Molecular studies on *ex-situ* conservation of rare and endangered Polish plants

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Abstract

Implementing Target 8 of the Global Strategy for Plant Conservation (GSPC) in 24 Polish botanical gardens, 275 taxa out of 446 National Red List threatened plant species (61,7%) are available as living plant collections or seed bank holdings. The effectiveness of genetic diversity conservation in *ex situ* collections should be monitored. We have chosen three endangered species in Poland and endemic or relict species to compare the genetic structure of *ex situ* and *in situ* populations using microsatellite-based DNA markers (ISSR and SAMPL). The particular aims of our studies were to examine the level of genetic variation between an artificial *(ex situ*) and its source *(in situ*) population and to analyze the efficiency of *ex situ* conservation of living plants in addressing issues to conserve genetic variation at the species level. The results of a comparative molecular analysis of investigated species showed significant changes in genetic diversity between populations from botanical gardens and natural localities. It was concluded that the *ex situ* collections just partially represent the primary genetic variation, however more than 70% of total gene pool of each species was captured in *ex situ* populations.

Keywords

Botanical garden, conservation genetics, genetic diversity, ISSR, living collections, SAMPL

Introduction

To achieve the GSPC Targets 8 and 9, the Botanical Garden of the Polish Academy of Sciences in Warsaw has gathered data based on gene-level diversity of *ex situ* conserved collections of three endangered in Poland and endemic or relict species to compare the genetic structure of artificial versus source populations using microsatellite-based DNA markers (ISSR and SAMPL).

Cochlearia polonica (Brassicaceae) is a narrow endemic Polish scurvy-grass extinct in its primary locality known only from one transplanted population in southern Poland (Fig. 1) (Kwiatkowska 2001). Once classified as “extinct in the wild” (EW) it was listed as one of the species of the highest conservation concern in several conservation catalogues (global IUCN, Annex II and IV of the EU Habitat Directive, Bern Convention) (Kaźmierczakowa, 2004). In the late 1980s five individuals from a transplanted population were used to establish the artificial site in the Botanical Garden of the Polish Academy of Sciences in Warsaw.

Erysimum pieninicum (Zapał.) Pawł. (Brassicaceae) (Pieniny Mts. wallflower) is a narrow Polish endemic species with distribution restricted to Polish part of Pieniny Mountains (Pawłowski 1946). Given its vulnerable status, *E. pieninicum* has been considered as a priority species for conservation in the Pieniny NP and listed in the Annex II and IV of the EU Habitat Directive and covered by the Bern Convention (Korzeniak, 2001). Throughout its small geographic range, *E. pieninicum* occurs as discrete populations varying in size from 10 to 1,000 plants (Fig.1.) (Vončina and Wróbel, 2004). The artificial population was introduced to the Botanical Garden of the PAS in Warsaw in 2001.

Dendranthema zawadzkii (Herbich) Tzvelev *(*Asteraceae*)* (Zawadzki’s chrysanthemum) is a very rare plant because its pleistocene relict sites in Poland are separated by a significant

disjunction from the compact range, which is Eurosiberian with the centre in central and east Siberia (Zarzycki, 1976). This species, protected by law in Poland and in Europe by the Bern Convention refers to a few described from few populations ranging in size (from 12 to 100 individuals) in the Pieniny Mts. (Fig.1), two of which were sampled to create an *ex-situ* population in the Botanical Garden of the PAS in 2001.

In recent years empirical studies have employed the comparison of both types of population structure using molecular markers in order to further understand the genetic changes that occurred during the period of preservation in the botanical garden. The particular aims of our studies were to examine the level of genetic variation between an artificial *(ex situ*) and the source *(in situ*) populations and to analyze the efficiency of the *ex situ* conservation method in addressing issues to conserve species genetic variation

Materials and methods

Seven populations of three species were studied (*in situ* and *ex situ* for each species). All of the individuals were sampled from *ex situ* conserved populations of three analyzed species (Tab.1.). Samples from *in situ* populations were randomly taken from flowering plants. About 3–4g of fresh leaves per plant were collected and stored in zip–lock plastic bags with silica gel until thorougly dried. Total genomic DNA was extracted from the dried tissue following the protocol of A&A GenomicMiniAXPLANT kit (A&A Biotechnology).

