I. STRUCTURAL AND FUNCTIONAL DIVERSITY OF PLANT ORGANISMS

GENETIC TRAITS IN PINUS PALLASIANA D.DON TREES WITH DIFFERENCES IN RADIAL GROWTH IN THE PLANTATIONS OF SOUTH-EAST OF UKRAINE

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Abstract. Using 15 isozyme loci, analysis of 220 trees of Pinus pallasiana D.Don with different radial growth rates (stem diameters) aged 9 to 40 years was performed. The deficit of heterozygous genotypes (5.7 – 32.1%) was found for all of the studied trees samples. The tendency of increasing the level of multilocus heterozygosity in groups of older age trees (40 years) and the larger stem diameter in comparison to smaller stem diameter trees was observed. This tendency was also observed in the analysis of individual loci (namely Got-1, Mdh-2, Mdh-3). Nei's genetic distances coefficient (DN) between groups of trees with larger and smaller stem diameter was insignificant and ranged from 0.003 to 0.008.

INTRODUCTION

Productivity is the most valuable feature of the stand quality. Therefore, management of plantations is carried out to increase the intensity of growth of the most high grade trees (Koba, 2009). In pine stands tending felling is carried out in phases, starting from the age of young trees (20 years), when fast and slow-growing trees become evident in the stand, and ending at the age of 70-80-years (Koba, 2009). Slow-growing trees are usually to be eliminated in the events on clearing of stands.

The role of the genetic component in the productivity of plantations and individual trees has not been fully elucidated yet (Novikov, Sheykina, 2012). The situation is more difficult with variability of many economically important quantitative traits, as it may have adaptive value (Ivanovskaya, 2012). According to some researchers, adaptive and neutral variability exists in the populations and both of its types should be considered in preserving genetic resources of species (Ledig, 1988; Geburek, Turok, 2005; Eriksson et al., 2006; Ivanovskaya, 2012).

However, not knowing how to organize complex economic and important features and properties of the plants is almost impossible to create an adequate tool of genetic analysis (Kochergina, 2009). One set of approaches and methods for genetic analysis that exists today, does not always reflect the real situation. However, the link between molecular and phenotypic variation of several economically important quantitative traits is often complex (Szulkin et al., 2010; Chhatre et al., 2013; Koralewski et al., 2014).

In population genetics of conifers, the isoenzymes are often used as molecular genetic markers up to the present time, despite the rapid development of DNA technology (Geburek, Turok, 2005; Politov, 2007; Korshikov, Privalikhin 2007; Korshikov et al. 2007; Radu 2014). They have established themselves as a convenient tool for early diagnosis of the quality of timber at the stage of seedlings, it is very important for forest species characterized by long periods of cultivation (Zhigunov, 2013). Therefore, the search for the relationship between the nature of variability of molecular genetic markers (isoenzymes) and the parameters of growth of the stem, as well as the quality of wood is one of the priorities of forestry.

The aim of this study was to reveal the genetic characteristics of trees of *Pinus pallasiana* D. Don, which differ in terms of growth rates using isozymes as molecular genetic markers.

The following tasks were set to achieve the objective:

1. To identify the relationship of the level of observed heterozygosity *P. pallasiana* trees from plantations of south-east of Ukraine with variability of the stem diameter.

2. To identify loci that can be used as genetic markers to assess the intensity of the radial growth of *P. pallasiana* trees.

MATERIAL AND METHODS

An analysis for 220 trees of *Pinus pallasiana* D.Don aged 9-40 years was carried out. Material for the study was collected in the Donetsk Botanical Garden of NAS of Ukraine (DBG), as well as in Petrovskoe, Amvrosievskoe and Shakhterskoe forestries. The diameter of each tree was measured at the height of 1.3 m. In the course of data processing the trees were grouped depending on the stem diameter: the first group – the trees with a smaller diameter, the second group – with a larger diameter (Table 1).

