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LONG-TERM SEED CRYOPRESERVATION OF RARE AND ENDANGERED POLISH PONTO-PANONIAN PLANT SPECIES

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ABSTRACT: During "FlorNaturOB" conservation project seeds of 23 rare and threatened species representing Ponto-Panonian element of Polish flora had been collected at natural sites and secured in BG PAS-CBDC Seed Bank at Warsaw-Powsin. Sixteen species e.g.: *Arabis recta, Carlina onopordifolia, Thymus praecox, Veratrum nigrum* etc. do not exhibit dormancy, five species (*Allium rotundum, Bupleurum tenuissimum, Dictamnus albus*, *Oxytropis pilosa* and *Stipa joannis*) produced completely dormant and two (*Adenophora liliifolia* and *Veronica praecox*) partially dormant seeds. Freezing test have shown that they maintain viability after 30 days of LN2 storage. There were no significant differences in germination ability between immersion and slow freezing (0,5°C/min.) in the majority of examined samples. Our experiments proved that cryopreservation is a reliable method for *ex situ* conservation of the rarest Ponto-Panonian species native for Poland.

KEY WORDS: cryopreservation, *ex situ* conservation, dormant seeds, Ponto-Panonian element

Introduction

Ponto-Panonian floristic elements occur as xerothermophilous relics at scattered localities in whole Poland. The richest regions with those steppe plants are: Lublin Upland, Małopolska Upland and Pomerania (Pawłowska 1972; Zając M. and Zając A. 2009; Paul 2010a,b). Some interesting sites can be also found at Pieniny and Holy Cross massifs (Cyunel 1959; Zarzycki 1976; Bacler-Żbikowska 2012). According to Polish geographers and botanists steppe plant species colonized the territory of nowadays Poland after maximum glaciation by 3 to 5 routes (main ecological corridors):

(1) from Podolia region (modern Ukraine) along the northern Carpathian slopes

(2) from Panonia region (modern Hungary, SE Slovakia, SW Romania, W Serbia) through the Beskid Niski subsidence and/or the Moravian Gate

(3) from Türingen by the Toruń-Eberwsalde Urstromtal,

(4) from Prealpine Piedmont and southern Bayern along the northern Sudeten slopes

(5) from south-western Siberia/north-eastern Europe along the central and north-eastern European Lakelands.

Present dry grassland species distribution can be an effect of several different migration waves from western Byelorussia, eastern Bohemia and eastern Germany by above mentioned 3-5 routes (Raciborski 1916; Pawłowski 1925; Kozłowska 1931; Cyunel 1959; Tacik 1959; Zając M. and Zając A. 2009; Paul 2010a,b). Among Polish "steppic" species, identified as Pontic-Panonian floristic element and belonging to xerothermophilous ecological group there are probably present 3 different migration-historic elements:

(i) cryoxeric relics of "stepotundras/cold steppes" of interglacial protocratic phase,

(ii) natural, spontaneous migrants from SE glacial refugia and

(iii) archeophytes spreading further north-west due to open-biotope promotion by early agriculture (Paul 2010b).

There are numerous projects dedicated to *in situ* conservation of critically endangered and Community important Panonian species and archaeophyta (e.g. Nowak A. and Nowak S. 2011; Barańska et al. 2013; Kozub et al. 2013), but only few programs focused on their *ex situ* protection (Łuszczyńska 2003; Chernetskyy et al. 2013; Kolasińska and Wójtowicz 2013). Key role in modern, *ex situ* conservation of those resources play "*Ex situ* conservation of native, protected and threatened species in Eastern Poland" – FlorNaturOB project" (Puchalski et al. 2010, 2013). During this program germplasm of 61 species from 161 natural localities were secured in liquid nitrogen at Polish Academy of Sciences' Botanic Garden – Center for BioDiversity Conservation (PAS BG-CBDC) Seed Bank (Puchalski et al. 2010,

2013). Among them there are many species typical for dry grassland and steppe-forests habitats, some of them vulnerable or even critically endangered in Poland, yet still not protected by law e.g.: *Veronica praecox* All., *Arabis recta* Vill., *Allium rotundum* L., *Bupleurum tenuissimum* L. etc. We store seeds of 165 endangered and rare species from 569 localities in PAS BG-CBDC Seed Bank.

Material and Methods

Species were chosen according to their threat categories and orthodox seed behavior. Seeds were collected according to ENSCONET manual. In laboratory conditions seeds were cleaned and dried to 6-8% moisture content (15°C, 20% RH). Next seeds of each species were tested in order to check its viability, germination biology, dormancy presence and methods of its breaking (germination tests in environmental chamber with controlled conditions of temperature, light and humidity). After viability testing, as germination ability, the successive test for freezing tolerance in LN2 was applied by: direct immersion in LN2 or slow freezing (0,5°C/minute) according to control, non-frozen sample. After 30 days of storage in vapor of LN2 (ca. -160°C) the seed germinability was again tested. In case of no differences between frozen and control samples the particular seed sample was transferred for long-term storage in cryogenic vaults.

