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Genetic differences among the four *Stipa* species endangered and protected in Poland

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Abstract: The distinct character of four species of *Stipa* genus, *S. borysthenica, S. capillata, S. joannis* and *S. pulcherrima* was manifested in the variability of three enzyme systems, including glutamate-oxalacetate transaminase (GOT), esterase (EST) and peroxidase (PX). The studies were conducted on two-month-old seedlings cultured under the same glasshouse conditions, obtained from seeds from the Botanical Gardens collection. Fifteen loci were described, of which eight proved to be polymorphic. The studies included also the complex species of *S. pennata* and the closely related species of *S. tirsa*.

Key words: *Stipa borysthenica*, *S. capillata, S. joannis, S. pulcherrima*, *S. pennata, S. tirsa,* enzyme, electrophoresis

1. Introduction

In Poland occur four species of the *Stipa* genus, including *S. borysthenica* Klokov, *S. capillata* L., *S. joannis* Čelak and *S. pulcherrima* K. Koch. All belong to the group of grasses covered by strict protection and, being endangered, they are included in the Polish Red Book of Plants (Kaźmierczakowa & Zarzycki 2001). Populations of *Stipa* species in Poland have a low frequency due to human activity as they were used them for decoration due to the exceptionally aesthetic habit of their panicles (Kozłowski 2002). For this reason, the species in some regions are endangered by extinction (Żukowski & Jackowiak 1995). Occurrence of the species is linked to xerothermic grasses of a steppe type in the *Festuco-Brometea* class. These may change their character both in a natural way and due to spontaneous succession of bushes and trees which leads to shadowing and disappearance of heliophilic species (including those of *Stipa* genus). It may also be due to human activity in the form of excessive pasturage or effects of eutrophic waters flowing down from the neighbouring fields under cultivation (this pertains particularly *S. joannis*).The increasingly rare biotopes of *Stipa* genus species are protected in preserves which play the role of living gene banks. The most frequently protected are species of *S. capillata* and *S. joannis* (Piękoś-Mirkowa & Mirek 2002).

S. borysthenica grows in Poland very infrequently. The species is known to occupy only five xerothermic stands along the lower Odra River and in Wielkopolska (Ceynowa-Giełdon 2001a). *S. capillata* is a species typical for the *Potentillo-Stipetum capillatae* community. It grows in small populations along Lower Odra River, in northwestern Poland, in the Toruń-Eberswalde proglacial streat valley, in Nida Lowland, Małopolska and Lubelska Highlands (Filipek 1974; Górska-Zajączkowska & Węglarski 1993; Piękoś-Mirkowa & Mirek 2002; Towpasz & Mitka 2001). Individual stands of *S. joannis* can be encountered mainly along the lower Vistula River, in the Notecka proglacial stream valley, in Warta valley, and along the lower Odra River (Górska-Zajączkowska et al. 1989; Michalik 1991; Majtkowska & Majtkowski 2004). In the north of Poland the species forms the *Potentillo-Stipetum* community. *S. pulcherrima* is a very rare species in Poland, endangered by extinction. Along the lower Odra River it composes the *Linosyridi-Stipetum pulcherrimae* complex. It is thought to represent a typical species of xerothermic grasses of the *Festucetalia-valesiaceae* order, together with *S. joannis* (Matuszkiewicz 2001). It occupies a few stands by the Odra River and on the Sandomierska Highland (Ceynowa-Giełdon 2001b).

The progression of the degradation of biotopes in recent years, constrains botanists to undertake additional measures to preserve the gene pool of endangered species using *ex situ* culture in botanical gardens. Taking into account that inter-specific differences, according to biochemical character, were not investigated earlier

in the genus *Stipa,* we have decided to take advantage of such collections for this preliminary study.

2. Material and methods

The species were represented by the following samples of seeds:

S. borysthenica – 2 samples: Botanical Garden, Adam Mickiewicz University; the seeds originated from exchange with Botanical Garden in Budapest;

S. capillata – 10 samples: Botanical Garden, Adam Mickiewicz University – the seeds originated from populations in the vicinity of Węgrzyce near Gorzów, from the Ojcowski National Park and St. Laurent's Mountain, and from the Botanical Garden, Frankfurt am Main; Botanical Garden, University of Łódź; Botanical Garden, University of Marie Skłodowska-Curie in Lublin – the seeds originated from natural populations in Wiślica and from an exchange program; Botanical Garden, University of Warsaw; Botanical Garden, Jagiellonian University in Cracow;

