

## IN VITRO SEED GERMINATION IN THREE RARE TAXA FROM THE ROMANIAN CARPATHIANS FLORA

CATANĂ Rodica, HOLOBIUC Irina, MOLDOVEANU Mirela

**Abstract.** Germination of the seeds is the primary step for plant development *in vivo* and also *in vitro* conditions. The seed germination rates vary between different species, altitudes of the habitats, site locations and years, but without clear trends. In the case of endangered plant species the number of seeds may be limited or they have problems concerning their germinability. In this context, an evaluation of the seed germination under *in vitro* (using different temperatures, media variants and pH values) and *ex vitro* conditions in three rare plant species belonging to different families was made. This aspect is important for conservative purpose. Rare plant species are considered of interest because of their ability to adapt to extreme or limitative environmental conditions. The studied species were *Erigeron nanus*, *Dianthus callizonus*, *Papaver alpinum* ssp. *corona-sancti-stefani*. In *ex vitro* conditions, only *E. nanus* and *D. callizonus* germinated. In *P. corona-sancti-stefani*, seed germination was induced only in *in vitro* conditions with low germination rate (10%) at 4°C. In the case of *E. nanus*, only media variant added with sucrose (30g) allowed a good germination rate (66.66%). The seeds of *D. callizonus* germinated better on the media variant with sucrose (30g/l) and higher pH value (6.8).

**Keywords:** rare plants, seed germination, *in vitro*, *ex vitro* culture.

### Rezumat. Germinarea in vitro a semințelor la trei specii de plante amenințate din flora Carpaților din România.

Germinarea semințelor reprezintă prima etapă în dezvoltarea plantelor atât *in vivo* cât și *in vitro*. Rata de germinare a semințelor variază între specii, în funcție de altitudine, de locul și anul recoltării. Întrucât la speciile de plante rare pot apărea probleme privind viabilitatea și germinarea semințelor, a fost realizată evaluarea germinării semințelor în condiții *in vitro* (diferite temperaturi, variante de medii de cultură și pH) și *ex vitro*, la trei specii de plante din familii diferite. Acest aspect prezintă importanță conservativă. Aceste specii de plante amenințate sunt considerate de interes datorită capacității lor de a se adapta la condiții de mediu extreme sau limitante. Speciile luate în studiu au fost *E. nanus*, *D. callizonus*, *P. alpinum* ssp. *corona-sancti-stefani*. În condiții *ex vitro*, au germinat numai semințele de *E. nanus* și *D. callizonus*. La specia *P. corona-sancti-stefani* a fost indusă germinarea semințelor menținute la 4°C, în condiții aseptice însă cu rate mici de germinare (10%). În cazul semințelor de *E. nanus* o rată bună de germinare (66,66%) a semințelor în condiții *in vitro* a fost obținută pe mediu adiționat cu 30g/l și pH 5,8, fără factori de creștere. Semințele de *D. callizonus* au germinat mai bine pe varianta de mediu adiționată cu 30g/l, fără factori de creștere și pH 6,8.

**Cuvinte cheie:** plante rare, germinarea semințelor, condiții *in vitro*, *ex vitro*.

## INTRODUCTION

The seed represents the main way of plant reproduction and is one of the key factors that determine the species maintenance in time and space (VENABLE & LAWLOR, 1980). Its storage may cause the loss of its germinative capacity (AMORIM et al., 1997).

In the case of threatened, rare and endemic plant species, knowing the seed germination requirements is useful for their conservation and management. Seed germination is important for the preservation, especially for **ex situ** strategies and reproduction of rare and endangered plant species (PORTEOUS, 1993; CLEMENTE & HERNÁNDEZ, 1995; BURMEIER & JENSEN, 2008; KAGAYA et al., 2008). It is recommended to use seeds for propagation of the rare and endangered plant species (VAN WYK & SMITH, 1996). The success of rare plant conservation programs depends on the knowledge of the seed germination behaviour, being considered important in developing effective protocols for promoting **ex situ** conservation (FENNER & THOMPSON, 2005). Seed germination is also used as initial explant for obtaining *in vitro* tissue culture for secondary metabolites (VERPOORTE, 2000).

The rare plant species are that species whose taxa have small populations worldwide, which are currently not threatened but are at risk (due to their restricted area) (OLTEAN et al., 1994). Because the rare taxa are exposed to possible extinction (MACE & LANDE, 1991), studies carried on them are considered important both of practical point of view concerning biodiversity conservation (MYERS et al., 2000) and also of theoretical value due to their ability to adapt to extreme conditions.

In the case of rare plant species, some problems concerning seed viability and germination can occur.

The **ex situ** conservation means the preservation of plant species out of their natural habitats and it is ensured by **ex situ** collections (seed banks, gene banks, botanical gardens and the use of *in vitro* techniques) (IPGRI / FAO, 1996). The *in vitro* techniques can be used to optimize the culture conditions to enhance the seed germination.

