

PHENOTYPIC PLASTICITY AND NUCLEAR DNA POLYMORPHISM OF TWO DIFFERING *PINUS SYLVESTRIS* L. OPEN-POLLINATED FAMILIES ORIGINATING FROM THE SAME POPULATION

Aušra Juškauskaitė Oleg Yu. Baranov, Stanislav V. Panteleev, Virgilijus Baliuckas

Juškauskaitė A., Baranov O.Y., Panteleev S.V., Baliuckas V. 2015. Phenotypic plasticity and nuclear dna polymorphism of two differing *Pinus sylvestris* L. open-pollinated families originating from the same population. *Acta Biol. Univ. Daugavp.*, 15 (1): 95 – 103.

Trees originating from two open-pollinated families of one Lithuanian *Pinus sylvestris* L. population were studied with respect to phenotypic plasticity and nuclear DNA polymorphism at age 30. Phenotypic plasticity of families for total height, stem diameter, stem straightness, branch thickness and angle, spike knots, wood hardness was calculated based on the data from five field trials. Two most differing in trait plasticity families were selected among other 20 within the population. Needles from 84 trees of selected families were sampled in the three field trials, 14 trees per family in each trial. 5 SSR loci of nuclear DNA were used for assessment of family DNA polymorphism.

Family having significant ecovalences for four traits studied had much higher DNA polymorphism compared to the family showing no plasticity in any trait. This result was consistent through the trials.

Key words: *Pinus sylvestris* L., half-sib families, field trials, phenotypic plasticity, SSR molecular markers, DNA polymorphism.

Aušra Juškauskaitė. Institute of Forestry of Lithuanian Research Center for Agriculture and Forestry, Department of Genetics and Tree Breeding, Liepų str. 1, LT-53101 Girionys, Kaunas district, Lithuania, e-mail: juskauskaite@gmail.com.

Oleg Yu. Baranov, Stanislav V. Panteleev. Forest Institute of the National Academy of Sciences of Belarus, Laboratory of Genetics and Biotechnology, Proletarskaya str. 71, 246001 Gomel, Republic of Belarus.

Virgilijus Baliuckas. Aleksandras Stulginskis University, Faculty of Forest Science and Ecology, Institute of Forest Biology and Silviculture, Studentu str. 11, LT – 53361 Akademija, Kaunas distr. Lithuania.

INTRODUCTION

Forest tree species have experienced numerous environmental changes, during their evolutionary and ecological histories (Hamrick 2004). Many

characteristics of tree populations indicate that trees are uniquely adapted to withstand environmental changes. Longevity of trees together with phenotypic plasticity should allow individuals and populations to survive in harsh or altered environmental conditions (Hamrick 2004).

Phenotypic plasticity is the ability of an organism to express different phenotypes in response to various environmental conditions (Bradshaw 1965, Sultan 2000). Plants respond with changes in phenology, physiology, and reproduction, and such changes may have consequences for biotic interactions and community composition. The populations of forest trees could respond to an extremely variable environments by becoming both more plastic and more genetically variable. Different responses to climate change occur not only in populations throughout a species range but also between individuals within a population. Over the last decades, the study of phenotypic plasticity has received extensive attention from scientists (Matesanz et al. 2010). Plasticity could be the ideal solution to the problem of environmental heterogeneity: all that is needed is to select those genotypes that produces the best suited phenotypes across the range of environments (Pigliucci 2001a).

Heterozygosity is a measure of genetic variation within a population. High heterozygosity value may be due to long-term natural selection for adaptation, to the mixed nature of the breeds or to historic mixing of strains of different populations. A low level of heterozygosity may be due to isolation with the subsequent loss of unexploited genetic potential. One of the first authors who have started to examine the relationship between heterozygosity and plasticity was Lerner (1954). He proposed that highly heterozygous individuals have lower levels of phenotypic variation than do predominantly homozygous individuals. He presented a large body of evidence, primarily from domesticated animals and plants, to support his hypothesis that high levels of heterozygosity enhanced developmental homeostasis among individuals within populations.

