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# Intrapopulation variation of *Calamagrostis arundinacea* (L.) Roth revealed by electrophoretically detected ten enzyme systems

Maria Krzakowa[1*] & Zbigniew Celka[2]

[1]Department of Genetics, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, *e-mail: krzakowa@amu.edu.pl
[2]Department of Plant Taxonomy, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland

**Abstract.** One population of *Calamagrostis arundinacea* (L.) Roth was studied with ten electrophoretically separated, polymorphic (mean Pg = 0.54) enzyme systems: ACO, ADH, DIA, FDH, GDH, GOT, MDH, PGI, PGM, SKDH and PX. Each of 30 plants showed individual differences in respect of 16 loci. The average values of observed and expected heterozygosities were 0.48 and 0.46 respectively. Mean values of Wright's fixation index (F = -0.045) suggests that population remains in Hardy-Weinberg equilibrium.

**Key words:** *Calamagrostis arundinacea*, electrophoretically detected enzyme systems, population variability

## 1. Introduction

*Calamagrostis arundinacea* (Small-reeds; Reed Grass) is one of seven species of the genus *Calamagrostis*, and – like other amphimictics (*C. canescens*, *C. epigejos* and *C. varia*) – has an Eurasian range of distribution (Conert 1989; Colling 2005). In Poland, it occurs throughout the lowlands and up to the lower montane forest belt, usually on acid soil (Frey & Paszko 1999). This diploid (2n = 28) species is a tuft grass that does not propagate vegetatively and is a characteristic species for the associations *Calamagrostio arundinaceae-Quercetum* in the lowlands and *Bupleuro-Calamagrostietum arundinaceae* in the Karkonosze Mountains (Frey & Paszko 1999). Until now, Polish populations of Small-reeds have not been protected (Zarzycki & Szeląg 2006), although some species of *Calamagrostis* genus are listed in "Red Lists" (Zarzycki & Szeląg 2006; Paszko 2007). Earlier research on *C. arundinacea* (Krzakowa *et al*. 2005; Krzakowa & Dunajski 2007; Krzakowa & Celka 2008) concerned natural Mendelian populations. Each diploid biological species that reproduces sexually is regarded as a Mendelian population if its individuals can freely interbreed and have a common gene pool.

A population of *C. arundinacea* was 'restored' in greenhouse conditions, from seeds collected in the field and subjected to a complex analysis aimed to assess genetic differences between individual plants as well as the genetic structure of the population.

## 2. Material and methods

In acidophilous oak forest near the village of Kozia Wola (Barycz Forest District in the Świętokrzyskie province) mature panicles were collected randomly from 30 plants, similarly as it was practiced in earlier investigations on other grass species, e.g. *Phragmites australis* (Krzakowa 1996) and different species of the genus *Stipa* (Krzakowa *et al*. 2006). Seeds extracted from each panicle were sown and planted in greenhouse conditions, in trays filled with soil. The fresh leaves collected from individual seedlings were homogenized with a drop of double-distilled water (only for peroxidase) and with 0.12 M Tris-HCl pH 7.5 buffer for the other enzyme systems.

Electrophoresis was conducted in the following buffer systems: lithium – boric pH 8.1 (Ashton & Braden 1961), lithium-boric pH 8.3 and histidine-tris pH 7.0 (Wendel & Weeden 1989). Ten enzyme systems with reproducible patterns were analysed: ACO (aconitase EC 4.2.1.3), ADH (alcohol dehydrogenase, EC 1.1.1.1), DIA (diaphorase EC 1.6.4.3), FDH (formic

acid dehydrogenase, EC 1.2.1.1), GDH (glutamate dehydrogenase, EC 1.4.1.2), GOT (glutamate oxalacetate transaminase EC 2.6.1.1), MDH (malate dehydrogenase, EC 1.1.1.37), PGI (phosphoglucose isomerase, EC 5.3.1.9) PGM (phosphoglucomutase, EC 2.7.5.1), SKDH (shikimate dehydrogenase, EC 1.1.1.25), and PX (peroxidase, EC 1.11.1.7).