*Papaver alpinum* L. ssp. *corona-sancti-stefani* (ZAPAL.) BORZA (Papaveraceae family) (Fig. 1) is a rare (OLTEAN et al., 1994; OPREA, 2005; CIOCĂRLAN, 2009) and endemic plant species for South Eastern Carpathian Mountains (CIOCĂRLAN, 2009). The species has a scientific importance being the only representative of the genus in the alpine Flora. Also, the species plays an important role as pioneer plant fixing the detritus on the rocky substrate. The limitative factors for this species are tourism and detritus crumbling (DIHORU & PÂRVU, 1987).

*Erigeron nanus* SCHUR (Asteraceae family) (Fig. 1) is considered as vulnerable /rare species (OLTEAN et al., 1994; OPREA, 2005) and endemic plant species (CIOCĂRLAN, 2009; WITKOWSKI et al., 2003). Also, it was considered as a species of European concern (OZINGA et al., 2005).

*Dianthus callizonus* SCHOTT & KOTSCHY (Caryophyllaceae family) (Fig. 1) is an endemic rare alpine flower native in a small area of South Eastern Carpathians Mountains. It is valuable from a scientific reason because of its genetics and taxonomy and because of the possibility to form hybrids with *D. spiculifolius* and *D. tenuifolius* (DIHORU & PĂRVU, 1987).



Figure 1. *P. corona sancti stefani* (photo: Catană Rodica), *E. nanus* (source net), *D. callizonus* (photo: Holobiuc Irina) in natural habitats.

Our aim was to evaluate the seed germination in different conditions in the case of three rare plant species belonging to different families in order to improve the seed germination.

## MATERIAL AND METHODS

**The plant material** was represented by mature seeds. The plant material was collected in 2006 and 2008 from the natural habitats. The germination rate was tested in 2009 for all three species.

In the case of *E. nanus* the seeds were collected from Piatra Craiului Massif in 2006.

The evaluation of the seed germination was realized on the media variants added with different growth factors (Table 1), in two different temperatures and light/dark regime.

In the case of *P. corona-sancti-stefani*, part of the seeds was obtained from the Botanical Garden Cluj-Napoca "Al. Borza" in 2003 and also some of them were collected from Piatra Craiului Massif in 2008.

In the case of *D. callizonus*, *in vitro* germination of the seeds collected from two sites, in two years and on media variants with different pH values.

**The TTC test** was made in accordance with ISTA international standards. The TTC is used to differentiate metabolic active and inactive tissues and to check the seeds viability. 1% tetrazolium chloride (2,3,5-triphenyl-2H-tetrazolium chloride) was prepared in distilled water with pH 6.5. The seeds were immersed in TTC solution and kept at room temperature in the dark for 24 hours.

**In vitro seed sterilization.** The seeds were first washed for 2 hours in running tap water, followed by HgCl<sub>2</sub> 0.1 % treatment for 10 minutes and finally, three washing in sterile distilled water were done.

The **germination rate** (%) represents the number of germinated seeds/ the number of total seeds x100. The germination rate was tested *in vitro* (at different temperatures, media variants, pH value) and *ex vitro* conditions.

**For in vitro germination**, the sterilized seeds were inoculated on the Murashige and Skoog basal media variant (MURASHIGE & SKOOG, 1962) without plant growth factors at a 16/8 photoperiod 4°C and 25°C for *P. corona-sancti-stefani*.

In *E. nanus*, four media variants (Table 1) were tested at 4°C and 25°C.

Table 1. Media variants tested for *E. nanus* seed germination.

Media variants	Basal media	Sucrose (g/l)	Growth factors (mg/L)
S1	MS	30	BAP 1, NAA 0.1
S2	MS	30	-
S3	MS	-	-
S4	MS	30	GA <sub>3</sub> 100

**Legend:** MS - Murashige & Skoog medium, (MURASHIGE & SKOOG, 1962); BAP - benzylaminopurine; NAA - alfa-naphtyl acetic acid; GA<sub>3</sub> - gibberellic acid.

In the case of *D. callizonus*, MS without growth factors and three pH values was used.

**For ex vitro germination**, the seeds were placed in Petri dishes on the filter paper in distilled water. The Petri were kept in 25°C and a 16/8 photoperiod.

A number of 60 seeds per plant species was tested for the evaluation of the seed germination using three repetitions / treatment variant.

A seed was considered to be germinated when its radicle was emerged.

**Statistics.** The results were assessed using the 1-way ANOVA created by Daniel's XL Toolbox version 5.04. A Bonferroni-Holm posthoc test was used.

## RESULTS AND DISCUSSIONS

Knowing the seed germination rate is relevant for plant ecology. In the case of alpine plant species, the asynchronous germination rate may be considered as an adaptation to the environment, where medium controlled germination can be crucial to seedling survival (PELTON, 1956; AMEN, 1966; BLISS, 1971).

Previous studies concerning seed germination of the threatened plant species were done, but a specific methodology for germination of seeds belonging to the alpine taxa was not found (KORNER, 1999). The germination behaviour can vary within a single species from one population to another, from year to year and among individuals (URBANSKA & SCHUTZ, 1986).

A lot of studies concerning the evaluation of seed germination of rare plant species in non-sterile and sterile conditions were performed (CUEVAS & FIGUEROA, 2007; KANDARI et al., 2008; KADIS et al., 2010; SAYANIKA DEVI et al., 2012; ABDOLLAHI et al., 2012).

