IN VITRO CONSERVATION OF CERTAIN ENDANGERED AND RARE SPECIES OF ROMANIAN SPONTANEOUS FLORA

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Abstract

The in vitro method of breeding of vulnerable, rare and endangered species allows for their conservation and the repopulation of areas where they have become extinct or are in dange of disappearing. The in vitro culture can be initialized starting from different types of explants: apex (apical, lateral), inforescence (or bud), bulb (or bulb scales), leaves (segments of leaves), stem (sapling, tip, nodes), seeds, embryos etc. The culture mediums used can be simple (without hormones) , with supplemented hormonal balance according to the plants' nature (auxines or cytochinines in moderate doses of 0.1-0.5 mg/l) or with substitute hormonal balance via natural extracts (corn embryos, malt, coconut milk, pumpkin extract, etc.). The medium in which hormones are substituted by adding natural plant extracts are a very cost-effective way of breeding the respective species. The number of individual plants obtained from an explant depends on the nature of the species and of the explant, and on the composition of the medium. The micromultiplied species which have evolved through complete in vitro organogenesis (Dianthus spciculifolius Schur. (Doronicum, Fritillaria, Leontopodium, Lilium) adapted to the ex vitro environment up to 65-100%. Syringa josikaea J. Jack adapted up to 50%. Daphne, Drosera anglica and rotundifolia and Paeonia adapted unde 50%. Dianthus petraeus and Drosera intermedia adapted poorly or not at all, due to the fact that the neoplantules did not grow roots (chart 2). In this last case, we intervened by adding a complex hormonal balance with higher concentrations of hormones, in order to boost plant vigor, induce the growth of roots, control de normal organization of the plant and increase the adaptation percentage. Newly formed plantules can withstand mini-layering in vitro in order to increase the multiplication rate.

Key words: conservation, endangerment, *in vitro,* endemite, red lists, red book, regeneration, rooting, mini-layering, addaptation to *ex* vitro.

INTRODUCTION

The constant natural ratio between the number of species and the given area is at the base of biodiverity. Given moderate and balanced action of evironmental factors, the plant populations balance each-other out; however, if one of the environmental factors becomes overbearing, it begins to advantage some plant populations and works in the disadvantage of others (*Dihoru, Ghe., G. Negrean,* 2009). In any given area of plant life, some species are common and others are rare - the rare ones have always spiked the interes and concern of specialists, even when there were no endemic elements. The number of rare species in our country is high because they have continuous areal, but many of them exist at the edge of their respective areals (*Cristea, V.,* et all. 1996). The reasons for species' extinction, be they natural or caused by man, have several effects which generally lead to the

shrinking of habitat, diminnishing natural resources, environment degradation, species degeneration, decrease in biological potential, ecological dezasters, etc. (Flora RSR vol. XIII). Conservation pursuits for endangered species are aimed not only at society in general but also towards botanical, ecological and bio-technological specialists. Thus, **sozology** was created: the science concerned with rescuing and conserving all endangered organisms. The Berna Convention (1979) established concrete measures for the research and multiplication of rare species, *ecophilaxy* measures, for prevention of endangerment under the guidance of the International Union for the Conservation of Nature (I.U.C.N.), as well as some unconventional measures adopted by European states later, by the developement of gene banks (IBPGR, 1986).

It is estimated that in the last 50 years, around 300.000 species have disappeared(http://www.bbc.co.uk/nature/environment/conservationnow/global/biodiversity/page2.shtml), and between 20 and 40% of the global flora is in decline (*Farusworth, E.,* 2008). According to I.U.C.N. - 2006, the global rate of extinction is between 100 and 1000 times more alert than would be natural, the human factor placing one in ever 8 species of plants in danger of extinction. On a European level, statistics gathered in 1998 show that approximately 100 species out of those which are endangered are part of *in situ* restorations programs, with *ex situ* ulterior conservation measures, and approximately 35% of taxons are placed in a minimal protection program (*Maunder, M., S. Higgens.,* 1998). *In situ* conservation requires management, monitoring and protection plans for habitats which host the rare and vulnerable plant species, plans for revitalization of even one endangered species as well as *ex situ* conservation activity (*Blându, R., I. Holobiuc*, 2008). Along with protected areas, magazines, conferences and so forth, we would like to point out that Romania also hosts scientific-synthetic actions which consisted in the elaboration and actualization of "red lists" (*Dihoru, Fhe. A. Dihoru,* 1994; *Bo□caiu, N., et all.* 1994) and of the "Red Book" (*Dihoru, Ghe., G. Negrean,* 2009), containing the endangered species of Romania. The conservation of these species by *unconventional methods*, via *in vitro* micro-propagation is a high-interest concern (*De Langhe, E.A.L.,* 1984). The advantages of this method are the fact that in order to initiate the culture a single plant is needed (*Zăpâr□an, M.,* 1996), a single seed or even one single explant (the tip of the sapling, a floral bud, a segment of stem and leaf, etc.), thus plants in their natural habitat - already few and endangered - are not further affected by the collection of material from the source point.