Extracts were absorbed into Whatman chromatography 3MM paper wicks (4 mm × 5 mm) and electrophoresis was conducted in 11% starch gel (Sigma) at a constant voltage of 150V. The staining procedure closely followed that described by Lehmann (1997). Isozyme assays and corresponding electrophoretic buffers were identified that visualize gene products of single loci i.e., allozymes. The bands belonging to the same zone, having a typical homo- or heteromorphic allozymes composition were considered as belonging to the same locus. Mobility classes were arbitrarily designated for example as S and W for SDH: S1 and W1 migrating the farthest towards the anode from the start. Calculations of genetic parameters, such as observed heterozygosity (Ho), expected heterozygosity (He), the inbreeding coefficient F computed after Wright (1965) and genetic polymorphism index Pg (Kahler *et al*. 1980) enabled us to illustrate intrapopulation variation. Mean number of alleles per locus (A/L) and genotypes per locus (G/L) were also calculated. The results were subjected to the PCA statistics (Principal Components Analysis – STATISTICA 7.1), and dendrogram was constructed using unweighted pair groups method – UPGMA (Sneath & Sokal 1973).

## 3. Results and discussion

The population was characterized in respect of 16 loci and 42 allozymes (Fig. 1). Apart from peroxidase, for which the differences in frequency of genotypes have been analysed before in natural populations of *C. arundinacea* (Krzakowa *et al*. 2005; Krzakowa & Dunajski 2007; Krzakowa & Celka 2008), loci of other enzymes are described in this work for the first time.

*Aconitase* (ACO) is coded by two loci with two alleles. Band pattern of heterozygotes indicates that both loci are monomeric.

*Alcohol dehydrogenase* (ADH) is controlled by one locus coding for three allozymes which in heterozygotic stage show three-banded, i.e. dimeric band patterns.

*Diaphorase* (DIA) locus F is composed of two alleles which in heterozygotic stage configuration exhibits two-banded phenotype, therefore showing its monomeric behaviour.

*Formic acid dehydrogenase* (FDH). This enzyme system is controlled by one locus coding for three allozymes.

*Glutamate dehydrogenase* (GDH) is coded in *C. arundinacea* by one gene with three alleles.

*Glutamate oxalacetate transaminase* (GOT) shows two zones of activity. The faster migrating product of locus T is monomorfic, the slower migrating product of locus is controlled by a single gene G (with two alleles), that codes for a functionally dimeric protein.

*Malate dehydrogenase* (MDH) displayed three zones of activity with two alleles detected in each locus. Existence of heterozygotes composed of two bands in locus M allow us to suggest its monomeric character. Triple-banded heterozygotes of the two other loci indicate their dimeric subunit structure.

*Phosphoglucose isomerase* (PGI) has two loci. The faster migrating product of locus P was polymorphic. Heterozygotes show band patterns consistent with a dimeric subunit structure.

*Phosphoglucomutase* (PGM). Plants with one band of activity were recognized as homozygotes while others, with two-banded phenotypes, as heterozygotes.

*Peroxidases* (PX) are electrophoretically migrating in two directions: anodally migrating locus P and migrating cathodally of two loci: A (with four alleles) and C (with two alleles). Peroxidase in *C. arundinacea*, similarly to other plant species, is generally monomeric, however, dimeric peroxidase has been described for *Oryza sativa* (Shai *et al*. 1969) and *Phragmites australis* (Krzakowa 1996).

*Shikimate dehydrogenase* (SKDH) band patterns indicate the presence of two loci, each coding for three allozymes. Heterozygotes are double-banded indicating that SKDH is functionally monomeric in *C. arundinacea* as has been also observed in other plant species.

The population is polymorphic (Table 1) in respect of all enzyme systems. Individual plant heterozygosities ranged from 0.17 to 0.93 with the mean of 0.48.

