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The variability of common reed *Phragmites australis* (Cav.) Trin. *ex* Steudel populations growing in urban conditions

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Abstract. Differences between 13 common reed (*Phragmites australis*) populations, growing in urban conditions within the town of Poznań (western Poland), are described by 8 morphological traits of panicles' variability and the frequency of peroxidase (dimeric and monomeric) allozymes. Values of morphological characters were processed statistically using agglomerative clustering by the closest neighbours (UPGMA) method based on Euclidean distances. Proteins were separated in the starch gel electrophoretic procedure, showing cathodic migration. Populations are polymorphic and have a certain level of heterozygosity. The level of populations' diversity (DST = 0.097) is lower than the intra-population variability (GST = 0.187). The gene flow between populations is rather low (Nm = 1.090).

Key words: *Phragmites australis*, genetic variability, peroxidases, allozymes.

1. Introduction

The decay of common reed in natural water reservoirs of Europe, observed in the last decade, with parallel signs of the species expansion in other parts of the world, has brought about intensive investigations of the species (Van der Putten 1997; Clevering 1999).

Common reed is a variable species and has always been attracting the botanists' interest. The latter ones, aiming at introducing an order to the observed differences in the morphology and growth forms provided varieties with a taxonomic rank, e.g. distinguishing *Ph. australis* var. *stolonifera*, *Ph. australis* var. *rivularis*, *Ph. australis* var. *favescens* (2n = 48) and *Ph. australis* var. *gigantissima* (2n = 96), even if no direct relations have been detected between conditions of biotypes and the ploidy level. Common reed can grow in extremely variable biotype conditions and different osmotic pressures, also on soils with a significant salt content (Strogonov et al. 1970; Hellings & Gallagher 1992; Lisner & Schierup 1997).

The interest in common reed caused that studies have been undertaken not only on the morphology (Kraska et al. 1986; Kraska & Bobowicz 1987; Kühl et al. 1999; Krzakowa et al. 2003) but also on physiology (Armstrong & Armstrong 1988; Armstrong et al. 1996; Gessner et al. 1996; Kühl & Kohl 1993; Chambers et al. 1998; Cizkova & Bauer 1998; Ye et al. 1998; Grunfeld & Brix 1999; Klimes et al. 1999), genetics (Bahrman & Gorenflot 1983; Hauber et al. 1991; Kühl & Neuhans 1993; Neuhans et al. 1993; Krzakowa 1996; Kühl et al. 1999; Pellegrin & Hauber 1999; Krzakowa & Drapikowska 2000; Drapikowska & Krzakowa 2008) and on ecology (Zeidler et al. 1994; Clevering 1999; Kühl 1999) of the species.

Despite the constant hazard to biotype conditions, which change under the influence of anthropopressure, common reed grows within the limits of the Poznań town in as many as 109 biotypes (Jackowiak 1993). The extensive representation of common reed in the town is supported by its favourable geographic location, within the macroregion of the Wielkopolskie Lakeland (Jackowiak

1990). The region includes lowlands at the meander of the Warta River and the junction of the Warta–Odra Valley and the Toruń–Eberswalde Valley. The area of the town encompasses three lakes: Lake Kierskie (309.2 ha), Lake Strzeszyńskie (32 ha), Lake Malta (70 ha) and some small water reservoirs.

Common reed is generally thought to spread mainly by vegetative propagation (Kühl et al. 1999). However, since common reed also reveals a significant ability to reproduce generatively (Krzakowa & Drapikowska 2000), we decided to examine selected populations growing in urban conditions, in order to determine their genetic variability.

2. Material and methods

There are strong indications that common reed populations might be highly variable in respect of the clonal structure, therefore care was taken to ensure that the populations sampled were not connected with the water current. Mature panicles were collected in wintertime since the seed requires freezing and the ice table provided easy collection of randomly sampled panicles (30 from each population). The populations included those in: 1 – Lake Malta, 2 – the Ceglanka River, 3 – a water reservoir near Glinianka Str., 4 – the Młyński Pond, 5 – Lake Rudnicze, 6 – a pond by Hubalczyków Str., 7 – a water patch near Świątniczki Str., 8 – Łęgi Dębińskie, 9 – Różany Młyn, 10 – a water patch by Wołczyńska Str., 11 – Lake Strzeszyńskie, 12 – Lake Rusałka, 13 – Lake Kierskie. Each population was characterised according to eight panicle traits. The results of measurements were computed (using Statistica 7.1) for trait characteristics, multivariate analysis of variance and principal component analysis.

