Biodiv. Res. Conserv. 75: 27-34, 2024

BRC
www.brc.amu.edu.pl

DOI 10.14746/biorc.2024.75.3

Submitted 07.05.2024, Accepted 03.09.2024

Ex situ cultivation of the endangered savin junipers (*Juniperus sabina* L.) from the Western Carpathians

Katarzyna Jadwiszczak[1]*, Sylwia Parzonko[1], Małgorzata Mazur[2], Katarzyna Marcysiak[2], Adam Boratyński[3] & Agnieszka Bona[1]

[1]Faculty of Biology, University of Bialystok, Ciołkowskiego 1J, 15-245 Białystok, Poland; ORCID: KJ https://orcid.org/0000-0002-9345-8891; AB https://orcid.org/0000-0002-2359-517X

[2]Faculty of Biological Sciences, Kazimierz Wielki University, J. K. Chodkiewicza 30, 85-064 Bydgoszcz, Poland; ORCID: MM https://orcid.org/0000-0003-0043-3706; KM https://orcid.org/0000-0001-6162-6232

[3]Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland; ORCID: AB https://orcid.org/0000-0003-0678-4304

* corresponding author (e-mail: kszalaj@uwb.edu.pl)

Abstract. *Ex situ* conservation methods provide immediate insurance against extinction of relict trees and shrubs in the wild. To be well-managed, the living collection should be well-studied in respect of the place of origin of the individuals, their taxonomic status, and genetic variation. Using 12 nuclear microsatellite loci, we analysed 22 shrubs of *Juniperus sabina* L. var. *sabina* (savin juniper), cultivated in the Kórnik Arboretum (Poland) and sourced from a Tertiary relict population in the Pieniny Mts. (Western Carpathians). We found 2 clonal pairs of individuals and a pair of full siblings. The genetic diversity parameters were rather low: N_A = 2.5 alleles per locus, H_O = 0.316, H_E = 0.326, and the inbreeding coefficient was also very low (G_{IS} = 0.03). The individuals formed 3 groups in the principal coordinates analysis (PCoA), but 2 of these groups were genetically close. The Bayesian clustering analysis revealed that the specimens belonged to 2 genetic groups. We recommend that the cultivation of *J. sabina* var. *sabina* should be carefully protected, as it represents remnants of the Tertiary genetic diversity of the species.

Key words: conservation genetics, full-sibling, microsatellite, relict population, *Juniperus sabina* L. var. *sabina*

1. Introduction

Plants represent a very high rate of extinction in the wild (Gao *et al*. 2020), and as many as 34% of coniferous species are threatened with decline (Garndner 2003). Small-sized, genetically depauperate populations revealing low reproductive capacity and showing specialized niche demands are especially prone to extinction (Işik 2011; Griffith *et al*. 2021). Plant species and populations can be protected from becoming extinct by using the *in situ* and *ex situ* conservation approaches. *In situ* methods aim to protect organisms in their natural habitat by constituting national parks, biosphere and nature reserves or wildlife sanctuaries. It is worth noting that the natural disasters and anthropogenic pressures, whether within or adjacent to the protected areas, can still pose a threat to the existence of rare plants (Li & Pritchard 2009). The *ex situ* conservation strategies include seed and gene banks, cryopreservation, and botanical gardens, where the plants can be preserved outside the place of origin. Seed banks are the most popular among the *ex situ* methods because seeds are usually easy to collect, can represent substantial genetic diversity if sampled from different individuals, and need little space for storage (Li & Pritchard 2009). However, immediate insurance for planned conservation of the endangered rare and relict tree and shrub species seems to be the well-managed *ex situ* cultivation (Kozlowski *et al*. 2012; Sharrock 2012; Christe *et al*. 2014).

A global survey of *ex situ* collections of the genus *Zelkova* Spach has demonstrated very important issues related to the conservation of Tertiary relict trees (Kozlowski *et al*. 2012). Firstly, the selected botanic gardens and arboreta should be located within the natural range of distribution of the species. Secondly, the origin and taxonomic status of individuals should

be well documented. Thirdly, the *ex situ* cultivation should reflect accurately the genetic diversity of natural stands (Kozlowski *et al*. 2012). The significance of botanical gardens and arboreta will probably increase in the future. According to Westwood *et al.* (2021), these green spaces can potentially become the sole means of access to nature for numerous people in the progressively urbanized communities.

