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Isoenzyme structure of *Festuca nigrescens (Gramineae)* from the Boatin National Reserve, Bulgaria

Abstract

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The isoenzyme structure of the *Festuca nigrescens* populations of the Boatin National Reserve was examined for esterase (11 isoforms), peroxidase (8 isoforms), and acid phosphatase (10 isoforms), and the results compared with average values from 6 other Bulgarian populations, previously studied. In spite of its vicinity to a copper refinery, the Boatin population has an isoenzyme structure that is stable over time and does not differ significantly from the Bulgarian average.

Introduction

The Boatin National Reserve is an experimental site in the Bulgarian system of background monitoring. *Festuca nigrescens* Lam. is one of the species used for biomonitoring. Bulgarian populations of *F. nigrescens* had been studied with regard to 13 isoenzyme systems, and their isoenzyme structure was revealed (Angelov 1986). The aim of the present study was to analyse the isoenzyme structure of the Boatin population, in its temporal changes, and compare it with the average isoenzyme structure which *F. nigrescens* presents in its Bulgarian populations.

Material and methods

A set of 50 plants of *Festuca nigrescens* from the Boatin population was studied for three consecutive years (B1, B2, B3). A set of 216 plants belonging to 6 other populations (P6) had been analysed in a previous study (Angelov 1986) to reveal the average isoenzyme structure of *F. nigrescens*. The isoforms of esterase (EST), peroxidase (PER), and acid phosphatase (ACPH) were electrophoretically resolved. Details of the experimental procedures are described elsewhere (Angelov 1986, 1992). Each isoform was given a numerical symbol reflecting its gel migration in mm (Shumaker & Babble 1980). The frequency of each isoform in a given population was calculated as the percentage of

Table 1. Isoenzyme structure of anodal esterase (EST) in *Festuca nigrescens*. – B1-3: Boatin population in 3 consecutive years; P6: average of 6 other Bulgarian populations.

Isoforms:	14	16	18	20	22	24	32	35	37	40	44
B1	0.04	0.30	0.48	0.90	1.00	0.10	1.00	1.00	1.00	1.00	1.00
B2	0.02	0.24	0.54	0.96	1.00	0.08	1.00	1.00	1.00	1.00	1.00
B3	0.10	0.20	0.50	0.86	0.90	0.20	1.00	1.00	1.00	1.00	1.00
P6	0.08	0.26	0.58	1.00	1.00	0.12	1.00	1.00	1.00	1.00	1.00

Table 2. Isoenzyme structure of cathodal peroxidase (PER) in *Festuca nigrescens*. – B1-3: Boatin population in 3 consecutive years; P6: average of 6 other Bulgarian populations.

Isoforms:	18	23	26	30	33	37	39	42
B1	1.00	1.00	1.00	1.00	1.00	0.66	0.74	0.62
B2	1.00	1.00	0.96	0.90	1.00	0.56	0.50	0.74
B3	1.00	1.00	1.00	1.00	1.00	0.88	0.72	0.52
P6	1.00	1.00	1.00	1.00	1.00	0.79	0.87	0.77

Table 3. Isoenzyme structure of acid phosphatase (ACPH) in *Festuca nigrescens*. – B1-3: Boatin population in 3 consecutive years; P6: average of 6 other Bulgarian populations.

Isoforms:	7	11	14	28	39	41	43	44	48	51
B1	1.00	1.00	1.00	1.00	1.00	1.00	0.30	0.30	0.46	0.10
B2	1.00	1.00	1.00	0.96	0.82	1.00	0.18	0.34	0.36	0.04
B3	1.00	1.00	1.00	0.80	1.00	1.00	0.16	0.42	0.50	0.08
P6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.37	0.42	0.06

plants possessing it. Pairwise comparisons between B1, B2, B3, and P6 were made and the differences were evaluated by the Wilcoxon signed-rank test (Hollander & Wolfe 1973) at a level of significance (P) of 0.05.

Results and Discussion

In total, 11 isoforms of anodal EST were resolved (Table 1). Isoforms 32, 35, 37, 40, and 44 were monomorphically fixed. Regarding isoforms 14, 16, 18, 20, and 24, there

were some frequency fluctuations, but the pairwise comparisons showed statistically insignificant differences between the isoenzyme structure of the Boatin population and the average isoenzyme structure of *Festuca nigrescens*.

In total, 8 isoforms of cathodal PER were found in *Festuca nigrescens* (Table 2). Isoforms 18, 23, 26, 30, and 33 were monomorphically fixed, except for isoforms 26 and 30 in B2. The frequency of isoforms 37, 39, and 42 varied, but these variations were statistically insignificant at $P < 0.05$. As a whole, the isoenzyme structure of the Boatin population did not differ from the average isoenzyme structure of *Festuca nigrescens* in Bulgaria.

The isoenzyme structure with respect to ACPH is presented in Table 3. In total, 10 isoforms were resolved. Some of them – isoforms 7, 11, 14, and 41 – were monomorphic. The frequency of isoforms 28, 39, 43, 48, and 51 fluctuated within the Boatin population, but the statistical test applied showed no significant differences at $P < 0.05$ between the average isoenzyme structure of the Boatin population and the average isoenzyme structure of *Festuca nigrescens* in Bulgaria.

In summary, it is evident that the isoenzyme structure of the Boatin population is stable, as indicated by the insignificant differences between B1, B2, and B3, and does not differ statistically from the average isoenzyme structure found for the Bulgarian populations of *Festuca nigrescens*. It is worth mentioning that the Boatin National Reserve is comparatively close (15 km) to the copper refinery of Srednogorie. In a previous study (Angelov 1993) it was shown that populations of *Dichanthium ischaemum* (L.) Roberty and *Chrysopogon gryllus* (L.) Trin. growing close (1-5 km) to the Srednogorie refinery were influenced by industrial pollution, as revealed by isoenzyme markers EST, cathodal PER and GOT. Applying isoenzyme markers, many authors (Mejnartowicz 1983, Scholz & Bergmann 1984, Bergmann & Scholz 1987, Geburek & al. 1987) demonstrated that industrial pollution can alter the genetic structure of plant species. The isoenzyme stability of the Boatin population, despite its closeness to the Srednogorie refinery, is therefore noteworthy. The results of this study show that isoenzyme markers can be used effectively as an additional means for controlling the environment by background monitoring.

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