ISSR (*Inter-Simple Sequence Repeat)* reactions were carried out in a volume of 10 µl containing 1.0 U of HotStart Taq Polymerase (Polgen), 1 x PCR buffer, 0.25 mM of each dNTP, 0.25 mM $MgCl_2$, 20 pM of a single ISSR primer and 15 ng of template DNA. Amplifications were performed in a Biometra ThermalCycler (TProfessional). Electrophoretic separation of the PCR products was done on an 1,5% agarose gel at 100 V for 3 hrs.

SAMPL (*Simple Amplified Microsatellite Polymorphic Loci)* profiles were generated following established procedures for SAMLP analysis (Spataro, 2007) with an amount of 125 ng of genomic DNA used in restriction. Preselective products underwent the selective amplification with a previous screening of 40 fluorescence-labelled primer combinations. Those revealing the clearest banding patterns were choosen. Selective amplified products were analyzed on 6% denaturing polyacrylamide gels. Electrophoresis was performed at a constant power of 50 W for 4 h on a Dual Dedicated Height Nucleic Acid Sequencer (C.B.S. Scientific Co.)

Electrophoresed PCR products were visualized using the ImageQuant 400 Imager and TyphoonTrio+ (GE Healthcare). SAMPL and ISSR profiles were scored for the presence or absence of a band in the 100 – 500 bp (SAMPL) and 100 – 1200 bp (ISSR) range for all individuals and recorded in a binary data matrix.

Data analysis

Genetic diversity for each population was estimated by a percentage of polymorphic bands (PPB), and Nei's gene diversity index using POPGENE version 1.31 (Yeh and Yang, 1999). The level of population differentiation between *ex situ* and *in situ* population was estimated using the coefficient of Nei's gene differentiation (G_{st}). The primary binary data were applied to obtain a Principal Coordinates Analysis (PCO) plot to visually represent the relative degree of genetic similarity among individuals and populations. PCO analysis was performed using an NTSYS package version 2.21c (Rohlf, 2006).

Results and Discussion

Having the facilities and expertise for growing plants means that botanic gardens should be in an ideal position to monitor plants in their collections and undertake experiments to answer questions relating to conservation. The study of living collections, especially of species that

are threatened with extinction, has a long tradition in botanic gardens and has contributed significantly to the body of knowledge on threatened species and their conservation (Donaldson, 2009).

The main aim of this study was to investigate the extent of genetic variation conserved in *ex situ* populations of three endangered plant species in Poland and endemic or relict plant species using two types of molecular markers. There were some differences between the level of gene diversity and genetic differentiation indices obtained with ISSR and SAMPL markers, because both methods generate differences from different part of the genome. The results in terms of the indices of genetic diversity and genetic differentiation however were consistent for both types of molecular marker systems used in this study (Table 1).

The results of a comparative molecular analysis of *ex situ* and *in situ* populations showed significant changes in the genetic diversity level. The garden populations exhibited lower genetic diversity in comparison to their source populations for all the analyzed species (Table 1).

The level of genetic differentiation indicated striking differences between plants conserved in the garden and their source populations (Table 1). When observed on PCO plot, the individuals coming from both types of conditions formed two separated groups (Figure 2).

All of the above results suggested differences in genetic composition of the analyzed populations and implied homogenization of genetic structure of *ex situ* conserved population. A possible explanation for these findings is that the differences in genetic diversity and structure are related to founder effect and genetic changes occurring during the period through which the *ex situ* collections have passed during their time of preservation in the botanical garden.

The *ex situ* preservation resulted in a decrease of the species genetic diversity, implying that artificial populations only partly represent the primary genetic variability found *in situ* for analyzed species. It is of note that the garden's populations preserved about 50% of the gene pool represented by bands with moderate frequency (Table 2). However, the amount of genetic variation conserved in *ex situ* populations seems to be enough to implement target No. 9 of GSPC and to sustain long-term survival.