Juniperus sabina L. (savin juniper; Cupressaceae) is one of the threatened conifer shrub species in Central Europe. Two varieties are distinguished in Europe: *J. sabina* L. var. *sabina* and *J. sabina* var. *balkanensis* R.P. Adams and A.N. Tashev (hereafter referred to as var. *sabina* and var. *balkanensis*). The latter occupies the southern European peninsulas, while var. *sabina* can be found in the Cantabrians, Alps, Eastern and Western Carpathians, Apuseni, Crimean, and Caucasus mountain ranges (Mazur *et al*. 2021; and references therein). Central European populations represent var. *sabina* and they constitute the northern margin of the species range (Mazur *et al.* 2021; and references therein). This variety is recognized as endangered (EN according to the International Union for Conservation of Nature, IUCN) in Poland and Germany, and as vulnerable (VU) in Slovakia (Wróbel *et al.* 2014). The German populations of var. *sabina* appear in the Berchtesgaden and Ammergauer mountain ranges of the Bavarian Alps (https://daten.bayernflora.de). In Poland and Slovakia, var. *sabina* occurs only in the Pieniny Mts. (Western Carpathians), in the areas of the Polish Pieniny National Park (*Pieniński Park Narodowy*, PPN) and the Slovakian Pieniny National Park (*Pieninský národný park,* PIENAP). In Poland, the juniper grows on 3 steep rock walls: Facimiech and Piecki in the Łysina massif and Głowa Cukru in the Sokolica massif in the PPN (Wróbel *et al.* 2014). In Slovakia, var. *sabina* forms 6 microgroups on the rock wall called Sedem mníchov, which is located on Holica Mt. in the PIENAP (Kunštárová *et al*. 2007). Most probably, this variety in the Pieniny Mts. is a Tertiary relict because that mountain range served as a refugium for many plant species as it was not covered by the ice sheet during the Pleistocene glaciations (Smólski 1937; Zarzycki 1976; Kunštárová *et al*. 2007; Zając & Zając 2009).

The habitat conditions in the Pieniny Mts. had to be advantageous for *J. sabina* because it was a more common shrub there in the past (Smólski 1937, 1960; Zarzycki 1981). Compared to adjacent mountain ranges, the climate in the Pieniny Mts. is mild, with relatively high mean annual temperature, average precipitation, shorter snow cover, and longer growing season (Jaguś 2015; and references therein). During the last centuries var. *sabina* was mostly extirpated in the Pieniny Mts., due to its properties used in medicine and veterinary treatments. Local residents collected significant quantities of the juniper branches and sold them in pharmacies (Smólski 1937, 1960). Today, the species grows in crevices of southern, sunny slopes, on the calcareous rocks between 400 and 720 m a.s.l. in the Pieniny massifs (Smólski 1937; Kunštárová *et al*. 2007; Wróbel *et al*. 2014). The area covered by var. *sabina* in the PPN and PIENAP is limited, and it ranges from 2 m^2 to 38 m^2 in the Slovak micro-stands (Kunštárová *et al*. 2007), and up to 24 m^2 in Poland (Wróbel *et al.* 2014). To the best of our knowledge, a prior assessment of genetic variation involved a total of 10 individuals from the PPN and PIENAP populations by utilizing enzyme marker systems (Kosiński & Wojnicka-Półtorak 2010). The cited authors revealed rather low values of diversity indices (55.6% of polymorphic loci, 1.66 alleles per locus, $H_O = 0.18$, $H_E = 0.20$). However, another study using 2 genome-wide molecular marker systems revealed low genetic diversity in most *J. sabina* populations (SilicoDArT: $H_O = 0.1160$, $H_S = 0.0977$; SNP: $H_O = 0.0392$, $H_S = 0.0607$; Jadwiszczak *et al.* 2023).

An inventory conducted in the PIENAP in 2006 revealed that the junipers were able to reproduce sexually. The number of seed cones was between 9 and 67 in the largest 3 micro-stands, but there were no seeds in the smallest 3 groups (Kunštárová *et al*. 2007). Tylkowski (2010) found only 1.1 filled seeds per cone in a sample of 417 seed cones collected from the PPN. The cited author used these seeds in a dormancy-breaking experiment. Many germinated seeds in that experiment decayed due to infection by pathogenic fungi (Tylkowski 2010). However, some seedlings still grew in the Kórnik Arboretum of the Institute of Dendrology Polish Academy of Sciences (ID PAS). Thus, along with the *in situ* protection of var. *sabina* in Poland, the *ex situ* cultivation of progeny derived from the wild population exists. Jadwiszczak *et al.* (2023) found 3 different chloroplast DNA (cpDNA) haplotypes in this collection, but the diversity of nuclear markers was not studied then. Given that var. *sabina* in the Pieniny Mts. is an endangered plant and may represent a remnant of Tertiary species variation, it is crucial to protect this population through *ex situ* cultivation. Therefore, our objectives were: (1) to examine the genetic polymorphism of savin junipers cultivated in the Kórnik Arboretum; and (2) to estimate the genetic relationships among individuals within this population.