To determine the genetic characteristics of selected groups of *P. pallasiana* trees isozymes of 7 enzyme systems were used as markers: glutamate dehydrogenase (GDH, E.C. 1.4.1.2), acid phosphatase (ACP, E. C. 3.1.3.2), glutamate oxaloacetate transaminase (GOT, E.C. 2.6.1.1.), superoxide dismutase (SOD, E.C. 1.15.1.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), leucine aminopeptidase (LAP, E.C. 3.4.11.1) and formiat dehydrogenase (FDH, E. C. 1.2.1.2).

Vegetative buds collected from individual trees in the dormant period were used for electrophoretic studies of isoenzymes. To isolate the enzyme tissue of bud was homogenized in 100 ul of extraction buffer prepared by earlier proposed method (Molotkov, 1982) with some modifications. Electrophoretic separation of isoenzymes and subsequent histochemical staining of gels were performed by standard procedure (Davis, 1964). Allele and loci nomenclature followed Prakash and co-authors (Prakash et al., 1969). Statistical analysis was performed using the software package GenAlex (Peakall, 2006).

RESULTS AND DISCUSSIONS

15 isozyme loci were used in the analysis of genetic polymorphism of *P. pallasiana* trees. Of these, four loci of superoxide dismutase (Sod-1, Sod-2, Sod-3 and Sod-4) were monomorphic for all investigated trees. Locus Fdh for the trees in the plantation of Shakhterskoe forestry was low-polymorphic, while it was monomorphic for all other studied plants. Null alleles have been found for DBG planting at loci Got-3 and Acp-2. Such alleles are absent in other samples. It is known that rare alleles may influence the growth of plants (Blumenröther et al., 2001). In our case, nevertheless, the trees with rare alleles had no differences in growth from other trees in a sample.

It should be noted that the loci Got-1, Gdh and Lap-2 for individual trees in the studied stands were low-polymorphic or monomorphic, meanwhile for a locus Lap-1 low polymorphism is observed in the plantations of Amvrosievskoe (16-year-old trees) and Shakhterskoe forestries. Locus Got-2 turned to be highly polymorphic for all investigated trees while Got-3, Mdh-2, Mdh-3, Acp-1 and Acp-2 loci were low-polymorphic.

The actual distribution of genotypes in all plantations corresponds to the theoretically expected according to the Hardy-Weinberg equilibrium, although there are deviations at some loci. Such deviations were more numerous for the trees with a smaller stem diameter than for the trees with larger diameter. Only a sample of 9-year old trees from Amvrosievskoe forestry is fully consistent with the theoretically expected distribution.

Mean observed heterozygosity (H_O) was lower than expected (H_E) heterozygosity for almost all trees, which is indicative of the evident deficit of heterozygous genotypes ranging from 8.5 to 32.1% (Table. 1). Perhaps this can be explained by the high inbreeding level of seeds used for laying of these plantations. Outstanding excess Wright's fixation index values in groups of trees with smaller stem diameter above groups of trees with a larger stem diameter are also noteworthy. Consequently, a significant excess of homozygous genotypes is typical for the first group (with smaller stem diameter).

It is known that inbreeding accelerates homozygosity level by main genes, which in its turn may reduce adaptability in the expression of deleterious partially recessive alleles and loss of heterozygosity (Hansson B., Westerberg L., 2002). The correlation between the growth rate and heterozygosity should be apparent only among the inbred progeny. This is because of the fact that crossbred trees are likely to have small amount of harmful recessive alleles (Robin, 1987).

Table 1 Values of the main characteristics of genetic polymorphism in plant samples Pinus pallasiana D. Don, differing in growth rate in plantations from the south-east of Ukraine