The storage conditions of the seeds are one of the many other factors which can influence the seed quality expressed as seed viability and vigour. It is known that there are differences between species concerning seed longevity (how long seeds can be stored under given conditions). This aspect is crucial for an effective management of seed conservation collections (PROBERT et al., 2009).

The TTC test is an important rapid seed viability test based on the activity of dehydrogenases that catalyse mitochondrial respiration. In the living tissues of the seeds, dehydrogenases convert the TTC to formazan (NETO et al., 1999) an insoluble red compound that stains in red the living tissues. The presence of formazan allows the differentiation between living and inactive metabolic tissues (FILHO, 1999).

The TTC test results in the case of the three threatened plant taxa are shown in Table 2.

Table 2. TTC test performed on the seeds of three different plant taxa.

Species	Collected data	Sources	TTC reaction
<i>P. corona-sancti-stefani</i>	2003,	Botanical Garden Cluj, Piatra Craiului	-
	2008		+++
<i>E. nanus</i>	2006	Piatra Craiului	++
<i>D. callizonus</i>	2004,	Piatra Craiului	+
	2006		+++

**Legend:** +++ good reaction, ++ low reaction, + very low reaction, - no reaction.

Germination of the seeds is a complex physiological process determined by the imbibitions of the tissues with water, after the dormancy mechanisms were delayed. The seed germination depends on the internal (endogenous plant hormones) and external conditions (temperature, water level, oxygen content and light or darkness regime) (RAVEN et al., 2005; SEN, 2010). Also, it is already known that there are differences concerning the germination requirements connected to the geographical distribution (PROBERT, 2000).

In our case, the seed germination in sterile conditions (*in vitro*) was higher than in non-sterile conditions (*ex vitro*) for the all three plant taxa (Table 3).

In *ex vitro* conditions, only *E. nanus* and *D. callizonus* have germinated.

In the case of *in vitro* conditions, the seed germination was induced at low rate in *P. corona-sancti-stefani* and improved in *E. nanus* and *D. callizonus*.

Table 3. Formation of seedlings and % of seed germination in the three threatened taxa.

Species	Conditions	No. of seeds	Mean no. of seedlings	% of seed germination	P value	F Critical
<i>P. corona-sancti-stefani</i>	<i>In vitro</i>	30	4	13.33*	0.01613	16
	<i>Ex vitro</i>	30	0	0		
<i>E. nanus</i>	<i>In vitro</i>	30	21	70*	0.03759	9.375
	<i>Ex vitro</i>	30	6	20		
<i>D. callizonus</i>	<i>In vitro</i>	30	24	80*	0.047421	8
	<i>Ex vitro</i>	30	14	46.66		

**Legend:** Values marked by \* are significantly different between *in vitro* and *ex vitro* conditions at  $p < 0.05$  using the posthoc test Bonferroni-Holm.

Some *in vitro* studies were done concerning regeneration in *Papaver* species starting from seeds (TISSERAT & BERHOW, 2009; ZAKARIE et al., 2011). Also, a comparative study of seed germination ecology in four *Papaver* taxa was made (KARLSSON & MILBERG, 2007).

In the case of *P. corona-sancti-stefani*, the seeds collected in 2003 showed no TTC positive reaction and no germination rate neither in *ex vitro* nor in sterile (*in vitro*) conditions (Table 2). The seeds collected in 2008 germinated only in sterile conditions. There are some studies concerning the germination requirements which differ from year to year (KAYE et al., 1999). The variation in germination characteristics could be interpreted as one of the most important survival strategies for species growing under unpredictable environmental conditions (GUTTERMAN, 1994; KIGEL, 1995).

Two different temperatures (25°C and 4°C) were tested for the seeds collected in 2008 to induce the germination *in vitro*. After 45 days, the germination rate of the seeds was low, with non-significant differences registered between the tested temperatures (Fig. 2).

Despite of the data which proved that the seeds belonging to *Papaver* genus germinate and growth at 20-25°C (TISSERAT & BERHOW, 2009; GORGOROV et al., 2011), in our case, *P. corona-sancti-stefani* seeds germinated better at 4°C.

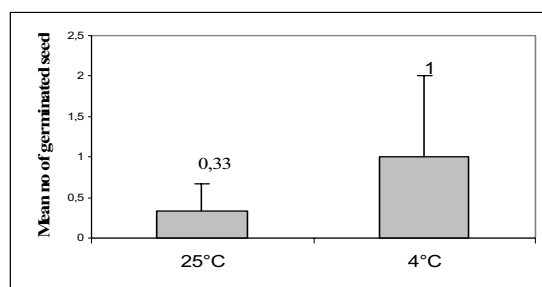


Figure 2. Mean number of *P. corona-sancti-stefani* seeds germinated at 25°C and 4°C.

Some studies in *Erigeron* species concerning seed germination in laboratory conditions were made (CUEVAS & FIGUEROA, 2007).