2. Material and methods

2.1. Laboratory analyses

We sampled leafy twig fragments of 22 shrubs in the living collection of var. *sabina* located in the Kórnik Arboretum, western Poland (52.24°N, 17.09°E; Fig. 1). We extracted the total DNA of each specimen by

Fig. 1. Location of the *ex situ Juniperus sabina* var. *sabina* collection in the Kórnik Arboretum and the natural population in the Pieniny Mts.

using an AX Plant Kit (A&A Biotechnology, Gdańsk, Poland), according to the manufacturer's procedure. To establish individual genotypes, we used 12 nuclear microsatellite loci: Sabv5, Sab6, Sabv8, Sabv15 (Geng *et al*. 2017), JS4, JS5, JS15, JS30, JS31, JS54, JS58, and JS61 (Lu *et al.* 2022). Geng *et al.* (2017) and Lu *et al.* (2022) described those loci on the basis of genetic material coming from Chinese populations of *J. sabina*. We amplified microsatellite fragments in three PCR multiplexes: M1 (JS5, JS15, JS54), M2 (JS4, JS30, JS31, JS58, JS61), and M3 (Sabv5, Sab6, Sabv8, Sabv15), under the following conditions: 95°C for 15 min; repeated cycles of 30 s at 94°C, annealing for 45 s (58°C for M1, 56°C for M2, 55°C for M3), and 30 s at 72°C; the final elongation cycle was 72°C for 7 min. The number of PCR cycles was 30 for M1 and M2, and 34 for M3. We conducted the amplification reactions using StartWarm HS-PCR Mix (A&A Biotechnology), based on the manufacturer's protocol. We used an ABI PRISM 3130 sequencer (Applied Biosystems, Foster City, CA, USA) to separate the fluorescently labelled microsatellite fragments, whereas GeneMapper 4.0 software (Applied Biosystems) to score these fragments.

2.2. Statistical analyses

To find the minimum number of microsatellite loci necessary to discriminate between individual genotypes, we generated the genotype accumulation curve by using the "poppr" R package (Kamvar *et al.* 2015). After identification of unique genotypes, the clones were removed from all further calculations. We searched for genotypic linkage disequilibria between all pairs of loci by using Genepop 4.7.5 software (Raymond & Rousset 1995; Rousset 2008). We verified the presence of null alleles with the help of MICRO-CHECKER 2.2.3 (van Oosterhout *et al.* 2004), and estimated the frequency of such alleles (FR_N) by using ML-NullFreq program (Kalinowski & Taper 2006). To calculate the genetic diversity indices, i.e. N_A = number of alleles per locus, H_O = observed heterozygosity, H_E = expected heterozygosity, and G_{IS} = inbreeding coefficient, we used GenoDive software version 3.06 (Meirmans 2020). We assessed significant departures from the Hardy-Weinberg equilibrium [*P(HWE)*] in the particular loci as well as in the total sample in Genepop. Recent reduction in effective sample size was tested using BOTTLENECK 1.2.02 software (Cornuet & Luikart 1996; Piry *et al.* 1999), based on 3 models: infinite alleles (IAM), stepwise mutation (SMM), and 2-phased mutational (TPM) model. In the last model, 70% single-step mutations were concerned. The one-tailed Wilcoxon test was employed to calculate the significance for heterozygosity excess under IAM, SMM, and TPM (Cornuet & Luikart 1996). We applied the sequential Bonferroni's correction (Rice 1989) to all multiple tests.

To show genetic relationships between individuals, we carried out the principal coordinates analysis (PCoA) based on genetic distances between all genotypes in GenAlEx 6.5 software (Peakall & Smouse 2006). To establish the number of genetic clusters based on *ad hoc* statistic ΔK (Evanno *et al.* 2005), Bayesian inference was performed with STRUCTURE v. 2.3.4 (Pritchard *et al.* 2000) with 10 independent runs for

K values ranging from 1 to 3 (20 000 burn-in periods, 200 000 iterations). The STRUCTURE results were summed up and visualized using CLUMPAK software (Kopelman *et al.* 2015). After employing locus-specific corrections of the null allele frequency, we verified the studied individuals in respect of being the full- or half-siblings at the probability level of ≥ 0.9 by using Colony 2.0.6.7 software (Jones & Wang 2010).

3. Results

In the studied sample of 22 shrubs of var. *sabina*, the genotype accumulation curve showed that the information produced by the 10 polymorphic loci allowed to identify 20 unique multilocus genotypes (Fig. 2). In the studied collection, we identified 2 pairs of clonal individuals; thus, 2 shrubs were removed from further consideration. After applying sequential Bonferroni's correction (Rice 1989), we did not detect any linkage disequilibria among the analysed pairs of loci. Most probably, loci JS31, JS54, and JS58 involved null alleles, with frequencies of 0.278, 0.204, and 0.126, respectively (Table 1). We found 30 alleles in total, with a mean of 2.5 alleles per locus. The values of H_O ranged from 0.000 in loci JS54, JS61, and Sabv15, to 0.500 in Sabv5 and Sabv6. The highest H_E = 0.588 was in locus JS15, whereas H_E = 0.000 was in JS61 and Sabv15. The mean values of H_O and H_E were 0.316 and 0.326, respectively.