For description of genetic variability, seeds from individual panicles were sown in identical glasshouse conditions; and when the seedlings reached the stage of three leaves (after approximately 6 weeks), they were subject to electrophoretic analyses. Peroxidases were stained in the cathode side of gel (Krzakowa 1996).

For each locus, in every population Ho and He, e.i. the observed and expected heterozygosities were defined. Two indices were calculated over polymorphic loci: the inbreeding coefficient (F) following Wright (1965) and the polymorphic index (Pg) according to Kahler et al. (1980). The gene flow, expressed as the average number of immigrants per generation (Nm), was estimated based on the overall allelic differentiation among populations.

Genetic differences between populations were described by peroxidase allozyme (Gregorius 1978) and genotype frequencies (Hedrick 1971).

3. Results and discussion

Trait characteristics (Tab. 1) show that the measures of panicles are very variable in all characters. The correlation coefficient calculated for pairs of all traits (Tab. 2) indicate that the highest correlation was observed between the first two characters. The inter-population variability, based on panicle traits, was reflected by the scatter diagram (Fig.1) of the studied populations on the plane of the two Principal Component axes (in total 80.94% of the information). Populations are divided into three groups; the most dissimilar is population 10. This separateness is mainly caused by the first trait, which in this population attains the highest values in comparison to other populations. Similarly, when relations between populations were characterised in the three-dimensional (Fig. 2) discrimination space (in total 84.99% of the information), population 10 is again very distant and populations 2 and 7, as well as populations 6 and 4 occupy nearer sites. Graphical presentation (Fig. 3) of populations' positions after the UPGMA procedure, based on Euclidean distances, shows tendency for clustering the populations into two main groups and population 10 is visibly different. It is probably caused by complex phenotypic traits, as this population is characterised the highest stalks and the largest panicles in comparison with other stands.

The electrophoretically separated allozymes migrated to the cathodal part of gel and pointed to the existence of three loci. Two of them (*A* and *C*) proved to be polymorphic, while the third locus *B* was monomorphic (and was excluded from comparisons) in all populations. The band patterns in locus *A* confirmed its dimeric behaviour (Krzakowa 1996). The allozyme band patterns in locus *C* indicate its monomeric character, as usual in the case of peroxidases.

Populations differed in allele frequencies in each locus (Fig. 4). They permitted to construct a dendrite (Fig. 5), which clearly illustrates that the populations are connected by similar distances.

Frequencies of genotypes in investigated populations are illustrated by Figure 6. Populations are significantly polymorphic (Tab. 3). Population 10 was the most polymorphic in respect of locus *A*, while populations 2 and 12 show the highest values of Pg indices in locus *C*. Low Ho values caused that some populations reached high fixation index *F*, for example in locus *A* for population 1 ($F = 0.82$), as well as in locus *C* for population 2 ($F = 0.94$) and 7 ($F = 0.86$), suggesting a high level of inbreeding. Two monomorphic populations in respect of locus *C* (populations 4 and 10) showed *F* coefficient values = 1.00.

When the populations were compared with each other based on genotype frequencies (Fig. 7), five populations clustered together, while the remaining 8 populations formed two sub-groups, each of them consisting of four elements.

Table 1. Characteristics for 8 panicle traits of *Phragmites australis*

Traits	x	SD	CV%	x_{min}	x_{max}
Panicle length	26.48	5.40	29.17	10.50	44.00
Panicle spread	22.80	5.67	32.19	9.50	46.00
The number of lateral ramifications	20.06	7.20	51.84	3.00	48.00
The number of top ramifications	6.57	3.67	13.51	2.00	20.00
The total number of whorls	8.42	2.83	8.06	3.00	17.00
The number of lateral branches in the first whorl	5.91	1.77	3.15	2.00	16.00
The number of lateral branches in the second whorl	4.91	1.71	2.93	2.00	12.00
The number of lateral branches in the third whorl	3.86	1.54	2.37	2.00	8.00

Table 2. Correlation coefficients for 1–8 traits of the reed (*Phragmites australis*): x – arithmetic means, SD – standard deviations, CV – variability coefficient, x_{min} – minimum and x_{max} – maximum values respectively, at $\alpha = 0.05$ level of significant correlation (**)

