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Genetic variation in populations of *Gentiana pannonica* (L.) Scop.

Vladislav Čurn*, Lenka Dolanská & Milena Haraštová

University of South Bohemia, Faculty of Agriculture,
Studentská 13, CZ-370 05 České Budějovice, Czech Republic
**curn@zf.jcu.cz*

Abstract
Molecular-genetic analysis of two populations of *Gentiana pannonica* (L.) Scop. in the Bohemian Forest was carried out using RAPD markers. Analysed populations are located in the central part of the Bohemian Forest near the villages of Horská Kvilda and Zhůří. Very low level of genetic variation was detected.

Key words: *Gentiana pannonica*, RAPD markers, population genetics

Introduction

Gentiana pannonica (L.) Scop. is an endemic species of Central Europe with a relatively small east Alpine area of distribution. The Bohemian Forest is the only area of its occurrence lying outside the Alps. The occurrence in other Czech mountains is not autochthonous. In the Bohemian Forest, *Gentiana pannonica* grows on mountain mat-grass pastures and meadows, and in glacial cirques of lakes. Most localities are situated in the central and north-western parts of the Bohemian Forest (both in the Bavarian and the Czech part of these mountains). Its occurrence in the south-eastern part of the Bohemian Forest is relatively rare. This species occurs mainly in the cirque of Plešné Lake (Plöckensteiner See) and in meadows on Plechý (Plöckenstein) and Třístoličník (Dreisesselberg) Mts. In the Austrian part of the Bohemian Forest this taxon has been found very rarely close to the Czech borders in the Plechý and Smrčina (Hochficht) region (Schönfelder & Bresinsky 1990, Kolektiv 1995–2001). Its distribution in the Bohemian Forest (pars Bohemiae, Austriae et Bavariae) is displayed in the map (Fig. 1).

The occurrence of *Gentiana pannonica* in the Bohemian Forest has been recorded since the beginning of its floristic investigation. The first reports on the occurrence of this taxon were those by Sternberg (1806) from Roklan (Rachel) and Kvilda (Aussergefild), Presl & Presl (1819) from Modrava (Mader), Opiz (1840) from Kvilda, Modrava, Zhůří (Heidelberg), Železná Ruda (Eisenstein), Javor (Gr. Arber) and Třístoličník, Jungbauer (1842) and Pfund (1842) from Plechý. Also Čelakovský (1883) reported in his "Prodromus" abundant occurrence of *Gentiana pannonica* in the Bohemian Forest. In the past, this gentian was intensively collected as a medicinal plant and became almost eradicated in the Bohemian Forest. After displacement of most of the ethnic Germans from the Bohemian Forest, the populations of *Gentiana pannonica* again increased in size (Chán & al. 1999). At present, less than one half of about 80 historical localities of this species still exist (Procházka in Čeřovský & al. 1999). In the 1990–2000, the data on the occurrence of this taxon were updated and this gentian was

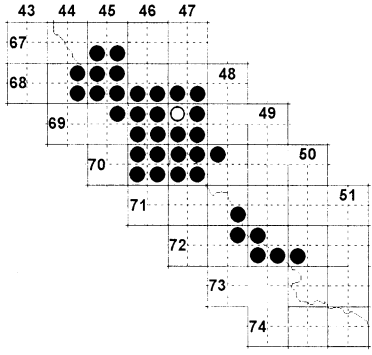


Fig. 1. – Occurrence of *Gentiana pannonica* in the Bohemian Forest (●) and the location of analysed populations (○).

also found on new localities thanks to an intensive systematic floristic investigation of the Bohemian Forest (KOLEKTIV 1995–2001).

Besides the classical morphological and demographic approach, genetic methods, especially analyses of isozyme and molecular (DNA) markers, are now used for detailed study of plant populations. Unfortunately, in small and genetically isolated populations of endangered species isozyme markers expressed only a small polymorphism or none at all (BARRET & KOHN 1991).