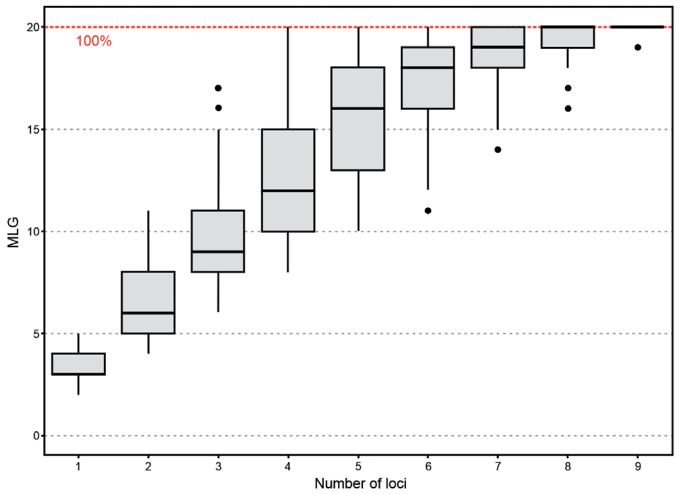

Fig. 2. Genotype accumulation curve showing 20 unique multilocus genotypes (MLGs) among 22 samples of *Juniperus sabina* var. *sabina* in the Kórnik Arboretum

Table 1. Genetic diversity parameters of *Juniperus sabina* var. *sabina* cultivated in the Kórnik Arboretum

Locus	N_A	FR_N	H_O	H_E	G_{IS}	*P*(HWE)
JS4	2	0.000	0.100	0.097	-0.027	1.000
JS5	2	0.000	0.450	0.482	0.066	1.000
JS15	4	0.000	1.000	0.588	-0.700	0.000*
JS30	2	0.000	0.300	0.387	0.224	0.273
JS31	3	0.278	0.143	0.266	0.464	0.206
JS54	2	0.204	0.000	0.189	1.000*	0.001*
JS58	3	0.126	0.350	0.587	0.404	0.009
JS61	1	0.000	0.000	0.000	-	-
Sabv5	4	0.000	0.500	0.530	0.057	0.356
Sabv6	3	0.000	0.500	0.409	-0.222	0.456
Sabv8	3	0.000	0.450	0.374	-0.204	0.711
Sabv15	1	0.000	0.000	0.000	-	-
Total sample	30	-	0.316	0.326	0.030	0.000*

Explanations: N_A – number of alleles per locus, FR_N – frequency of null alleles, H_O – observed heterozygosity, H_E – expected heterozygosity, G_{IS} – inbreeding coefficient, *P*(HWE) – probability for the Hardy-Weinberg equilibrium

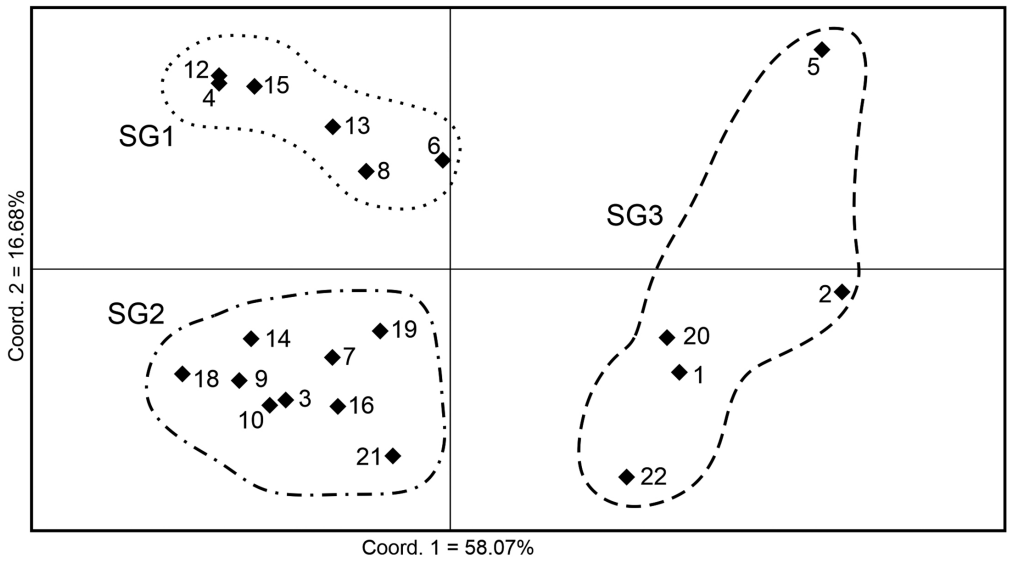

Fig. 3. Principal coordinates analysis (PCoA) showing genetic distances between 20 individuals of *Juniperus sabina* var. *sabina* from the Kórnik Arboretum

Explanations: SG1, SG2, SG3 – group codes

A significant value of inbreeding coefficient was in JS54 locus, but G_{IS} in the total sample was low (0.030). Two loci, JS15 and JS54, as well as the total sample showed significant departures from the HWE. Three models implemented in BOTTLENECK software did not reveal any signs of recent reduction in effective sample size: $P = 0.21582$ for IAM, $P = 0.83887$ for SMM, and $P = 0.61523$ for TPM.

