*, only one seed, kept in light, started to germinate after 15 days from the inoculation. Unfortunately this plantlet did not regenerate. We mention that the experiments were done both with seeds freshly harvested in the autumn and seeds that were kept at +4ºC during the winter, for vernalization.

There are only few studies concerning the in *vitro* culturing for ***Aquilegia*** genus. Until present, bibliographical references were found only for *A. formosa* Fisch. [1, 2, 3, 30] and *A. canadensis* L. (1999, American Journal of Botany, Internet cited source). Starting from floral buds, only the regeneration of the flower or floral stems was achieved *in vitro* by this authors.

Conclusions

In the case of the species *Centaurea reichenbachii*, endemic and rare, it can be said that its micropropagation was achieved, with a multiplication rate of 10-15 neoplantlets/inoculum, and its acclimatization, also. A characteristic for this species is the appearance, in time, of the callus at the base of the explants, a phenomenon that disturbs the micropropagation.

Regarding *Aquilegia nigricans* ssp. *subscaposa*, taking into consideration the few and irrelevant data found at present in the literature about *in vitro* multiplication, the low germination capacity for the seeds of this genus, along with the fact that our attempts to initiate the *in vitro* culture of this taxa, starting from seeds, led to the total infection of the culture medium, we

consider that, for it multiplication, a series of preliminarily experiments have to be done in order to eliminate the exo- and endogenous infections of the seeds and obtain a satisfactory multiplication. If the initial vegetal material is the carrier of some pathogenic germs and the germination capacity of the seeds is low, it is very difficult to induce a sterile culture. The best method of sterilization and for inducing the germination of the seeds *in vitro*, for this species, was presterelizing and stimulating the germination with H_2O_2 4%, for 12 hours followed by sterilization with H_2O_2 10%, for 18 minutes and their culture on a medium with 100 mg/l GA_3.

Based on our previous results, it can be inferred that, in the case of some aseptic culture inductions there is a great variability depending mainly on the species, the vegetation phase of the sampled material, as well as the habitat from where they were harvested.

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MICROPROPAGAREA UNOR SPECII VEGETALE, ENDEMICE ŞI RARE DIN MUNŢII GILĂU – MUNTELE. MARE (MUNŢII APUSENI, ROMÂNIA)

(Rezumat)

Biotehnologia culturilor vegetale *in vitro* a dus la reconsiderarea conceptelor clasice legate de multiplicarea, ameliorarea, protejarea, conservarea sau chiar obţinerea unor noi specii. Cercetările prezentate în acest articol au fost efectuate în cadrul grantului CNCSIS-ANSTI-A18, CNCSIS-B20 şi CNCSIS A103/52 intitulat „Studiul populaţiilor unor specii vegetale endemice rare şi periclitate din M-ţii. Gilău-M-tele. Mare (M-ţii. Apuseni) în perspectiva stabilirii strategiilor optime de conservare". În cazul speciei *Centaurea reichenbachii* DC., endemică [4] şi rară [7, 15, 21, 23], ocrotită în rezervaţia Scărişoara-Belioara [8, 12, 22] se poate afirma că s-a realizat micropropagarea ei cu o rată de multiplicare de 10-15 neoplante/inocul şi de asemenea, aclimatizarea ei. O caracteristică pentru această specie este însă apariţia în timp, a calusului la baza explantelor, fenomen care deranjează micropropagarea. *Aquilegia nigricans* Baumg. ssp. *subscaposa* (Borb.) Soó, este un taxon endemic, cu areal carpatic restrâns la România [25], considerată ca rară în flora spontană a ţării [5, 18, 21] sau vulnerabilă/rară [15]. Ţinând cont de faptul că încercările noastre de a iniţia cultura *in vitro* a acestui taxon, pornind de la seminţe s-au soldat de fiecare dată cu infectarea totală a mediului de cultură, considerăm, că pentru micropropagarea ei, vor trebui efectuate o serie de variante experimentale preliminare care să aibă în vedere eliminarea infecţiilor exo- şi endogene ale seminţelor. Deoarece în cazul ambilor taxoni, datele găsite până în prezent în literatura de specialitate referitoare la multiplicarea *in vitro* sunt foarte puţine şi irelevante, şi deoarece capacitatea germinativă a seminţelor, mai ales a genului *Aquilegia* este redusă, s-au testat mai multe variante de sterilizare, bazate pe hipoclorit de sodiu, alcool etilic sau apă oxigenată. În cazul ambilor taxoni, cele mai bune rezultate de dezinfecţie au fost realizate folosind ca agent de presterilizare şi de sterilizare apa oxigenată (4% şi respectiv 10%). Pe baza studiilor efectuate, se poate deduce că, în cazul inducerii unor culturi aseptice există o mare variabilitate de răspuns, dependentă în principal de specie, de stadiul de vegetaţie în care se află materialul recoltat, precum şi de habitatul din care a fost recoltat acesta.