Non-coding DNA sequences are often used as molecular markers. These sequences are less conservative, are subject to relatively rapid evolution and should express a high level of polymorphism, which is necessary for genetic analyses at the meta- and intrapopulation levels (PARKER & al. 1998). In the last decade, RAPD (FISCHER & MATTHIES 1998) and microsatellite markers (SSRs – simple sequence repeats and ISSR- inter simple sequence repeats) are used in the population biology of wild-growing plants (GUSTAFSSON 2000, HOLLINGSWORTH & al. 1998).

Suitable for phylogenetic study are more conservative DNA regions such as the chloroplast, mitochondrial and nuclear ribosomal DNA (CRUZAN 1998). GIELLY & TABERLET (1996) and GIELLY & al. (1996) reported results of phylogenetic analysis of European species of the genus *Gentiana*, based on polymorphism of chloroplast DNA (*trn*L (UAA) intron). YUAN & KÜPFER (1995, 1996) studied phylogenetic relationships in the family Gentianaceae using another approach – polymorphism in ITS sequences.

The combination of genetic methods/approach and classical ecological methods should result in a new and more complex view on genetic structure of selected populations of *Gentiana pannonica*. This combined approach can help us to understand the ecology and dynamics of these populations, and to establish new models of management of these endangered species.

Materials and Methods

The objective was to verify RAPD markers for detection of genetic polymorphism in selected populations of *Gentiana pannonica* (L.) Scop. Two relatively large populations were selected for observation in the Bohemian Forest. The first population was located near village Horská Kvilda – about 1 km north of the village in a *Nardetum* on the edge of a meadow enclave. This part of the meadow enclave is unmanaged now, former it was pastures and mesophytic meadows. Second population was located in Zhůří enclave – 1 km north-west of former settlement Zhůří, on the northeastern slope of the Hutská hora Mt., in a mesophytic meadow. Extensive and long term study of the impact of different types of management of mountain meadows was performed on these localities and our study on *Gentiana pannonica* was part of this project: 50 plants from each population were analysed. In addition five plants from the Krkonoše Mts. (Modrý důl) and five from the Hrubý Jeseník Mts. (Petrovy kameny) were observed, too. These plants were analysed as outgroup plants to populations from Bohemian Forest and their further study is suggested.

The method used for isolation of total DNA was the DNeasy (™) Plant Mini Kit (QIAGEN) according to standard protocol (Sáková & al. 2000). DNA samples were stored at –20°C prior to their use.

The protocol for RAPD analysis was adapted from that of Williams & al. (1990) and Sáková & Čurn (1998). The volume of the final PCR reaction (25 µl) composed of 1× buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 1% Triton X-100), 100 µM dNTP, 10 pM of primer (Operon Technologies, series A, B and F), 1 U DyNAzyme II *Taq* polymerase (Finzyme) and 25 ng of template DNA. Amplifications were carried out in an MJ Research Thermocycler PTC 100 with 45 cycles of 1 min at 92°C, 2 min at 35°C and 3 min at 72°C. PCR products were separated on 1.5% agarose gel in TAE buffer, and DNA bands were visualized after ethidium bromide staining under UV light.

RAPD analysis was performed with a complex of random primers. For digital analysis of electrophoretic data software *BioProfil 1D* ++ was used. RAPD data were statistically analysed (*Statistica*). Genetic similarity coefficients were scored for detection of intrapopulation variation or for finding genetic variation level between particular populations. Cluster analysis was performed using the unweighed pair group method with arithmetic means (UPGMA) and its results were expressed as dendrograms.

