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The *Salix lapponum* L. (downy willow) among-population genetic diversity in the Polesie Lubelskie Region

Genetyczne zróżnicowanie międzypopulacyjne *Salix lapponum* L.
(wierzby lapońskiej) na Polesiu Lubelskim

SUMMARY

In Poland the downy willow (*Salix lapponum* L.) is a glacial relic, subject to strict legal protection. It is shown as an endangered species in *The Polish Red Book of Plants.* The aim of our research was to determine the among-populations genetic diversity of *Salix lapponum* in the stations in the Polesie Lubelskie Region and in the comparative area the Karkonosze Mountains, as well as to study the relations between the genetic distance and the distance between these populations in space. The material for genetic analysis was sampled in July 2003 as population mixes containing plant materials in the form of leaves from several specimens from five isolated *Salix lapponum* populations in the Polesie Lubelskie Region and one population functioning in The Karkonosze National Park. The genetic analyses were carried out using AFLP technology because of its high resolution and credibility and also a small amount of plant material needed for study. The researches on *Salix lapponum* among-populations genetic diversity in Polesie Lubelskie indicated that a genetic distance between populations of the studied species generally does not correspond to the distance between them in space. The downy willow population in the Karkonosze Mountains comparative area clearly differs in genetic terms from the majority of the Polesie Lubelskie populations.

STRESZCZENIE

W Polsce wierzba lapońska (*Salix lapponum* L.) jest reliktem glacjalnym, gatunkiem objętym całkowitą ochroną prawną, a w *Polskiej Czerwonej Księdze Roślin* wykazanym jako gatunek zagrożony. Celem badań było określenie genetycznego zróżnicowania międzypopulacyjnego *Salix lapponum* w stanowiskach na Polesiu Lubelskim i w terenie porównawczym (Karkonosze), jak również wykazanie związku między dystansem genetycznym a dystansem między tymi populacjami w przestrzeni. Materiał do analiz genetycznych został pobrany w lipcu 2003 r. w postaci mixów populacyjnych zawierających materiał roślinny w postaci liści kilku osobników, z każdej z pięciu populacji *S. lapponum* na Polesiu Lubelskim oraz jednej funkcjonującej w Karkonoskim Parku Narodowym. Analizy genetyczne zostały przeprowadzone metodą AFLP ze względu na jej dużą rozdzielczość i wiarygodność, ale również na mało inwazyjny charakter przyżyciowego pobierania prób materiału roślinnego do analiz. Wyniki badań międzypopulacyjnego zróżnicowania genetycznego *S. lapponum* na Polesiu Lubelskim wskazały na dystans genetyczny dzielący populacje badanego gatunku, który nie zawsze wiąże się z ich wzajemną odległością w przestrzeni. Populacja ze stanowiska porównawczego (Karkonosze) wyraźnie różni się pod względem genetycznym od większości populacji z Polesia Lubelskiego.

K e y w o r d s: *Salix lapponum*, among-population genetic diversity, AFLP

INTRODUCTION

The history of distribution range development of many relict plant species is related to long-term climatic changes which in tuand asexual reproduction, and adjustment of hybrid specimens, their initial forms and the effect of their bio-geographic past on the structure of the population (Avise1989; Falińska 2002).

At present, molecular taxonomy, including phylogenetic analysis based on genetic data, is indispensable in determining the endangered species protection priorities and in maintaining biological diversity. Gern resulted in changes in the topography, chemical composition of the substratum, etc. Boreal species that have survived until today outside of the dense distribution range populate only small areas which have retained their original character. The relict species have reached their present distribution also thanks to numerous changes in the ecological properties occurring constantly in nature as a result of active evolutionary processes (Podbielkowski 2002).

Currently DNA markers are widely applied in population ecology to provide insights into the diversification patterns of various growth type plant populations, adjustment of specimens arising as a result of sexual netic data are used to document the distribution and degree of hybridization, as well as to monitor demographic and genetic processes which can lead to a species becoming extinct (Soltis & Gitzendanner 1999; Stephan et al. 2003; Wu et al. 2004).