Schlichting (1986) reviewed the two hypotheses about the relationship between heterozygosity and plasticity. The first hypothesis states that phenotypic plasticity should increase as the amount of heterozygosity decreases, due to the increase in developmental instability caused by deleterious homozygous recessive genes. This hypothesis arises from assumption that there

is a direct relationship between plasticity and instability (i.e. unstable genotypes will be more plastic). The second hypothesis proposes that an inverse relationship between the amount of phenotypic plasticity and heterozygosity may exist, because they represent alternative methods of dealing with environmental heterogeneity. A number of studies was carried out to test these hypothesis, mostly with herbaceous plants and animals.

Scots pine (*Pinus sylvestris* L.) is the most common conifer species in Lithuania. It is one of the most important forest tree species in Europe both economically and ecologically. Forest genetic resources are important as a source of evolutionary potential for adaptation to changes in climate and other ecological factors. If environmental change is directional and continuous, trees in particular may have the potential to adapt genetically (Hamrick 2004). Unraveling the forces shaping genetic variation within and between species has long been of interest in population genetics. An understanding of the genetic structure of a population is the key to our understanding of the importance of the genetic resources and the importance of genes for the conservation of species and biodiversity. Scots pine is one of the most genetically studied forest tree species. The importance of the species for timber production has resulted in tree breeding programmes with Scots pine being carried out in most European countries.

The aim of the study was to assess the phenotypic plasticity and genetic diversity of half-sib open-pollinated families originating from one Lithuanian of *Pinus sylvestris* L. population using genetic polymorphism analysis of nuclear DNA loci.

MATERIAL AND METHODS

The object of molecular genetic study were open-pollinated half-sib families of one Lithuanian *Pinus sylvestris* L. population. The offspring of two families (No.462 and No.479) were studied with respect to phenotypic plasticity

and nuclear DNA polymorphism at age 30. Field trials with open-pollinated progeny of Lithuanian *Pinus sylvestris* L. populations were established in different provenance regions in 1983. (Fig. 1). Phenotypic plasticity of families for total height, stem diameter, stem straightness, branch thickness and angle, spike knots, wood hardness were calculated based on the data

from five field trials. Two most differing in trait plasticity families were selected among other 20 within the population. The significance of ecovalece values of families in analysis of genotypic stability was tested using the method developed by Shukla (1972). Pine needles were used for DNA extraction. Needles from 84 trees of selected families were sampled in the three

Table 1. SSR markers used in this study: locus code, sequence of primers and size

Locus code	Sequence of primers (5'-3')	Size bp
PfTX 3116	CCTCCCAAAGCCTAAAGAATCATAAAG	170
	GCCTTATCTTACAGAA	
PfTX 4001	CTATTTGAGTTAAGAAGGGAGTC	236
	CTGTGGGTAGCATCATC	
PfTX2123	GAAGAACCCACAAACACAAG	190
	GGCAAGAATTCAATGATAA	
PfTX3013	GCTTCTCCATTAATAATTCTA	119
	TCAA AATTGTTTCGTAAAACCTC	
PfTX3020	GTCGGGGAAGTGAAAGTA	180
	CTAGGTGCAAGAAAAGAGTAT	