**Table 1.** Genetic parameters for *Calamagrostis arundinacea* population. He – expected heterozygosity, Ho – observed heterozygosity, F – Wright's fixation index, Pg – polymorphism coefficient

| Locus | He | Ho | F | Pg |
|---|---|---|---|---|
| ACO C | 0.3394 | 0.2333 | 0.3126 | 0.4911 |
| ACO O | 0.4550 | 0.5000 | -0.0989 | 0.5800 |
| ADH L | 0.6394 | 0.4667 | 0.2702 | 0.7867 |
| DIA K | 0.4978 | 0.6667 | -0.3393 | 0.4978 |
| FDH F | 0.5950 | 0.9000 | -0.5126 | 0.4400 |
| GDH N | 0.6294 | 0.6333 | -0.0062 | 0.7356 |
| GOT G | 0.2061 | 0.1667 | 0.1914 | 0.3311 |
| MDH H | 0.1528 | 0.1667 | -0.0909 | 0.2778 |
| MDH I | 0.1528 | 0.1667 | -0.0909 | 0.2778 |
| MDH M | 0.4950 | 0.7667 | -0.5488 | 0.3800 |
| PGI P | 0.6228 | 0.9333 | -0.4987 | 0.6244 |
| PGM B | 0.6250 | 0.7667 | -0.2267 | 0.7044 |
| PX A | 0.4861 | 0.3333 | 0.3143 | 0.6622 |
| PX C | 0.1528 | 0.0333 | 0.7818 | 0.1844 |
| PX D | 0.5928 | 0.5333 | 0.1003 | 0.7467 |
| SHDH S | 0.5950 | 0.4333 | 0.2717 | 0.7667 |
| SHDH W | 0.5128 | 0.4000 | 0.2199 | 0.6578 |
| Mean values | 0.4559 | 0.4765 | -0.0452 | 0.5379 |

