VARIABILITY OF *PINUS MUGO* TURRA CLONES FROM OSTRY WIERCH PEAT BOG

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ABSTRACT

Present study aimed at determining genetic structure of the *Pinus mugo* Turra population, growing at a peat bog, on Ostry Wierch slopes in the High Tatra mountains, in respect to the number of genotypes present in the population, and at defining variabilities of morphological and anatomical traits. In the examined plants, isoenzymatic analysis demonstrated existence of only two genotypes (A and B), which formed relatively vast clones. Mathematical-statistical analysis of 13 anatomical-morphological traits of the needles proved that the examined clump consisted of two clearly distinguishable groups, corresponding to the two earlier electrophoretically identified clones A and B. The obtained data allowed to conclude that the method based on anatomical-morphological measurements may, at least in some cases, permit identification of *Pinus mugo* clones.

KEY WORDS: *Pinus mugo*, morphology, clones, isoenzymes, variability, genotypic structure.

INTRODUCTION

Slopes of the Carpathians and the Sudeten mountains above the tree line, i.e., above altitude of 1400-1870 m represent the natural biotope of *Pinus mugo* Turra in Poland. The pine forms at first compact thickets and then, going upwards, the thickets disperse into small groups and individual shrubs. *Pinus mugo* Turra can also be encountered in lower parts of the mountains and foothills in Podhale but only in the peat bogs. The isolated stands represent late-glacial relics and survival of the plant in the stands has been possible due to the absence of competition from other tree species, reflecting the unfavourable for trees edaphytic conditions (Szafer 1959).

Importance of the *Pinus mugo* Turra belt in the mountains results from its capacity: 1) to fix the screes, 2) to promote formation of soil layer on the screes, 3) to prevent abrupt erosion and to decrease the hazard of avalanches.

Pinus mugo Turra exhibits great ability to propagate in a vegetative way which affects shaping of the individual structure of the population. The ability permits also wide spread of genotypes, capable to grow in difficult environmental conditions and, despite the difficulties in pollination and fructification, in sites in which its individuals grow in isolation or in which weather conditions prevent effective generative propagation. It seems that this ability presents for this pine species an important adaptive strategy.

In the past, *Pinus mugo* Turra was burnt a mass scale in mountain meadows to increase pasture area (Sokołowski

1936) but in the sites with difficult access as, e.g., in the fields of the scree, on steep slopes or on peat bogs, many natural populations survived in an unchanged genetic composition of populations and with unchanged genetic and demographic structure. For this reason, we have chosen for our studies, a most probably natural population of *Pinus mugo* growing on peat bog, on the west slope of Ostry Wierch in the High Tatra mountains (Fig. 1).

Fig. 1. Schematic distribution of clones A and B of (*Pinus mugo*) on the peat bog of Ostry Wierch.

The present study aimed at examining genetic structure of the population in respect to the number of genotypes present in the group of plants and at defining its variability the respective morphological-anatomical traits.

Genetic structure of the population was tested using analysis of isozyme variability at some enzymatic loci while the morphological-anatomical variability was studied using biometric techniques.

MATERIAL AND METHODS

The studies were performed on a compact clump of *Pinus mugo* Turra, size about: 8 m x 25 m, growing on peat bog on the west slope of Ostry Wierch in the High Tatra mountains (altitude 1345 m). Twenty seven shoots were examined, collected along transect every 1 m (Map no. 1). From each shoot, the apex bud was taken for electrophoretic studies and 27 two-years old needles were collected for anatomical-morphological studies. The needles were preserved in 70% ethanol.

Variability at 10 enzymatic loci was tested, including: alcohol dehydrogenase (ADH, E.C.1.1.1.1.), shikimate dehydrogenase – two loci (ShDH, E.C.1.1.1.25), malate dehydrogenase – two loci (MDH, E.C.1.1.1.37), glucose-6-phosphate dehydrogenase (G6PD, E.C.1.1.1.44), fluorescent esterase (FEst, E.C.3.1.1.23), diaphorase (DIA, E.C.1.6.99), glutamate oxaloacetate transaminase (GOT, E.C.2.5.1.1.), glutamate dehydrogenase (GDH, E.C.1.4.1.2.)