In the case of *E. nanus* SCHUR, four media variants, two different temperatures and light/dark regime were tested to increase the germination rate. A high percentage of seed germination of *E. nanus* (66.66%) was obtained at 25°C in the presence of light, while at 4°C in the dark, the seed germination rate was inhibited (Fig. 3). According to the literature, more than 86% of the species require light to germinate without requiring cold treatment. The data obtained are in concordance with studies on seed germination ability in other species of rare plants (KOORNNEEF et al., 2002).

The seed germination is genetically determined and affected by endogenous plant hormones (BENTSINK & KOORNNEEF, 2002). The applications of exogenous plant growth factors (PGR) have been extensively used for enhancing the seed germination and the development of seedlings in the laboratory. There are several studies regarding the influence of cytokines on the germination capacity of seeds in different species (NIKAM & BARMUKH, 2009; NIKOLIĆ et al., 2006). The gibberellins are most prominent growth regulator used (SHUE-LOCK, 1968; CERABOLINI et al., 2004).

In our case, the addition of BAP (1mg/l) in one medium variant had no significant effect on the seed germination, this being lower than the rate obtained in the case of S2 medium variant (MS medium without PGR).

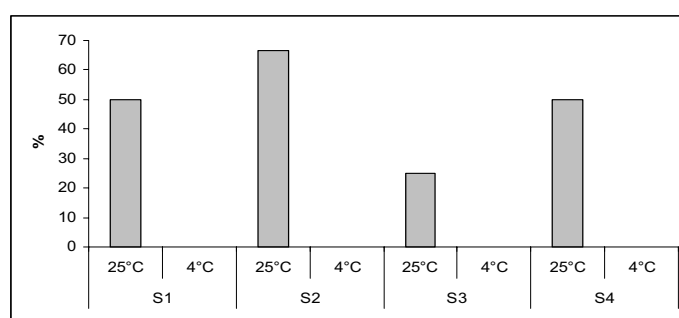


Figure 3. The seed germination rate of *E. nanus* at 25°C and 4°C and light/dark regime.

S4 variant added with 100mg/l GA<sub>3</sub> allowed about 20% of the seeds to germinate (Fig. 4). Between the four media variants tested for seed germination there are statistically significant differences ( $p < 0.05$ ).

The four tested media variants (S1-S4) (Table 2) allowed the seeds to germinate with different rates, with good results on the S2 media variant added with 30g/l sucrose (Fig. 4).

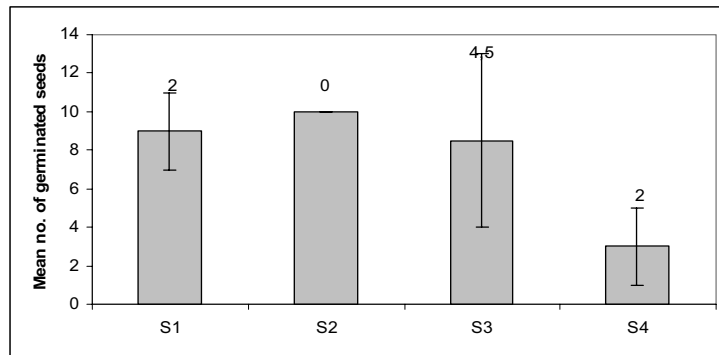


Figure 4. The mean number of *E. nanus* seeds germinated on S1-S4 media variants.

There are some studies concerning the seed germination in Caryophyllaceae family. A comparative research concerning germination of the seeds collected from different side of Europe was done (THOMPSON, 1970). Other studies of the seed size variation in the populations in relation to habitat conditions were performed in *Silene dioica* (L.) CLAIRV (THOMPSON, 1981). Studies concerning *in vitro* seed germination in *Dianthus* species were made in *D. nardiformis* JANKA (HOLOBIUC et al., 2009), in *D. ciliatus* ssp. *dalmaticus* and *D. giganteus* ssp. *croaticus* (RADOJEVIĆ et al., 2010), in *D. henteri* (CRISTEA et al., 2010), in *D. barbathus* (LĂPĂDĂTESCU et al., 2012).

In our case, the *D. callizonus* seeds tested in *ex vitro* conditions showed over 40% germination rate. These data are in accordance to those obtained by MICLE in 1967, who reported that the germination of *D. callizonus* seeds in natural habitat was around 35-50% (MICLE, 1967).

Comparing the germination rate of the seeds collected in 2004 and 2006, the seeds collected in 2004 lost their germinability (Fig. 5). These data are in accordance with the data of KAYE et al., 1999.

The seeds collected in 2006, are originated from two different sites of Piatra Craiului Massif: Piatra Craiului Mică (1811 m altitude) and Padina Popii (2018 m altitude). The distance between the two sites is ~ 20 km. In our case, non-significant differences were obtained concerning the seed germination rate from the two sites (Fig. 4). The same results were reported by GIMÉNEZ-BENAVIDES et al., 2005 in 20 endemics species of the Iberian Peninsula who did not find a consistent pattern in the germination rate related to altitude variation.