As molecular methods develop, it is possible now to determine the origin and spread of various varieties (Taberlet et al. 1998; Brzosko et al. 2002; Rivera-Ocasio et al. 2002; Maldano et al. 2003; Wróblewska et al. 2003). Researches combining genetic analysis and population ecology lead to the establishment of a reliable basis determining the rules of rare species protection policies (Soltis & Gitzendanner 1999). The genetic diversity and evolutional potential of species seem to be important for surviving of rare taxons which often occur in small and isolated populations (Gajewski 1987; Lembicz 1998).

All these factors affect the intra- and interpopulation genetic diversification, and together with demographic and environmental factors determine the survival of the population. In the light

of these data, species protection should be based on information about genetic diversification among populations and within a single population.

Salix lapponum L. as the object of research

The downy willow (*Salix lapponum* L.) is an inconspicuous bush (nanofenerophyt – life form according to Raunkiaer), one meter high and exceptionally, when competing for light with other bushes, up to two meters high. Brown shoots and leaves with silvery grey napped underside are a typical feature of the species (Reichholf & Steinbach 1995). *S. lapponum* is a dioecious plant, it blooms in May/June. It is an entomophilous species. It can develop hybrids with several other willow species (*S. aurita*, *S. caprea*, *S. cinerea*, *S. dasyclados*, *S. myrtilloides*, *S. nigricans*, *S. purpurea*, *S. rosmarinifolia*, *S. vinimalis*) which displace the genetically pure form (Rutkowski 1998). In Poland however, the downy willow vegetative reproduction is mainly observed (Kaźmierczakowa & Zarzycki 2001).

Salix lapponum is a species that is commonly found in peat bogs of the subarctic and boreal areas of Eurasia, mainly in northern and north-eastern Europe and western Siberia (Bolliger et al. 1998; Podbielkowski 2002), in isolated stands it also grows in Scotland and in some of the mountain ranges in Central and Southern Europe. The south-western confines of the species distribution run through Poland (Kaźmierczakowa & Zarzycki 2001).

In Poland the downy willow is a glacial relict, legally protected, listed in *The Polish Red Book of Plants* as an endangered species, with a rapidly declining number of stands located in Poland (Szafer et al. 1986; Kaźmierczakowa & Zarzycki 2001; Andrzejewski & Weigle 2003). The listed population of *S. lapponum* was based on some 60 stands in lowland areas in north-eastern and mid-eastern Poland and in the Karkonosze Mountains, however the majority of these stands no longer exist (Kaźmierczakowa & Zarzycki 2001). At present, *S. lapponum* stands exist only in mid-eastern Poland, mainly in the Polesie Lubelskie Region (Wojciechowski & Fijałkowski 1998; Pogorzelec et al. 2001; Urban & Wawer 2001; Fijałkowski & Izdebski 2002; Nowicka-Falkowska 2002; Radwan 2002; Pogorzelec 2003, 2004; Urban & Gawlik 2003) and in the Karkonosze Mountains (Fabiszewski et al. 1996; Goczoł-Gontek & Gontek 2002; Wojtuń & Żołnierz 2002).

In the natural lowland stands of Polesie Lubelskie, *S. lapponum* grows on valley and blanket peat bogs (Fijałkowski 1994; Fijałkowski & Lorens 1998). Shoots of the downy willow usually grow singly or in small clusters (Pogorzelec 2003, 2004). In mountain stands, in The Karkonosze National Park, *S. lapponum* grows on the subalpine floor at 1,400 m above sea level (Raj 2001). It forms there dense thickets of the endemic *Salicetum lapponum* association W. Mat. 1965, covering flat or slightly sloping insides and edges of postglacial cirques (Fabiszewski et al. 1996).