S. joannis – 4 samples: Botanical Garden, Adam Mickiewicz University – the seeds originated from collection of our own, from the Barbarka population near Toruń and Skorocice population near Busko; Botanical Garden, Institute of Plant Culture and Acclimation in Bydgoszcz: the seeds originated from Chorągiewki population;

S. pennata – 2 samples: Botanical Garden, University of Warsaw; Botanical Garden, Adam Mickiewicz University: the seeds originated from Frankfurt am Main;

S. pulcherrima – 2 samples: Botanical Garden, Institute of Plant Culture in Bydgoszcz – the plants were developed from seeds obtained by exchange with the Botanical Garden in Berlin-Dahlem; Botanical Garden, Adam Mickiewicz University: seeds of plants originating from Ukraine;

S. tirsa – 2 samples: Botanical Garden, Adam Mickiewicz University: the seeds originated from exchange with the Botanical Garden in Berlin and our own collection.

Seedlings originating from Botanical Gardens seed collections were cultivated in identical glasshouse conditions. A crude extract of individual plants was subjected to electrophoresis in 11% starch gel in

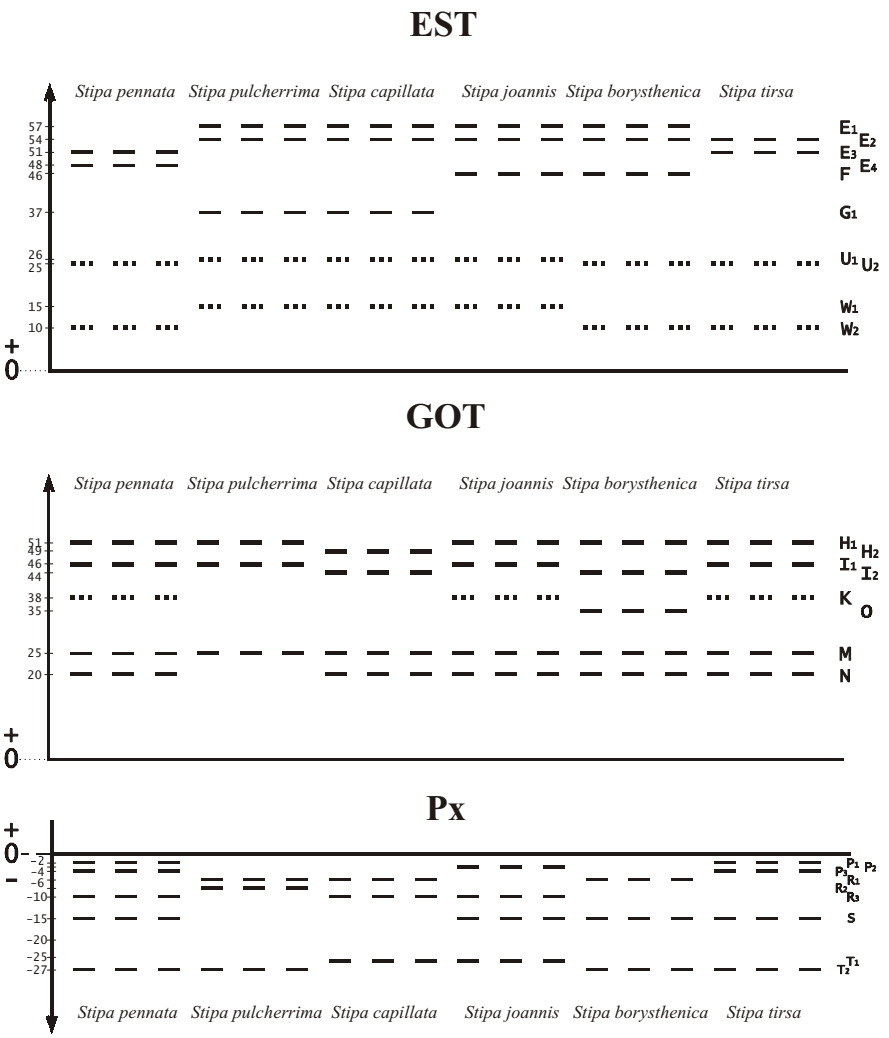

Fig. 1. Schematic diagrams of electrophoretically detected allozymes for the examined 6 species

lithiumborate buffer system, pH 8.3. The gels were stained specifically (Show-Prasad 1970) for three enzymes: glutamate-oxalacetic transaminase (GOT, EC.2.6.1.1), esterase (EST, EC 3.1.1.2), peroxidase (PX, EC 1.11.1.7). Interspecific differences were illustrated by Sphi coefficient (Leuschner 1974), principal component analysis and aglomerative clustering using the unweighted-pair-group method (UPMGA). In this way, six species were compared, using the nomenclature of the Botanical Gardens from which the seeds came.

