

# In Vitro Conservation of Some Drosera Taxons From the Protected Areas of Pnma (Romania). Drosera Anglica Hudson a Specie Critically Endangered with Extinction



## Environmental Science

**KEYWORDS :** Drosera anglica Hudson critically endangered (CR), in situ, in vitro, conservation, community SITE, regeneration, multiplication, acclimatization ex vitro, ecological reconstruction.

Eliza Maria Agud

University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru, St., 410048 Oradea; Romania

### ABSTRACT

Our research encompasses ample studies of in vitro conservation of some taxons from Drosera specie, endangered with extinction, from the protected area of PNMA (Romania). Drosera anglica Hudson specie is found in our country only in Transylvania in the Gil u Mountains, a rarity of botany, a geoelement with poor populations, critically endangered (CR). Conservation through in vitro multiplication was made starting from the very young bud cultivated on the Murashige and Skoog, 1962 (MS) medium, with the variants: V<sub>0</sub> = MS; V<sub>1</sub> = MS+0.5mg/l AIB+1.0mg/l BA; V<sub>2</sub> = MS+0.5mg/l AIB+1.0mg/l2iP; V<sub>3</sub> = MS+0.5mg/lAIB= 20mg/lAd.SO<sub>4</sub> (table 1). After 45-50 days of in vitro culture we followed: the percentage of regeneration, rooting, multiplication and acclimatization (by evaluating the average of the number of neo-plantlets/explants). In vitro evolution is favoured by the presence of cytokinins in the medium, in the case of this experiment BA and 2iP, 1.0mg/lm and also of AdSO<sub>4</sub> - 20mg/l (which can substitute cytokinins), combined with an auxine in a small dose (0.5mg/l AIB). After about 3 weeks of ex vitro preculture of the neo-plantlets there are obtained vigorous plants which can ensure the repopulation of the protected area through their planting in the area of origin.

### INTRODUCTION

The degeneration of the environment leads to the decrease of the biological potential of the vegetable world and finally to the disappearance of an entire population of species and taxons of plants (Flora României vol. XIII, 1952-1974). The disappearance of the species of plants on the globe intensified being of 100 up to 1000 times greater, mainly due to the activity of man (one of 8 species of plants is threatened with extinction, according to IUCN, 2006). In the last 50 years about 300.000 plants disappeared, between 20-40% from the world flora is in decline (Farusworyh., 2008): the species of plants which are threatened on our continent are included in programmes of in situ restoration and even with ex situ conservation measures, and the majority of the endangered taxons can be found in a programme of minimal protection. In In situ conservation is based on plans of monitoring the endangered habitats, plans of recovery even of a single endangered specie through activities of ex situ conservation (Y. Bajaj, 1986). Information on the zoological estate of the species are ensured by the lists and the red books (N. Bo caiu et al., 1994) which encompass the endangered species from all over the country.

F. Engelman, 1997 sustains that the conservation of the species of plants endangered with extinction through unconventional methods through biotechnologies, the method being initially experimented at the horticultural species and also at the botanical elements with a scientific value from the spontaneous flora (M. F. Fay, 1992) on the fact that for the initiation of the culture it is necessary a single plant, a seed, a single explants: apex, meristem, bud, section of a leaf, stem, etc. (E. Agus, 2014). The research concerning in vitro techniques at the rare, endangered and endemic species from Romania, for their conservation were applied at a great number of species (V. Laslo et al., 2011b), especially at the species of plants which have a difficulty in multiplying through the classical method, then the field was extended to the photoautotrophs cultures in vitro, to the venerable, endangered species and even to some endemics threatened with extinction ( Victoria Cristea et al. 2004).

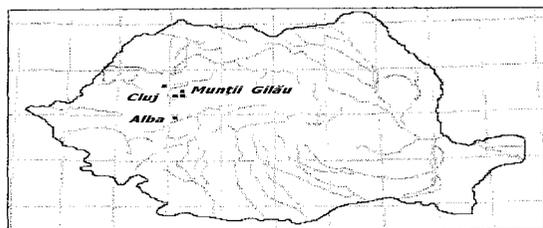


Fig.1 Prevalence areal in Romania for Drosera species

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(Dihorul, Negreanu, 2009)

Drosera anglica Hudson (specie taken by us for analysis) is a biological rarity with a scientific and ornamental importance, to which initially, there were applied in situ conservation measures through creating reservations in Gilău Mountains, PNMA massive from the Western Carpathians (fig. 1). In the conditions from Romania Drosera taxons were conserved in situ and in protected areas, greenhouses, botanical gardens, etc., and the seeds through stocking them into gene banks. Drosera anglica Hudson specie in the Europaea Flora is considered a "sterile hybrid", and in Romania's Flora it is treated as a full entitled specie and it is signalized only in the mires from PNMA, and also in Băile Tuşnad, Mohoş Mire (Ghe. Dihoru and G. Negrean, 2009, etc.). Missing in many areas of the country and now encountered only in Transylvania in the Gilău Mountains from the Apuseni Mountains Natural Park from the massive mountain of the Western Carpathians (N.Boşcaiu et. all., 1994)