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Table 1. Genetic diversity and genetic differentiation indices within the *ex situ* and *in situ* populations

species	population	No. of individuals	Polymorphic bands (%)		Nei's gene diversity		Genetic differentiation	
			ISSR	SAMPL	ISSR	ISSR	ISSR	SAMPL
Cochlearia polonica	*In situ*	85	104 (44.2)	127 (51.2)	0.1558	0.1674	0.2015	0.1286
	Ex situ	26	68 (28.9)	65 (26.2)	0.1007	0.0761		
Erysimum pieninicum	*In situ*	47	65 (36.1)	125 (50.6)	0.0921	0.2044	0.1044	0.0702
	Ex situ	33	34 (18.8)	112 (45.3)	0.0508	0.1656		
Dendranthema zawadzkii	*In situ*	32	196 (81.3)	143 (42.8)	0.2840	0.1894	0.3654	0.1263
	Ex situ	12	45 (18.6)	101 (42.8)	0.0461	0.1214		

Table 2. The amount of genetic variation conserved in *ex-situ* populations of analyzed species

		N p>0.05 (p=1)		**Gd (%)**		**Gd (%) >0.05**		**1>Gd >0.05**	**(%)**
		ISSR	SAMPL	ISSR	SAMPL	ISSR	SAMPL	ISSR	SAMPL
Cochlearia polonica	*Ex situ*	176 (131)	211 (111)	73.7	89.4	74.8	96.7	65.7	50.4
	In situ	235 (31)	218 (85)						
Erysimum pieninicum	*Ex situ*	155 (125)	235 (131)	90.9	98.8	93.4	95.9	57.7	85.3
	In situ	166 (114)	245 (123)						
Dendranthema zawadzkii	*Ex situ*	176 (131)	211 (111)	73.7	89.4	74.8	96.7	22.1	54.6
	In situ	235 (31)	218 (85)						

N p>0.05 number of amplified bands with a frequency >0.05 (band with frequency p=1)
Gd (%) percentage of genetic variation conserved in each population

Gd (%) >0.05 percentage of genetic variation conserved in each population - for bands with p>0.05
1>Gd (%) >0.05 percentage of genetic variation conserved in each population - for bands with 1>p>0.05