3. Results and discussion

For each enzyme system, band patterns on the gels permitted the establishment of separate loci and their allozymes (Fig. 1).

GOT. Assuming that, similarly to other plants, the glutamate-oxalacetic transaminase is a dimer (Hillis & Moritz 1990), the two-banded phenotypes reflect expression in two separate loci. Each of bands containing two allozymes (in cases of H and I loci) are documented by separate, single-banded loci as well as for M and N (showing no hybrid bands).

EST. The band pattern pointed to the monomeric character of the enzyme. The five distinguished loci well characterized the species.The most polymorphic proved to be the E locus with four alleles. At this locus the species demonstrated fixation of the heterozygous condition, frequently termed „the fixed heterozygosity" which, nevertheless, was typical for the species. Thus, four species (*S. pulcherrima, S. capillata, S. joannis* and *S. borysthenica*) demonstrated E1E2 phenotype, *S. tirsa* showed E2E3 phenotype while *S. pennata* demonstrated E3E4 phenotype or differed from each other even if, as closely related, they shared the common E3 allele. It should be recalled that *S. pennata* as a morphologically variable species served as a common species for a few other species the taxonomic identity of which underwent periodic modifications. Material for our studies originated from botanical garden collections, included small numbers of seeds and, therefore, monomorphism of the samples. Even if they

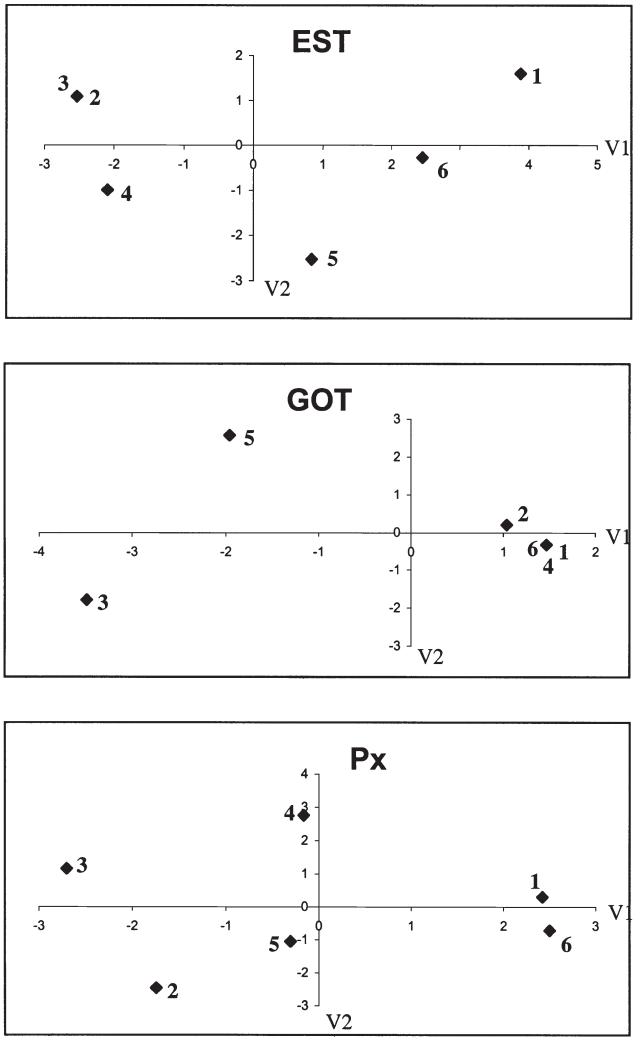

Fig. 2. Scatter diagram for the species investigated on the plane of the first two axes of the Principal Components. 1 – *S. pennata*, 2 – *S. pulcherima*, 3 – *S. capillata*, 4 – *S. joannis*, 5 – *S. borysthenica,* 6 – *S. tirsa*

are heterozygotic, they might occasionally result from their original form, a single plant. Ambiguities of the type may be resolved only by appropriate population studies.

