FORMS OF PRESERVATION OF SOME BOTANICAL ELEMENTS: DIANTHUS SEROTINUS WALDST & KIT., VAR. TRANSILVANICUS NOVÁK

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Abstract

Preservation through in vitro multiplication of the specie Dianthus serotinus Waldst & Kit., var. transilvanicus Novák, was followed, a specie critically threatened (CR), a Dacian-Pannonian endemism, a species from the country's flora, also important for its ornamental value. At the initiation of the in vitro culture we started from the juvenile flower bud, gathered from the field from Alba County (Râpa Roşie, Lancrăm) from young plants at the first flowering. Explants were cultivated on Murashige Skoog (1972) medium with the following variants: D_o = MS; D_1 = D_o + 3g/l CV + 1g/l EGP; D_2 = D_o + 0,1mg/l AIB + 0,1mg/l BA + 825mg/l NH_4NO_3; D_3 = D_o + 0,1mg/l AIB + 2mg/l BA + 40mg/l $AdSO_4$, which after three months of in vitro culture evolved depending on the composition of the medium. The best evolution took place on D_1 (economical medium devoid of phytohormones), on which there were obtained completely conformed plants. On the variants with phytohormones and additional additives there were reported undesired phenomena, vitrification or hyperhidricity of the neoplantlets due to the additional additive of 825 mg/l NH_4NO_3; or the differentiation of a weak Radicular System (short roots and few in number), but a large number of neoplantlets, about 85 neoplantlets/explant, due to the presence of BA and of $AdSO_4$ (D_3). Completely conformed neoplantlets (Photo. 1) acclimatized in a percentage of 80%, in compliance with the stages of acclimatization (Laslo, 2013). During the first year they were kept in greenhouses or in cold seedbeds, then in the spring of the second year they were planted in a definitive place (Cluj-Napoca Botanical Garden, Râpa Roşie Reserve, Alba County), a year in which they gained forcefulness. We believe that form of ex situ conservation through in vitro multiplication is in the pipeline and also it is of interest for the conservators and for the species threatened with extinction and which also have an ornamental value. For Dianthus serotinus Waldst & Kit., var. transilvanicus Novák, sozological, being critically endangered specie (CR), Dacian-Pannonian geoelement and endemism, in vitro micropropagation technique makes possible its conservation in a botanical garden, in a reserve or in its area of origin, following periodically the capacity to adapt of the new plantlets at their in vitro transfer.

Keywords: Dianthus serotinus, in situ, ex situ conservation, severely endangered, Dacian element, Dacian-Pannonian endemism, regeneration, in vitro micropropagation, ex vitro acclimatization

INTRODUCTION

In the last two decades there were introduced on the red list over 8300 species of plants with different degrees of endangerment (Sarasan, V et. al., 2006), and numerous natural habitats and plant populations have dropped heavily as number of individuals due to the anthropic activity (Laslo, 2013). According to the IUCN Report from May 2006 the rate of extinction of the species is of 100 up to 1000 times higher than the natural one, therefore appreciating that more than 50% of the species have gone extinct in the last 20 years, first of all due to the climate changes. (http://www.natureserve.org/aboutUs/PressReleases/IUCN_Red_List_release.pdf).. Global Strategy for

Plant Biodiversity (CBD) elaborated at Hague in 2002 within the Convention for Biodiversity, has the task of maintaining the plants' natural patrimony and supports researches in different fields of conservation, establishing preservation protocols of the plant species, also supporting, as main objectives, measures for the *ex situ* conservation (http://www.bgci.org.uk/files/7/0/global_strategy.pdf).

Table 1

Brief presentation of some measures and forms of plant conservation

No. Crt.	Conservation	Form	Categories
A.	***„in situ"***	Maintenance of the species in their natural ecosystem and habitat	PROTECTED AREAS, which include 10 categories: 1.Scientific Reserves; 2.National Parks; 3.Monuments of nature; 4.Reserves of nature preservation; 5.Marine and terrestrial landscapes; 6.Natural resource reserves: 7.Natural biological regions and anthropological reserves; 8.Natural regions designed for multiple uses; 9.Biosphere Reserves; 10. World treasures.
B.	***„ex situ"***	a. Thematic collections	Active and basic work collections: in Botanical Gardens or in other institutions in the field
		b. Gene banks	*In vitro* cultures of the seeds and of the tissues; Cryostorage of the germplasm and tissues obtained *in vitro* at N - liquid temperature
		c. Multiplication of plants and their conservation through "in vitro" cultures and collections	1. *In vitro* collections with periodical passing and rejuvenation of the material; 2. *In vitro* cultures with the acclimatization of the *ex vitro* plants and their relocation in the space of origin