In the PCoA, the first and second axes explained 58.07% and 16.68% of the total genetic variation, respectively (Fig. 3), and 20 specimens formed 3 groups: SG1, SG2, and SG3. The STRUCTURE analysis revealed 2 genetic clusters ($K = 2$; Fig. 4). The first cluster consisted of individuals 1, 2, 5, 20, and 22, which were categorized as the SG3 group in PCoA. The second cluster included all the other specimens, with individual 6 exhibiting a significant admixture from the first cluster. The analysis conducted in Colony software revealed that individuals 2 and 22 could be full-siblings, with $P = 0.9$. There was no half-sibling pair in the studied sample.

4. Discussion

Marshall and Brown (1975) suggest that for successful conservation of genetic resources, the *ex situ* collection has to comprise at least one copy of 95% of the alleles that appeared in the original population with a frequency ≥ 0.05. In the cultivation of var. *sabina* in the Kórnik Arboretum, we detected 30 alleles in 12 loci, yielding 2.5 alleles per locus. In consequence, the observed and expected values of heterozygosity were not high ($H_O = 0.316$, $H_E = 0.326$). Due to a lack of assessment of the genetic polymorphism of the population

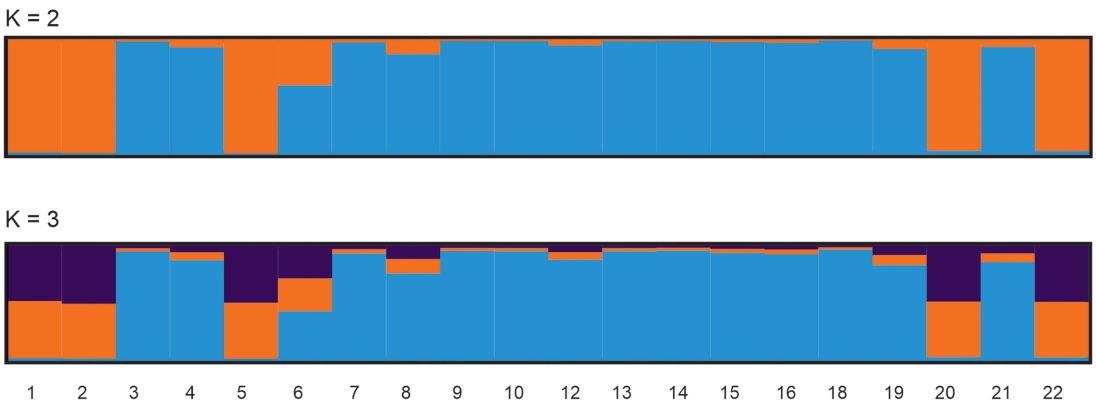

Fig. 4. Bayesian clustering of 20 *Juniperus sabina* var. *sabina* individuals (K = 2 and K = 3) sampled in the Kórnik Arboretum

of this species in the Pieniny Mts. by using nuclear microsatellites, we are not able to determine if the *ex situ* collection conforms to the criterion of Marshall and Brown (1975). Nuclear microsatellites usually reveal a high level of variation (Ashley 2010), so the number of alleles and the heterozygosity measures in the living collection of var. *sabina* in the Kórnik Arboretum seems to be low. However, the average number of alleles per locus in 11 Chinese populations of *J. sabina* ranged from 2.8 to 4.3 (Lu *et al.* 2022). As mentioned earlier, the Kórnik collection was derived from the relict population inhabiting the Polish part of Pieniny Mts. Wróbel *et al.* (2014) stated that 27 groups of var. *sabina* in the PPN were found in 1997-1998, but the number of distinct genetic individuals (genets) was unknown. In the PIENAP, Kunštárová *et al*. (2007) detected 6 microstands covering 2-38 m^2, but the number of specimens was not assessed, either. Individuals of *J. sabina* can reach up to 100 m in diameter, with a median value of 13.9 m (Wesche *et al*. 2005). As the species intensively grows clonally, and individual shrubs often outgrow each other, it is sometimes difficult to distinguish one specimen from another.