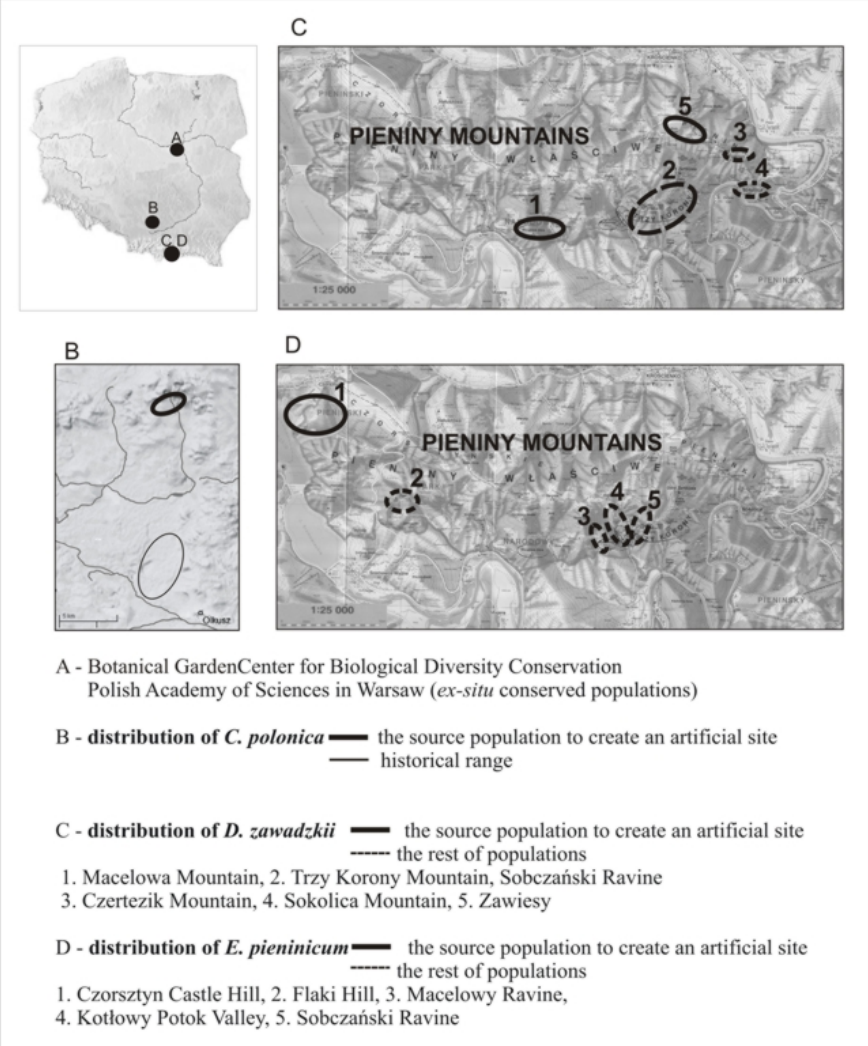

Fig.1. Origin and geographic information about the collection sites of the examined populations

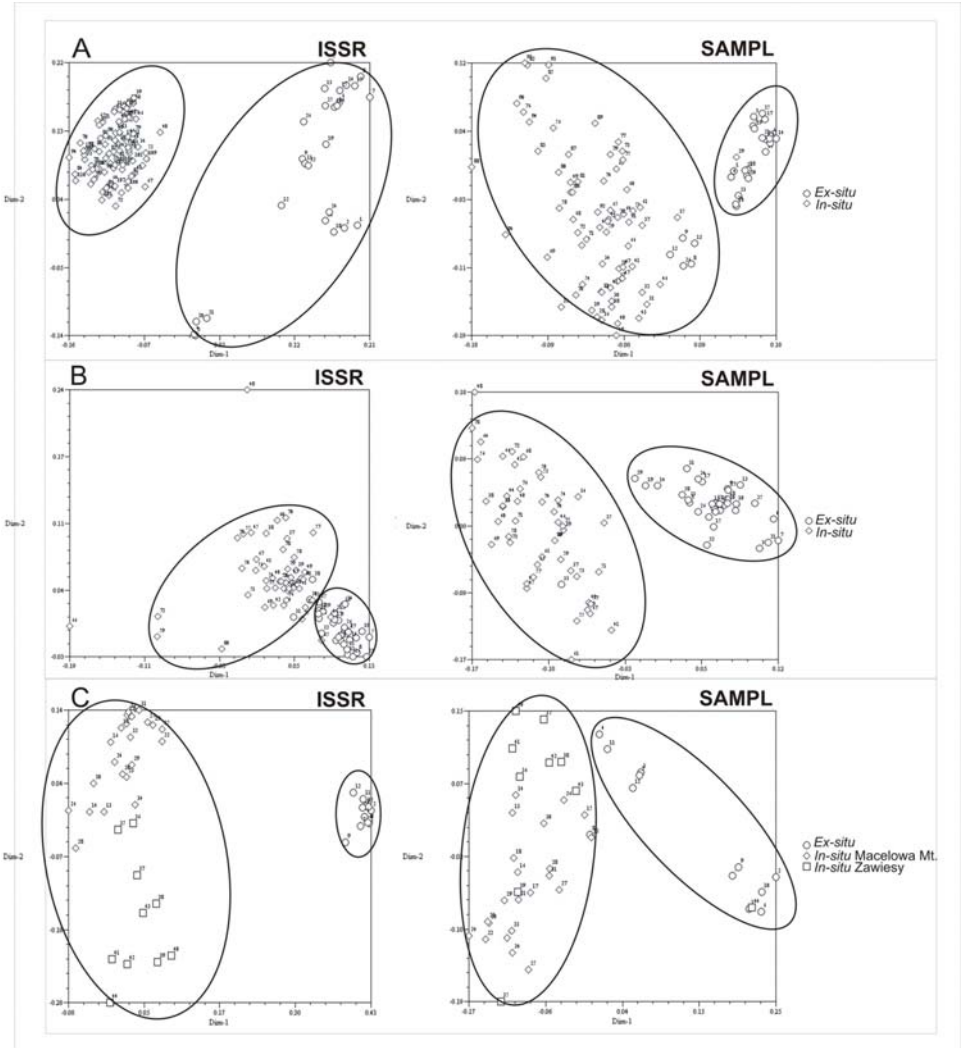

Fig.2. Principle coordinate analysis plot of individuals sampled based on ISSR and SAMPL data (A. *C. polonica* B. *E. pieninicum* C. *D. zawadzkii*)