In 1995 there was established Planta Europa, an organization concerned with the spontaneous flora conservation on the European space, which established the need for long-term conservation of plant diversity, for restoring habitats and ecosystems through programmes and measures presented in Table 1. *In situ* conservation of plants, according to some researchers, ensures the protection of the species threatened with extinction through the conservation of the biological community and of the ecosystems belonging to them, considered until the middle of the last century as being the only way of conservation (Boşcaiu, 1985). *Ex situ* conservation is an alternative to the conservation and protection of biodiversity, highlighting the independence between ecology, economy and social life (Seager, 1995), in order to restore de populations of some endangered species through measures taken into another ecological ambiance than the one within the own habitat, through the protection of the endangered elements (Cristea, and Denaeyer, 2004). Due to the isolation of the tissue cultivated *in vitro* and to its growth in different conditions towards the ones of the natural life, through *in vitro* cultures somaclonal variability is induced (Zăpârţan, 1995), that may be due to the method of preservation, to the type of explant, to the medium, genotype, age of the explant donor plant, etc. (Blându and Holobiuc, 2008). After the genomic analysis of the plant material obtained *in vitro* and after the *ex vitro* acclimatization there can be initiated repopulation actions of the endangered habitats, after a prior consultation with the specialists from the natural reserves and from the protected areas (Cristea, V. et. al., 2004;). The method of preservation through *in vitro* collections takes into account the slowing of some growth and multiplication processes (Withers, 1990a), *in vitro* collections cannot be compared to a gene bank because the material obtained *in vitro* must be conserved at the highest standards (Laslo, 2011a and b) and even at standards of biological security (Martin and Postman, 1999), and the morphological description of the material must be accompanied, as a general rule, by molecular, biochemical analysis, etc. (Withers, 1990b; Halmagyi and Keul, 2007). The shortcomings of the conservation in gene banks or in clonal field

collections made grew interest for *in vitro* conservation, the method becoming certitude, making possible a link between the classical method and the modern one, both of them complementing each other (Engelman, F., 1991; Asmore, 1997), being considered a solution for the conservation of the species that raise issues (Bajaj, 1986; Cachiță et. al., 2007).

Plant species conserved *ex situ* included a wide range of species of economic interest, as the case of *in vitro* obtaining of the planting material at some autochthonous varieties (Agud et. al., 2013; Köteles, 2013), using economical methods of *in vitro* culture (Agud, 2011), or at some ornamental species, as for example, *in vitro* conservation of some collections of roses using repeated subcultures on fresh mediums (Movchan, et. al., 2004), or other species from the spontaneous flora threatened with extinction (*Leontopodiu alpinum*, *Lilium sp.*, *Fritilaria sp.* etc.) ensuring a higher degree of protection of the plant material thus obtained than of the material obtained *in situ* (Zăpârțan, 1996; 1997;). A large number of *Dianthus* species were conserved through the *in vitro* technique, either from germplasm or from the tissue conserved in gene banks (Holobiuc et. al., 2010), or multiplied *in vitro* and acclimatized for their introduction into the collections (Cristea et. al., 2004; Zăpârțan, 2000) and planted after acclimatization at their place of origin (Zăpârțan, 1994), or following *in vitro* photoautotroph cultures at some *Dianthus* species from the spontaneous flora of the country (Cristea, Victoria 2010).

MATERIAL AND METHOD

Dianthus serotinus Waldst & Kit. specie, var. *transilvanicus* Novák from Caryophyllaceous family, has other synonyms too, depending on the area they populate (Flora RSR). It is a species of scientific interest because it is a Dacian-Pannonian endemism, with a small area and with very poor populations, being a decorative plant, considerations for which it received sozological statute of critically threatened specie (Boşcaiu et. al., 1994). Taxonomically, it is a heliophile plant, perennial, with white or cream flowers, slightly perfumed (Dihoru and Negreanu, 2009). It is a geoelement from the Pannonian basin and the center of Romania, located in several places in Transylvania, in the Olt Defile, etc. (Dihoru and Dihoru, 1994). At Râpa Roşie the specie is protected because here it is located in the reserve, in the other areas *in situ* or *ex situ* conservation measures must be applied; botanical gardens, gene banks, etc. (Fig. 1). Living on a small area and in populations which are poor in specimens, it was resorted to *in vitro* micromultiplication and to the repopulation of the area of origin.

