

European needlegrass (*Stipa capillata* L.) population found in the New Zoological Garden in Poznań, characterised by electrophoretically detected peroxidase and esterase variability

Adam Wysocki¹, Maria Krzakowa² & Marcin Michalak²

¹Department of Plant Taxonomy, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, e-mail: wysocki@amu.edu.pl

²Department of Genetics, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, e-mail: krzakowa@amu.edu.pl

Abstract: Individual plants of *Stipa capillata* L. originating from the population in the New Zoological Garden in Poznań city (West Poland) were examined by electrophoresis of 2 enzyme systems: peroxidases and esterases. The results were compared to those obtained for populations originating from 2 natural stands near Chełmno (ca. 120 km away from Poznań). The populations proved to be monomorphic with respect to peroxidases and only slightly polymorphic with respect to esterases. The prevalence of G2G2 phenotype in the population from the zoo suggests that this population is of some distant origin.

Key words: *Stipa capillata*, populations, peroxidases, esterases, electrophoresis

1. Introduction

Stipa capillata is a species of a very wide distribution. Its natural range includes the southeastern Europe, Caucasus, southwestern parts of Russia, central Asia and southwestern parts of Siberia. Asia is the original homeland of *Stipa* species, particularly its steppe regions and the adjacent steppes of Europe (Koczwara 1949). In Poland, *Stipa capillata* is a legally protected species, growing mainly in xerothermic grasslands of the Lublin Upland, Miechów-Sandomierz Upland, on steep banks of the lower course of the Odra River (Filipek 1974) as well as on dry and warm banks of the Vistula River close to Toruń (Koczwara 1949; Ceynowa 1968; Głazek 1994, 1996).

Only few local populations of the species can be found in midwestern Poland, i.e. in the Wielkopolska region (Górska-Zajączkowska & Węglarski 1993; Żukowski & Jackowiak 1995). Occurrence of *S. capillata* in the urban agglomeration of Poznań was first noted already in 1971 by Professor Waldemar Żukowski on the slopes close to the train station of Poznań-Franowo (Jackowiak 1993). Another population of *S. capillata* in the New Zoological Garden of Poznań was localised in 1998 (Wysocki 2002). These two findings

have probably no common history although they may point to variable pathways of the spread of the species. The long awn of the seed promote its dispersal with wind and by animals as well as help the caryopsis to settle in the soil. The caryopsis of *S. capillata* can get screwed into the skin of animals, e.g., sheep, resulting in ulcers and sometimes even deadly diseases, while the perforated skins become worthless (Falkowski 1974).

An earlier analysis of the succession rate of the population from the New Zoological Garden indicated that the species appeared there in the early 1990s, probably imported with some animals or with forage for livestock (Wysocki 2002). The demographic trends within the population have been monitored since 1999. In the first year of the observation the population included 27 tufts of plants with inflorescence shoots. Since then the area inhabited by the population has increased from 12.5 m² to around 15 m², and in 2005 the population already consisted of 141 tufts.

Isolated populations can show a unique genetic structure, so in this study we aimed to investigate the genetic structure of this population of *S. capillata*. Its existence as a temporarily appearing species in the urban flora of Poznań has provided a good reason to become the topic of our study.

2. Material and methods

The population of *S. capillata* is situated in the southeastern part of the exposition area of the New Zoological Garden in Poznań (53°10'N, 18°35'E), on a sandy slope with southeastern exposure and inclination of over 30°. From the North this site borders on a pine forest, from the South and the West it is neighbouring on an animal-run, and in the East it is delimited by a walking lane. Because of the absence of trees from the South and the West, the stand can be exposed to sunlight nearly all day long.

Enzymatic analysis was conducted for individual plants collected from the population in the zoo (80 plants) and from 2 natural populations located about 120 km east of Poznań, near the village of Chełmno (52°28'N, 17°00'E), i.e. from Saint Laurence Mount (Góra Św. Wawrzyńca, 32 plants) and from the Kiełpski Ravine (34 plants), separated from each other by a distance of about 10 km.

From every plant, leaf tissue was crushed and crude extract was absorbed into filter paper wicks (5 mm × 5 mm, Beckmann 319329). The electrophoresis was conducted in 11% starch gel (Starch Art.Co.) in lithium-borate buffer system, pH 8.3. The staining procedure strictly followed that described by Show & Prasad (1970) for peroxidase (EC 1.1.1.17) and esterase (EC 3.1.1).

3. Results and discussion

Locus E of esterases in *Stipa* species consists of 4 allozymes (Krzakowa *et al.* 2006). In the studied populations of *S. capillata*, 2 alleles are present (E1 and E2), which in most cases appear in heterozygous form (E1E2). Some plants from the zoo, for example Nos. 9, 10 and 14 (Fig. 1), have shown no E2 activity, suggesting the homozygous E1E1 status.