### MATERIAL AND METHODS

It was followed the in vitro conservation of Drosera anglica Hudson specie, from the Şes Mountain protected area, a site of community importance (SCI). The research literature also recalls other endangered species from the area of Transylvania (Bihor region) and from the surrounding areas and successfully conserved in vitro, hence we recall the species from Piatra Craiului massive (R. Blându, I. Holobiuc., 2007, 2008), Gilău Mountains (V. Cristea, at. all., 2004). Also, in our works there was always an interest for the in vitro multiplication and conservation and of other taxons of Drosera, derivating from the protected areas of Transylvania, as for example Drosera rotundifolia (E. Agud, 2015a; 2015b) from the PNMA areas and the ecological reconstruction of those endangered areas.

Var.	MB	ANA mg/l	BA mg/l	2iP mg/l	AdSO <sub>4</sub> mg/l
V <sub>0</sub>	MS	-	-	-	-
V <sub>1</sub>	MS	0.5	1.0	-	-
V <sub>2</sub>	MS	0.5	-	1.0	-
V <sub>3</sub>	MS	0.5	-	-	20

Table 1 Medium for the in vitro multiplication of Drosera anglica Hudson specie (MS = medium according to Murashige-Skoog; AIB = β - indole butyric acid; AB = benzyl adenine; 2iP= 2 izopentyladenine; Ad.SO<sub>4</sub> = adenine sulphate)

The young floral bud of about 0.3-0.4mm Ø, was harvested from plants from the recalled protected area and cultivated on MS

culture medium (Murashige-Skoog, 1962) with the hormonal variants presented in table 1, with moderate and even small doses and with additional additives for obtaining the new vegetal material at a low cost price. It was followed the micro-multiplication in vitro of the specie via explant – floral bud and the reconstruction of the area of origin, the method ensuring the obtaining of plantlets directly from the explant, on mediums with balanced doses of phytohormones.

**RESULT AND DISCUSSION**

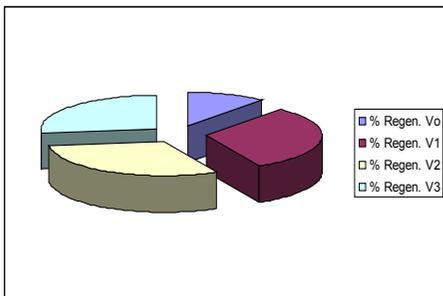
After 45-50 days of in vitro incubation it was followed the evolution of the juvenile bud of *Drosera anglica* Hudson on the medium variants from table 1, following: the regenerative capacity of the explants (%), the average number of plants/explant, the rooting percentage of the neo-plantlets, the multiplication percentage of the explant and the ex vitro acclimatization percentage of the neo-plantlets. Value average of the of the analysed parameters is presented in table 2.

Specie	Var.	% Rege.	Average no. pl./ expl.	% Rooting	% Multi.	% Ac-clim.	Evolution/ notes
<i>Drosera anglica</i> Hudson	V <sub>0</sub>	32	3	5	40	20	xx
	V <sub>1</sub>	92	38	51	100	55	xxxxxx
	V <sub>2</sub>	80	27	45	100	48	xxxxxx
	V <sub>3</sub>	78	25	39	92	52	xxxx

**Table 2 The values of the analysed parameters at the in vitro culture of *Drosera anglica* Hudson bud (after 45-50 days)**

Another specie of *Drosera* (*Drosera intermedia*) from the same protected area, on the variants with high doses of cytokinins (2 up to 5mg/l) differentiated embryogenic callus which subcultivated generated plantlets: at some variants, callus differentiated at the beginning of explants' incubation stopped the differentiation and multiplication of the *Drosera* explant (V. Laslo et al., 2013), this is why we used them in the experiments with other taxons of *Drosera*, moderate (and even small) doses of phytohormones which favour in vitro micro-multiplication via – explant.

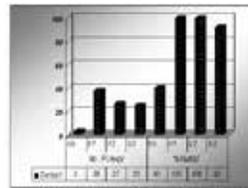
Table 2 presents the average of the analysed parameters, the in vitro evolution of the explant of *Drosera anglica* bud and the value differences of the parameters depending on the nature of the culture medium. The best in vitro regeneration is obtained in the presence of cytokinins benzylaminopurine (BA) and 2- izopentyladenine (2iP), with 92% and respectively 80% regeneration (figure 2). Over 78% regeneration was also obtained on the medium with AdSO<sub>4</sub>, whereas on the witness sample V<sub>0</sub>(MS) regeneration is of about 32%.



