

# Seed banking of Polish endangered plants – the FlorNatur Project

Jerzy Puchalski\*, Maciej Niemczyk, Piotr Walerowski,  
Wiesław Podyma & Adam Kapler

Polish Academy of Science Botanical Garden – Center for Biological Diversity Conservation in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland  
\* corresponding author (e-mail: bgpas@obpan.eu)

**Abstract:** Among the 2750 species of the Polish vascular flora, about 500 species are threatened with extinction and 430 of them are strictly protected by national law. The FlorNatur project for the *ex situ* conservation of the most endangered species was started in 2009. The aim of the project is to collect seeds of 61 species from 161 sites in eastern Poland and store them in the Seed Bank of the Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Warsaw-Powsin. A complementary program is being carried out by the Forestry Gene Bank at Kostrzyca in western Poland. Their task is to collect 58 species from 129 natural sites in the western part of Poland. To date, seeds of 31 species from 56 populations have been collected, tested and stored in liquid nitrogen.

**Key words:** seedbank, threatened plants, Polish flora, cryo-conservation, *ex situ* conservation, dormancy, *Fritillaria meleagris*

## 1. Introduction

Strict area protection is a commonly used method for the preservation of endangered species (Pimm & Lawton 1998; Bruner *et al.* 2001; Brooks 2004). Paradoxically sometimes it leads to local extinction or significant decreases in the numbers of some rare species due to secondary succession (habitat evolution), and the altering of open habitats suitable for protected species into more shady forest habitats (Michalik 1990; Kucharczyk & Kucharczyk 2008).

Well known examples are the *Adenophora liliifolia* Bess. population from the Kampinos National Park, the *Thymus praecox* Opiz population from the Ojców National Park and the *Pulsatilla patens* (L.) Mill. population from the Białowieża National Park, which significantly decreased in number. The observed habitat changes were as follows: light oak wood supporting *A. liliifolia* changed into shady lime-hornbeam forest (Rapa 2009, 2011; Otręba *et al.* 2010; Plan Ochrony KPN 2012); light *Cladonio-Pinetum* pine forest suitable for abundant *P. patens* stands evolved into dark pine forest (Karczewska 2009; Okołów 2012); and open limestone grasslands supporting *T. praecox* become

dark thickets (Sołtys-Lelek & Barabasz-Krasny 2009). Habitat change also led to the total disappearance of *Saxifraga hirculus* (L.) Scop. from wet meadows at the Białowieża NP and *Dracocephalum ruyschiana* L. from the Wigry National Park (Romański M. – pers. communication 2010; Okołów 2012).

In the cases of the rare plant species mentioned above, *ex situ* conservation is a necessary conservation strategy to augment populations that are declining and/or to restore lost, historic localities (Rapa 2009, 2011; Karczewska 2009; Sołtys-Lelek & Barabasz-Krasny 2009; Otręba *et al.* 2010; Okołów 2012; Plan Ochrony KPN 2012).

Conservation outside of natural localities is also very important in the case of endangered plant species occurring in unprotected areas eg.: *Carex secalina* Wahl. from pastures, meadows and cattle pond edges at Kujavia, *Ligularia sibirica* Cass. from the Pakosław mire, etc. (Olaczek 2004; Żukowski *et al.* 2005; Nowak *et al.* 2008; Nobis & Piwowarczyk 2008; Tabor & Tabor 2009; Lembicz *et al.* 2009, 2011).

The Seed Bank in the Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Warsaw-Powsin (PAS BG-CBDC)

was established in 1991 for long-term seed storage studies. The main goal of the Department of Plant Biodiversity Research and Conservation of PAS BG is collecting and preserving the gene pools of the rarest and most endangered populations of the native Polish flora, primarily, species listed on the National Red List (Zarzycki & Szelaǵ 2006) or regional Red Data Lists, such as Kucharczyk & Wójciak (1995), Żukowski & Jackowiak (1995), Kaćki *et al.* (2003), Jackowiak *et al.* (2007), Nowak *et al.* (2008) etc.

Whereas most European seed banks use low temperatures, -20°C and drying technology (Gómez-Campo 1972; Puchalski 2004; Pérez-García *et al.* 2007), the authors use ultra-low temperature storage in liquid nitrogen for long-term germplasm preservation. This method is rather uncommon for plant materials, however, cryopreservation of seeds and spores has become more and more popular in such countries as the US, Australia, Japan and Russia (Puchalski *et al.* 2010; Voronkova & Kholina 2010; Ashmore *et al.* 2011). Cooling generally enhances dry seed longevity and cryopreservation may ensure long-term (10-100 years) storage of short-lived orthodox seeds (Walters *et al.* 2004; Pritchard & Nadarajan 2008). Studies carried out at the PAS BG-CDBC have shown that cryopreservation is a reliable storage method. For example, seeds from 12 endangered Polish species (e.g. *Polemonium coeruleum* L., *Linum flavum* L., *L. austriacum* L., *Leontopodium alpinum* Cass.) have maintained their viability during 10 years of cryostorage (Muranyi unpublished).

