EX SITU PLANT BIODIVERSITY CONSERVATION STRATEGIES AND SOLUTIONS

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Abstract.

Ex situ conservation is comprised of a complex of complementary measures, developed in a different ecological ambient than the original habitat, in order to rebuild and perpetuate the populations of certain species. The adopted strategies and solutions must take into account the situations in which some species have fallen to a number of individuals which is much smaller than their minimum effective, a limit under which self-restoration and self-development is no longer possible. The paths which impose ex situ conservation are based on the in vitro propagation technique for spontaneous species and on specific aspects of the in vitro organ-genesis, growth and development processes of these species. The in vitro multiplication technique of endangered spontaneous species ensures the obtainment of a large number of individuals, identical to the parent plant, thus ensuring the repopulation of the specie's original areal. Our research throughout five years has proven that spontaneous species are suitable for ex situ conservation, starting from culture initiation from a single plant, seed or explant and using a simple culture medium, sometimes without hormones, or with natural additives or extracts suitable for replacing phytohormones, which are very expensive, thus ensuring economic efficiency of the method.

Key words: Ex situ , in vitro ,neo-plantlet,differentiation,phytohormones

INTRODUCTION

Now, when technological progress has reached such a high level, when human rights ignore the reciprocity of rights and obligations, when the abusive exploitation of nature by man, especially by the western industrial society, has degenerated, when the Earth in its complexity is being threatened, it is necessary to implement a sound moral principle, an ecological morality, as the parent of biodiversity, E. O. Wilson, calls it (Jonas, H, 2000). With the end of the ONU Decade against povery (1997-2006) and after the Johanesburg Summit (August, 2002), the age's motto "Water for food" warns that water is becoming a global issue (Dorst, J. 1999), that "Humanity will die of thirst before it dies of starvation", a statement based on the fact that in the first decade of this century, circa 1 billion people did not have access to potable water and circa 10 million were dying from illnesses generated by its absence (Seager, J. 1995). In such a situation, we believe any measure meant to ensure the conservation of biodiversity in its entirety, or of highly endangered areas, is necessary, because it is a proven fact that biodiversity is of tremendous value when it comes to the durable development of humanity into the future.

Table 1

Ex situ conservation systems (Cristea V., and Denaeyer S., 2004)

No. Crt.	**Conservation system**	**Characteristics**
1	***Thematic collections***	Collections which require a great deal of work and energy consumption. They can be: 1. *working* collections, comprised for the duration of the experiment; 2. *active* collections, for exchange with similar institutions; 3. *base* collections, for long-term conservation
2.	*Gene banks* (Germplasm conservatories)	Comprised of seeds, fruit, in vitro cultures, embryos etc., which are cryogenically stored. The following types of collection emerge: **1**. of materials which are easily dehydrated and can withstand temperatures from 0 to -20^{o}C; **2**. Of refractory materials, which cannot withstand low temperatures; **3.** Lyophilating materials which can be stored only after initial vacuum dehydration; **4.** embryos and certain plants obtained in vitro can be store cryogenically at – 196^{o}C (long-term), at – 70^{o}C to – 100^{o}C (short term), or at 1-9^{o}C (temporary)
3.	*In situ conservatories*	- Also belong to *ex situ* conservation, conceived for specific bio-geographical regions, they are: orchards, cereal, vegetable, animal breed conservatories and so on, most of which are financed by the international community. An example is the Cluj Fruit Tree Research and Production Station, with a collection of 963 varieties. For Europe, the EUCARPIA program was established, which is comprised of five conservation centers: 1. for the North-West of the continent (in Brauschweig); 2. fot Russia (in St. Petersburg); 3. for Scandinavia (in Lund); 4. for Eastern Europe (in Gatersleben); 5. for the Southern and Mediterranean region (in Bari).