MATERIAL AND METHODS

The beginnings of use of *in vitro* techniques for rare, endangered and endemic species in Romania, aiming for their conservation, are connected to

the last years of the last century, 1994 being the first year (*Zăpârţan, M., C. Deliu*., 1994; *Zăpârţan, M*., 1996). During the same period, at the VIIth International Congress of Plant Tissue and Cell Culture, held between 12 and 17 of June in Florence, the Romanian researchers' interest in this issue is brought to an international audience (*Zăpâr an, M.,* 1995). The results of our research are then published in conservation magazines in Europe and Romania ((*Zăpârţan, M.,* 1996 şi 2001) or in publications concerned with ornamental species (Fritillaria meleagris L. -*Zăpârţan, M.,* 1997). The Cluj Botanical Garden researcher's efforts for *in vitro* conservation and restoration of the *Astragalus peterfii* Jav. species (*Şuteu, A., et all*. 1997-1998), of certain Dianthus rare and endemic taxons (*Cristea, V*., et all., 2004; *Zăpârţan, M*., 1995) and of certain endangered species from the Gilău - Muntele Mare area (*Cristea, V*., et all., 2004) are also entitled to accolades. The conserved species list is further enriched with some vulnerable (*Banciu, C*., et all., 2006; *Blându, R., I. Holobiuc*., 2006) or rare species from the Piatra Craiului mountains (*Blându, R., I. Holobiuc*., 2007). Table 1 shows the species upon which we have conducted experiments, organized based on risk category, area, chronology and importance.

The biological material was represented by explants of different size and nature, ranging from portions of leaf and stem up to flowers, floral organs, seeds, etc. The succes of regeneration and multiplication *in vitro* of plants ***depends*** heavily on the nature and source of the explant, on the age of the donor plant, considering the fact that the youth of the plant is directly linked to the potency of the cells. It has been proven that *in vitro* regeneration takes place more easily from young tissue (generally concerning woody plants). Other plants show accelerated regeneration from tissue in full morphological activity (plants which regenerate and multiply from buds and floral organs). Others yet show best results from tissue in full physiological maturity (especially annual plants), or even from tissue in a state of repose, such as bulous plants (see Table 2).

Initialization and incubation of the *in vitro* culture has to be performed under prefectly sterile conditions, beginning with the sterilization of the material obtained from the mother plant with the aid of certain substances (in the case of the species included in Table 1, sodium hypochlorite in 3-10% concentration, according to the explant type, its structure or age, with a duration of treatment ranging from 8 to 30 minutes, relative to the texture of the tissue). According to each particular case, one can perform a preliminary treatment by submerging the source tissue in 70% alcohol for 30 seconds and then submerging it in the sterilizing substance, followed by successive washings with sterile distilled water. A great number of *in vitro culture mediums* were used: (Gautheret – 1942, Heller – 1953, Nitsch & Nitsch - 1956, Wood & Braun – 1961, Whire-1963 şi 1942, Eriksson – 1965,

Gamborg, Miller & Ojuma(B_5) – 1968, Nagata & Takebe – 1971, Schenk – Hidebrand - 1972), however the best results were obtained on the *Murashige-Skoog*- 1962 (MS) and *Schenk-Hidebran*-1972 (SH) mediums. Generally all experimental species have shown a good reaction to the MS medium, better than any of the others, because of that our final conclusions, in Table 2, contain the mean measurements on the MS medium, with certain hormonal balances.