The main advantages of ultra low temperature storage technology are:

- 1) Reduction of biological activity and seed aging rate – the lower temperature of seed storage results in prolonged life of the seeds. Work on dry lettuce seeds has suggested half-lives of 500-3400 years in cryostorage (vapour-phase and liquid-phase) (Walters *et al.* 2004).
- 2) Reduction in the need for regeneration – the method can decrease risks related to seed regeneration. When viability of collected seeds fall to “regeneration standard” it is necessary to generate new seed lots. This involves risks to the genetic integrity of the accessions due to selection, genetic drift or hybridization. Because seeds need to be regenerated less often, cryopreservation reduces the risks connected with this process and allows maintenance of genetic fidelity (Engelmann 2004; Ashmore *et al.* 2011; Puchalski *et al.* 2014a, 2014b).

The method is particularly suitable and is recommended for seed storage of endangered or endemic species for which only small amounts of seeds are available (Pérez-García 2008; Ensconet 2009).

## 2. Material and methods

The “*Ex situ* conservation of wild endangered and protected plants in Eastern Poland – FlorNaturOB” project encompasses many activities, and uses a combination of different approaches tailored for particular purposes. The major objectives of the project are long-term *ex situ* plant conservation through seed banking, seed biology research and establishment of field collec-

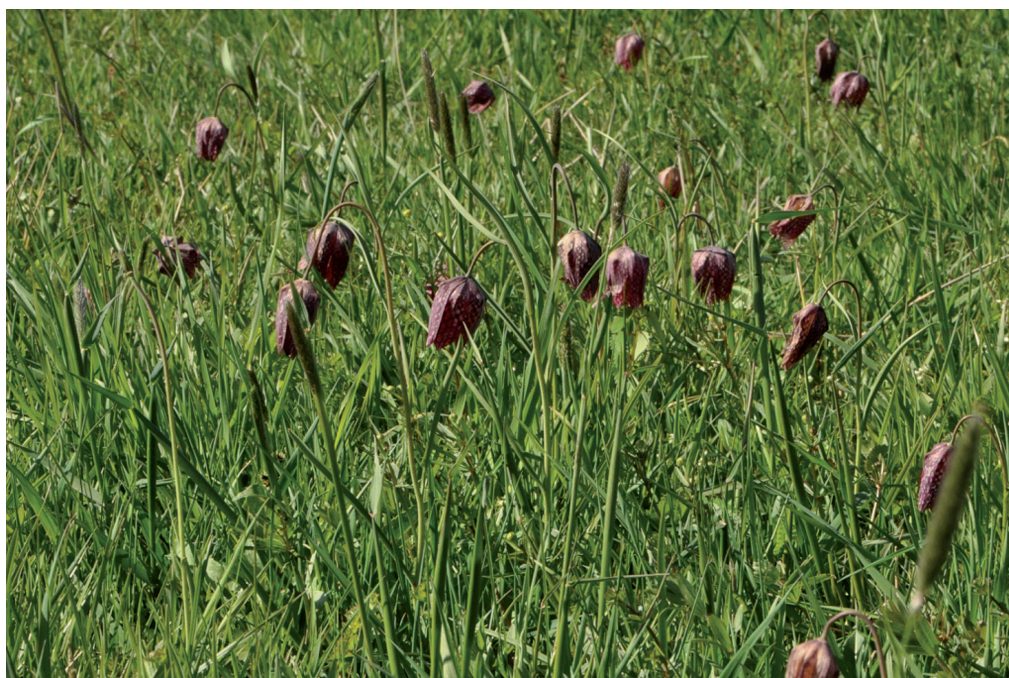
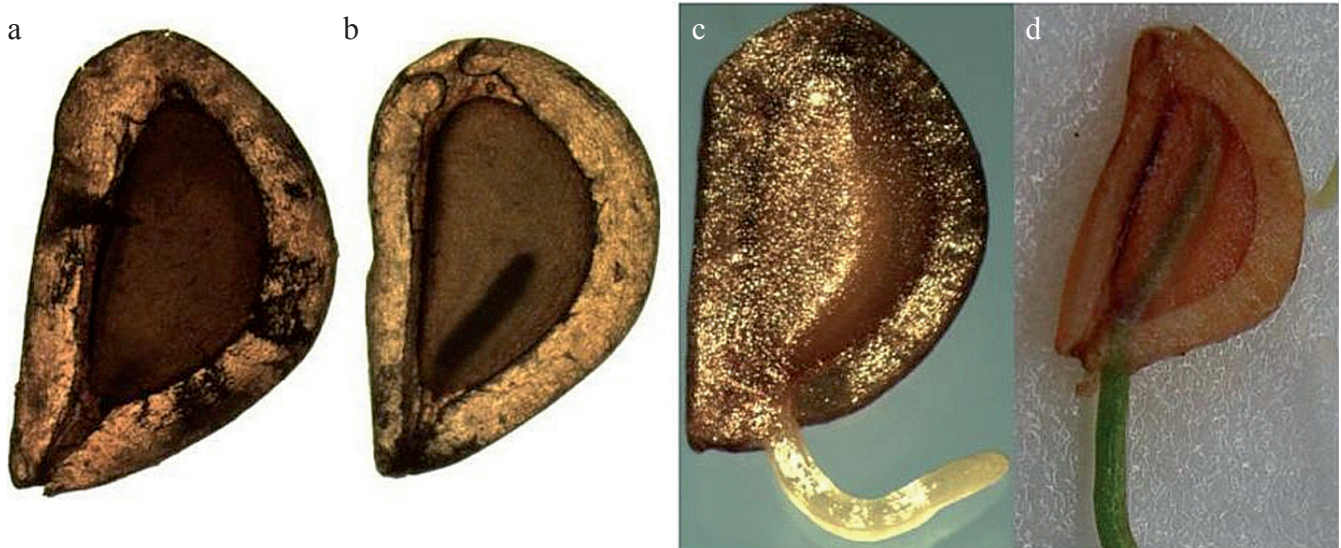