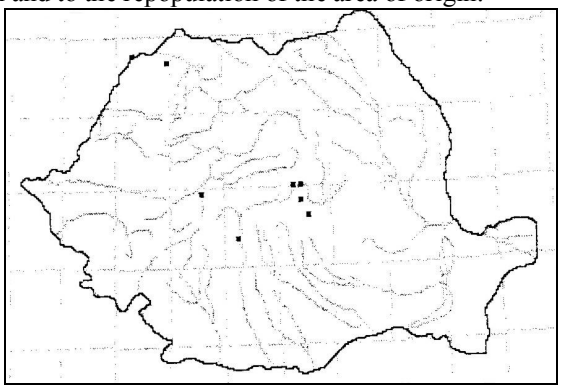

Fig. 1. Mapping areal of *Dianthus serotinus* Waldst & Kit. specie, on the Romanian territory (Dihoru and Negreanu 2009 p. 211)

From the young plant in bud of *Dianthus serotinus* Waldst & Kit., var. *transilvanicus* Novák, isolated from Râpa Roşie and Lancrăm (Alba County) explants were detached consisting of *juvenile buds* which were cultivated *in vitro* on Murashige-Skoog (MS) medium with the following variants: **D_o** = MS; **D_1** = D_o + 3g/l CV + 1g/l EGP; **D_2** = D_o + 0,1mg/l AIB + 0,1mg/l BA + 825mg/l NH_4NO_3; **D_3** = D_o + 0,1mg/l AIB + 2mg/l BA + 40mg/l $AdSO_4$.: CV = vegetal coal; EGP = natural extract of corn germ. We regularly watched the evolution of the explants until the organization of true *in vitro* cultures, following over time the capacity of organogenesis and acclimatization of the neoplantlets.

RESULTS AND DISCUTIONS

Following the evolution of the explant formed from juvenile bud detached from *Dianthus serotinus* Waldst & Kit. specie, var. *transilvanicus* Novák, after three months of *in vitro* culture there were observed the following: the regeneration of *in vitro* plantlets took place on the control medium (D_o), in a somewhat smaller number, with a slow but existing evolution; on variant D_1, the evolution is very good, the presence of the vegetal coal (CV) and also the natural extract of corn germ (EGP) in the administered concentration has a beneficial effect, the medium being also recommended by the economical formula of structure, and also by the lack of phytohormones; on medium D_2 *in vitro* differentiation of neoplantlets takes place in a good number but the phenomenon of hyperhidricity (vitrification) occurs and inhibits plant growth and the formation of a corresponding Radicular System. Vitrification is due to the phytohormones from the medium, associated with a large amount of NH_4NO_3 (825mg/l); - variant D_3 records a good evolution, over 80 neoplantlets/bud of over 1 cm height but with short and frail roots, the addition of $AdSO_4$ associated with a high dose of BA (2mg/lBA) produced a slight inhibition of the forcefulness of the Radicular System (Table 2).

Climatic factors from the growth chamber were adjusted according to the requirement of the specie, the light intensity with duration of 8 hours light from 24 hours, humidity from the vegetation chamber situated at about 80%, and temperature between 18 – 22^oC, with automatic adjustment.

The evolution of the juvenile bud detached from *Dianthus serotinus* specie and cultivated *in vitro* (after about 3 months) was followed in fig. 2 and 3 from which we can see that the largest number of neoplantlets was obtained on D_3 (MS+0,1mg/lAIB+2mg/l BA+40mg/l $AdSO_4$), about 85 plantlets/bud (Fig. 2). The best Radicular System and the best evolution take place on (MS + 3g/l CV + 1g/l EGP) and with the highest capacity of acclimatization (photo 1).

Table 2

The evolution of the juvenile floral bud of *Dianthus serotinus* cultivated on MS medium

Var.	No. pl / length (cm)	No. Roots / length (cm)	Observations	Bonification
D_o	5.5 / 4.0	7.5 / 2.0	Slow evolution	xx
D_1	15.0 / 4.8	35.0 / 2.0	Very good evolution, very good multiplication and rooting	xxxxxx
D_2	24.0 / 1.5	6.0 / 1.0	After a good multiplication vitrification occurs and affects the formation of a good Radicular System, it is necessary to transfer them on a risogen medium	xxxx
D_3	85.0 / 1.5	12.0 / 0.5	Good evolution, very, very good multiplication, less roots, shorter and frailer, it is necessary to transfer them on a simple risogen medium	xxxxx

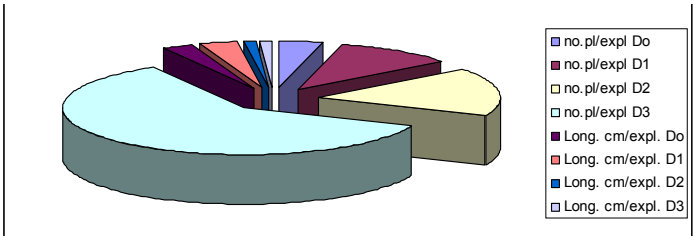

Fig. 1. The evolution of the number of plants and of their length at the bud of *Dianthus serotinus* Waldst & Kit., var. *transilvanicus* Novák (after three months)