The decline of the species population and reduction of the number of its natural stands is mainly attributable to changes in habitat conditions resulting from habitat dehydration and succession processes occurring in the stands. So far it has not been however determined whether or not the decline of the population is affected by processes within the population, manner of reproduction of the specimens in the various stands and the sexual structure of the population.

The purpose of this work was to determine the *Salix lapponum* among-population genetic diversity in the selected stations of the Polesie Lubelskie Region and in the comparative area (the Karkonosze Mountains) and to confirm the relationship between the genetic distance and the distance between these populations in space.

MATERIAL AND METHODS

Plant material and DNA isolation

The material for genetic analysis was sampled in July 2003 according to directions included in Minister's of the Environment decision (DOPweg-410–1401/03/jk – 25.03.2003) which allowed to collect the plant material (leaves) from selected stations. The plant materials were population mixes (intended to reflect the gene pool of a population) from several specimens (depending on their number in a station) from five *Salix lapponum* populations in the Polesie Lubelskie Region (four located in The Polesie National Park – on the peat bogs of lakes Długie, Moszne, Karaśne and the Blizionki forest peat bog and one in the Park's buffer area – on the peat bog of Bikcze Lake) and one population functioning in the mountains of The Karkonosze National Park (Kocioł Małego Stawu) described as *Salicetum lapponum* plant association stands. A small amount of plant material sampled did not affect adversely the functioning of the various specimens of *S. lapponum.*

Immediately after the sampling, the whole plant material was disinfected with a 70% ethanol solution. DNA isolation was performed according to the CTAB procedure (Murray & Thompson 1980; Rogers & Bendich 1985; Doyle & Doyle 1990).

AFLP analysis

The genetic analysis has been conducted using the AFLP method (*Amplified Fragment Length Polymorphism*) at the Plant Breeding and Acclimatization Institute at Radzików. AFLP technology combines the power of restriction of the fragment length polymorphism (RFLP) with the flexibility of PCR-based technology by ligating primer–recognition sequences (adaptors) to the restricted DNA.

For the AFLP reactions, sets of chemicals provided by Applied Biosystem were used. The analyses were conducted with fluorescent primers, using ABI-PRISM 377. The 12 most polymorphic primers were chosen for the investigation proper (Table 1).

Table 1. Selection of AFLP primer combinations for the *Salix lapponum* investigation

	Mse I CAA	Mse I CAC	Mse I CAG	Mse I CAT	Mse I CTA	Mse I CTC	Mse I CTG	Mse I CTT
EcoR I ACT								
EcoR I ACA	X			X				
EcoR I AAC				X		X		
EcoR I ACC								
EcoR I AGC	X	X						
EcoR I AAG		X	X	X			X	
EcoR I AGG								X
EcoR I ACG							X	

The minimum height of the peak taken for the analysis was 100 points.

The range of the analysis was 35–500 bp. Electrophoresis was conducted on a 36 cm-long 4.5% polyacrylamide denaturing gel, for 4 h at 2400 V. Pictures of bands were studied using Genescan software. The zero-one template was generated using Genotyper software.

Statistic analysis

The analysis of the frequency distribution (of the occurrence or the lack) of bands (alleles) in the plant material collected from six various stations and examined using the AFLP method, was carried out using the χ^2 non-parametric test. As the significance level in all tests it was assumed that $\alpha = 0.05$, which means that the verified zero hypothesis (about the lack of differences between the results) was rejected if the maximum of its probability did not exceed 5%. In such a case an alternative hypothesis was accepted as true. Thus the possibility of making the so-called type 1 error (i.e. rejecting a hypothesis although it is true) was reduced to 5%.

The findings of the research were analysed using an IBM PC and the software STATISTICA 5.0 (Statsoft, Polska).

RESULTS AND DISCUSSION

The research on the genetic diversity of *Salix lapponum* in various stations of Polesie Lubelskie indicated a genetic distance between populations of the studied species does not always correspond to the distance between them in space. In genotype terms, the Polesie Lubelskie Region populations turned out to be quite diverse, but there are also populations that demonstrate a statistically significant similarity (> 70%) between them.

