SHORT NOTE

GENETIC CONTROL OF DIAPHORASE IN SCOTS PINE FROM UKRAINE

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Received May 5, 1996; accepted September 28, 1996

ABSTRACT

Allozyme polymorphism of DIA were studied by means of PAAG techniques in six marginal Scots pine populations from Ukraine. DIA is encoded by at least four loci, three of them, *Dia–2, Dia–3* and *Dia–4* are sufficient for interpretation. All three loci are polymorphic and have five, three and two alleles, respectively. The polymorphism of the locus *Dia–4* is firstly reported.

Keywords: *Pinus sylvestris*, diaphorase, allozyme polymorphism, Ukraine

INTRODUCTION

For the last decade some attention of population geneticists have been given to the study of genetic structure of marginal and isolated populations of *Pinus sylvestris* (GONCHARENKO *et al.* 1993; PRUS-GŁOWACKI & STEPHAN 1994).

Isozymes of diaphorase (EC 1.6.4.3) are among ones used in population genetics of Scots pine. Despite this fact their description is incomplete and erratic, especially regarding the Ukrainian populations. There are only a few articles describing diaphorase loci in some marginal Scots pine populations and provenances from the Ukraine (GONCHARENKO *et al.* 1993, 1995; PRUS-GŁOWACKI & BERNARD 1994). Additionally, these data cannot be compared adequately, because the authors have not given zymograms, so that it is elusive which loci and alleles were meant.

This work presents data on genetic control of diaphorase allozymes in Scots pine populations from the Ukraine, and attempts to compare them to those reported for *P. sylvestris* of other territories.

Cones were collected from 140 trees growing in six natural populations of Scots pine in the Ukraine (Table 1). Seeds were extracted from cones for each tree separately.

For the isozyme study 10–20 seeds were analysed from each tree. Macrogametophyte tissue was isolated from the seeds and homogenised with 0.025 ml of 0,2 M tris-glycine buffer, pH 7.5. The homogenates were subjected to vertical polyacrylamide gel electrophoresis (BREWER 1970). Diaphorase was histochemically detected according to HARRIS and HOPKINSON (1976) with minor modifications (NBT was used instead of MTT). The solution for the gel plates incubation was composed of 0.1% 2,6-dichlorophenol-indophenol (fresh) – 1 ml, 0.025 M tris-HCl-buffer, pH 8.5 – 50 ml, NADH – 10 mg, and NBT – 7.5 mg.

The loci observed were designated as follows. One specifying the most anodally migrating isozymes was indicated as 1, the next as 2, and so on. Within each locus, the totally most frequent allele was assigned the value of 100. Other alleles of the locus were marked according to their relative mobility to the most frequent allele (PRAKASH *et al.* 1969).

Due to the small number of the seeds analysed from each tree, Mendelian segregation was examined in single trees by χ^2 test only for rare genotypes occurring in unique trees, while for the most common ones – pooled over all trees.

In our experiments generally four zones of the enzyme activity were observed. However, the most anodally migrating, *Dia–1*, manifested itself instably probably due to its low activity. This fact did not permit sufficient interpretating the alleles, so that this zone was excluded from the analysis. So, the three regularly developed zones with sufficient enzymatic activity – *Dia–2, Dia–3* and *Dia–4* – were then interpreted according to the above mentioned methodics.

All three loci are polymorphic, especially *Dia–2*, that has five alleles. *Dia–3* and *Dia–4* are less polymorphic and have three and two alleles respectively (Table 2).

Dia–2

Totally five active alleles were observed for this locus. Four of them are two-banded and the least mobile,

Table 1 Scots pine populations used in the study

Population		Number of trees sampled	Latitude (N)	Longitude (E)
Nova Radcha 1	(N1)	23	51° 23'	29° 24'
Nova Radcha 2	(N2)	24	51° 23'	29° 24'
Bryukhovichi	(LB)	24	49° 55'	23° 55'
Stradch	(LS)	24	49° 55'	23° 45'
Neteshin	(HA)	22	50° 21'	26° 44'
Izum	(IH)	24	49° 09'	37° 11'

Table 2 Allelic frequencies at diaphorase loci in Scots pine populations from the Ukraine