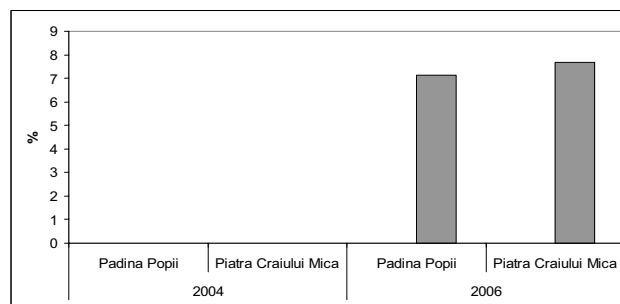


Figure 5. The germination rate of *D. callizonus* seeds collected in 2004 and 2006 from two different sites.

Other important factor that can influence the seed germination is the pH value. Three different pH values (4.8; 5.8 and 6.8) were tested. The higher number of germinated seeds was obtained on the medium variant with 6.8 pH (Fig. 6). The differences between the three used pH values were statistically non-significant ( $p < 0.005$ ). These results are in accordance with the data recorded in the natural habitats, *D. callizonus* being an alpine species growing on calcareous substrate with 6-7.5 pH (ONETE, 2011). Our data in *D. callizonus* are similarly to the experiment made in *Salvia* sp. where the 5.0 to 6.5 pH values increased seed germination (SHOEMAKER & CARLSON, 1990).

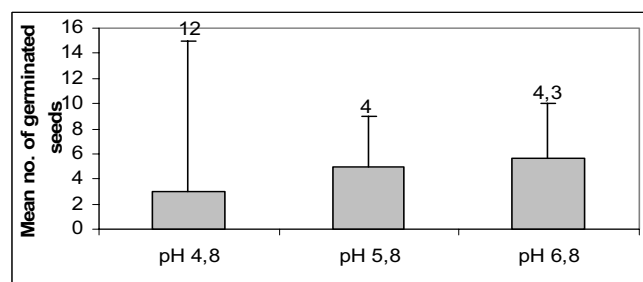


Figure 6. The mean number of germinated seed of *D. callizonus* at different pH values.

The plants obtained through *in vitro* seed germination were used for the establishment of short-term preservation protocol in *E. nanus* and *D. callizonus* (HOLOBIUC et al., 2005; HOLOBIUC & BLÎNDU, 2006; BLÎNDU & HOLOBIUC, 2007), for *in vitro* conservation under slow-growth conditions during medium-term (HOLOBIUC & BLÎNDU, 2006; CATANĂ et al., 2010a). In *E. nanus*, *in vitro* regenerants obtained from one germinated seed was used to assay the genetic stability (CATANĂ et al., 2010b).

### CONCLUSIONS

The seed germination rates were improved in the case of *in vitro* conditions in all three taxa tested.

Seeds of *P. corona-sancti-stefani* germinated better at 4°C.

Seeds of *E. nanus* germinated at 25°C in the presence of light on the medium variant added with 30g/l sucrose, without plant growth factors and 5.8 pH value.

For *D. callizonus* seeds germination, it is suitable a medium added with 30g/l sucrose without plant growth factors, at 6.8 pH.

*In vitro* germinated seedlings were used for further studies concerning *in vitro* preservation during short and medium-term preservation.

### ACKNOWLEDGEMENTS

This study was funded by CNCIS Research projects for scientific research/artistic creation youth candidates for a doctor's degree, no. 227/1.10.2007 entitled "The use of *in vitro* techniques for the conservation of rare plants from Piatra Craiului Massif". We gratefully thank to Dr. Onete M. for the collection of the seeds.