	2	3	4	5	6	7	8
1	0.80 **	0.41 **	−0.08	0.40 **	0.37 **	0.36 **	0.37 **
2		0.34 **	−0.18 **	0.40 **	0.40 **	0.39 **	0.41 **
3			−0.31 **	0.00	0.25 **	0.16 **	0.15 **
4				−0.38 **	−0.29 **	−0.20 **	−0.23 **
5					0.29 **	0.36 **	0.46 **
6						0.66 **	0.52 **
7							0.71 **

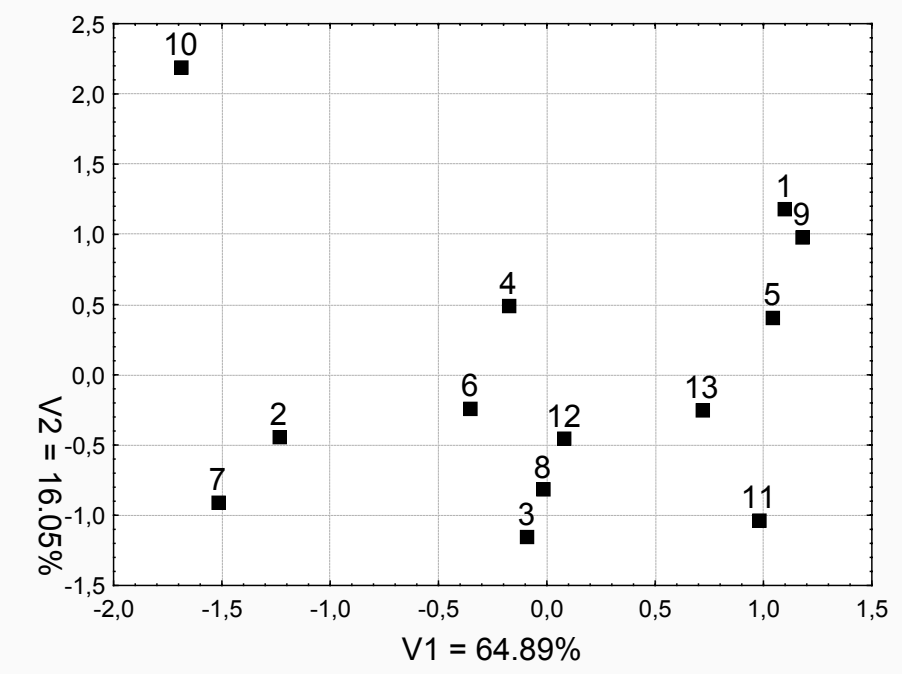


Figure 1. Scatter diagram for 13 reed populations on the plane of the two first principal components axes

Table 3. Parameters of genetic variability coefficients of 13 reed populations: He – expected heterozygosity, Ho – observed heterozygosity, F – inbreeding coefficient, Pg – percentage of polymorphic loci

Locus	Populations	He	Ho	F	Pg
PXA	01	0.3343	0.0606	0.8187	0.3893
PXA	02	0.4352	0.5600	−0.2868	0.5248
PXA	03	0.4728	0.1667	0.6475	0.5978
PXA	04	0.4978	0.5333	−0.0714	0.6044
PXA	05	0.4933	0.6538	−0.3253	0.5059
PXA	06	0.4644	0.4000	0.1388	0.6244
PXA	07	0.4638	0.2692	0.4195	0.6243
PXA	08	0.4328	0.3667	0.1528	0.5978
PXA	09	0.4950	0.6000	−0.2121	0.5550
PXA	10	0.5000	0.4400	0.1200	0.6496
PXA	11	0.3848	0.1200	0.6881	0.4832
PXA	12	0.4352	0.2400	0.4485	0.5888
PXA	13	0.4712	0.4400	0.0662	0.6208
PXC	01	0.4642	0.1818	0.6083	0.5859
PXC	02	0.6584	0.0400	0.9392	0.6816
PXC	03	0.0950	0.0333	0.6491	0.1267
PXC	04	0.2778	0.0000	1.0000	0.2778
PXC	05	0.3913	0.1154	0.7051	0.4822
PXC	07	0.5658	0.0769	0.8641	0.6213
PXC	08	0.6528	0.2667	0.5915	0.7822
PXC	09	0.5237	0.2500	0.5227	0.6400
PXC	10	0.2112	0.0000	1.0000	0.2112
PXC	11	0.5976	0.2000	0.6653	0.7104
PXC	12	0.4856	0.5200	−0.0708	0.6720
PXC	13	0.3648	0.4000	−0.0965	0.5248
PXC	06	0.0000	0.0000		0.00000”