The populations were also nearly monomorphic in respect to G1 and G2 alleles. Both populations near Chełmno were monomorphic with respect to the G1

allele, representing G1G1 homozygotes. The population from the zoo manifested a prevalence of G2G2 phenotypes as shows individual No. 24 manifested the presence of the G1G1 phenotype in the locus. Such a G1G1 phenotype was detected in only 2 plants out of the 80 (2.5%) plants examined. Since there is virtually no chance of gene flow from distant metapopulations, the homozygous G2G2 genotype will probably persist until the G1 allele, which is rare in this population, increases its frequency due to random hybridization.

The population in the zoo undoubtedly represents an isolated population, showing all symptoms of adaptation to the biotope, as demonstrated by the increase in the number of individuals manifesting the potential for generative reproduction. The nearly monomorphic character of esterases in locus G (more precisely, in the G2 allele), not encountered in other populations of *S. capillata*, may suggest a distant origin of the diaspores from which the population originated.

The low polymorphism in the G locus is an important discovery, indicating that the population was probably initiated from a single plant or from seeds including at least one being a G1G2 heterozygote. In cases of monomorphism in small populations, it is described as the 'founder effect', since the founder may be even a single diaspore.

The persistence of the heterozygous status of esterases (E1E2) represents a completely different problem: it may result from self-pollination, so important for the tufted xerophytes, in which this mechanism promoting formation of large seed numbers is a prerequisite of survival (Stebbins 1958).

As regards peroxidases, the populations of *S. capillata* proved to be fully monomorphic both with respect to the anodally migrating locus A and the cathodally migrating loci R3 and T1 (Fig. 2). Locus T1 is characteristic exclusively of the species *S. capillata*, while the other manifested in Poland species of *Stipa* genus are characterized by the common for them locus T2. The R3 allele, one of the alleles in R1 locus, is present

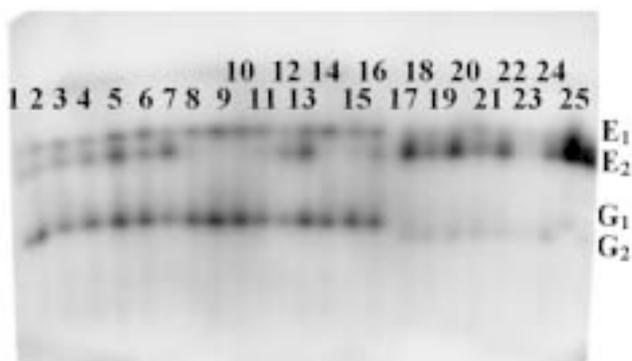


Fig. 1. Electrophoretically detected esterases. Plants 1-16 belong to the *Stipa capillata* population from Saint Laurence Mount near Chełmno, while plants 17-25 represent the population from the New Zoological Garden in Poznań

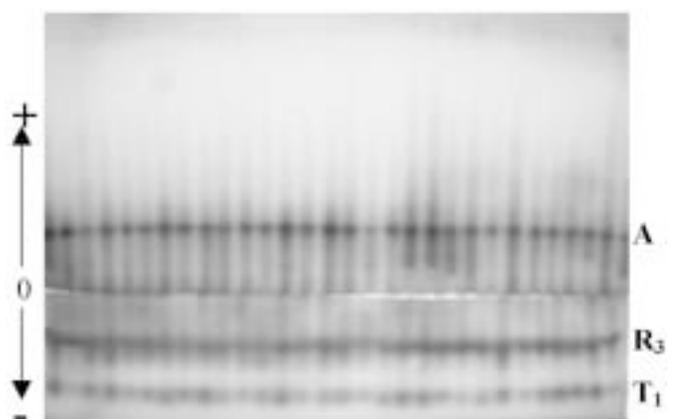


Fig. 2. An example of peroxidase band patterns, monomorphic in the 3 populations investigated

not only in *S. capillata* but also in *S. joannis* and *S. pennata* (Krzakowa *et al.* 2006).

At the present state of our knowledge on the level of enzymatic variability in *S. capillata* species, the population studied by us undoubtedly exhibits a stable but unique gene pool, which distinguishes it from other populations. However, even more unexpected is the monomorphism of peroxidases, since in grasses peroxidases manifest extensive polymorphism (Krzakowa & Kraupe 1981; Krzakowa 1996; Krzakowa & Mikulski 1997; Krzakowa *et al.* 2003, 2005).

In addition, the identical peroxidase patterns in the population found in the zoo and in the populations located over 100 km away is surprising. We cannot exclude (even if it seems improbable) that the absence of peroxidase polymorphism characterizes not only the studied populations but the entire species. However, since isolated populations very frequently manifest

a unique gene pool, which distinguishes them from populations in the centre of the range, the other populations may prove to be polymorphic. It also cannot be excluded that vegetative propagation, promoting the maintenance of homozygous genotypes, plays a principal role in the studied populations. Fixed homozygosity used to be an exponent of inbreeding and the latter may restrict the potential for adaptation but, on the other hand, in small populations it may reduce the genetic load and in this way it may preserve the hitherto observed fitness of the population.