Kosiński and Wojnicka-Półtorak (2010) analysed the genetic diversity of 10 savin juniper individuals from the Pieniny population by using the enzyme loci, and found rather low values of genetic parameters. The cited authors revealed 15 alleles in 9 loci; thus, this marker system was less proper to distinguish particular individuals in a population, as compared to the microsatellites. We recognized 20 genets among 22 sampled shrubs in the *ex situ* collection. We found only one pair of full-siblings in the studied sample. A recent genetic bottleneck was not confirmed in this *ex situ* collection, but the collection significantly deviated from HWE (P = 0), most likely as a result of the presence of null alleles in loci JS31, JS54, and JS58. PCoA showed clearly that the individuals were distinct to some extent, and they clustered into 3 groups. As it was expected based on the amount of genetic diversity explained by the first axis (58.07%) in PCoA, the most genetically similar were specimens from groups SG1 and SG2, while group SG3 seemed to be distinct to some extent. This result was confirmed by the Bayesian clustering showing 2 genetic clusters (K = 2). The first cluster was congruent with SG3, and the second cluster consisted of all the other individuals. Hence, potential artificial pollination trials aimed at seed production should be carried out between SG3 and either SG1 or SG2. Such combinations of parental individuals will help prevent the occurrence of inbred progeny, which may suffer from reduced fitness (inbreeding depression). The cultivation of var. *sabina* in Kórnik revealed a slight excess of heterozygotes (H_E = 0.326), as compared to homozygotes (H_O = 0.316), resulting in a low inbreeding index (G_{IS} = 0.03). Kosiński and Wojnicka-Półtorak (2010) reported a small excess of homozygotes (H_O = 0.20, H_E = 0.18) in the sample of var. *sabina* collected from the Pieniny Mts. This implies that the natural relict population can be more threatened with inbreeding than the *ex situ* collection. Inbreeding depression, caused by the accumulation of deleterious alleles, poses a significant risk to small, isolated populations consisting of closely related individuals (Frankham 1995).

The savin junipers cultivated in the Kórnik Arboretum derived from seeds collected in the wild population that were further used in an experiment on dormancy breaking (Tylkowski 2010). Unfortunately, there is no detailed information about the maternal origin of savin juniper seeds used by that author. It cannot be excluded that the germinated seeds came from a small number of mother shrubs. In 2006, a large number of seed cones was observed in one group of the savin juniper specimens in the PPN, but the remaining shrubs had single fruits only (Wróbel & Wróbel 2008).

In a relict population of *Betula humilis* Schrk. located in north-eastern Poland, more than half of sprouting seeds came from 3 individuals only (Bona *et al.* 2019). Tylkowski (2010) analysed 697 seeds extracted from 417 seed cones, but 33% of the seeds lacked ovules. Compared to the sexual reproduction efficiency in the relict populations of *Betula nana* L. located in the Sudetes, the number of filled seeds in the population of var. *sabina* in the Pieniny Mts. was high. In locations of *B. nana*, the contribution of empty seeds reached up to 99% (Jadwiszczak *et al*. 2017). However, the abundance of savin juniper seeds collected in 2006 was an unusual phenomenon (Tylkowski 2010). In the PIENAP population, the seed cones were noted in the biggest shrub micro-groups only in 2006 (Kunštárová *et al*. 2007).

Smólski (1937, 1960) implied that *J. sabina* was widespread in the Pieniny Mts. after the last glaciation, but the extensive use by the local inhabitants has brought the species to the brink of extinction in the Western Carpathians. Despite the relatively low variation of nuclear microsatellite markers in var. *sabina* in the Kórnik Arboretum, the very high scientific value of this collection is unquestionable because it may represent the remnants of Tertiary genetic variation. The individuals grown there form 2 quite distinct genetic groups that can be used to produce seeds, so the collection should be carefully protected. To increase genetic variation of cultivated var. *sabina* and decrease potential dissimilarity between the *ex situ* and *in situ* stands (see Forgiarini *et al*. 2023), we recommend to enrich the gene pool of the *ex situ* collection with individuals coming from the PPN and PIENAP populations. To implement this plan, we intend to carry out genetic analyses in the natural populations of var. *sabina* in the Western

Carpathians in the future. This will allow us to assess the level of polymorphism in the *in situ* populations and potential gene exchange between the PPN and PIENAP localities as well as to identify suitable specimens for reproduction in the *ex situ* collection.

Acknowledgements. This study was supported by the Polish Minister of Science and Higher Education under the program "Regional Initiative of Excellence" in 2019-2023 (grant no. 008/RID/2018/19).