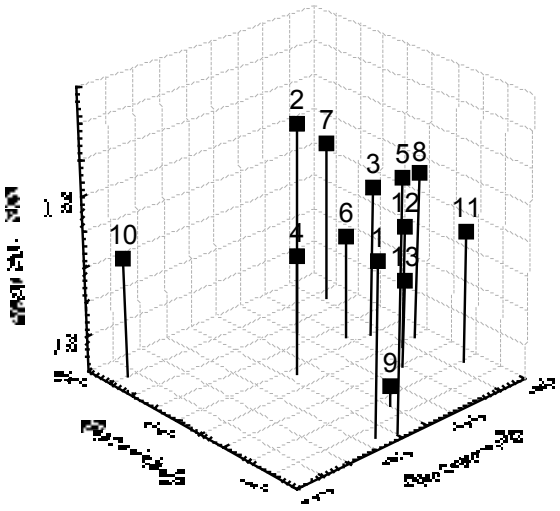


Figure 2. Results of discriminance analysis for 13 populations in the space of the first three discriminant variables: U1, U2, U3 containing 84.99% information of the applied set of eight panicle traits

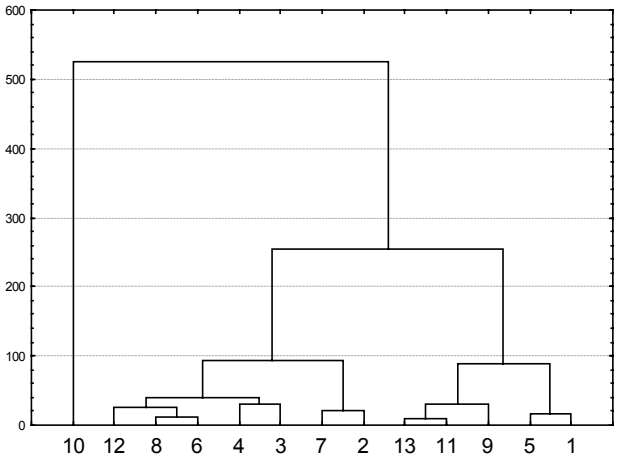


Figure 3. UPGMA clustering of 13 populations of *Phragmites australis* based on Euclidean distances