For a long time it was believed that *in situ* conservation remains the optimal, ideal and sole solution for the conservation of the biological communities and the ecosystems to which they belong (Boşcaiu, N., 1985; Botnariu, 1989), a prevalent method both for protectionist specialists' work and in sustainable development. However, the Convention on biodiversity in 1992 offers a sum of principles and strategies synthetized under the motto *"think globally, act locally"* (Rio de Janeiro, 1992). *Ex situ* conservation is comprised of conservation systems listed in Table 1, but the mention of some elements which are mandatory in the use of this technique is crucial (Dessauer, H.C., et al. 1996): the exact establishment of a taxonomy of conserved plants (cormophytes, inferior plants etc.), localizing the great bio-

geographical region from which the material comes (European, Oriental, Australian etc.) and determining the form under which the different species will be conserved (tissue cultures, plasmids, seeds, fruit, spores etc.). Out of the species listed as extinct in red lists and books, circa 10% are endangered, and for most of these we still do not know their role and normal function in the ecosystem in which they are integrated (Bouchet, P., 2000).

The adopted strategies and solutions should take into account the situation in which some species' individual numbers have fallen way below the inferior limit of effectiveness, beyond which self-restoration and self-development is no longer possible. The research collectives at the Biology, Agronomy, Environmental Protection Faculties and at the Botanical Gardens remain the most important institutions with a role in *ex situ* biodiversity conservation, a role which will become as important as their educational aspect. Naturalists, biologists, agronomists and zootechnicians have the fundamental task in the *ex situ* conservation mission. With the term "naturalist" we mean not only botanists, faunists and ecologists, but all those who study nature, from a macro-systemic to a micro-systemic approach, including study at a molecular level. *Ex situ* conservation is a major endeavor for European research institutes (F. Fay, 1992), as well as in Australia, India, Brazil etc. Our country complies with demands imposed by programs signed at certain international convention regarding the protection of natural resources (Bern, 1979, Sofia, 1991, Rio, 1992, Aarhus, 2001, Hague, 2001). In Romania, the conservation of endangered spontaneous flora is of interest for the above mentioned institutions, and in the cases where the research collectives have focused on the capitalization of existing resources and applying ex situ conservation technologies for biological material (Şuteu A et al, 1999; Cristea, V et al., 2002, 2004; Laslo V et al. 2010, 2011; Blându, R and Holobiuc, 2006, 2007 and 2008; Zăpârţan, M et al. 1994, 1995, 1996, 1997, 2000, 2001). *Ex situ* conservation is an alternative for the contemporary world, where social, ecological and economic problems are interdependent; engaging in this work, we fulfill a duty to future generations. We present some strategies and research paths of this method in Table 2 (Seager, J. 1995).

Table 2

Strategies and directions imposed in *ex situ* conservation (Seager, J. 1995)

No. crt.	Strategy	Characteristics
1.	The identification of the location in which the taxon survives (specie, variety, breed)	Establishing the population structure and its degree of vulnerability.
2.	Deciphering the genetic structure of the specie, population, infra-taxons, etc.	Methods already widely used by geneticists and biochemists
3.	Studying the biology and ecology of these taxons (species, subspecies, varieties, breeds)	The highlighting of the role these taxons play in the functioning of the ecosystem and the future economic value
4.	Establishing Ex situ conservation methods, respecting international methodology	Identifying the taxon, encoding and storing in the condition specific to each taxon
5.	The triggering of experiments for the restoration of natural populations	Starting from the biological material obtained through modern or classical multiplication techniques
6.	The involvement of inhabitants, human population, where necessary	For taxons with decorative, sentimental or traditional-cultural value
7.	The development of national and international programs	With the participation of universities, botanical gardens, zoos, agricultural, horticultural, zootechnical research stations
8.	Constructing certain collectives	Multidisciplinary research which can offer viable long term conservation solutions and methods

The advantages of the *ex situ* method of conservation for biological material are real, and we shall restrict the number of them we will mention in this study to the following: the obtainment of a high number of individuals which are identical to the parent plant; the short time required to obtain the material; it has the advantage of being the only method of multiplication for plants which cannot multiply via seeds; the methods can be made possible directly via *in vitro* organ-genesis or via somatic embryo genesis. The technique can also present certain disadvantages, such as the fact that while manipulating the material, unwanted somatic variations can occur in the case of spontaneous species, because the occurrence of mutations can modify the biology of the plant and its adaptive ability, or

such as the risk of reducing the genetic stock of the population (when restoring the population, a small number of genotypes is used), as well as the high cost of the technique when it comes to laboratory equipment, necessary materials and specialist training. In order to reduce the possibility of occurring mutations, we've attempted to diminish the concentration of hormones in the medium, or eliminating them completely, given the fact that hormones in the culturing environment can be mutagenic factors in the multiplication of vegetal cells.