The in vitro culture conditions after explant innoculation and during its period in the growing chamber take note of lighting, temperature and humidity, conditions which are programmed based on the needs of each species, in modern chambers. Thus, light varies according to purpose and species. We used fluorescent diffuse light with an intensity of 2-10 klux, according to the developement stage of the neo-plantules, and later on with an addition of continual light or mixes of flourescent light with red-violet, which certain types of inocules need for organogenesis to begin. Lighting time was generally 8 out of 24 hours. Growth chamber temperature is generally maintained betwee 25 and 27 degrees Celsius but some species, during incubation period, might require a different regimen - in these cases we used special acclimatized chambers, capable of ensuring the needed lighting period.

RESULTS AND DISCUSSION

Following inoculation of explants on culture medium with a hormonal balance specified in Table 2, the flasks were keps in previously described growth chamber conditions. After aproximately 2 months, measurements and observations were conducted on the 15 experimental species, regarding the explant regeneration percentages, multiplication, growth of root system, number of neo-plantules/explant and also the adaptation process for each species. Table 2 contains the mean number of roots and differentiated plantules, as well as the regeneration, multiplication and adaptation percentages.

The regeneration percentage is shown in Fig. 1, out of which we derive the superior regenerative capacity of *Dianthus petraeus* Wald., *Dianthus spiculifolium* Schur., *Fritillaria meleagris* L. şi *Syringa josikaea* J. Jacq. (70-100%), approximately 50% and even above for *Daphne cneorul* L., *Daphne mezereum* L., *Drosera anglica* Huds. şi *Leontopodium alpinum* L. (Cas.) and between 18 and 40% in the case of the remaining species *Drosera rotundiflolia* L., *Drosera intermedia* Hayne., *Lilium jankae* L. A. Kern., *Lilium martagon* L, *Paeonia tenuifolia* L. şi *Paeonia officinalis* L. ssp. banatica. Research has proven that the regenerative process of *in vitro* tissue follows the natural biological cycle of each species and that the time of year in which the plant has the highest regenerative capacity depends on

the nature of the species. The time of years which is favorable for multiplication is early spring for most perennial or annual species and late autumn (end of November) for species which multiply via bulbs (Lilium, Fritillaria).

Tabel 1

Experimental species, vulnerability category and area (*Dihoru, Ghe., G. Negreanu*., 2009) (Sozological categories: VU= vulnerable; CR =critically endangered; LR = low risk; NE= non-evaluated)

Nr. crt.	Experimental species	Sozological categories	Chronology	Areal	Importance
1.	Daphne cneorul L. (fam. Thymeleaceae)	VU (rară)	Europa	Element european mediteranean	- ornamental, medicinal
2.	Daphne laureola L. (fam. Thymeleaceae)	VU (f. rară)	Defileul Dunării, SV României	Element atlantic mediteranean	- decorative, medicinal
3.	Dianthus petraeus Wald & Kit ssp. orbelicus (Velen) (fam. Caryophyllaceae)	CR (rară)	Centru României	Element daco – Balcanic, cu areal restrâns	- scientific,
4.	Dianthus spiculifolium Schur. (fam. Caryophyllaceae)	CR (rară)	Centru României	Endemit daco – panonic cu areal restrâns	- scientific - decorative
5.	Drosera rotundiflolia L (fam. Droseraceae)	VU	Europa, M-ții Gilău	Element circumboreal	- scientific - decorative
6.	Drosera anglica Huds. (fam. Droseraceae)	CR (rară)	Numai în Transilvania	Element circumboreal, european	- scientific Decorative
7.	Drosera intermedia Hayne (fam. Droseraceae)	CR (rară)	Europa, M-ții Gilău	Element circumboareal	- scientific Decorative
8.	Doronicum orientale Hoffm. (fam. Asteraceae)	CR	SE Europei, Carpați, Italia, Anatolia, Caucaz	Element submediteranean	-decorative, researched in order to find the cause of seed sterility
9.	Fritillaria meleagris L. (fam. Liliaceae)	VU (rară)	Zone temper. Jud. Bistrița	Element carpato-pontic	- ornamental, Vernal flora element
10.	Leontopodium alpinum L. (Cas.) (fam. Asteraceae)	VU	Europa, masivele muntoase	Element carpatic	- ornamental, researched in order to find cause of endangerment
11.	Lilium Jankae L. A. Kern (fam. Liliaceae)	VU	Sporadin în C-ții Occidentali şi Meridionali (în cohabitare)	Endemit european	- ştiințific, - ornamental, New variety amelioration research
12.	Lilium martagon L (fam. Liliaceae)	VU(rară)	M-ții Cozia (în cohabitare)	Element daco-balcanic	- ornamental value
13.	Paeonia tenuifolia L. (fam. Paeoniacea)	VU (f. rară)	Cen. Transilvani. SV Olteneie, Rezervații în Zaul de Câmpie	Europa, Asia, element continental	- decorative vernal medicinal
14.	Paeonia officinalis L ssp. banatica (fam. Paeoniaceae)	CR (f. rară)	- Ungaria, Serbia, SV şi V României	Element panonic	- decorative and Medicinal
15.	Syringa josikaea J. Jacq. (fam. Oleaceae)	LR	- M. Apuseni, Valea Arieș, Bihor-Ciucea, Valea Someşului	Relict, endemit carpatic.Vicariantă a Sp. Syringa emondii, din Himalaia	- ornamental - scented flowers - blooms after Syringa vulgaris