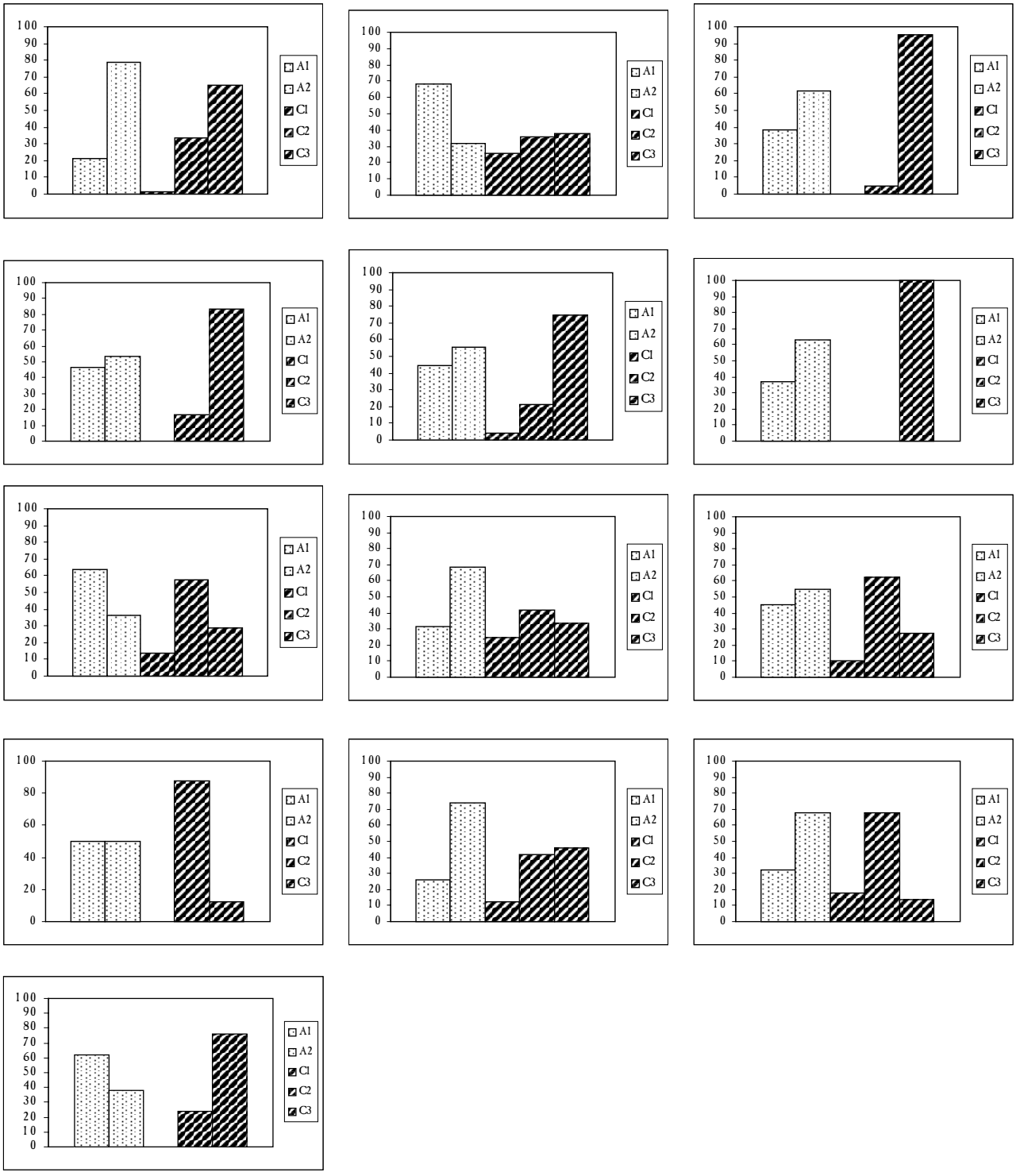


Figure 4. Histograms of allozymes frequency in 13 populations investigated

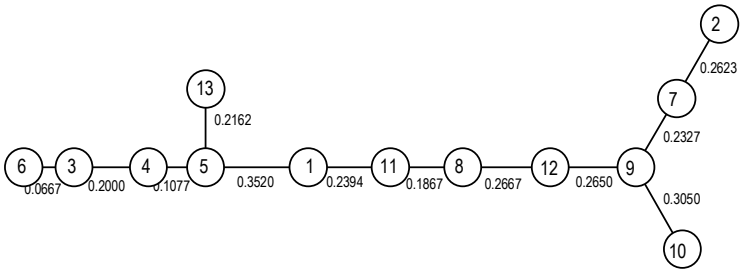


Figure 5. Minimum spanning tree for 13 examined populations of *Phragmites australis* constructed on the basis of genetic distances after Gregorius (1978)