Group	Percentage of polymorphic loci, P_{99}	Mean number of alleles per locus, A Expected, H_E	Mean heterozygosity, Mm		Wright`s fixation index, F
			Observed, H_O		
Shakhterskoe forestry 40 years					
1	0.667	1.800	0.169±0.050	0.131±0.044	0.225
2	0.733	1.800	0.196±0.048	0.145±0.040	0.260
Whole of the stand	0.733	1.867	0.184±0.049	0.137±0.041	0.255
Amvrosievskoe forestry 16 years					
1	0.533	1.800	0.113± 0.039	0.084± 0.034	0.257
2	0.600	1.733	0.144± 0.044	0.119± 0.036	0.174
Whole of the stand	0.600	1.867	0.131±0.041	0.103±0.034	0.214
Petrovskoe forestry 16 years					
1	0.467	1.600	0.153±0.051	0.106±0.042	0.307
2	0.467	1.533	0.134±0.047	0.113±0.045	0.157
Whole of the stand	0.467	1.667	0.144±0.048	0.110±0.043	0.236
Amvrosievskoe forestry 9 years					
1	0.533	1.733	0.153±0.047	0.140±0.041	0.085
2	0.400	1.533	0.109±0.043	0.119±0.047	-0.092
Whole of the stand	0.533	1.800	0.141±0.045	0.133±0.042	0.057
DBG 9 years					
1	0.533	1.733	0.137±0.044	0.093±0.036	0.321
2	0.400	1.467	0.108±0.041	0.079±0.040	0.269
Whole of the stand	0.571	1.733	0.129±0.043	0.087±0.037	0.326
Group 1					
	0.733	2.400	0.152±0.045	0.111±0.0333	0.270
Group 2					
	0.733	2.333	0.153±0.043	0.119±0.038	0.222

Footnote. 1. – group of trees with small stem diameter 2. – group of trees with large stem diameter

It should be noted that a slight excess of heterozygotes (9.2%) is detected for the group of trees with larger diameter (Group 2) from plantation in Amvrosievskoe forestry (9-year-old trees). A tendency to higher heterozygosity is observed in more mature trees (aged 16 and 40) with a larger diameter (Group 2) compared to the smaller diameter trees (group 1). For younger trees the trend is the contrary, as evidenced by the published data. For example, F. Ledig indicated that the heterozygosity-growth ratio changes from strongly positive (for old plants) to negative (for the young ones) (Ledig, 1983). Also, the degree of influence of heterozygosity on plant growth is strongly dependent on age.

In general, an increase in the level of heterozygosity with age, characteristic of most species of conifers, is related to elimination of inbred progenies and balancing selection toward heterozygotes, which is a key factor in maintaining population polymorphism (Belokon M. et al. 2010).

There have been several studies on genetic differences among trees with different parameters of growth and productivity. Novikov et al. analyzed (using 6 ISSR-primers at 178 loci) the variability of *Pinus sylvestris* L. individuals of different breeding grades in the plus stands of educational-experimental forestry in the Republic of Mari El (Novikov, Sheykina, 2012). Total of 88 trees was analyzed. Their findings showed that plus trees differed in genetic distance from minus (Nei's genetic distance 0.0824) and normal (Nei's genetic distance 0.0520) ones. However, no specific DNA fragments for each category

of individuals in tree breeding were found. Blumenröther et al. (2001) investigated a correlation between genetic characteristics and growth parameters of 47-year-old trees of *Pinus sylvestris* L. in Germany at 16 gene loci using horizontal starch gel electrophoresis (Blumenröther et al., 2001). The authors found differences in average observed heterozygosity between the high-grade and ordinary Scots pine trees and also noted the impact of heterozygosity at 6-PGDH and MDH-C loci on the stem diameter.

Despite the trend towards an increase of heterozygosity in mature trees of *P. pallasiana* with large stem diameter, no significant differences in the level of heterozygosity between trees with different diameters have been detected. According to previously published data, the trend in the change of heterozygosity in trees with larger and smaller stem diameter may vary depending on the particular enzyme locus (Kartavtsev and Zhdanova, 2012). Therefore, we investigated differences in heterozygosity among sampled groups of trees for specific enzyme loci (Table 2).