Analyzing the *multiplication percentage* of experimental rare and endangered species, we have concluded that the maximum value has been reached by the same species as in the case of regenerative ability. Thus, multiplication percentages range from 60-100% in the case of *Dianthus petraeus* Wald & Kit ssp. Orbelicus and *spiculifolium* Schur., *Drosera anglica* Huds., *Doronicum orientale* Hoffm. and around 50% in the case of *Syringa josikaea* J. Jacq., *Drosera rotundiflolia* L. and *intermedia* Hayne., *Fritillaria meleagris* L., *Leontopodium alpinum* Cas., *Lilium martogon L. and Syringa josikaea* J. Jack. The other species contained in Table 2, although unmentioned here, have a 30-35% multiplication percentage. The maximum multiplication percentage is attained on the MS medium, in the presence of adenine sulphate.

Analyzing Figure. 3, a good percentage of adaptation can be observed, save for the *Dianthus petraeus* Wald & Kit ssp. Orbelicus and *Drosera intermedia* Hayne. species, where the percentage is 0, due to the lack of a root system, caused by a lack of auxine in the culture medium. It appears these two species need stimulents in the environment to take root, although there are many species which take root easily on simple MS 1/2 medium (with vegetal charcoal or without). Bulbous species (*Fritillaria meleagris* L, *Lilium jankae* A. Kern. and *martagon* L.) have a 100% acclimation, regardless of the size of bulbs which have been regenerated *in vitro*, whereas the other species acclimate only between 40 and 80%, depending on the species. After *ex vitro* acclimation of the explant, the species' original area must be repopulated, which makes it very important to know the origin of the plant and its' developement conditions.

Tabel 2

Aspects regarding *in vitro* micro-propagation of certain rare, vulnerable and endangered species

Nr. crt.	Experimental species	Explant type	MB + hormones(mg/l)	Regen. %	Pl nr../ expl.	Root nr./ expl.	Multi pl.%	Ada pt%
1.	Daphne cneorul L.	Bud, apex, knot	SH + 1g/l EP	50	5	4	30	20
2.	Daphne mezereum L.	Bud, apex, knot	SH + 1mg/l AIB + 1g/ EP	55	6	8	38	35
3.	Dianthus petraeus Wald & Kit ssp. Orbelicus	Apex, knot	MS1/2 + 3 g/l Cv. + 40mg/l $AdS0_4$	100	20	-	100	-
4.	Dianthus spiculifolium Schur.	Apex, knot	MS + 2mg/AIB +2m/l BA + 40mg/l $AdS0_4$	80	90	40	100	80
5.	Drosera rotundiflolia L	Floral bud	MS + 1mg/lANA + 0,1 mg/l Z	22	34	2	50	40
6.	Drosera anglica Huds.	Floral Bud	MS + 1mg/l ANA + 0,2 BA	54	30	6	70	44
7.	Drosera intermedia Hayne	Floral bud	MS + 1 mg/l BA	20	10	-	50	-
8.	Doronicum orientale Hoffm.	Knot, rhizome	MS + 1 mg/l BA +0,1 mg/l ANA + 825mg/l NH_4N0_3	45	5	6	60	60
9.	Fritillaria meleagris L.	Bulb (scales)	MS + 1mg/l AIA + 1mg/lBA + 40mg/l Ad. $S0_4$	72	10-15 Bulbs/ expl.	3	50	100
10.	Leontopodium alpinum L. (Cas.)	Seed, bloom	Ms + 2 mg/l AIB + 2mg/l BA + 40mg/l Ad.$S0_4$	66	84	10	50	72
11.	Lilium jankae L. A. Kern	Bulb (scales)	MS1/2 + 0,5mg/l ANA + 2mg/l 2iP	14	4 bulbs/ Expl.	2	23	100
12.	Lilium martagon L	Bulbs (scales)	MS1/2 + 1mg/l ANA + 0,5mg/l 2iP	40	12 bulbs/ expl.	3	50	100
13.	Paeonia tenuifolia L.	Very young bloom	MS + 0,5mg/l AIB + 2mg/l Z	22	5	2-3 rhizomes	33	45
14.	Paeonia officinalis L ssp. banatica	Very young bloom	MS + 0,5mg/l AIB + 2mg/l K	18	5	3 rhizomes	35	40
15.	Syringa josikaea J. Jacq.	Knot, apex	MS + 1mg/AIB + 0,1 mg/ BA	70	3	3-4	52	50