### REFERENCES

- ABDOLLAHI J., EBRAHIMI M., RAMSHINI H. A., JAAFARI A. A., EFTEKHARI M., MANSOURI M. A., GOHARRIZI MASB. 2012. *Seed germination as the major conservation issue of endemic Iranian salvia species*. Journal of Medicinal Plants Research. Academic Journals, DOI: 10.5897/JMPR11.690. **6**(1): 37-46.
- AMEN R. D. 1966. *The extent and role of seed dormancy in alpine plants*. Quarterly Review of Biology. **41**: 271-28.
- AMORIM I. L., DADIVE A. C., CHAVES M. M. F. 1997. *Morphology of fruit, seed and germination pattern of Tremma micrantha (L.) BLUM*. Cerne. Universidade Federal de Lavras. Brasil. **3**(1): 138-152.
- BENTSINK L. & KOORNNEEF M. 2002. *Seed dormancy and germination*. The Arabidopsis book (Eds. C. R. Somerville and E. M. Meyerowitz), <http://www.aspb.org/publications/arabidopsis/> American Society of Plant Biologists, Rockville, MD. DOI 10.1199/tab.0050 (Accessed: February 6, 2007).
- BLÎNDU RODICA & HOLOBIUC IRINA. 2007. *Contributions to ex situ conservation of rare plants from Piatra Craiului Massif using biotechnology*. Conference Proceedings The 1<sup>st</sup> International Conference Environment-Natural Sciences-Food Industry in European Context Ensfi. Baia Mare. **1**: 483-788.
- BLISS L. C. 1971. *Arctic and alpine plant life cycles*. Annual Review of Ecology and Systematics. USA. **2**: 405-438.
- BURMEIER SANDRA & JENSEN K. 2008. *Is the endangered Apium repens (JACQ.) LAG. rare because of a narrow regeneration niche?* Plant species biology. Wiley Blackwell. Germany. **23**: 111-118.
- CATANĂ RODICA, CIUCĂ MATILDA, HOLOBIUC IRINA. 2010a. *Using RAPD techniques to check the genetic stability of Erigeron nanus Schur regenerants in the ex situ conservation context*. Analele Universității din Oradea. Fascicula Biologie. University of Oradea Publishing House. **2**: 230-234.
- CATANĂ RODICA, MITOI ELENA MONICA, HELEPCIUC FLORENȚA, HOLOBIUC IRINA. 2010b. *In vitro conservation under slow growth conditions of two rare plant species from Caryophyllaceae family*. Electronic Journal of Biology. <http://www.ejbio.com/pps/2010/86.pdf>. **6**(4): 86-91. (Accessed: March 3, 2013).
- CERABOLINI B., ANDREIS R., CERIANI R. M., PIERCE S., RAIMONDI B. 2004. *Seed germination and conservation of endangered species from the Italian Alps: Phsoplexis comosa and Primula glaucoscapa*. Biological Conservation. Elsevier Ltd. **117**: 351-356.
- CIOCĂRLAN V. 2009. *Flora ilustrată a României. Pteridofita et Spermatophyta*. Edit. Ceres. București. 1141 pp.
- CLEMENTE M. & HERNÁNDEZ E. 1995. *A conservation plan for the Andalusian flora*. Plant Talk Magazine. London. **2**: 16-18.
- CRISTEA VICTORIA, BRUMMER A.T., JARDA LILIANA, MICLĂUȘ L. 2010. *In vitro culture initiation and phytohormonal influence on Dianthus henteri- a Romanian endemic species*. Romanian Biotechnological Letters. **15**(1): 25-3.
- CUEVAS JAIME G. & FIGUEROA J. A. 2007. *Seed germination of species of the Juan Fernández archipiélago under laboratory conditions*. Gayana Botánica. Universidad de Concepción. Chile. **64**(1): 60-80.
- DIHORU G. & PÂRVU C. 1987. *Plante endemice în Flora României*. Edit. Ceres. București. 179 pp.
- FENNER M. & THOMPSON K. 2005. *The ecology of seeds*. Cambridge. Cambridge University Press. 260 pp.
- FILHO M. J. 1999. *Testes de Vigor: Importância e utilização*. In: Krzyzanowski F. C., Vieira R. D., França-Neto J. B. (Eds.). Vigor de sementes: conceitos e testes. Abrates. **1**: 1-21.
- GIMÉNEZ-BENAVIDES L., ESCUDERO A., PÉREZ-GARCIA F. 2005. *Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability*. Ecological Research. Springer. **20**: 433-444.