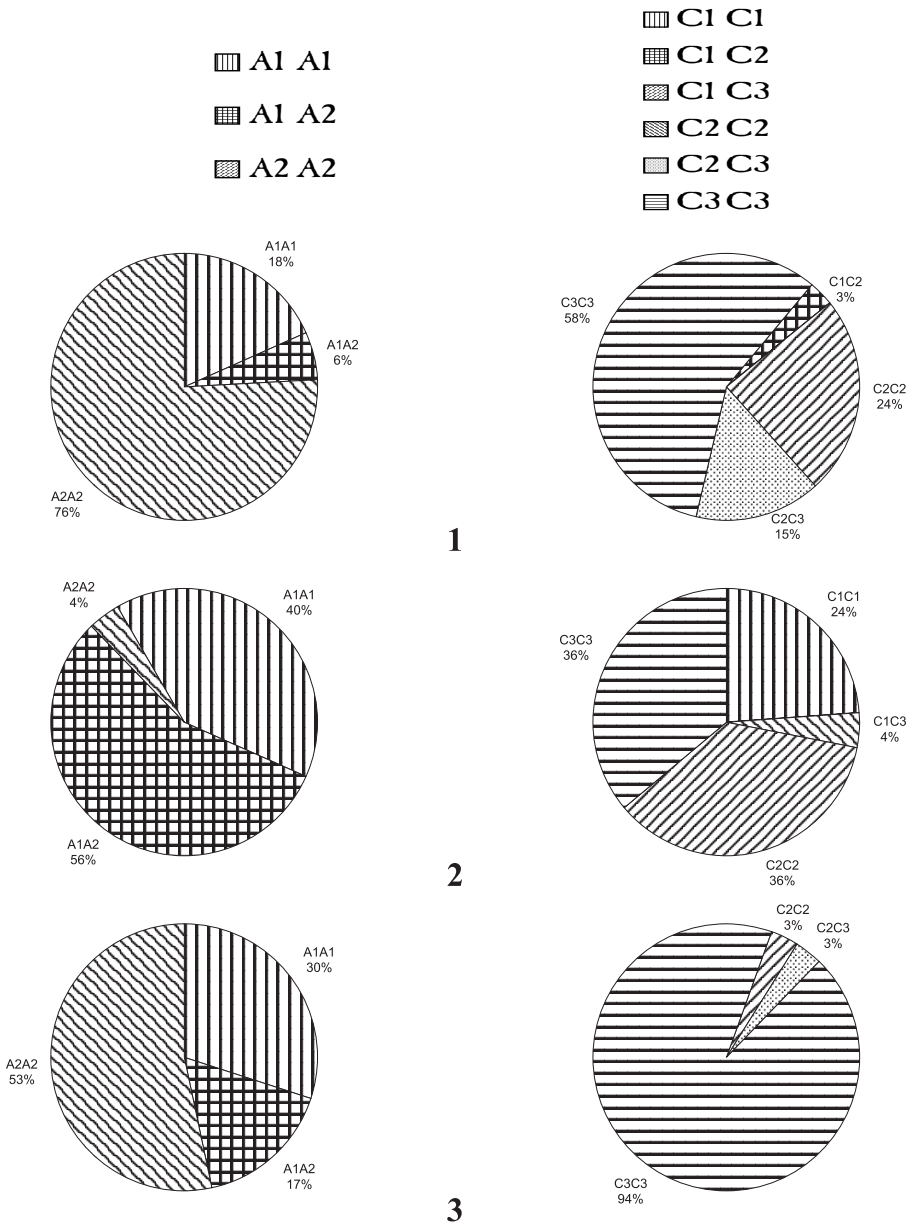
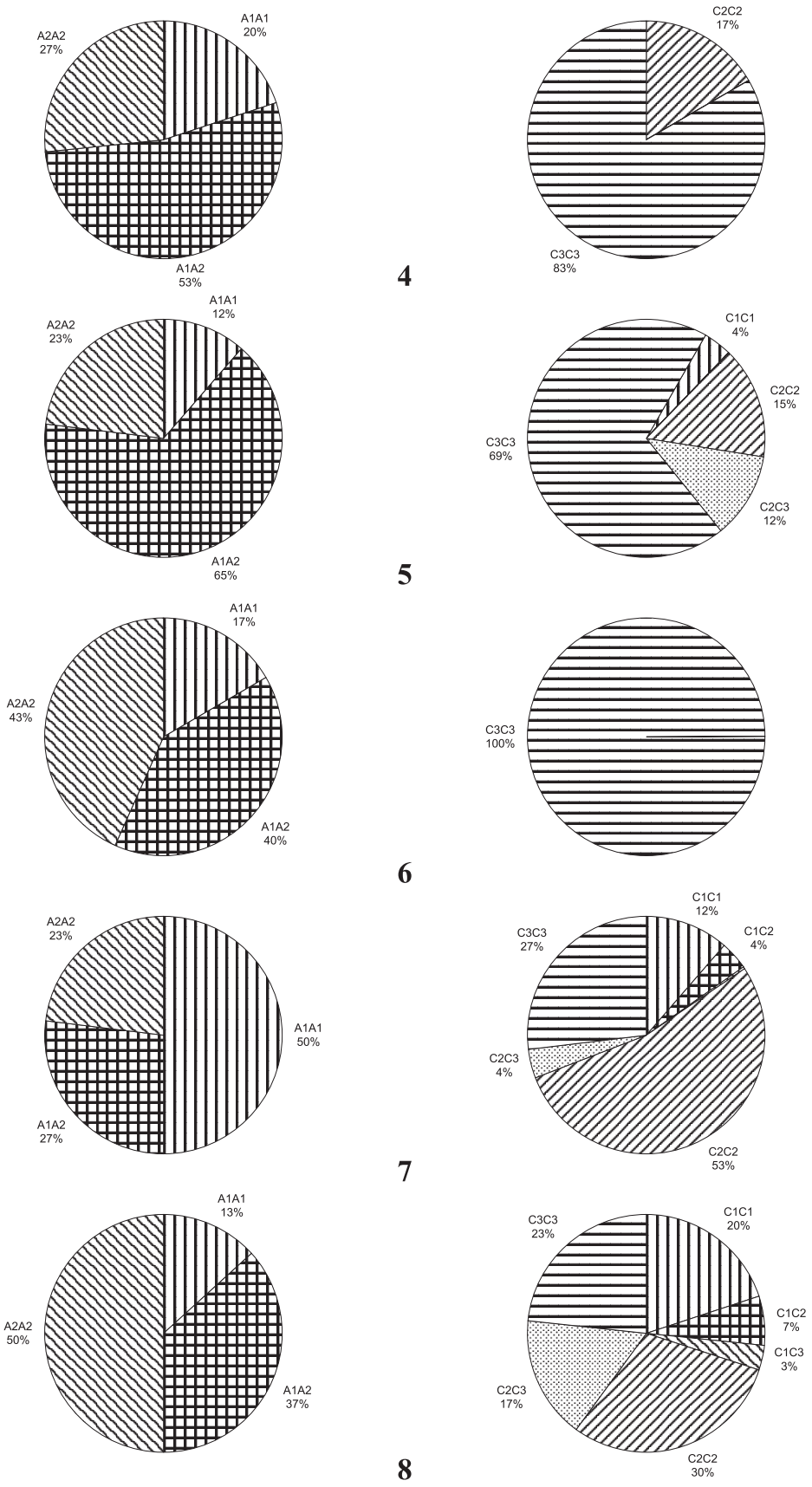