(MS = base culture medium; MS = Muraschige-Skoog; SH = Schenk – Hidebran; AIB = acid indolil butiric; ANA = acid naftil acetic; AIA = acid indolil acetic; BA = benzil adenină; Z = zeatină: 2iP = 2 izopentil adenină; $Ad.SO_4$ = sulfat de adenină; Cv = cărbune vegetal; EP = extract din germeni de porumb)

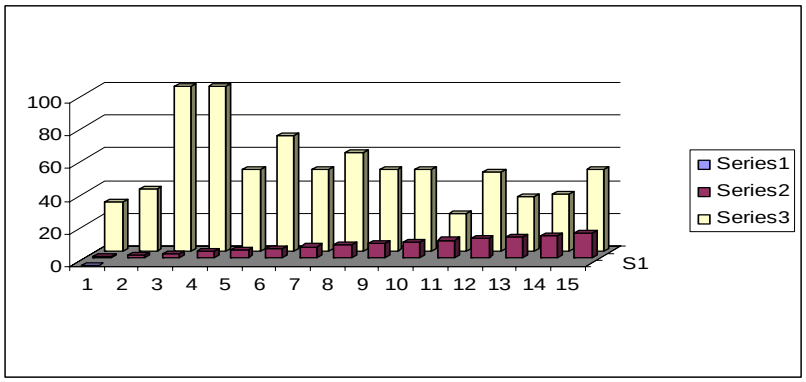

Fig. 1 Regeneration percentages of endangered species mutliplied *in vitro*
(1- 15 = species according to table numbering)

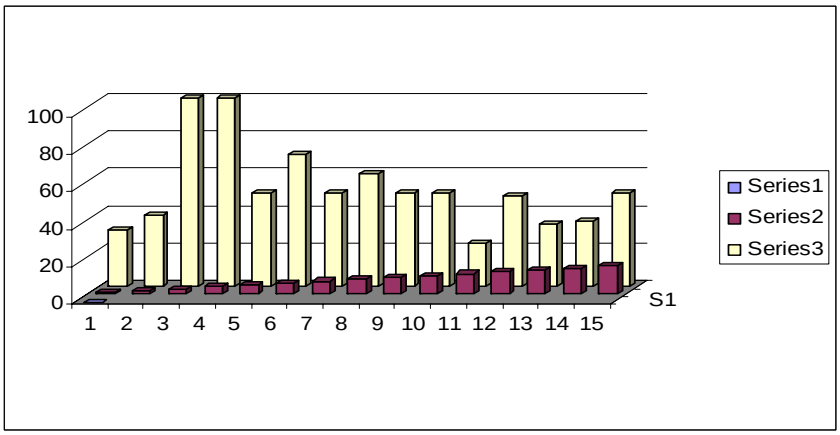

Fig. 2 Multilpication percentage for endangered species during *in vitro* breeding
(1-15 = species according to table numbering)