Cross-sections through the needle mid-point were prepared manually. They were preserved in polyvinyl alcohol. A total of 13 morphological-anatomical traits were examined, including:

1. Number of stomatal rows on the abaxial (convex) side of the needle;
2. The same on the adaxial (flat) side of the needle;
3. Stomatal row index (value of trait #1 divided by value of trait #2);
4. Mean number of stomata in a 2 mm long fragment of the abaxial side of the needle;
5. The same on the adaxial side of the needle;
6. Number of resin canals;
7. Thickness of the epidermis on the adaxial side of the needle [μm];
8. Mean width of the epidermal cell, measured as an average width of three neighbouring cells [μm];
9. Width of the needle measured on cross-section [μm];
10. Thickness of the needle measured on cross-section [μm];
11. Thickness to width ratio (ratio of trait 10 to trait 9);
12. Distance between vascular bundles [μm];
13. Marcet's coefficient (value of trait #9 x value of trait #12 divided by value of trait #10; Marcet 1967).

Fig. 2. The way of measuring anatomical traits on the transverse section of the needle.

Traits ## 6, 7, 8, 9, 10, 12 were measured under a microscope while traits ## 1, 2, 3, 4, 5 were measured using a stereo microscope. Results of the measurements were subjected to statistical-mathematical analysis in the Main Computing Centre, Adam Mickiewicz University in Poznań.

The results were subjected to analysis of discriminant variables (Caliński, Kaczmarek 1973; Krzyśko 1979, 1982) and to analysis of principal components (Caliński, et al. 1975).

RESULTS

In the first approach we examined isoenzymatically 50 randomly collected samples along the clump of *Pinus mugo*, then samples were collected as described above, every 1 m. Results of analysis of variability at 10 enzymatic loci demonstrated that two multilocus genotypes could be distinguished among the 27 examined samples. The distribution of genotypes in the clump is shown in Fig. 1.

The examined material of two groups of which one comprised 17 genetically identical samples (genotype A) while the other was formed by the remaining 10 samples (genotype B). With a very high statistical probability it could be concluded, that the two groups represent two clones and the analysed samples represent ramets originating from two genetically distinct individuals (Shea, Grant 1986). Isoenzymatic data pertaining to structure of individuals within the studied population are presented in Table 1.

TABLE 1. Genotypic structure of *Pinus mugo* population from Ostry Wierch peat bog in the Tatra Mts. 1/1; 2/2 – homozygotes. 01/1; 1/2; 1/3; 1/5 – heterozygotes.

LOCUS	GENOTYPES	FREQUENCY	CLONE
FEst	01/1	17	A
	1/1	10	B
ShDH A	1/5	17	A
	1/2	10	B
ShDH B	1/1	27	A, B
GOT B	1/2	17	A
	1/1	10	B
Diaf. B	1/2	17	A
	1/1	10	B
MDH A	1/1	27	A, B
MDH C	1/2	17	A
	1/1	10	B
6PGD	1/3	17	A
	1/1	10	B
GDH	2/2	17	A
	1/1	10	B
ADH	1/1	27	A, B

Mathematical-statistical analysis of 13 anatomical-morphological traits, examined in 27 shoots, demonstrated also that the compact clump of *Pinus mugo* Turra could be subdivided into two clear-cut groups, which correspond to the electrophoretically identified clones A and B, as presented in the diagrams of discriminant variables (Fig. 3) and principal components (Fig. 4).

DISCUSSION AND CONCLUSIONS

Individuals belonging to distinct clones may be distinguished from each other using several techniques. The ap-

Fig. 3. Result of discriminant analysis (first and second variables) for 13 needle traits of 27 plants. A – the first clone of *Pinus mugo*; B – the second clone of *Pinus mugo*.

Fig. 4. Result of principal component analysis (first and second) for 27 plants of 13 needle traits. A – the first clone of *Pinus mugo*; B – the second clone of *Pinus mugo*.

proach applied in this study has involved biochemical techniques. Such techniques include electrophoresis, which permits to determine particular genotypes of individuals, also chromatography of low molecular weight compounds and serology (Prus-Głowacki – unpublished data).