Figure 6. Diagrams of genotypes frequency in 13 populations of *Phragmites australis*



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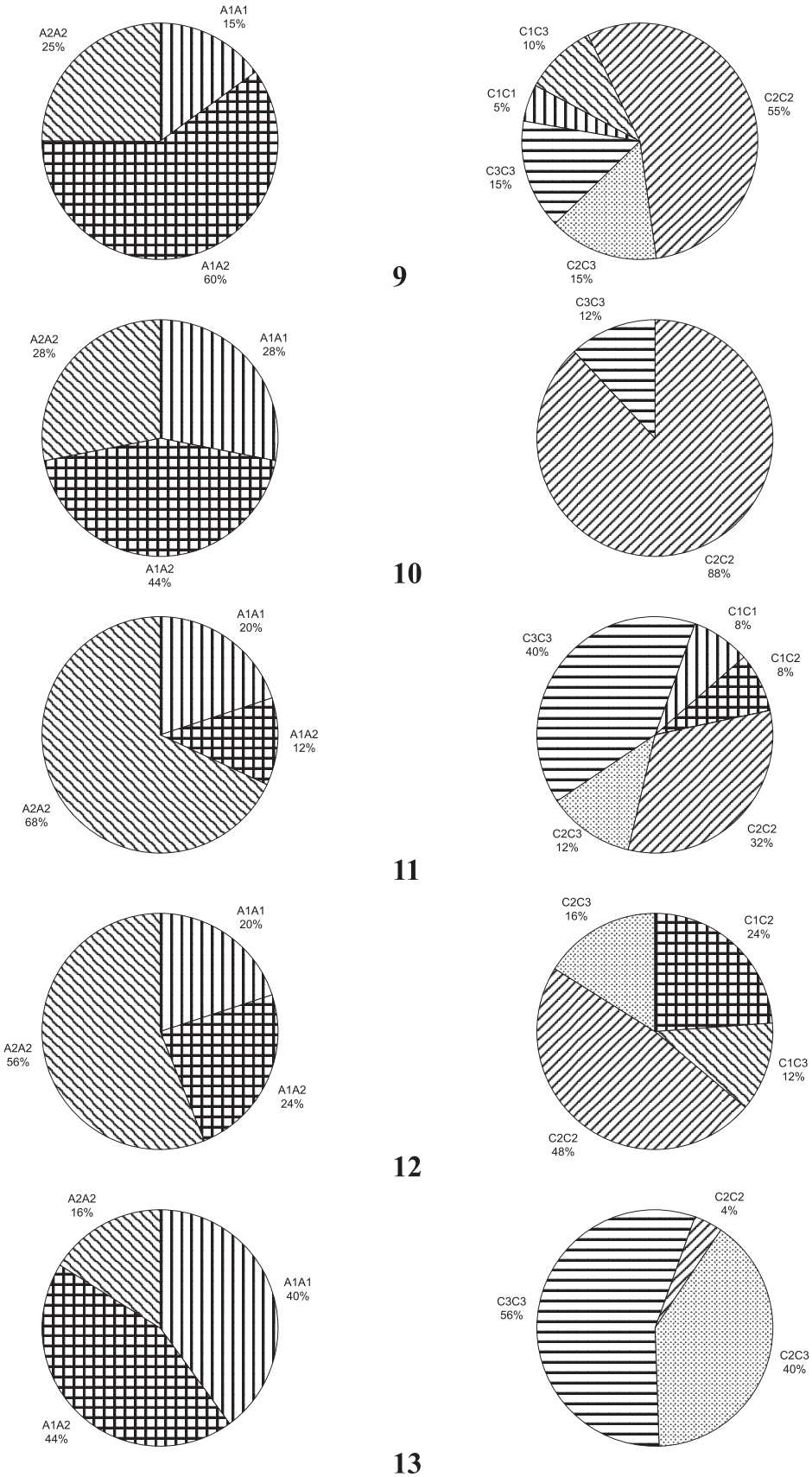