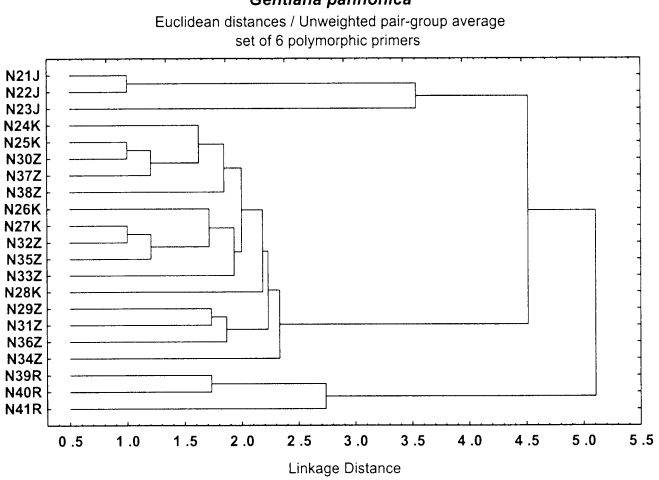
Results and Discussion

A total of 60 random primers were surveyed. About 70% primers exhibited no or only low level of polymorphism (polymorphic bands had weak staining intensity); 6 primers (OPA-04, OPA-07, OPA-08, OPB-15, OPB-17, OPB-18) (10%) detected a minimum of 3 polymorphic bands. These polymorphic primers amplified 83 bands and 52 (63%) from this number were polymorphic.

Relatively small differences were detected in the spectrum of RAPD markers in the studied Bohemian Forest populations. This low level of genetic variation detected with RAPD markers reflects the results which were acquired with microsatellite analysis. SSR markers were in most analyses uniform and no or very low level of polymorfism was detected (Čurn & Sáková 2001).

Also isozyme markers expressed low level of polymorphism and their possible utilisation is undertaken in further study and detection of a broader profile of isozyme loci (Čurn & Sáková 2001).

Fig. 2 shows the results of cluster analyses of RAPD fingerprints. Dendrogram 2a demon-

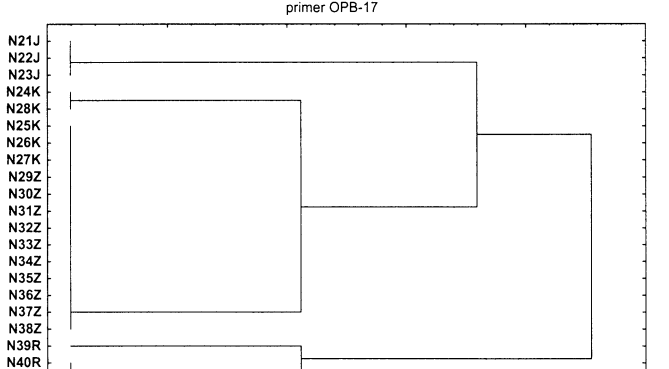


N..K – Bohemian Forest – Horská Kvilda
N..Z – Bohemian Forest – Zhůří
N..R – Krkonoše Mts.
N..J – Jeseníky Mts.

Fig. 2a. – Analysis of genetic distances of assessed populations of *Gentiana pannonica*. 2a – result generated with set of six primers, 2b – result generated with only one primer OPB 17.

strates the results of 6 RAPD analyses (6 fingerprints with different polymorphic primers) in populations from the Bohemian Forest and in analysed sample plants from the Krkonoše and Jeseníky Mts. It shows not only small differences between the analysed plants originating from the Bohemian Forest populations, but also mutual penetration and coincidence of the observed two populations. Relatively significant is the fact that populations from the Krkonoše and Jeseníky Mts. express significant differences in RAPD marker profiles and are easily distinguishable from the Bohemian Forest populations. Dendrogram 2b shows the result generated with only one primer (OPB 17); it demonstrates low level of polymorphism and requires more total analysis and employment of further RAPD analysis.

Intrapopulation and interpopulation variation in the taxon *Gentiana pannonica* can be distinguished on the basis of the respective RAPD profiles. Nevertheless, the genetic polymorphism of *Gentiana pannonica* is quite small. Further study will be focused on different types of molecular markers (new set off microsatellite primers, noncoding sequences – ITS and *trn*(UAA) intron). We also intend to analyse a more extensive set of populations from the Bohemian Forest.



N..K – Bohemian Forest – Horská Kvilda
N..Z – Bohemian Forest – Zhůří
N..R – Krkonoše Mts.
N..J – Jeseníky Mts.

Fig. 2b. – Analysis of genetic distances of assessed populations of *Gentiana pannonica*. 2a – result generated with set of six primers, 2b – result generated with only one primer OPB 17.

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