In selected cases, such as aspen (Kemperman and Barnes 1976) or corals (Diamond 1986) clones may occasionally be distinguished by easily noticeable morphological traits. Individual clones may differ in leaf glaze (in the aspen) or in the general pattern of growth and width of branches (in corals). In the case of *Pinus mugo*, individuals frequently differ also in such traits as needle colour, length of dwarf shoots, shape of winter buds, shape and colour of cones. The traits may be helpful in identifying clones. However, it should be born in mind that expression of such traits may be significantly affected by environment. Therefore, identification of individuals (clones) based on measurements of defined morphological and anatomical traits of leaves, cones, seeds, fruits, etc., which are then subjected to mathematical-statistical analysis need to be supported by biochemical techniques.

In this study, identification of individuals has been based not only on electrophoresis of isoenzymes but also on biometrical techniques. Results obtained with each of the techniques have proven compatible. They have shown that the compact clump of *Pinus mugo* Turra on the slopes of Ostry Wierch in the High Tatra mountains contains only two clones. The obtained data allow to conclude that the method based on measurements of morphological-anatomical traits may serve also for identification of *Pinus mugo* clones, as illustrated by diagrams of discriminant variables and of principal components analysis (Fig. 3 and 4).

Even if associated with a significant labour cost, the mathematical-statistical method is relatively easy and economic. The result may prove of importance for selecting appropriate methods and strategy of sampling: if cloning is a common phenomenon in *Pinus mugo* populations, it should be taken

into account both in studies on population variability of *Pinus mugo* and in collection of seeds for recultivation of destroyed *Pinus mugo* populations to preserve genetic biodiversity of the species as large as possible. The results indicated that the biometric techniques may find broader application as mentioned above.

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LITERATURE CITED

- CALIŃSKI T., KACZMAREK Z., 1973. Methods of complex analysis of multitrait experiment (in Polish). Wyd. V PAN and PTB, Warszawa-Wrocław
- CALIŃSKI T., CZAJKA S., KACZMAREK Z., 1975. Analysis of principal components and its application (in Polish). Roczniki Akademii Rolniczej w Poznaniu, Algor. Biometr. Statyst. 36: 159-185
- DIAMOND J.M., 1986. Clones within a coral reef. Nature, 323: 109.
- KEMPERMAN J.A., BARNES B.V., 1976. Clone size in American aspens. Canadian Journal of Botany 54: 2603-2607
- KRZYŚKO M., 1979. Discriminant variables. Biometrical Journal 21: 227-241
- KRZYŚKO M., 1982. Discriminant analysis (in Polish). Wyd. UAM in Poznań, Ser. Matematyka 6: 1-98.
- MARCET E. 1967. Über den Nachweis spontaner Hybriden von *Pinus mugo* Turra und *Pinus sylvestris* L. auf Grund von Nadelmerkmalen. Ber. Schweiz. Bot. Ges. 77: 314-361.
- SHEA K.L., GRANT M.C., 1986. Clonal growth in spire-shaped Engelmann spruce and subalpine fir trees. Can. J. Bot. 64: 255-261.
- SOKOŁOWSKI S., 1936. Tatra mountains forest (in Polish). WPN Muzeum Tatrzańskie, Zakopane.
- SZAFER W., 1959. Plants in Poland (in Polish). PWN Warszawa.

ZMIENNOŚĆ KLONÓW *PINUS MUGO* TURRA POCHODZĄCYCH Z TORFOWISKA POD OSTRYM WIERCHEM

STRESZCZENIE

Celem pracy było zbadanie struktury genetycznej populacji *Pinus mugo* Turra, rosnącej na torfowisku, na zboczu Ostrego Wierchu w Tatrach Wysokich, pod kątem liczby występujących tu genotypów oraz określenie zmienności cech anatomiczno-morfologicznych. Analiza izoenzymatyczna wykazała, istnienie jedynie dwóch genotypów wśród przebadanych roślin, tworzących dość obszerne klony: A i B. W wyniku analizy matematyczno-statystycznej cech igieł, stwierdzono również, że badana kępa składa się z dwóch wyraźnie różniących się grup, odpowiadających wcześniej zidentyfikowanym elektroforetycznie klonom A i B. Na podstawie uzyskanych danych, można stwierdzić, że metoda oparta na pomiarach anatomiczno-morfologicznych może służyć, przynajmniej w niektórych przypadkach, do identyfikacji klonów *Pinus mugo*.

SŁOWA KLUCZOWE: kosodrzewina, klony, morfologia, izoenzymy, zmienność, struktura genetyczna.