Locus/Allele	Population					
	Nova Radcha N1	Nova Radcha N2	Bryukhovichi LB	Stradch LS	Neteshin HA	Izum IH
Dia–2						
85	0.000	0.000	0.000	0.000	0.000	0.058
90	0.148	0.246	0.200	0.288	0.323	0.275
95	0.000	0.000	0.008	0.000	0.000	0.000
100	0.811	0.712	0.792	0.712	0.677	0.621
105	0.041	0.042	0.000	0.000	0.000	0.046
Dia–3						
95	0.017	0.000	0.021	0.000	0.000	0.000
100	0.926	0.977	0.854	0.912	0.850	0.987
105	0.057	0.023	0.125	0.088	0.150	0.013
Dia–4						
80	0.022	0.004	0.092	0.029	0.032	0.067
100	0.978	0.996	0.908	0.971	0.968	0.933

Table 3 Observed allozyme segregation in endosperms of heterozygous trees and χ^2 tests for goodness of fit to 1:1 ratio among the employed populations

Locus	Tree	Allelic combination	Observed segregation	Deviation χ^2	P
Dia–2	joint	90 / 100	256 : 274	0.611	0.43
	LB 19	90 / 95	15 : 5	5.000	0.03
	IH 16	85 / 100	8 : 12	0.800	0.36
	joint	100 / 105	35 : 15	8.000	<0.01
	IH15	90 / 105	7 : 13	1.800	0.16
Dia–3	joint	95 / 100	9 : 12	4.800	0.03
	joint	100 / 105	80 : 60	2.857	0.09
Dia–4	joint	80 / 100	48 : 62	1.782	0.18

Table 4 Literature references on diaphorase in Scots pine

Number of loci	Number of alleles per locus	Tissue	Author(s)
1	3	M*	SIEDLEWSKA & PRUS-GŁOWACKI (1994)
	4	M	PRUS-GŁOWACKI *et al.* (1993)
			PRUS-GŁOWACKI & BERNARD (1994)
			SZWEYKOWSKI *et al.* (1994)
	5	M	PRUS-GŁOWACKI & STEPHAN (1994)
2	2, 2	M	GONCHARENKO (1989)
2	5, 3	M	GONCHARENKO *et al.* (1993)
2	3, 1	M	SHIGAPOV *et al.* (1995)

*) M – macrogametophytes

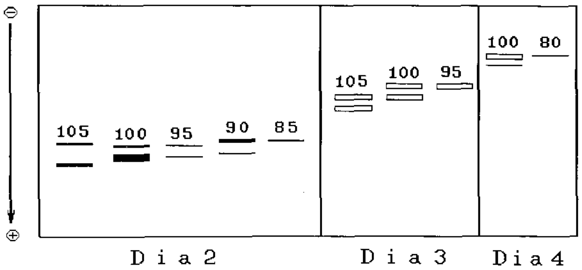

Figure 1 Schematic zymograms of diaphorase allozymes occuring in Scots pine populations from the Ukraine. The number above bands refers to the relative mobility (see text for details). Black edged bands represent faint staining alleles.

Dia–2$_{85}$, is one-banded (Figure 1). Two most common alleles, 90 and 100, appeared in all studied populations, and the others – only in some. Alleles 85 and 95 occurred only in one population each.

Dia–3

All three alleles of this locus are faint staining. Alleles 100 and 105 are two-banded, allele 95 manifests one band of activity (Figure 1).

Dia–4

This locus carries two alleles, the most common of them is two-banded (Figure 1).

Segregation of alleles showed significant deviations from Mendelian ratio (1:1) for some combinations of allelic variants (*Dia–2*$_{90/95}$, *Dia–2*$_{100/105}$ and *Dia–3* $_{95/100}$), when analysed using χ^2 test for goodness of fit (Table 3).

The different number of diaphorase loci and alleles were reported before (Table 4). Based on our data, we can conclude about at least four loci coding diaphorase. However, because of low activity of *Dia–1* we could not determine its allelic structure.