Author Contributions:
Research concept and design: K. Jadwiszczak, K. Marcysiak, A. Boratyński
Collection and/or assembly of data: M. Mazur, K. Marcysiak
Data analysis and interpretation: S. Parzonko, K. Jadwiszczak, A. Bona
Writing the article: K. Jadwiszczak, K. Marcysiak
Critical revision of the article: K. Jadwiszczak, S. Parzonko, M. Mazur, K. Marcysiak, A. Boratyński, A. Bona
Final approval of article: K. Jadwiszczak, S. Parzonko, M. Mazur, K. Marcysiak, A. Boratyński, A. Bona

References

Ashley M.V. 2010. Plant parentage, pollination, and dispersal: how DNA microsatellites have altered the landscape. Crit. Rev. Plant. Sci. 29: 148-161. https://doi.org/10.1080/07352689.2010.481167

Bona A., Kulesza U. & Jadwiszczak K.A. 2019. Clonal diversity, gene flow and seed production in endangered populations of *Betula humilis* Schrk. Tree Genet. Genomes 15: 50. https://doi.org/10.1007/s11295-019-1357-2

Christe C., Kozlowski G., Frey D., Fazan L., Bétrisey S., Pirintsos S., Gratzfeld J. & Naciri Y. 2014. Do living ex situ collections capture the genetic variation of wild populations? A molecular analysis of two relict tree species, *Zelkova abelica* and *Zelkova carpinifolia*. Biodivers. Conserv. 23: 2945-2959. https://doi.org/10.1007/s10531-014-0756-9

Cornuet J.-M. & Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144: 2001-2014. https://doi.org/10.1093/genetics/144.4.2001

Evanno G., Regnaut S., Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14: 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Forgiarini C., Parzefall F. & Reisch C. 2023. The impact of ex situ cultivation on the genetic variation of endangered plant species – implications for restoration. Biol. Conserv. 284: 110221. https://doi.org/10.1016/j.biocon.2023.110221

Frankham R. 1995. Inbreeding and extinction: a threshold effect. Conserv. Biol. 9: 792-799. https://doi.org/10.1046/j.1523-1739.1995.09040792.x

Gao J.G., Liu H., Wang N., Yang J. & Zhang X.L. 2020. Plant extinction excels plant speciation in the Anthropocene. BMC Plant Biol. 20: 430. https://doi.org/10.1186/s12870-020-02646-3

Garndner M.F. 2003. The international conifer conservation programme. Acta Hortic. 615: 405-409. https://doi.org/10.17660/ActaHortic.2003.615.45

Geng Q.F., Qing H., Ling Z.R., Jeelani N., Yang J., Yoshikawa K., Miki N.H., Wang Z.S. & Lian C.L. 2017. Characterization of polymorphic microsatellite markers for a coniferous shrub *Juniperus sabina* (Cupressaceae). Plant Spec. Biol. 32: 252-255. https://doi.org/10.1111/1442-1984.12152

Griffith M.P., Cartwright F., Dosmann M., Fant J., Freid E., Havens K., Jestrow B., Kramer A. T., Magellan T.M., Meerow A.W., Meyer A., Sanchez V., Santiago-Valentín E., Spence E., Sustasche-Sustache J.A., Francisco-Ortega J. & Hoban S. 2021. *Ex situ* conservation of large and small plant populations illustrates limitations of common conservation metrics. Int. J. Plant Sci. 182: 263-276. https://doi.org/10.1086/713446

Işık K. 2011. Rare and endemic species: why are they prone to extinction? Turk. J. Bot. 35: Article 11. https://doi.org/10.3906/bot-1012-90

Jadwiszczak K.A., Kłosowski S., Zalewska I., Banaszek A. & Chrzanowska A. 2017. Genetic diversity and sexual reproduction in relict populations of *Betula nana*. Silva Fenn. 51: 1-18. https://doi.org/10.14214/sf.5643

Jadwiszczak K.A., Mazur M., Bona A., Marcysiak K. & Boratyński A. 2023. Three systems of molecular markers reveal genetic differences between varieties *sabina* and *balkanensis* in the *Juniperus sabina* L. range. Ann. Forest Sci. 80: 45. https://doi.org/10.1186/s13595-023-01211-w

Jaguś A. 2015. Charakterystyka regionalna Pienin na potrzeby terenowej edukacji przyrodniczej. Inżynieria Ekologiczna 41: 46-60. https://doi.org/10.12912/23920629/1828

Jones O.R & Wang J. 2010. COLONY: A program for parentage and sibship inference from multilocus genotype data. Mol. Ecol. Resour. 10: 551-555. https://doi.org/10.1111/j.1755-0998.2009.02787.x

Kalinowski S.T. & Taper M.L. 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conserv. Genet. 7: 991-995. https://doi.org/10.1007/s10592-006-9134-9

Kamvar Z.N., Brooks J.C. & Grünwald N.J. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Front. Genet. 6: 208. https://doi.org/10.3389/fgene.2015.00208

Kopelman N.M., Mayzel J., Jakobsson M., Rosenberg N.A. & Mayrose I. 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15: 1179-1191. https://doi.org/10.1111/1755-0998.12387

Kosiński P. & Wojnicka-Półtorak A. 2010. Wstępne badania nad zróżnicowaniem allozymowym *Juniperus sabina* L. (Cupressaceae) na reliktowym stanowisku w Pieninach Centralnych. Pieniny – Przyroda i Człowiek 11: 39-44.