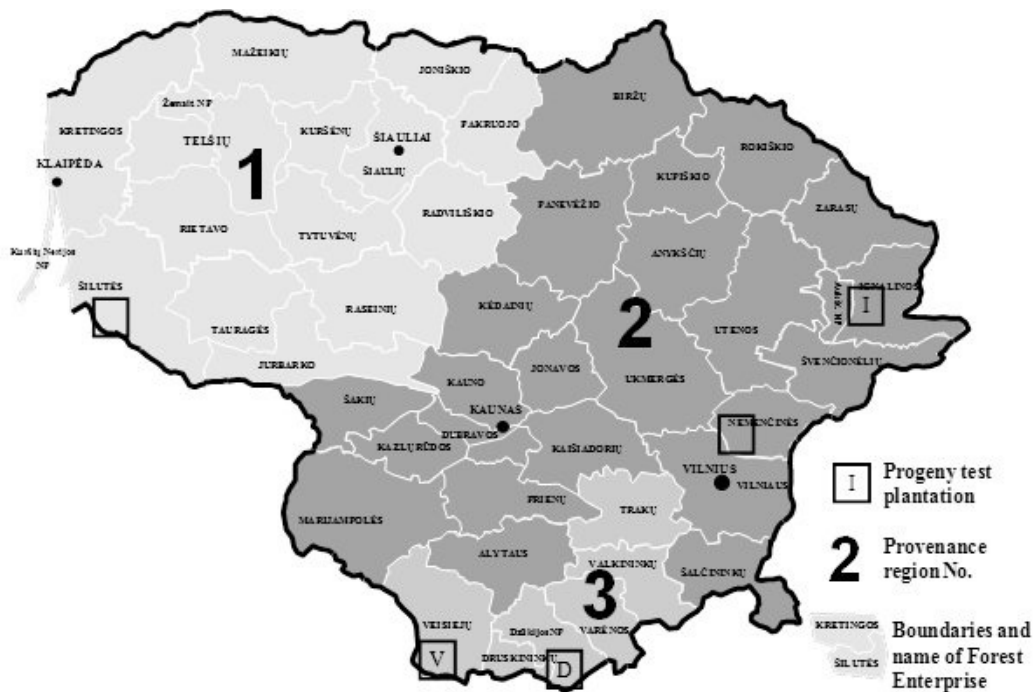


Fig. 1. Field trials with open-pollinated progeny of Lithuanian *Pinus sylvestris* L. populations, established in 1983. The tree samples for DNA analysis were taken from the labeled field trials.

field trials, 14 trees per family in each trial. DNA samples isolated using modified CTAB procedure. 5 SSR loci of nuclear DNA were used for assessment of family DNA polymorphism (Table 1). Results were analyzed using the software package GeneMapper 4.0 (Applied Biosystems). Dendrogram was constructed by applying UPGMA (unweighted pair-group method of arithmetic averages).

RESULTS

39 alleles were identified at five microsatellite loci, with an average of 7.8 alleles per locus. The highest diversity was found at locus PtTX 4001 with 14 alleles. The loci PtTX3013, PtTX 3116, PtTX3020 and PtTX2123 had 3, 13, 6, and 3 alleles, respectively.

The presence of a wide range of polymorphism was revealed among the studied genes. Average heterozygosity at each locus and for each family can be estimated from allele frequencies at each locus. Locus heterozygosity is estimated by summing the heterozygosity at all loci for each family and averaging this quantity over all families. The highest level of heterozygosity was detected for 2 loci: PtTX3116 and PtTX4001, (H_E exceeded 60%, Table 2). The next group, which included 2 locus (PtTX3013 and PtTX2123), has an average level of variability, since the value of expected heterozygosity ranged from 27% to 43%. Locus PtTX3020 characterized

by low variability, for which the value of H_E did not exceed 10%. This turned out to be least polymorphic locus in all the investigated objects (462I, 479D, 479V).

The most important parameter is H_E , characterizing half-sib offspring family No.462 (high ecological plasticity), regardless of regional origin of the locus (Fig. 2). The expected heterozygosity (also called gene diversity) is calculated from individual allele frequencies (Nei 1987).

The highest average value of expected heterozygosity (H_E) was obtained in 462V Scots pine family (Fig. 2). 462D and 462I families have similar expected heterozygosity. The lowest average values was calculated for 479D and 479V families.

Fig. 3 shows that locus PtTX 3116 has the highest value in 462D family. That probably indicates the location of the marker in linkage group with loci associated with ecological plasticity number of morphological parameters of Scots pine. The lowest values, as in previous figure, was calculated to 479D and 479V families.

Analysis of the distribution parameters in observed heterozygosity among the investigated objects also revealed certain regularities in the variation of the H_O index. The observed heterozygosity is defined as the percentage of loci heterozygous per individual or the number of individuals heterozygous per locus.

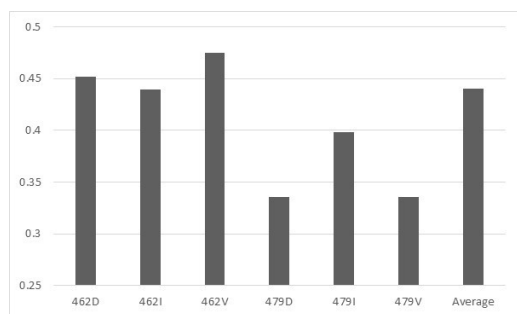


Fig. 2 Distribution of parameter values of expected heterozygosity (H_E) at 5 loci among the investigated objects of Scots pine.