Fig. 1. *Fritillaria meleagris* L. in the “Krówniki” reserve (photograph by Arkadiusz Nowak)





**Fig. 2.** Stages of *Fritillaria meleagris* germination

Explanations: a – fresh seed, b – seed during storage in 7°C (visible embryo), c – seed germination, d – seedling

tions at Powsin Botanical Garden. We use the following procedures:

- monitoring and detailed documentation of natural localities of threatened native plants (photographic, phytosociological, climatic and soil parameters),
- seed collecting (according to the ENSCONET manual. See [http://ensconet.maich.gr/PDF/Collecting\\_protocol\\_English.pdf](http://ensconet.maich.gr/PDF/Collecting_protocol_English.pdf)),
- seed cleaning and desiccation,
- viability and freezing tolerance tests (according to PAS BG CBDC' original methods, see below),
- long-term storage in LN2 (according to PAS BG CBDC' unique methods, see below).

The methods were developed and adapted for statutory PAS BG CBDC' conservation tasks (Graniszewska *et al.* 2004; Puchalski *et al.* 2010) and in "FlorNaturL BG" project (Gugała 2010; Jałowska 2011).

Seed germination tests are the first step for long-term seeds storage in PAS BG-CBDC. The main aim of these tests is seed viability evaluation. It is very important to know seed viability before seeds are deposited in the seed bank. Germination tests also give us information about the biology of germination (dormancy, required temperatures etc.). That information can be useful in the future for eventual reintroduction projects.

When a seed sample is viable and tolerates freezing to liquid nitrogen temperatures (this factor is evaluated in different experiments) it can be stored in the cryobank.

### 3. Results

For the species whose seeds have been collected since 2010, 18 have been identified as dormant, 6 as

partially dormant and 26 as non-dormant (Table 1). Non-dormant seeds are mostly associated with thermophilous oakwood and grassland habitats, for example the *Potentillo albae-Quercetum* Libb., *Festuco-Brometea* Br.-Bl. et R. Tx. and *Dendranthemo-Seslerietum variaae* Grodz. et Jas. in Dzwonko et Grodz. communities.

In most cases dormancy was broken successfully by using 400 ppm gibberellic acid (GA3). Stratification was the optimal method for 5 species (mainly partly-dormant species, e.g. *Saxifraga hirculus* L.). There were also 5 species producing hard coated seeds (*Allium rotundum* L., *Dictamnus albus* L., *Oxytropis pilosa* DC., *Scheuchzeria palustris* L. and *Stipa joannis* Čelak.) that germinated after scarification and water imbibition. *A. rotundum*, *D. albus*, and *O. pilosa* were scarified by chipping the seed coat by scalpel, *S. palustris* by rubbing with sandpaper. *S. joannis* required the removal of the covering structure.