- GORGOROV ROSSEN N., YANKOVA ELINA P., BALDJIEV G. A., APOSTOLOVA IVA I, YURUKOVA-GRANCHAROVA PETKA D., STANILOVA MARINA I. 2011. *Reproductive capacity and in vitro cultivation of the glacial relict Papaver degenii (Papaveraceae)*. Phytologia balcanica. Bulgarian Academy of Sciences. Sofia. **17**(3): 333-339.
- GUTTERMAN Y. 1994. *Strategies of seed dispersal and germination in plants inhabiting deserts*. Botanical Review. The New York Botanical Garden, New York. **60**: 373-425.
- HOLOBIUC IRINA, PAUNESCU ANCA, BLINDU RODICA. 2005. *Ex situ conservation using in vitro methods in some Caryophyllaceae plant species from the Red List of the vascular plants in Romania*, Romanian Journal of Biology. Plant Biology. București. **49-50**: 3-16.
- HOLOBIUC IRINA & BLINDU RODICA. 2006. *Improvement of the micropropagation and in vitro medium-term preservation of some rare Dianthus species*. Contribuții Botanice. Cluj-Napoca. **41**(2): 143-151.
- HOLOBIUC IRINA, BLINDU RODICA, CRISTEA VICTORIA. 2009. *Researches concerning in vitro conservation of the rare plant species Dianthus nardiformis Janka*. Biotechnology & Biotechnological Equipment. **23**(2): 221-224.
- KADIS C., KOUNNAMAS C., GEORGHIOU K. 2010. *Seed germination and conservation of endemic, rare and threatened aromatic plants of Cyprus*. Israel. **58**: 251-261.
- KAGAYA M., TANI T., KACHI N. 2008. *Effect of gravel conditions on seedling emergence of the endangered monocarpic perennial Aster kantoensis (Compositae) on a floodplain*. Plant Species Biology. Wiley Blackwell. Germany. **23**: 47-50.
- KANDARI L. S., RAO K. S., MAIKHURI R. K., CHAUHAN KUSUM. 2008. *Effect of pre-sowing, temperature and light on the seed germination of Arnebia benthamii (WALL. ex G. DON): An endangered medicinal plant of Central Himalaya, India*. African Journal of Plant Science. Academic journals. **2**(1): 005-011.
- KARLSSON LAYLA M. & MILBERG PER. 2007. *A Comparative Study of Germination Ecology of Four Papaver Taxa*. Annals of Botany. Oxford Journals. **99**(5): 935-946.
- KAYE T. N., KIRKL M., TESTA N. 1999. *Growing endangered plants to save them: Germinating, propagating and restoring pink sandverbena*. In: R. Rose and D. Haase (eds.). Native plants: Propagating and planting. Forest Research Laboratory. Oregon State University: 100-107.
- KIGEL J. 1995. *Seed germination in arid and semiarid regions*. In: Kigel J, Galili G (Eds). Seed development and germination. Marcel Dekker Press Inc. New York: 645-699.
- KOORNNEEF M., BENTSINK L., HILHORST H. 2002. *Seed dormancy and germination*. Current Opinion in Plant Biology. **5**: 33-36.
- KÖRNER C. 1999. *Alpine plant life: functional plant ecology of high mountain ecosystems*. Springer. Berlin Heidelberg New York. 338 pp.
- LĂPĂDĂTESCU SIMONA, PETOLESCU CERASELA, VELICEVICI GIANCARLA, BĂLA MARIA. 2012. *In vitro culture initiation and phytohormonal influence on ornamental plants*. Journal of Horticulture, Forestry and Biotechnology. Timișoara. **16**(2): 203-205.
- MACE G. M. & LANDE R. 1991. *Assessing extinction threats: toward a reevaluation of IUCN threatened species categories*. Conservation Biology. Elsevier Ltd. **5**: 148-157.
- MICLE F. 1967. *Studiul germinăției unor specii din familia Caryophyllaceae în funcție de vârsta semințelor*. Contribuții Botanice. Cluj Napoca: 217-221.
- MURASHIGE T. & SKOOG F. 1962. *A revised medium for rapid growth and bio-assays with tobacco tissue cultures*. Physiol Plant. Dartmouth Journal Services. Waterbury. **15**(3): 473-497.
- MYERS N., MITTERMEIER R., MITTERMEIER CRISTINA G., DA FONSECA GAB., KENT JENNIFER. 2000. *Biodiversity hotspots for conservation priorities*. Nature. doi:10.1038/35002501. **403**: 853-858 (Accesed: March 6, 2013).
- NETO F. J. B., KRZYŻANOWSKI F. C., COSTA N. P. 1999. *Metodologia do teste de tetrazólio em sementes de soja*. In: Krzyzanowski, F. C., Vieira, R. D., França Neto, J. B. (Eds.). Vigor de sementes: conceitos e testes. Londrina: Abrates. **8.5**: 1-28.
- NIKAM T. D. & BARMUKH R. B. 2009. *GA<sub>3</sub> enhances in vitro seed germination in Santalum album*. Seed Science and Technology. International Seed Testing Association. **37**(2): 276-280. (Accesed: February 10, 2012).
- NIKOLIĆ R., MITIĆ N., MILETIĆ R., NEŠKOVIĆ M. 2006. *Effects of cytokinins on in vitro seed germination and early seedling morphogenesis in Lotus corniculatus L*. Journal Plant Growth Regulation. Springer Germany. **25**: 187-194.
- OLTEAN M., NEGREAN G., POPESCU A., ROMAN N., DIHORU G., SANDA V., MIHĂILESCU SIMONA. 1994. *Lista roșie a plantelor superioare din România*. Studii, Sinteze, Documentații de Ecologie. Academia Română. București. 26 pp.
- ONETE MARILENA. 2011. *Studiul ecologic al unor populații de Dianthus callizonus și Dianthus gelidus*. Editura Ars Docendi. București. 236 pp.
- OPREA A. 2005. *Lista Critică a Plantelor Vasculare din România*. Edit. Universității "Al. Ioan Cuza". Iași. 231 pp.
- OZINGA W. A. & SCHAMINÉE J. H. J. (Eds.). 2005. *Target species - Species of European concern*. A database driven selection of plant and animal species for the implementation of the Pan European Ecological Network. Wageningen, Alterra. Alterra-report. **1119**. 193 pp.
- PELTON J. 1956. *A study of seed dormancy in eighteen species of high altitude Colorado plants*. Butler University Botanical Studies. **13**: 74-84.
- PORTEOUS T. 1993. *Native forest restoration. A practical guide to landowners*. Queen Elizabeth II National Trust. Wellington. New Zealand. 184 pp.