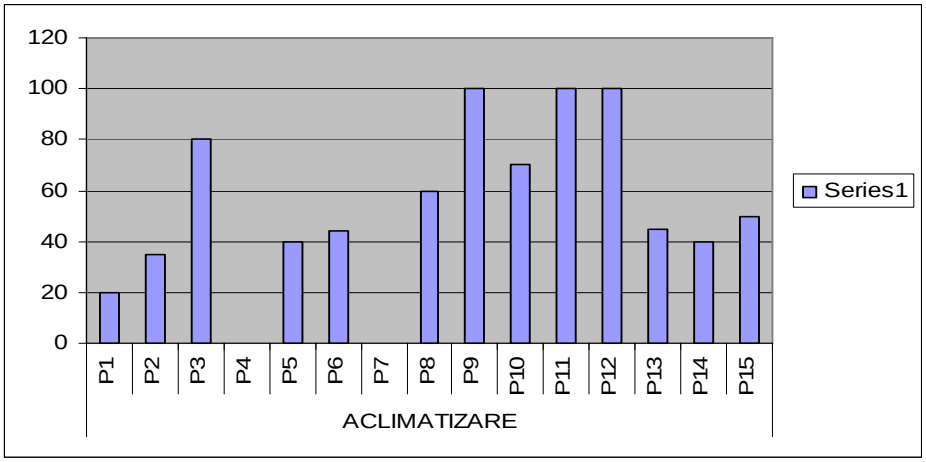

Fig. 3 Acclimation percentage of endangered species multiplied *in vitro*
(P_1 - P_{15} = species according to table numbering system)

CONCLUSIONS

1. One must make sure that the donor plant's origin and genetic variability within the population are known and preserved respectively;
2. Knowledge of the sozological category, chronology and spread area of a species ensure the success of repopulation for the original area/locus;
3. *In vitro* multiplication and regeneration is dependant on the species, on its' capcaity to adapt to *in* vitro conditions and its' metabolism's ability to reboot;
4. Essential roles in micropropagation are held by the nature, origin and age of the explant donor plant, the tissue's regenerative ability, as well as the time of year when the culture is initialized;
5. The regenerative capavity (or potency) of the cell is directly proportional to the youth of the explant donor plant, using each organ, seed or part of the plant (root, leaf, stem, bloom etc.), each with its' own multiplication and regeneration ability;
6. Climate factors in the growth chamber (light duration and intensity, humidity, temperature) must be adjusted according to each species' needs;
7. Acclimation is successfully ensure if one takes into account the intermediary stages which neo-plantules must follow in order to attain an optimal *ex* vitro survival percentage.

REFERENCES

1. Banciu, C., A., Brezeanu., M., Paucă-Comănescu., 2006, Reactivitatea *in vitro* a speciei vulnerabile *Crocus flavus* Weston, Micropropagarea speciilor vegetale. în: Lucrările celui de al XV lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale, Iaşi, 2006, p. 50-59
2. Blându, R., I., Holobiuc, 2006, *Armeria maritima* ssp. Alpina - *ex situ* conservation using *in vitro* techniques, Acta Universitans Cibiniensis, Univ. Lucian Blaga, Sibiu,
3. Blându, R., Holobiuc, I., 2007, Contributions ti *ex situ* conservation of rare plants from Piatra Craiului massif using biotechnology, Conference Proceedings The 1st Interna. Confer. Environment – Natural Sciences – Food Industrx in European Context Ensfi 2007, 1st edition, p. 483-788.
4. Blându, R., Holobiuc, I., 2008, *Conservarea ex situ a speciilor de plante din lista roşie a plantelor superioare în România*., în: Lucrările celui de al XVI-lea Simozion Naţi. de Cult. de Ţesuturi şi Cel. Vegetale., Cluj-Napoca, Ed. Risoprint, pp. 153 – 168.
5. Boşcaiu, N., Coldea Gh., Horeanu C., 1994, Lista roşie a plantelor vasculare dispărute, periclitate, vulnerabile şi rare din flora României, Ocrot. Nat. Med. Înconj., 38 (1); p. 45-56
6. Cristea, V., Miclăuş M., Puşcaş M., Deliu C., 2004, Conservative micropropagation of some endemic or rare species from the Dianthus L. genus. In: In vitro Cult. and Hortic. Breeding, Fifth IV CHB Symposium Biotehnology, as therorz and Practice in Horticulture, 2004, p. 3-13