A particularly interesting group was the species with specific germination temperature requirements. *Allium victorialis* L., *Fritillaria meleagris* L., *Muscari comosum* Mill., *Scandix pecten-veneris* L. and *Ranunculus arvensis* L. did not germinate under the standard germination temperatures used in the PAS BG-CBDC seed bank, i.e. 25°C/15°C (16h light/ 8h darkness). *A. victorialis* germinated at a constant temperature of 22°C (16h light/ 8h darkness). *F. meleagris*, *M. comosum*, *S. pecten-veneris* and *R. arvensis* germinated in darkness at temperatures below 10°C.

Most interesting of all was the germination biology of *F. meleagris*. Seeds of this species did not germinate at 25°C/15°C (16h light/ 8h darkness) or at constant temperature 7°C (24h darkness) – even with addition of GA3. In order to germinate them it was necessary to

**Table 1.** Dormancy and its breaking for the “FlorNaturOB” species

	Taxon	Natural locality	Dormancy	Optimal method for dormancy breaking		
				Stratification	GA <sub>3</sub>	Scarification
1	<i>Aconitum</i> sp.	Bieszczadzki NP, Grojec, Gilowice, Matyska, Piłsko, Śnieżnik, Babiogórski NP, Cergowa Góra	D			
2	<i>Adenophora lilifolia</i>	Krzemionki Opatowskie, Kisielany	P			
3	<i>Agrimonia pilosa</i>	Wigierski NP,	N			
4	<i>Allium rotundum</i>	Wola Zagojska, Charsznica, Jędrzejów, Busko Zdrój	D			Sc
5	<i>Allium victorialis</i>	Environs of Ustrzyki Górne, Glinianka	N			
6	<i>Arabis recta</i>	Wały, near Broniny, Wola Zagojska	N			
7	<i>Artemisia pontica</i>	Miechów, Pińczów, Trzebinia	N			
8	<i>Betula nana</i>	Linje	D			
9	<i>Campanula serrata</i>	Tatrzański NP, environs of Ustrzyki Górne	D			
10	<i>Carex pediformis</i>	Wdowie Skały, Dolina Będkowska, Grodzisko, Krzyżowa	N			
11	<i>Carex secalina</i>	Dulsk, Turzany, Jacewo, Złotniki Kujawskie	N			
12	<i>Carlina onopordifolia</i>	Pińczów	N			
13	<i>Cerastium alpinum</i>	Babiogórski NP	D			
14	<i>Cochlearia polonica</i>	Centuria, Rajecznicza	N			
15	<i>Dendranthema zawadzkiei</i>	Pieniński NP	N			
16	<i>Dictamnus albus</i>	Kulin, Grabowiec	D			Sc
17	<i>Dracocephalum ruyschiana</i>	Biebrzański NP, Puszcza Knyszyńska	D			
18	<i>Eleocharis carniolica</i>	Environs of Ustrzyki Górne	D			
19	<i>Erysimum pieninicum</i>	Pieniński NP	N			
20	<i>Euphorbia epithymoides</i>	Podwarpie, Bukowa Góra	D			
21	<i>Euphorbia palustris</i>	Tyszowce	D			
22	<i>Fritillaria meleagris</i>	Krówniki, Stubno	N			
23	<i>Galium cracoviense</i>	Olsztyn	N			
24	<i>Galium valdepilosum</i>	Dąbie, Wały, Kalina-Lisinieć	N			
25	<i>Irys aphylla</i>	Czumów, Izbica, Podgrodzie	N			
26	<i>Kickxia elatine</i>	Krzanowice, Nowa Wieś Królewska	P			
27	<i>Ligularia sibirica</i>	Pakoślaw, Bagno Serebryskie, Roskosz, Brzeźno	P			
28	<i>Lindernia procumbens</i>	Biała Nyska	D			
29	<i>Minuartia setacea</i>	Pieniński NP	N			
30	<i>Muscari comosum</i>	Wrocław	N			
31	<i>Ostericum palustre</i>	Zwierzyniec, Terlików, Dolina Łabuńki	P			
32	<i>Oxytropis pilosa</i>	Skorocice, Wola Zagojska	D			Sc
33	<i>Pedicularis palustris</i>	Biebrzański NP, Ostrowiec Świętokrzyski	D			
34	<i>Pedicularis sceptrum-carolinum</i>	Biebrzański NP, Antoniówka	D			
35	<i>Peucedanum alsaticum</i>	Kąty	N			
36	<i>Pinguicula vulgaris</i> subsp. <i>bicolor</i>	Bagno Serebryskie, Brzeźno, Pogoria, Śniatycze, Kotlina Orawsko-Nowotarska	D			
37	<i>Pulsatilla patens</i>	Puszcza Augustowska, Berźniki, Sejny, Myszyniec	N			
38	<i>Pulsatilla pratensis</i>	Biebrzański NP, Wigierski NP, Puszcza Knyszyńska	N			
39	<i>Pulsatilla vernalis</i>	Environs of Tuchola	N			
40	<i>Ranunculus arvensis</i>	Niemodlin, Opole	N			
41	<i>Rhododendron luteum</i>	Kołacznia, Ciechanowiec	N			
42	<i>Saxifraga hirculus</i>	Biebrzański NP, Wigierski NP, Szeszupa, Giby, Rospuda, Krąg	P			
43	<i>Scandix pecten-veneris</i>	Wolbrom, Kielce, Janów, Busko Zdrój, Pilica	N			
44	<i>Scheuchzeria palustris</i>	Zabrodzie, Galwica, Jezioro near Drozdowa, Supraśl, Nożegary, Klimontek, Smolak Mały	D			Sp
45	<i>Serratula lycopifolia</i>	Skorocice	N			
46	<i>Stipa joannis</i> ( <i>S. pennata</i> )	Skorocice, Skowronno, Przypust, Bałtów	D			Csr
47	<i>Thymus praecox</i>	Góra Koronna, Dziurawiec, Trzaska, Wieża	N			
48	<i>Veratrum nigrum</i>	Łabunie, Kąty	N			
49	<i>Veronica praecox</i>	Gniazdowice, Busko Zdrój, Wola Zagojska	P			
50	<i>Viola epipsila</i>	Białowiecki NP, Wigierski NP	D			