- PROBERT J. R. 2000. *The Role of Temperature in the Regulation of Seed Dormancy and Germination*. CAB International 2000. Seeds: The Ecology of Regeneration in Plant Communities, 2nd edition. Ed. Fenner M (CABI, Wallingford). 261 pp.
- PROBERT J. R., DAWS M. I., HAY R. FIONA 2009. *Ecological correlates of ex situ seed longevity: a comparative study on 195 species*. Annals of Botany. Oxford Journals. **104**(1): 57-69.
- RADOJEVIĆ L., DUŠICA Ć. D., JOVANKA Š., BRANKA S., STEVANOVIĆ V. 2010. *In vitro propagation of Dianthus ciliatus ssp. dalmaticus and D. giganteus ssp. croaticus (Caryophyllaceae) from stem segment cultures*. Botanica serbica. Belgrade. **34**(2): 153-161.
- RAVEN P. H., RAY F. E., EICHHORN SUSAN. 2005. *Biology of Plants, 7<sup>th</sup> Edition*. New York: W. H. Freeman and Company Publishers: 504-508.
- SAYANIKA DEVI W., BENGYELLA L., SHARMA G. J. 2012. *In vitro seed germination and micropropagation of edible bamboo Dendrocalamus giganteus Munro using seeds*. Biotechnology. URL: <http://scialert.net/abstract/?doi=biotech.2012.74.80>. **11**: 74-80. (Accessed: December 3, 2011).
- SEN S. 2010. *S-nitrosylation process acts as a regulatory switch for seed germination in wheat*. American Journal of Plant Physiology. Elsevier GmbH. **5**: 122-132.
- SHOEMAKER CANDICE A. & CARLSON W. H. 1990. *pH affects seed germination of eight bedding plant species*. Hort Science. **25**(7): 762-764.
- SHUE-LOCK L. A. M. 1968. *Interaction of temperature and gibberellin on potato seed germination*. American Journal of Botany. Castleman Ave. St. Louis. **55**(2): 193-198.
- THOMPSON P. A. 1970. *Germination of species of Caryophyllaceae in Relation to their Geographical Distribution in Europe*. Annals of Botany. Oxford Journals. **34**: 427-449.
- THOMPSON P. A. 1981. *Variations in Seed Size within Populations of Silene dioica (L.) Clairv. in relation to Habitat*. Annals of Botany. Oxford Journals. **47**: 623-634.
- TISSERAT B. & BERHOW M. 2009. *Production of pharmaceuticals from Papaver cultivars in Vitro*. Engineering in Life Sciences. **9**(3): 190-196.
- URBANSKA K. M. & SCHÜTZ M. 1986. *Reproduction by seed in alpine plants and revegetation research above timberline*. Botanica Helvetica. Swiss Botanical Society. **96**: 43-60.
- VAN WYK B. E. & SMITH G. 1996. *Guide to the Aloes of South Africa*. Briza Publications. Pretoria. South Africa. 304 pp.
- VENABLE D. L. & LAWLOR L. 1980. *Delayed germination and dispersal in desert annuals: escape in space and time*. Oecologia. **46**: 272-282.
- VERPOORTE R. 2000. *Secondary metabolism*. In: Verpoorte R, Alfermann AW (Eds). Metabolic engineering of plant secondary metabolism. Kluwer Academic Publishers, London: 1-29.
- ZAKARIA R. A., HAGHIGHAT HOUR MARYAM, NASER ZARE. 2011. *Callus production and regeneration of the medicinal plant Papaver orientale*. African Journal of Biotechnology. Academic Journals. **10**(54): 11152-11156.
- WITKOWSKI Z. J., KRÓL W., SOLARZ W. (Eds.). 2003. *Carpathian List Of Endangered Species*. WWF and Institute of Nature Conservation, Polish Academy of Sciences, Vienna-Krakow. Published by: WWF International Danube-Carpathian Programme, Vienna, Austria and Institute of Nature Conservation. Polish Academy of Sciences. Krakow. Poland. 84 pp.
- \*\*\*. ANOVA created by Daniel's XL Toolbox version 5.04. <http://xltoolbox.sourceforge.net> (accessed: March 1, 2013).
- \*\*\*. IPGRI/FAO. 1996. *International Technical Conference on Plant Genetic Resources: Preparatory process for Europe*. International Plant Genetic Resources Institute/Food and Agricultural Organization, Rome, Italy. <http://www.fao.org/sd/wpdirect/wpre0012.htm> (Accessed: October 2008).
- \*\*\*. ISTA (International Seed Testing Association). 1999. *Rule 9.5.8. Low constant temperature oven method*. Seed Science and Tehnology. (Suppl. International Rules for Dees testing Rules 1999). Switzerland. **27**. 49 pp.
- \*\*\*. *Erigeron nanus*. [http://lh3.ggpht.com/\\_pJ787NOjpdK/SV\\_BfHuHMGi/AAAAAAAAAoKA/3psnUREogWE/erigeron+nanus.jpg](http://lh3.ggpht.com/_pJ787NOjpdK/SV_BfHuHMGi/AAAAAAAAAoKA/3psnUREogWE/erigeron+nanus.jpg) (Accessed: October 14, 2008).

**Catană Rodica**

Institute of Biology Bucharest,  
Romanian Academy,  
296 Splaiul Independenței, 060031  
Bucharest, Romania.  
E-mail: catanarodica@yahoo.com

**Holobiuc Irina**

Institute of Biology Bucharest,  
Romanian Academy,  
296 Splaiul Independenței, 060031  
Bucharest, Romania.  
E-mail: irina.holobiuc@ibiol.ro

**Moldoveanu Mirela**

Institute of Biology Bucharest,  
Romanian Academy,  
296 Splaiul Independenței, 060031  
Bucharest, Romania.  
E-mail: mirela.moldoveanu@ibiol.ro

Received: March 19, 2013  
Accepted: April 18, 2013