MATERIALS AND METHODS

Our research has shown that the *ex situ* multiplication and conservation technique ensures the obtainment of a high number of phenotypically and genotypically identical individuals from the studied species, and that endemic Romanian species, rare, vulnerable and endangered species in our flora are suitable for this type of multiplication in order to expand their presence in their original areas. Initiating the *in vitro* culture began with a single plant, seed, explant (bud, knot, meristem, apex etc.), given the fact that the metabolism and heredity of vegetal cells allow for manipulation, within the limits of unaltered conservation of cell totipotency. (Cachița C-D, 2006).

Table 3

Spontaneous species conserved *in vitro* according to the conservation status and origin

No. Crt.	SPECIE	Botanical family	UICN[1] conservation cathegory	Source
1.	***Arnica montana*** *L*	Asteraceae	**V**	Fânațele Clujului; Cluj–NapocaBotanical Garden
2.	***Campanula carpatica*** *Jacq.*	Campanulaceae	**V**	PiatraFântânele(Tiha Bârgăului , BN county)
3	***Dianthus spiculifolius*** *Schur.*	Caryophylaceae	**AR**	Banat Mountains, Cheile Nerei
4.	***Drosera rotundifolia*** *L.*	Droseraceae	**R**	Gilău Mountains
5.	***Drosera intermedia*** *Hayne*	Droseraceae	**R**	Gilău Mountains
6.	***Fritillaria meleagris*** *L.*	Liliaceae	**V/R**	Lechinței hills (BN County)
7.	***Leontopodium alpinum*** *(L) Cass.*	Asteraceae	**V/R**	Retezat Mountains
8.	***Syringa josikaea*** *Jacq.*	Oleaceae	**E**	Drăganului valley, Ciucea (Cluj County)

V = vulnerable; **AR** = endemic to Romania alone, with small global populations; **R** = rare; **V/R** = vulnerable and rare; **E** = in danger of extinction

[1] UICN = International Union for Nature Conservation

Some of the species studied are listed in Table 3, which lists the botanical family, the conservation status and the origin (source) of the vegetal material. The majority of vegetal material obtained *in vitro* is available at the faculty's biotechnology laboratory. The culture medium used varied according to the nature of the specie, from those widely used from the very beginnings of *in vitro* culturing techniques (Murashige-Skoog (MS), 1962; Gamborg (B_6), 1968; Schenk-Hidebrandt (SH), 1973), to medium formulas with enriched compositions (specific for almost each individual plant group), or economical medium formulas, with halved macro and micro-elements, and even mediums without phytohormones (MS1/2) or with supplementary additives (natural extracts, vegetal coal, macro-element surplus etc. – see Table 4).

RESULTS AND DISCUSSION

After inoculating the explants collected from species experimented on and listed in this study, on mediums with compositions listed in Table 4, they were kept in growing chamber conditions (8 hours of darkness/16 hours of light, at 25-27^{0}C temperature and humidity of circa 75-89%).

Table 4

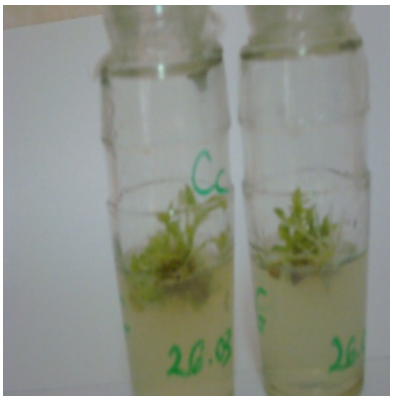
Evolution of some plant species conserved in our lab