These interesting results encourage us to undertake broader studies, in particular with natural populations representing a wider geographic range.

Acknowledgements. Scientific work financed from the resources earmarked for science in years 2005-2007 as the Research Project no. 2 P04C 095 28.

References

- CEYNOWA M. 1968. Zbiorowiska roślinności kserotermicznej nad Wisłą. Stud. Soc. Sci. Torun. 8(4): 1-155.
- FALKOWSKI M. 1974. Trawy uprawne i dziko rosnące. 598 pp. PWRiL, Warszawa.
- FILIPEK M. 1974. Kserotermiczne zespoły murawowe nad dolną Odrą i Wisłą na tle zbiorowisk pokrewnych. Bad. Fizjogr. Pol. Zach. seria B-Botanika 27: 45-82.
- GŁAZEK T. 1994. Projektowany rezerwat stepowy 'Dwikozy' na stromej krawędzi doliny Opatówki. Chrońmy Przyr. Ojcz. 50(1): 51-60.
- GŁAZEK T. 1996. Projektowany rezerwat stepu ostnicowego 'Kamień Plebański' koło Sandomierza. Chrońmy Przyr. Ojcz. 52(1):16-21.
- GÓRSKA-ZAJĄCZKOWSKA M. & WĘGLARSKI K. 1993. Ostnica włosowata *Stipa capillata* L. – rzadki i zagrożony gatunek flory północno-zachodniej Polski. Biuletyn Ogrodów botanicznych 2: 5-14.
- JACKOWIAK B. 1993. Atlas of distribution of vascular plants in Poznań. Publications of the Department of Plant Taxonomy of the Adam Mickiewicz University of Poznań 3: 1-409.
- KOCZWARA M. 1949. Ostnice Polski. Chrońmy Przyr. Ojcz. 4(5-6): 25-30.
- KRZAKOWA M. 1996. Genetic diversity of *Phragmites australis* (Cav) Trin. ex Stued. revealed by electrophoretically detected differences in peroxidases. In: C. OBINGER, U. BURNER, R. EBERMANN, C. PENEL & H. GREPPIN (eds.). Plant Peroxidases: Biochemistry and Physiology, pp. 184-189. University of Geneva.
- KRZAKOWA M., CELKA Z. & DRAPIKOWSKA M. 2005. Genetic variability of *Calamagrostis arundinacea* populations growing in *Calamagrostis arundinacea-Quercetum petraeae* community. In: L. FREY (ed.). Biology of grasses, pp. 23-30. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.
- KRZAKOWA M., JAŃCZYK-WĘGLARSKA J. & ŚLIWIŃSKA E. 2003. Peroxidase polymorphism in *Calamagrostis epigejos* (Poaceae) indicating interspecific hybridization. In: ZWIERZYKOWSKI Z., SURMA M. & KACHLICKI P. (eds.). Application of Novel Cytological and Molecular Techniques in Genetics and Breeding of the Grass, pp. 109-114. Institute of Plant Genetics PAS, Poznań, Poland.
- KRZAKOWA M. & KRAUPE A. 1981. Isozyme investigations of natural populations of the cheatgrass (*Bromus tectorum* L.). Bot. Jahrb. Syst. 103(1-4): 393-399.
- KRZAKOWA M., MICHALAK M. & JUDEK M. 2006. Genetic differences among the four *Stipa* species endangered and protected in Poland. Biodiv. Res. Conserv. 1-2: 45-49.
- KRZAKOWA M. & MIKULSKI W. 1997. Peroxidase as marker in pure lines of perennial ryegrass (*Lolium perenne* L.). In: Z. STASZEWSKI, W. MŁYŃCIEC & R. OSIŃSKI (eds.). Ecological aspects of breeding fodder crops and amenity grasses, pp. 322-326. Proceedings of 20th Meeting of EUCARPIA, Fodder and Amenity Grass Section, October 1996, Radzików, Poland.
- SHOW C. R. & PRASAD R. 1970. Starch gel electrophoresis of enzymes, a compilation of recipes. Bioch. Gen. 4: 297-320.
- STEBBINS G. L. 1958. Zmienność i ewolucja roślin. 252 pp. Wyd. Nauk. PWN, Warszawa.
- WYSOCKI A. 2002. Przemiany flory roślin naczyniowych Ogrodu Zoologicznego na Malcie w Poznaniu w latach 1987-2001. MSc Thesis, Department of Plant Taxonomy, Adam Mickiewicz University, Poznań.
- ŻUKOWSKI W. & JACKOWIAK B. 1995. List of endangered and threatened vascular plants in Western Pomerania and Wielkopolska (Great Poland). In: W. ŻUKOWSKI & B. JACKOWIAK (eds.). Endangered and threatened vascular plants of Western Pomerania and Wielkopolska. Publications of the Department of Plant Taxonomy of the Adam Mickiewicz University of Poznań 3: 9-96. Bogucki Wyd. Nauk., Poznań.