GONCHARENKO *et al.* (1993) reported about two polymorphic loci of DIA detected in Ukrainian populations of Scots pine. Those are apparently the same as *Dia–2* and *Dia–3* described in the present work. However, we have not observed a null allele in the samples, while the above authors mentioned one for two populations which are refugia. On the other hand, allele *Dia–2*$_{95}$ is apparently firstly described by us. The other researchers (Table 4) studying genetic structure of Scots pine populations in the other parts of its range (Spain, Poland, Russia, Germany, Latvia) reported about an only polymorphic locus of the enzyme that is definitely *Dia–2*. Probably polymorphism of loci *Dia–3* and *Dia–4* is unique to marginal Scots pine populations.

It should be noted that all the genotypes with the observed violation of Mendelian segregation are rare. Only one tree with genotype *Dia–2*$_{90/95}$ (in population LB) was found, three trees with *Dia–2*$_{100/105}$ genotype (all in population N1), and three – with genotype *Dia–3*$_{95/100}$ (two trees in LB and one – in N1).

Clear and intensive stain of the described Dia variants (especially those of *Dia–2* locus) in macrogametophyte samples as well as enough polymorphism make this enzyme useful as a genetic marker of *Pinus sylvestris*, in particular, for studying its geographically marginal populations.

ACKNOWLEDGEMENTS

This work was partially supported by the Central European University within the Research Support Scheme, grant # 1304 / 93/ 1095.

REFERENCES

BREWER, Y. J. 1970: An Introduction to Isozyme Techniques. Academic Press, New York – London.

GONCHARENKO, G. G. 1989: The results of applying the methods of isozyme genetics to breeding of conifers. *In:* Improvement of Forest Industry in Byelorussia. pp. 103–119. Moscow [in Russian].

GONCHARENKO, G. G., SILIN, A. E. & PADUTOV, V. E. 1993: Study of genetic structure and level of differentiation of *Pinus sylvestris* L. in central and isolated populations of Eastern Europe and Siberia. *Genetika* **29**:2019–2038 [in Russian].

GONCHARENKO, G. G., SILIN, A. E. & PADUTOV, V. E. 1995: Intra- and interspecific differentiation in closely related pines from *Pinus* subsection *Sylvestris* (*Pinaceae*) in the former Soviet Union. *Plant Systematics and Evolution* **194**:39–54.

HARRIS, H. & HOPKINSON, D. A. 1976: Handbook of Enzyme electrophoresis in Human Genetics (with Supplements). Amsterdam: North-Holland Publ. Co., New York – Oxford, American Elsevier Publ. Co.

PRAKASH, S., LEWONTIN, R. C. & HUBBY J. L. 1969: A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations *of Drosophila pseudoobscura*. *Genetics* **61**:841–858.

PRUS-GŁOWACKI, W. & BERNARD, E. 1994: Allozyme variation in populations of *Pinus sylvestris* L. from a 1912 provenance trial in Puławy (Poland). *Silvae Genetica* **43**:132–138.

PRUS-GŁOWACKI, W. & STEPHAN, B.R. 1994: Genetic variation of *Pinus sylvestris* from Spain in relation to other European populations. *Silvae Genetica* **43**:7–14.

PRUS-GŁOWACKI, W., URBANIAK, L. & ZUBROWSKA-GIL, M. 1993: Allozyme differentiation in some European populations of Scots pine (*Pinus sylvestris* L.). *Genetica Polonica* **34**:159–176.

SHIGAPOV, Z. KH., BAKHTIYAROVA, R. M. & YANBAEV, YU. A. 1995: Genetic variation and differentiation in natural populations of the common pine *Pinus sylvestris* L. *Genetika* **31**:1386–1393 [in Russian].

SIEDLEWSKA, A. &PRUS-GŁOWACKI, W. 1994: Allozyme variability of putative hybrid swarm population (*Pinus mugo* Turra x *P. sylvestris* L.) from Topielisko peat-bog near Zieleniec. *Genetica Polonica* **35**:285–302.

SZWEYKOWSKI, J.,PRUS-GŁOWACKI, W. & HRYNKIEWICZ, J. 1994: The genetic structure of Scots pine (*Pinus sylvestris* L.) population from the top of Szczeliniec Wielki Mt., Central Sudetes. *Acta Soc. Bot. Pol.* **63**:315–324.