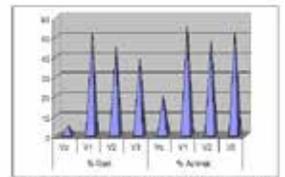
**Fig. 2 In vitro regeneration capacity of *Drosera anglica* Hudson specie (after 45-50 days)**

Figure 3 presents the average number of neo-plantlets/bud in relation with the percentage of in vitro multiplication of *Drosera*

*anglica* specie (after 45-50 days) from which we can also see the superior values and at the same parameters on V<sub>1</sub> (MS+1.0mg/LBA+0.5mg/LAIB), the average number over passing 38 neo-plantlets/explant with a multiplication capacity of over 100%, followed by V<sub>2</sub> with 27 neo-plantlets/explant with a similar multiplication capacity of 100%. In the presence of AdSO<sub>4</sub> the evolution is slightly inferior, about 25 neo-plantlets/explant with a good multiplication capacity, of 92%. On the witness variant (V<sub>0</sub>) the bud of *Drosera anglica* regenerates only 2-3 neo-plantlets/explant, with a multiplication capacity of less than half (about 40%). The percentage of differentiation of the radicular system is influenced by the presence of auxine (AIB) in small doses (0,5mg/l) and of cytokinin in moderate doses of 1mg/l and it is in a tight relation to the capacity of acclimatization ex vitro of the neo-plantlets, and it is suggestively presented in fig. 4. The rooting percentage is of 51%, and respectively of 45% (on V<sub>1</sub>, respectively on V<sub>2</sub>), with a percentage of acclimatization of 55%, and respectively 48%; in the presence of AdSO<sub>4</sub> the differentiation of the radicular system reaches about 40%, followed by a very good capacity of acclimatization (a value close to the one on the medium with BA -V<sub>1</sub>), of over 50%. Following fig. 4, we can see that the acclimatization of *Drosera anglica* neo-plantlets is directly proportional with the value of the radicular system and it reaches on the variants with phytohormones to 55-48%; on the variant with AdSO<sub>4</sub>, about 52%; and on the witness variant (V<sub>0</sub>) the acclimatization is of only 20% because the rooting percentage is also small (about 5%). For the differentiation of the radicular system at *Drosera anglica* Hudson specie, the presence of AIB is beneficial.



**Fig. 3 Average number of plants/bud in relation to the percentage of in vitro multiplication of *Drosera anglica* Hudson specie (after 45-50 days)**



**Fig. 4 In vitro rooting percentage of *Drosera anglica* Hudson neo-plantlets in relation to the species of acclimatization**

In supporting in vitro multiplication of *Drosera anglica* Hudson specie we recommend the presence of cytokinins in the basal medium in a moderate dose of about 1 mg/l and of adenine sulphate of 20mg/l associated with a small dose of auxine (AIB 0,5mg/l), for obtaining a great number of *Drosera* neo-plantlets, completely organized, well rooted and with an increased capacity of acclimatization (of over 55%).

**CONCLUSIONS**

1. Micro multiplication in vitro of *Drosera anglica* Hudson specie via explant (bud), presents the advantage of obtaining the plants at an advantageous low cost price by using in the culture mediums balanced doses of phytohormones.
2. The explant consisting of *juvenile bud* of *Drosera* presents a good in vitro evolution; the parameters analysed as a whole have superior values in the presence of cytokinins in the MS medium in a moderate dose of 1mg/l(BA and 2iP) in combination with an auxine in a small dose of 0,5mg/l (AIB), and in the presence of AdSO<sub>4</sub> (20mg/l).
3. We signalize a regenerative reaction and a satisfactory evolution on the witness sample too (V<sub>0</sub>) on the MS medium in the absence of the phytohormones, the purpose of obtaining the a cheap vegetal material being reached.
4. For a successful in vitro multiplication of *Drosera anglica* specie, we recommend the medium according to Murashige-Skoog with 1mg/LBA and 0,5mg/LAIB, whose presence ensures in vitro multiplication and differentiation of completely organized neo-plantlets, with a vigorous radicular system and with a good capacity of acclimatization (of over 55%).

5. Indolyl  $\beta$  butyric acid (AIB) is the auxine which in the case of this specie too, in a small dose, manifested its superiority in stimulating the differentiation of the radicular system that reaches percentage values of over 50%.
6. Ex vitro acclimatization of the neo-plantlets is ensured by running over the binding steps of acclimatization (protection towards the direct sun's rays, temperature adequate to the specie, moderate soil and atmosphere humidity, etc.), by obtaining a vigorous vegetal material, well rooted plants which can repopulate and rebuild the area of origin of *Drosera anglica* Hudson specie.



Photo 1. Uniform evolution of *Drosera anglica* Hudson explant on medium with cytokinin and salicylic sulphate (after about 50 days)



Photo 2. Number of neo-plantlets obtained in vitro from a leaf of *Drosera anglica* on medium V<sub>1</sub> (with B.A.), after about 50 days



Photo 3. Acclimatized *Drosera anglica* Hudson plantlet

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