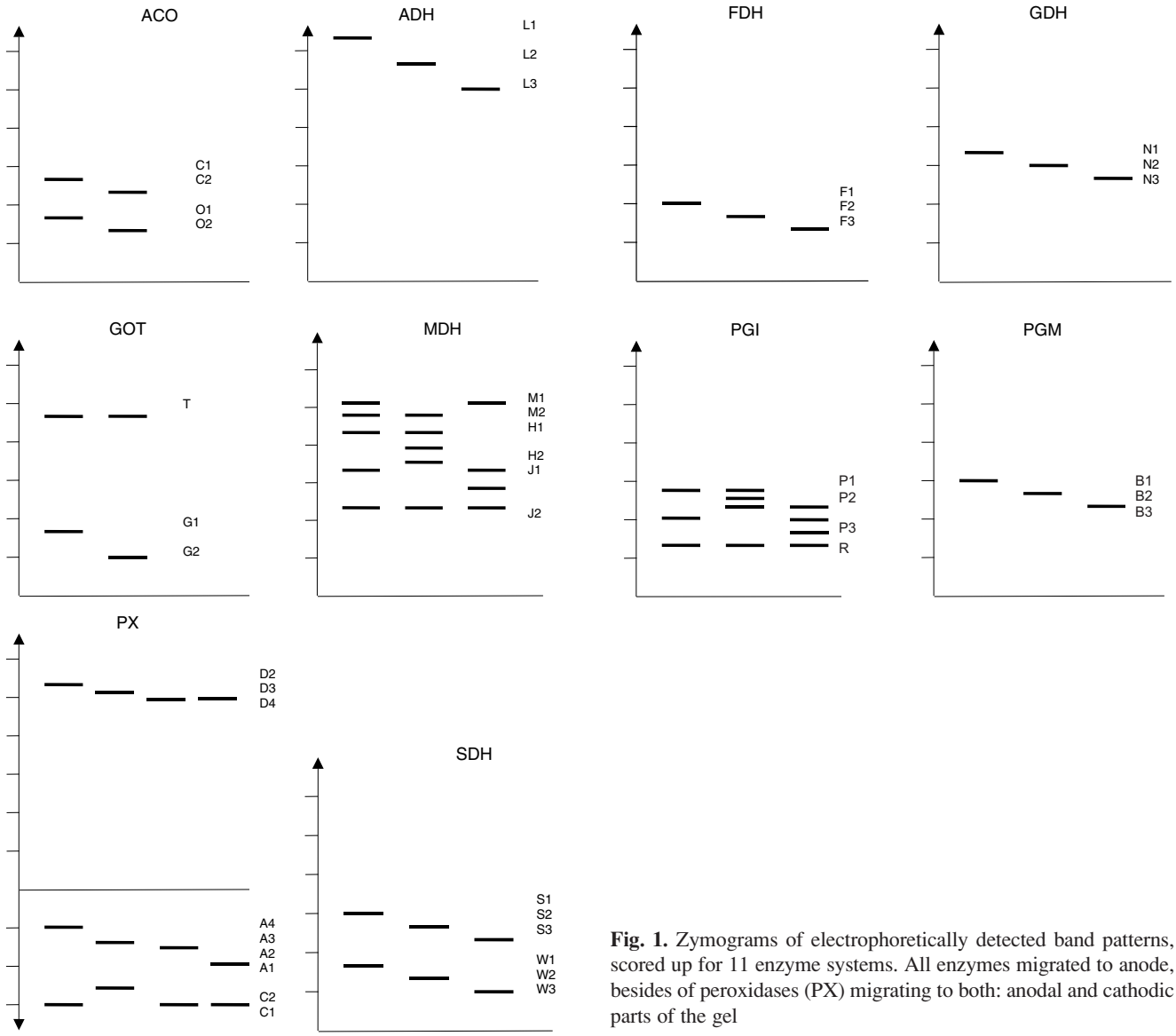


**Fig. 1.** Zymograms of electrophoretically detected band patterns, scored up for 11 enzyme systems. All enzymes migrated to anode, besides of peroxidases (PX) migrating to both: anodal and cathodic parts of the gel