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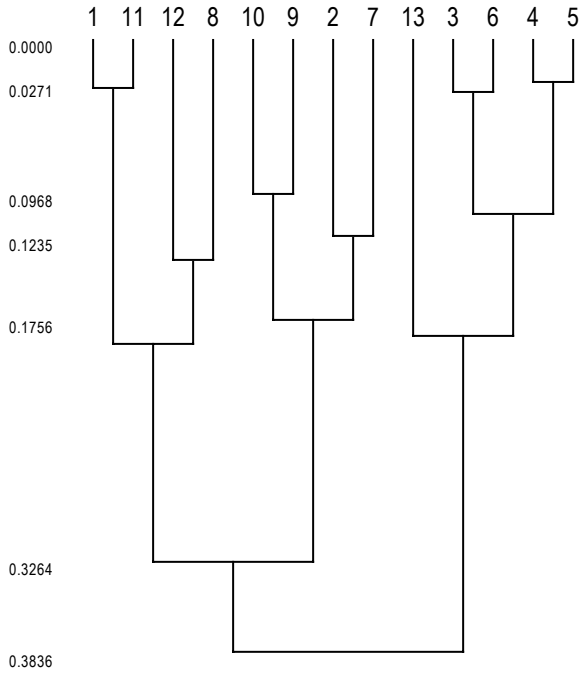

Figure 7. UPGMA cluster analysis of Hedrick's distances based on genotypes frequency

The inter-population differences pointed out a significant effect of generative reproduction on the population genetic structure. The level of diversity among populations (mean DST = 0.0973) is lower as compared to intra-population variability (GST = 0.1866). It is also interesting that the gene flow between populations was relatively low (Nm = 1.090). In such situation, when the gene flow is limited, adaptive divergence to different selective environments may occur (Schluter 2000). It might concern common reed populations growing in different environments and distributed across an ecological gradient.

Some populations, which demonstrate a low polymorphism level, are probably composed of few clones, which in the case of small water reservoirs are exchanging alleles between the same genotypes quite frequently and what more, might simulate self-fertilization in this way. It can also be explained by the "founder effect".

Dimeric peroxidases are rare in the plant kingdom. One of such interesting cases involves dimeric peroxidases in *Oryza sativa* (Shahi et al. 1969; Pai et al. 1973) and *Phragmites australis* (Krzakowa 1996). Since both species grow in similar aquatic conditions, it is very possible that this trait carries some similar physiological importance of a common, unrecognised origin.

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