Statistical, comparative analysis of the different DNA bands occurrence frequency which were amplified (present = 1) or not amplified (absent = 0) in the plant material samples collected in the Polesie Lubelskie Region indicates that the highest genetic similarity there is between the populations situated on the peat bogs of Moszne and Bikcze lakes (genetic conformity 78% events), and next between Moszne and Blizionki forest peat bog (75%), and Bikcze and Blizionki (72%; Fig. 1).

The close genetic similarity amongst those three groups of *Salix lapponum* does not reflect the distance in space because the stations near Bikcze and Moszne lakes are situated 10 km from each other, Bikcze and Blizionki – about 5.5 km, Moszne and Blizionki about 5 km in a straight line (Fig. 1).

The populations from the most distant stations on the peat bogs of Bikcze and Długie lakes (12.5 km) are characterized by high genetic similarity – 68%. It is worth to note that the populations from the nearest stations show a significantly lower genetic similarity (54%, Moszne-Karaśne 2.75 km) simultaneously, it was found that there is no basis to confirm the genetic similarity between the populations Blizionki-Karaśne (the distance in space 3 km). It should be also

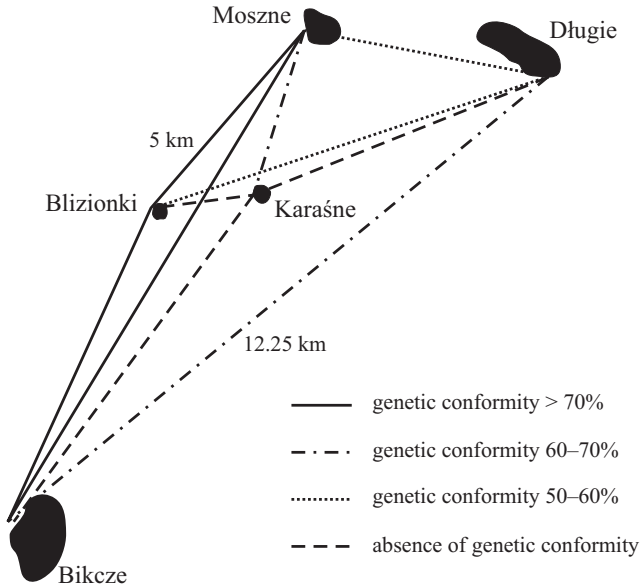

Fig. 1. The downy willow (*Salix lapponum* L.) among-populations genetic and space distance in the Polesie Lubelskie Region

noted that statistical analyses showed neither significant genetic similarity nor the lack of significant genetic similarity of the *Salix lapponum* species between the populations near Karaśne lake and the Karkonosze Mountains.

The population in the Karkonosze Mountains clearly differs in genetic terms from the majority of the Polesie Lubelskie populations (absence of genetic conformity < 50%; Fig. 1), which is most likely due to a significant spatial isolation and different habitat conditions in the stations of the compared populations. The statistically high similarity between the Polesie Lubelskie populations and the absence of that similarity in respect of the Karkonosze population may indicate that the currently small isolated populations were in the past a single dense "west-Polesie" population. This conclusion is also confirmed by the fact that in the 1950's *S. lapponum* was still more large and it populated the majority of bog peat ecosystems functioning at that time (Fijałkowski 1958). It seems that besides the changes in the habitat conditions and possibility of generation the interspecific hybrids, the stand isolation has affected also the decline of *S. lapponum* population size. The lack of gene flow between the isolated populations may result in low diversity inside the population (Pogorzelec 2004).

Since the research was conducted only on a limited number (6) of *Salix lapponum* stations and it was the first research of the genetic differentiation of this species (there is no point of reference) the results can be changed with es-

sential errors. The problems of the *Salix lapponum* population ecology and biology require many years of further studies, whose results will allow to explain the reasons for the decrease in the number of its stands and their overall number in our country.

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