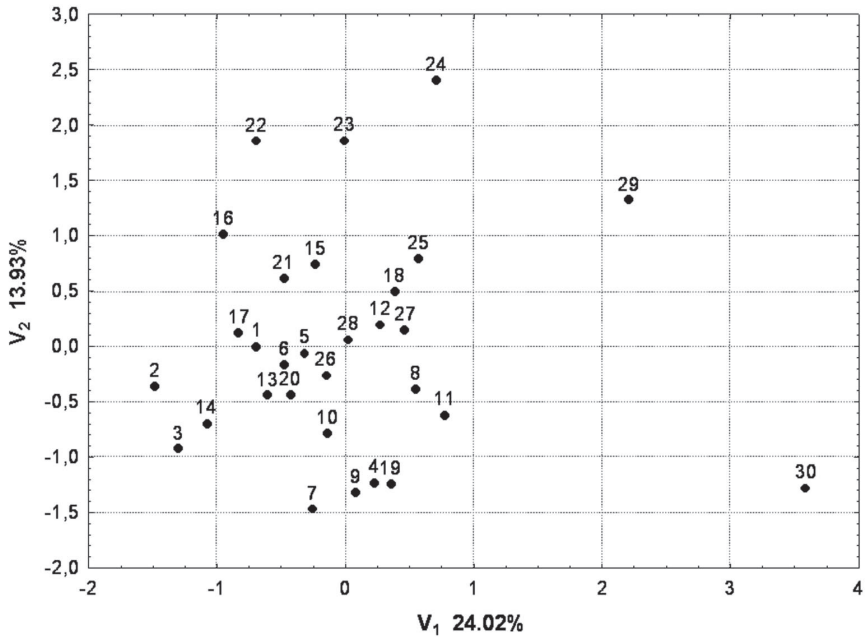


**Fig. 2.** Distribution of individuals on the plane of the two principal component axes (V1 and V2)

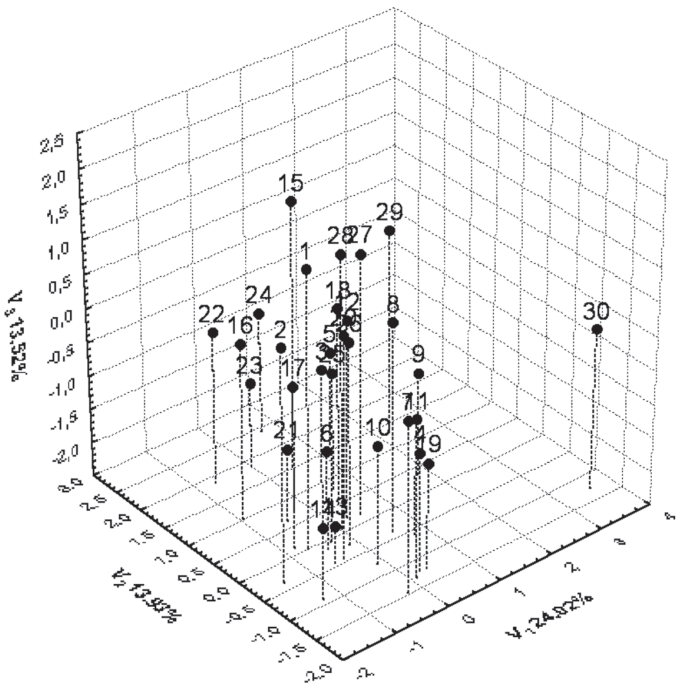

**Fig. 3.** The investigated plants situated in the space of the three principal component axes V1, V2 and V3 (51.27% of information)

Polymorphic coefficient values (Pg) range from 0.18 for PXC to 0.79 for ADH. The population is in Hardy-Weinberg equilibrium, since most of F values are close to zero, with some tendency to high level of heterozygosity, while stabilized homozygosity is observed in the nearly monomorphic peroxidase locus C (F = 0.78). Completely monomorphic locus T in GOT and locus R in PGI were excluded from comparisons. All together, alleles were recognized and the average number of alleles per locus was 2.71. When the observed proportions of heterozygotes were compared with those expected (He) from a population in Hardy-Weinberg equilibrium slight differences were detected and no difference has been found for GDH (chi-square=0).

The distribution of individuals on the plane of the two first Principal Components (Fig. 2) shows that there are differences between individual plants. Most plants form a generally compact group, and only individual no. 30, having rare GOT and MDH alleles, is clearly distinct from the others. Similarly, in Fig. 3, which illustrates the distribution of plants in the space of the three Principal Components, the distinctness of individual no. 30 is very conspicuous.

The dendrogram constructed on the basis of agglomerative clustering by the method of closest neighbourhood UPGMA (Sneath & Sokal 1973) shows the existence of small groups of individuals within the population, and the most separate genotypes are those of nos. 25 and 30 (Fig. 4).

We can compare these results only with our earlier investigation of peroxidase variability in *C. arundinacea* populations, since no other enzyme systems were included. Generally, the whole population shows the high level of heterozygosity, what can be an advantageous feature for the species, as there is an opinion that highly heterozygous organisms are able to cope better with fluctuating environmental conditions (Ellstrand & Elm 1993).

Nevertheless, in some populations, allelic frequency can undergo occasional fluctuations in next generations leading to fixation or loss of some alleles. Since sexual reproduction of *C. arundinacea* is abundant and some

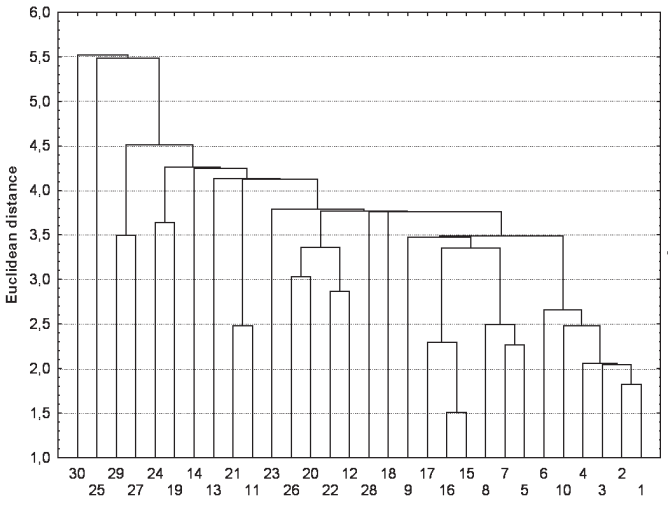


**Fig. 4.** Unweighted pair groups method (UPGMA) dendrogram based on Euclidean distances, calculated for all enzyme systems genotypes shows some genetic differences between individual plants in the population

gene flow among populations is observed (Krzakowa *et al*. 2005), it can exert higher effect on changes, if any, in population variability level.

It cannot be excluded that enzyme polymorphism can be similar for closely related species of *Calamagrostis*. Therefore, further population studies on variability within the genus may also become very useful for description of inter-specific relation, including hybridization.

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