Results

Recently we managed to examine 23 Ponto-Panonian species of Polish flora (Tab. 1). Most of them produced non-dormant seeds (16 species), but there were also species with dormant (5) or partially dormant (2) seeds. Freezing test have shown that tested species maintain its viability after 30 days of LN2 storage. In the majority of tested seed samples, there were no significant differences in germination ability between immersion and slow freezing (0,5°C/min.) Our experiments has shown that cryopreservation is a reliable method for *ex situ* conservation of rare Ponto-Panonian species occurring in Poland. Therefore this material stored in seed bank could be used for reintroduction into natural biotopes in the future.

Discussion and conclusions

Although already Pawłowski (1925) and Pawłowska (1953) demanded to retain traditional, extensive management at reserves and national parks, majority of steppe plots undergone strict protection in reserves or National Parks. Lack of mowing, grazing, grass burning, trees and shrubs removal etc. led to natural succession and loss of their unique, conservational

values (Kornaś 1981; Loster and Gawroński 2004, 2005; Michalik 2009; Sołtys-Lelek and Barabasz-Krasny 2009). Rare, xerothermophilous vascular plant species e.g. *Carlina onopordifolia* Bess., *Linum flavum* L., *L. hirsutum* L. etc., usually form short-living, soil seed bank and most of their seedlings die due to competitiveness of perennials and woody plants. The chance of spontaneous reestablishment of xerothermophilous vegetation only from the natural, soil seed bank is hardly possible. That is the reason why *in situ* protection of remaining dry grasslands and thermophilous oakwoods supported by *ex situ* cultivation, seed banking, enforcement of weak population and creation of substitute localities of rare plant species associated with those habitats are highly desirable (Banach 2010; Loster 2013; Ślizowski 2013). Obtained results of the "FlorNaturOB" project will be very useful during cooperation between PAS BG CBDC and the Hungarian "Panon Seed Bank (PSB)" at Vacratot preserving the seeds of wild vascular flora of the Panonian biogeographical region. Priority will be given to protected, rare and endangered species, Panonian endemics, indicators, key-stone species of unique, Panon plant communities, medicinal plants, crop wild relatives and drought-tolerant fodder plants (Katalin, pers. comm.).

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Conservation project „ *Ex situ* conservation of native, protected and threatened species in Eastern Poland. Polish: Ochrona *ex situ* dziko rosnących, zagrożonych i chronionych roślin w Polsce wschodniej - FlorNaturOB" has been funded by the European Union at the framework of the **The Operational Programme Infrastructure and Environment (OPI&E)**, Priority V, Action 5.1.2 „Species *ex situ* conservation, protection of genetic resources and establishment of the animal rehabilitation centers", and from the Polish government funds provided by the **National Fund for Environmental Protection and Water Management.**

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Tab. 1. Steppe and steppe-forest species stored in PAS BG seed bank (D – dormant, P – partly dormant, N – non-dormant, imm. LN_2 – immersion in LN_2, sl. LN_2 – slow freezing, con. – control sample:

No	Species	Locality	Dorm.	Viability		
				imm. LN_2	sl. LN_2	con.
1	*Adenophora lilifolia*	Kisielany	P	52	43	50
2	*Allium rotundum*	Wola Zagojska	D	80	76	70
3	*Arabis recta*	Wały	N	88	80	82
4	*Artemisia pontica*	Pińczów	N	100		100
5	*Aster amellus*	Kąty	N	60		72
6	*Bupleurum tenuissimum*	Busko Zdrój	D	96		90
7	*Carlina onopordifolia*	Pińczów	N	95	100	95
8	*Carex pediformis*	Grodzisko	N	60		80
9	*Dictamnus albus*	Kulin	D	82	84	58
10	*Erysimum pieninicum*	Góra Zamkowa	N	72	78	80
11	*Galium cracoviense*	Olsztyn, Skałki Lipowskie	N	71	63	72
12	*Galium valdepilosum*	Kalina Lisiniec	N	60	50	56
13	*Minuartia setacea*	Trzy Korony	N	48	28	70
14	*Muscari comosum*	Wrocław	N	80	74	74
15	*Oxytropis pilosa*	Wola Zagojska	D	96	91	92
16	*Peucedanum alsaticum*	Kąty II	N	25	40	43
17	*Pulsatilla pratensis*	Biebrzański N.P.	N	64	52	79
18	*Serratula lycopifolia*	Skorocice	N	72	75	75
19	*Sisymbrium polymorphum*	Wola Zagojska	N	94	92	93
20	*Stipa joannis*	Skorocice	D	100	92	100
21	*Thymus praecox*	Wieża	N	70		72
22	*Veratrum nigrum*	Łabunie	N	76	76	66
23	*Veronica praecox*	Wola Zagojska	P	94	92	98

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