Kozlowski G., Gibbs D., Fun H., Frey D. & Gratzfeld J. 2012. Conservation of threatened relict trees through living ex situ collections: lessons from the global survey of the genus *Zelkova* (Ulmaceae). Biodivers. Conserv. 21: 671-685. https://doi.org/10.1007/s10531-011-0207-9

Kunštárová V., Kĺč V. & Wróbel S. 2007. Borievka netatová (*Juniperus sabina* L.) v Pieninách. Bulletin Slovenskej botanickej spoločnosti 29: 79-82. Bratislava

Li D-Z. & Pritchard H.W. 2009. The science and economics of ex situ plant conservation. Trends Plant Sci. 14: 614-621. https://doi.org/10.1016/j.tplants.2009.09.005

Lu D., Huang H., Wang A. & Zhang G. 2022. Genetic evaluation of *Juniperus sabina* L. (Cupressaceae) in arid and semi-arid regions of China based on SSR markers. Forests 13: 231. https://doi.org/10.3390/f13020231

Marshall D.R. & Brown A.H.D. 1975. Optimum sampling strategies in genetic Conservation. In: O.H. Frankel & J.G. Hawkes (eds.). Crop Genetic Resources for Today and Tomorrow, pp. 53-80. Cambridge University Press, Cambridge.

Mazur M., Boratyński A., Boratyńska K. & Marcysiak K. 2021. Weak geographical structure of *Juniperus sabina* (Cupressaceae) morphology despite its discontinuous range and genetic differentiation. Diversity 13: 470. https://doi.org/10. 3390/ d1310 0470

Meirmans P.G. 2020. GENODIVE version 3.0: Easy-to-use software for the analysis of genetic data of diploids and polyploids. Mol. Ecol. Resour. 20: 1126-1131. https://doi.org/10.1111/1755-0998.13145

Peakall R. & Smouse P.E. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6: 288-295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Piry S., Luikart G. & Cornuet J.M. 1999. BOTTLENECK; a computer program for detecting recent reductions in the effective population size using allele frequency data. J. Hered. 90: 502-503. https://doi.org/10.1093/jhered/90.4.502

Pritchard J.K., Stephens M. & Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959. https://doi.org/10.1093/genetics/155.2.945

Raymond M. & Rousset F. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. 86: 248-249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223-225. https://doi.org/10.1111/j.1558-5646.1989.tb04220.x

Rousset F. 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Mol. Ecol. Resour. 8: 103-106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Sharrock S. 2012. The global strategy for plant conservation. https://www.bgci.org/wp/wp-content/uploads/2019/04/GSPC_flyer.pdf (accessed on 15.01.2024)

Smólski S. 1937. Jałowiec sawina (*Juniperus sabina* L.) w Pieninach. Ochrona Przyrody 17: 216-221.

Smólski S. 1960. Pieniński Park Narodowy. Polska Akademia Nauk, Zakład Ochrony Przyrody, Kraków, pp. 273.

Tylkowski T. 2010. Dormancy breaking in savin juniper (*Juniperus sabina* L.) seeds. Acta Soc. Bot. Pol. 79: 27-29. https://doi.org/10.5586/asbp.2010.004

van Oosterhout C., Hutchinson W.F., Wills P.M. & Shipley P. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4: 535-538. https://doi.org/10.1111/j.1471-8286.2004.00684

Wesche K., Ronnenberg K. & Hensen I. 2005. Lack of sexual reproduction within mountain steppe populations of the clonal shrub *Juniperus sabina* L. in semi-arid southern Mongolia. J. Arid Environ. 63: 390-405. https://doi.org/10.1016/j.jaridenv.2005.03.014

Westwood M., Cavender N., Meyer A. & Smith P. 2021. Botanic garden solutions to the plant extinction crisis. Plants People Planet 3: 22-32. https://doi.org/10.1002/ppp3.10134

Wróbel I. & Wróbel S. 2008. Jałowiec sabiński, *Juniperus sabina* L. In: Z. Mirek & H. Piękoś-Mirkowa (eds.). Czerwona Księga Karpat Polskich. Rośliny Naczyniowe (Red Book of the Polish Carpathians. Vascular Plants), pp. 57-59. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Wróbel I., Wróbel S. & Zarzycki K. 2014. *Juniperus sabina* L. Jałowiec sawina. In: R. Kaźmierczakowa, K. Zarzycki & Z. Mirek (eds.). Polska Czerwona Księga Roślin (Polish Plant Red Book), pp. 78-80. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Zając M. & Zając A. 2009. Elementy geograficzne rodzimej flory Polski. 94 pp. Institute of Botany, Jagiellonian University, Kraków.

Zarzycki K. 1976. Małe populacje pienińskich roślin reliktowych i endemicznych, ich zagrożenie i problemy ochrony. Ochrona Przyrody 41: 7-76.

Zarzycki K. 1981. Rośliny naczyniowe Pienin: rozmieszczenie i warunki występowania. 257 pp. PWN, Warszawa.