Persistence of „fixed heterozygosity" might reflect also the biology of the studied species since xerophytic

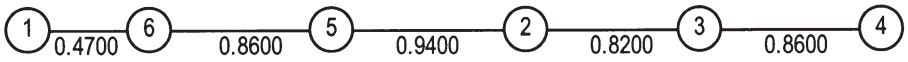

Fig. 3. Dendrite of the shortest connections between *Stipa* species constructed on the basis of the summarised data obtained from all enzyme systems
Explanations: 1 – *S. pennata*, 2 – *S. pulcherima*, 3 – *S. capillata*, 4 – *S. joannis*, 5 – *S. borysthenica,* 6 – *S. tirsa*

tuft grasses of genera such as *Stipa* and of other stable species of grasses with the patchy growth, e.g., those of *Bromus* genus, are mostly autogamic due to the presence of cleistogamic flowers (Stebbins 1958).

PX. Band patterns indicated that, similarly to other grasses (Krzakowa & Kraupe 1981; Krzakowa 1996; Krzakowa & Mikulski 1997; Krzakowa *et. al.* 2003, 2005) including the studied species of *Stipa* genus, the enzyme system shows its monomeric behaviour. Two loci could be distinguished, each with three alleles, including loci P and R as well as locus T with two allozymes. Locus S, not detected in studied samples of *S. capillata* and *S. pulcherrima*, in the remaining species was present in the form of a single band.

In order to illustrate reciprocal relationships between the species, the technique of principal components was applied for individual enzymes (Fig. 2). In respect to esterases (EST), *S. pennata, S. borysthenica* and *S. tirsa* formed a single species group. The most pronounced similarity was manifested by two species: *S. pulcherrima* and *S. capillata*, which occupied the same space, while *S. joannis* was quite distinct.

In respect to glutamate-oxalacetate-transaminase (GOT), here species including *S. pennata*, *S. joannis* and *S. tirsa*, proved similar. Worth noting, *S. capillata* and *S. borysthenica* manifested a distinct character. The species variability pattern in the space formed for peroxidases (PX) by the two principal components confirmed the distinct character of *S. capillata*, linked *S. pennata* and *S. tirsa* and pointed to a similar character of *S. pulcherrima*, *S. joannis* and *S. borysthenica*.

The total dendrite constructed for all the populations (Fig. 3) on the basis of the lowest values of the Sphi coefficient demonstrated close taxonomic similarity between *S. pennata* and *S. tirsa* while the other species are connected by similar taxonomic distances.

Grouping by the closest neighbourhood technique (UPGMA) pointed to phylogenetic relationships between the species (Fig. 4). It resulted in the formation of two groups of species: the first including *S. pennata* and *S. tirsa* with *S. borysthenica* attached to them and the other formed by *S. pulcherrima* and *S. joannis*, while *S. capillata* proved its separate character.

In all the schemes a noticeable tendency appears for links between *S. pennata* (1) and *S. tirsa* (6) samples. Inclusion in our studies of samples representing *S. pennata* complex, which still persists in the world

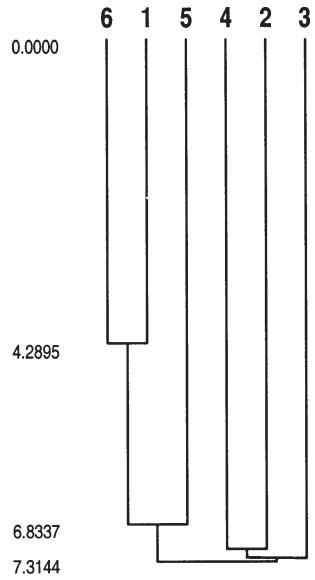

Fig. 4. Dendrogram depicting hierarchial structure of genetic relatedness among *Stipa* species
Explanations: 1 – *S. pennata*, 2 – *S. pulcherima*, 3 – *S. capillata*, 4 – *S. joannis*, 5 – *S. borysthenica,* 6 – *S. tirsa*

nomenclature (Strid & Tan 1991), allows the recollection that races of *S. joannis, S. tirsa* and *S. pulcherrima* have been isolated from the *S. pennata* species (Hegi 1906), and later reached the status of species (Hegi 1992). Also *S. borysthenica* has originated from *S. pennata* subsp. *sabulosa* (Rybcov 1972; Hegi 1992). The inter-specific differences demonstrated in the present study are sufficiently interesting to prompt further studies on natural populations as long as they are still available.

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