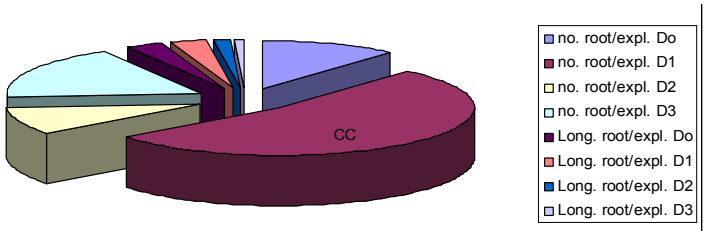

Fig. 2 The evolution of the Radicular System at the plantlets of *Dianthus serotinus* Waldst & Kit., var. *transilvanicus* Novák (after three months)

Analyzing the evolution of the bud on the medium variants it is necessary to make a few recommendations in order to find the best and most advantageous formula of medium for obtaining forceful neoplantlets and with a corresponding Radicular System, an essential condition for ensuring *in vitro* acclimatization. Vitrified neoplantlets resulted on variant D_2, for their good organization they must be transferred on a medium with another dose (1mg/lAIB) without additional NH_4NO_3, or administered alone without cytokinin. Plantlets differentiated on D_3 must be transferred on a medium with auxin in a moderate dose (0,5mg/lAIB) and only with BA or with $AdSO_4$ (not in mixture).

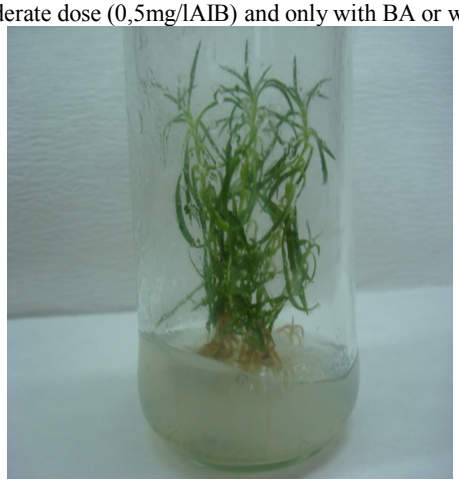

Photo. 1. The evolution of the bud of *Dianthus serotinus* Waldst & Kit., var. *transilvanicus* Novák (after three months of *in vitro* culture)

CONCLUSIONS

1. *Dianthus serotinus* Waldst & Kit. specie, var. *transilvanicus* Novák from the endemic flora of the country sozologically framed in the critically endangered group (CR), was successfully preserved through in vitro multiplication ensuring the repopulation of some spaces and areas.
2. *Dianthus serotinus* Waldst & Kit. specie, was located at Râpa Roşie and Lancrăm (Alba County), from where explants were harvested for the *in vitro* culture, with the purpose of maintaining the genetic variability within the specie.
3. Totipotency of the tissues detached from the mother plant is the bigger the donor plant of explants is younger, this is why juvenile bud harvested from the areal of the *Dianthus serotinus* Waldst & Kit. species, var. *transilvanicus* Novák., in May was used as explant.
4. The culture medium used was according to Murashige-Skoog with three variants and the control sample. The variant with additional CV and GEP, D_1 (D_o + 3g/l CV + 1g/l EGP), without hormones proved to be the best, this is why we recommend it for an advantageous *in vitro* micropropagation of the specie.
5. On the variants with phytohormones in different doses there were reported undesirable phenomena (vitrification, the lack of roots), in order to end these phenomena, neoplantlets are transferred on a fresh medium with auxin in small concentrations and without cytokinin.
6. Dacian-Pannonian geoelements, endemic for our country, critically endangered, are also preserved *ex situ*, through the *in vitro* multiplication of the specie and through the attempt to repopulate the areas of origin where they can be found in a small areal and with poor populations.
7. The success of *in vitro* multiplication and regeneration depends on the specie, nature and age of the donor plant of explants, each type of tissue having its own capacity of *in vitro* regeneration and multiplication, on the capacity to adapt to the *in vitro* conditions and to resume the metabolic processes of the regenerated neoplantlet.
8. Acclimatization of neoplantlets took place after two years of observations of the capacity to adapt of the material stored in a cold greenhouse or seedbed, therefore the percentage of acclimatization has topped 80%.

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