Explanations: D – dormant, N – non-dormant, P – partially dormant, Stratification – 30 days on moist filter paper in 4°C, Scarification – chipped with scalpel (Sc), rubbed with sandpaper (Sp) or covering structure removed (Csr)





It is commonly accepted that fresh *Fritillaria*' seeds have undeveloped embryos (Zhang 1983). The embryo in this genus develops further only when temperatures are low and the seed becomes wet (Fig. 2b). That is the reason why traditional storage methods are not suitable for this species because its seeds quickly lose their viability. Post-dispersal embryo development requires imbibition and the rate of embryo growth increases at lower temperatures. Overall, the temperature preferences for *F. meleagris*' (as well as other *Fritillaria*s associated with open, moist habitats) germination and seedling recruitment are such that in natural localities germination occurs under snow cover or in late winter-early spring coinciding with the snow melt. Gibberellic acid ( $GA_3$ ) does not promote embryo growth and development (Zhang 1983; Carasso *et al.* 2011).

The results of our *F. meleagris* experiments show a strong correlation with the life cycle of this species in natural sites. Seeds are shed in June, but germination occurs in next year's early spring. That is why optimal germination temperatures are 7°C, and no germination took place at 25°C/15°C (16h light/ 8h darkness). Interestingly, seeds placed directly at 7°C on moist filter papers, did not germinate. Seeds germinate only when we preceded 7°C germination by 25°C/15°C (16h light/ 8h darkness) imbibition (Table 2). These results show that *F. meleagris* germination in the seed bank is burdensome because we must apply alternate warm (25/15°C) and cold (7°C) stratification in order to mimic the natural site's conditions in the laboratory.

In some cases, we could try to define species' dormancy or germination requirements (temperature,

photoperiod) using what we know about related species. For example most *Aconitum* and *Euphorbia* species have deep dormant seeds. In laboratory conditions we can break this dormancy only with gibberellic acid ( $GA_3$ ). However, sometimes we found big differences in germination biology within one genus – for example between lowland and mountain species of the *Pulsatilla* genus.

Most xerothermophilous and dry grassland species tested in the PAS BG-CBDC seed bank (about 80%) show no dormancy and their germination is quite straightforward. But there are also dry grassland species with dormant seeds e.g. *Oxytropis pilosa*, *Dracocephalum ruyschiana* L. On the other hand, most peatland and wet sites' species produce dormant or partly dormant seeds – e.g. *Betula nana* L., *Eleocharis carniolica* W. D. J. Koch, *Ligularia sibirica* Cass., *Ostericum palustre* Bess., *Pedicularis palustris* L., *Saxifraga hirculus*, *Viola epipsila* Ledeb. But in this group there are also species like *Cochlearia polonica* Frohl. and *Carex secalina* which produce non-dormant seeds, that germinate at a wide range of temperatures.

In conclusion, there is not always a clear correlation between a plant's ecology in the wild and its germination biology, so it is necessary to study each collected seed sample.

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