Table 2 Mean observed heterozygosity of 11 polymorphic loci in plants *Pinus pallasiana* D. Don. differing in growth rates in plantations from South-East of Ukraine

Locus	Heterozygosity									
	Shakhterskoe forestry (40 years)		Amvrosievskoe forestry (16 years)		Petrovskoe forestry (16 years)		Amvrosievskoe forestry (9 years)		DBG (9 years)	
Group	1	2	1	2	1	2	1	2	1	2
Sample size	27	23	27	33	22	30	20	9	18	11
Got-1	0.000	0.043	0.074	0.212	0.045	0.067	0.000	0.000	0.167	0.091
Got-2	0.333	0.304	0.481	0.424	0.500	0.567	0.400	0.444	0.444	0.364
Got-3	0.185	0.217	0.037	0.091	0.318	0.267	0.350	0.222	0.111	0.000
Mdh-2	0.037	0.087	0.111	0.212	0.045	0.100	0.200	0.222	0.222	0.000
Mdh-3	0.370	0.522	0.222	0.273	0.318	0.400	0.400	0.556	0.333	0.455
Acp-1	0.556	0.348	0.148	0.303	0.091	0.200	0.200	0.111	0.056	0.273
Acp-2	0.185	0.217	0.148	0.212	0.273	0.100	0.300	0.222	0.056	0.000
Gdh	0.037	0.043	0.037	0.030	0.000	0.000	0.050	0.000	0.000	0.000
Fdh	0.148	0.217	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lap-1	0.074	0.130	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000
Lap-2	0.037	0.043	0.000	0.000	0.000	0.000	0.200	0.000	0.000	0.000

As far as more mature trees are concerned, heterozygosity at Got-1 and Mdh-2 loci was higher for large diameter trees (exclusion DBG) compared to the smaller diameter ones. For a sample of trees from Shakhterskoe forestry, we did not found heterozygotes at Got-1 locus in a group of trees with small diameter. For trees with a large diameter from Amvrosievskoe and Petrovskoe forestries, heterozygosities at locus Got-1 were more than 2.9 times and 1.5 times higher compared to smaller diameter trees. As far as plantations of Shakhterskoe, Amvrosievskoe and Petrovskoe forestries are concerned, heterozygosities at Mdh-2 locus were 1.9; 2.2 and 2.4 times higher for the groups of trees with larger diameters than for the group with smaller diameters.

In the overall sample of trees, heterozygosities were 2 times (Got-1), 2.1 times (Lap-1) and 1.2 times (Mdh-3) higher for tree groups with a large stem diameter (Table 3). Heterozygosity at Lap-2 locus was 4.9 times greater in trees with a smaller stem diameter than for a large diameter trees. This may be explained by the fact that loci Lap-1 and Lap-2 were polymorphic only in some plantings that may lead to misunderstanding of the relationship between heterozygosity at these loci and growth parameters.

Table 3 Mean observed heterozygosity of 11 polymorphic loci in combined sample of plants *Pinus pallasiana* D. Don. differing in growth rates in plantations of South-East of Ukraine

Locus	Observed heterozygosity		
	Group 1	Group 2	In the whole for combined sample
Gdh	0.026	0.019	0.023
Got-1	0.053	0.104	0.077

Got-2	0.430	0.434	0.432
Got-3	0.193	0.170	0.182
Mdh-2	0.114	0.132	0.123
Mdh-3	0.325	0.406	0.364
Acp-1	0.228	0.264	0.245
Acp-2	0.193	0.160	0.177
Lap-1	0.018	0.038	0.027
Lap-2	0.044	0.009	0.027
Fdh	0.035	0.047	0.041

Small differences (in 1.2–1.4 times) are revealed in the observed heterozygosity at Mdh-3 locus between large and small diameter trees in all stands (see Table 3). In this regard, a more detailed study is required on the possibility of using this locus as a marker to evaluate the growth rate of *P. pallasiana*.

Nei's genetic distances coefficient (DN) among cohorts of trees with large and small diameters of the trunk in each of the plantings ranged from 0.003 to 0.008 that indicates insignificant differences in the genetic structure of trees differing in diameter of the stem.

CONCLUSIONS

Thus, the use of such a parameter as mean observed heterozygosity as a marker in selection of the best tree growth is rather difficult, due to the fact that the heterozygosity-stem diameter dependence directly correlates with the age of trees, among which a selection is performed. For this purpose it is better to apply the average observed heterozygosity at individual loci. There is a need in a detailed study of applicability of Mdh-3 enzyme locus as a marker locus to assess the intensity of stem diameter growth of *P. pallasiana*.

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