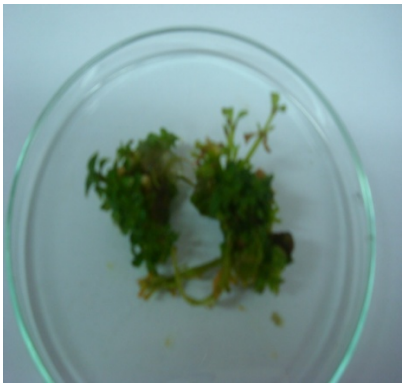
No. Crt.	SPECIES	Experimental culture mediums	Supplementary additives	Explant types	Regeneration and multiplication (%)
1.	***Arnica montana** L*	MS ½; MS ½ + 3g/l Cv.	Vegetal Coal (Cv)	Seeds, meristem, apex	65%
2.	***Campanula carpatica** Jacq.*	B_6 + 0,5 mg/l AIB + 0,5g/l BAP	-	Node, floral bud	90-92%
3	***Dianthus spiculifolius** Schur.*	MS+2mg/BA+0,5mg/l AIB+40mg/lAd.SO_4	Adenine sulfate Ad.SO_4	Seeds, apex, node	70%
4.	***Drosera rotundifolia** L.*	MS+1mg/lANA+ 0,1mg/Z; MS1/2	-	Seeds, floral bud	42%
5.	***Drosera intermedia** Hayne*	MS+0.1 mg/lZ (ori BA ori K) + 1mg/l AIA	-	Floral bud	55%
6.	***Fritillaria meleagris** L.*	MS+ 5mg/l2iP + 1,0mg /AIA+ 80mg/lAdSO_4; MS+ 0,1mg/lAIB + 0,1 mg /lBA+ 825 NH_4NO_3	Ad$S0_4$; NH_4NO_3	Floral bud	30%
7.	***Leontopodium alpinum** (L) Cass.*	MS+40mg/lAdSO_4+ 1mg/l Ep.	AdSO_4; Ep = corn extract	Seeds, meristem, floral bud	40%
8.	***Syringa josikaea** Jacq.*	SH + 3g Cv; SH+1mg/l AIB + 0,1 mg/lAIB +170 ; SH+ 5ml/l Ep.	Vegetal coal (Cv); Ep.	Seeds, node, apex, floral bud	< 55%

(MS = Murashige-Skoog, 1962: MS1/2 = Murashige-Skoog + halved macro and micro-elements; SH = Schenk – Hildebrand, 1972; B_6 =Gamborg, 1968; Z = zeatine; BA = benzilaminopurine; K = kinetine: ANA = α naphtilacetic acid; AIB = ; β indolil acetic acid)

After 4-6 weeks, observations were made on the evolution of explants cultivated *in vitro*, following the regeneration ability of each species, according to the nature of the explant, the medium composition and the neo-plantlet organ-genesis (caulogenesis, root-genesis, multiplication etc.). The biological material obtained *in vitro* in corresponding percentage (see percentages in Table 4), must overcome the problems which occur during *ex vitro* acclimation of neo-plantlets, which leads to the necessity of aiming for the obtainment of a vigorous neo-plantlet root system. Thus, this process must unfold in stages; initially in a lab, under a bell jar, then in greenhouses or cold hotbeds. After fortification, the plantlets can be transferred into the field or in an landscaping architectural space (if the specie has ornamental value), or in a collection of plants of a certain degree of endangerment, and finally in the original area of the specie. In order for the acclimation process to be successful, each stage needs to have a specific set of conditions, ensured for each particular specie.

Campanula carpatica *Jacq*





Campanula carpatica *Jacq*

Dianthus spiculifolius *Schur*

Leontopodium alpinum *(L) Cass.*

Arnica montana L

CONCLUSIONS

The *ex situ* conservation system is comprised of a complex of complementary measure, developed in an ecological ambient different from the original habitat, to the end of restoring and perpetuating the populations of certain species. The *in vitro* multiplication technique for endangered spontaneous species ensures a way of conserving environment biodiversity, other than *in situ* conservation, via the obtainment of a large number of

individuals, identical to the parent-plant, thus ensuring the repopulation of the specie's original area. Our research, within the Environmental Protection Faculty of the Oradea University, undertaken over a period of five years, have proven that spontaneous species are suitable for *ex situ* conservation, starting from the initiation of a culture from a single plant, seed or explant. Is it well known that the technique is costly, but we have achieved higher cost-effectiveness by using simple basic environments and by replacing phytohormones in the culture medium with natural extracts with identical properties concerning the stimulation of regenerative processes.

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