7. Cristea V., Miclăuş M., Puşcaş M., Deliu C., Halmagyi A., 2004, The micropropagation of some endemic or rare taxa from Gilău, M-tele Mare massif. Contrib. Bot., XXXIX, Cluj – Napoca, p. 201-209.
8. Cristea, V., Denaeyer S., Herremans J.P., Goia I., 1996, Ocrotirea naturii şi Protecţia Mediului în România, Ed. University Press., Cluj – Napoca, p. 365.
9. Dihoru Ghe., Dihoru A.,, 1994, Plante rare, periclitate şi endemice din flora României – Lista roşie. Acta Bot. Hort. Bucureşti, pp. 173-197.
10. Dihoru Ghe., Negrean G., 2009, Cartea roşie a plantelor vasculare din România., Ed. Academiei Române, Bucureşti, pp. 9 – 17.
11. De Langhe, E.A.L., 1984, The rol of in vitro techniyues in germoplasm conservation, în: Crop Genetic resourrce: Conserv. and Evalution. Ed. Holden, J.H.W., Williams, J.T., Allen and Unwin, London, pp. 131-137.
12. Farusworth E., (Lead Author), Sahotra Sarkar (Topic Editor), 2007, Conservation and management of rare plant species. in: Encyclopedia of Earth. Eds. Culter J. Cleveland (Washington, D.C., Environmental Information Coalition, National Council for Science and the Environment).[Published in the Encyclopedia of Earth Ahgust 28, 2007; Retrieved January 16, 2008].
13. Maunder, M., Higgens S., 1998, A survey of Bern Convention taxa in Europan botanic gardens – initial findings. Bot. Gard. Conservation News 2(10), p. 29-33.
14. Murashige, T., Skoog F., 1962, A revised medium for rapid growth and bioassays with tobacco tissue culture. In: Physiol. Plant., 15, pp. 374-497.
15. Olteanu, M., Negreanu G., Popescu A., Roman N., Dihoru G., Sandală V., Mihăilescu S., 1994, Lista roşie a plantelor superioare din România, Studii Sinteze Documentaţii de Ecologie., Academia Română, Instit. de Biol. Bucureşti., pp. 16-31.
16. Şuteu A., Keul A.L., Mocanu S.,, Pârvu M., 1997- 1998, Research concerning *in vitro* micropropagation of Astragalus peterfii Jav., an endangered species of the Romanian Flora, Contrib. Bot., 209 – 213.
17. Zăpârţan M., Deliu C., 1994, Conservations of endemic, rare and endangered species in the Romania flora using in vitro methods Lilium martagon Kerner., in: Proceeding of the 8-th Nat. Symp. Of Indus. Microbiology and Biotehnology, Bucharest, p 423 – 434.
18. Zăpârţan M., 1994, The conservation of some rare and protected plants from Romania using in vitro methods, in: Vii-th INternational Congress of Plant Tissue and Cell, Culture, Firenze, June 12-17, p. 44.
19. Zăpârţan M., 1995, Specii endemice rare şi ocrotite conservate prin tehnici de cultură *in vitro* (*Dianthus spiculifolius* Schur.), Anuarul Univ. Oradea, Biologie II, p. 42-49.
20. Zăpârţan M., 1996, Conservarea of *Leontopodium alpinum* using *in vitro* culture techiques. Bot. Garden Micropropagation News, Kew, 2, p. 26-19.
21. Zăpârţan M., 1996 Rolul culturilor de ţesuturi în conservarea unor specii rare pentru salvarea şi extinderea lor în cultură, Contrib. Bot. Cluj – Napoca, p. 217-221.
22. Zăpârţan M., 1997, Fritilaria meleagris L. – specie rară şi vulnerabilă conservată prin tehnici de cultură in vitro. În: Actualităţi şi Perspective în Biotehnologiile Vegetale, Ed. Cachişa, D., Ardeleanu, A. and Crăciun, C., Ed. Universităţii „Vasile Goldiş", Arad p. 162-167.
23. Zăpârţan, M, 2001, Conservarea florei spontane prin înmulţire *in vitro*. Ed. Alc Media Group, Cluj –Napoca.

[XXX] Flora, Republicii Populare Române., T. Săvulescu, (ed.), de la Vol. I din 1952, până la vol. XIII. 1974

[xxx] IBPGR (International Broard for Plant genetic resources),1986, Design, planning and operation of in vitro genebanks, IBPGR, Rome
http://www.iucnredlist.org/info/2007RL_Stats_Table%202.pdf