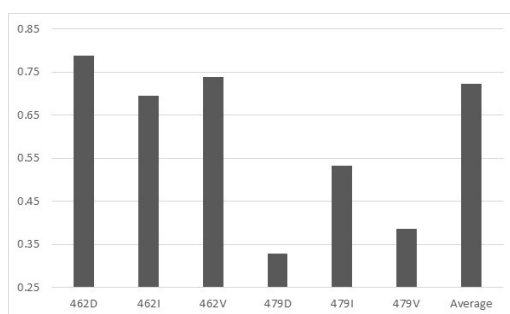


Fig. 3 Distribution of parameter values expected heterozygosity (H_E) at locus PtTX 3116 among the investigated objects of Scots pine.

Table 2. Distribution of parameter values of Scots pine families expected heterozygosity among the investigated objects

Object name	PtTX 3013	PtTX 3116	PtTX 3020	PtTX 2123	PtTX 4001	Average of 5 loci
462D	0.2619	0.7884	0.0714	0.4894	0.6481	0.4519
462I	0.2619	0.6958	0	0.5212	0.7196	0.4397
462V	0.1984	0.7381	0.328	0.4762	0.6323	0.4746
479D	0.254	0.328	0	0.4947	0.664	0.3357
479I	0.3889	0.5317	0.1402	0.3492	0.5815	0.3983
479V	0.3042	0.3862	0	0.1984	0.7884	0.3354
Average of objects	0.275	0.7225	0.0928	0.4306	0.6789	0.44

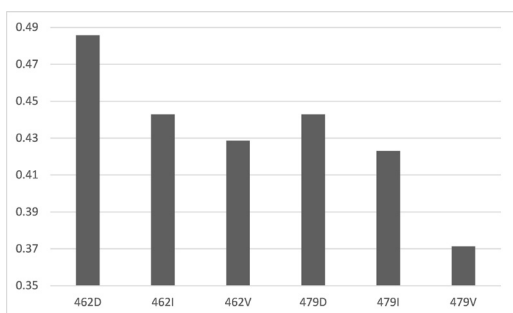


Fig. 4. Distribution of observed heterozygosity (H_0) at 5 loci among the investigated Scots pine trials.

As seen in Fig. 4, the value of H_0 for 479D family exceeded the value of 462I and 462V family subsets (the same family in two different trials). The analysis of groups from similar geographical regions showed a permanent excess of observed heterozygosity index of 462 family with high ecological plasticity in comparison with the values of H_0 for 479 family in each field trial. The obtained results seem to indicate the influence of growth conditions on the selection of genotypes and as a consequence on formation of the genetic structure in Scots pine trials. As in the previous case, the highest contribution to the structure of the diagram had locus PtTX 3116 (Fig. 5).

Distribution of genetic diversity values by Nei at 5 loci among the investigated Scots pine trials is presented in Fig. 6. The highest genetic diversity for family No.462 was revealed at the relatively

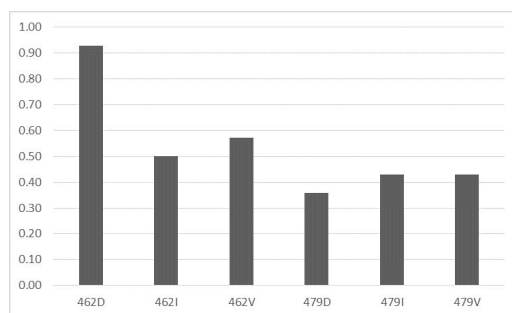


Fig. 5. Distribution of observed heterozygosity values (H_0) at locus PtTX 3116 among the investigated Scots pine trials.

richer by soil site. And the family No. 479 was genetically most diverse at the poorest site. Analysis of the overall genetic diversity by Nei also revealed the higher values for family No.462, characterized by a high level of ecological plasticity for a number of morphological characters. Comparative study of heterozygosity showed that family No.462 is characterized by the similar values of H_0 and H_E (Fig. 7).

UPGMA dendrogram generated from Nei's genetic distances of the two half-sib families allows to illustrate the degree of genetic differentiation among studied objects of Scots pine (Fig. 8). As can be seen from the structure of clustering, the data correspond to the basic laws of the distribution of genotypes and allelic structure of the studied sample. The same family (having a common origin) are characterized by

the similarity of the genetic structures regardless of growing site. Investigated half-sib families formed two distinct groups. In the group of 462 family, the smallest genetic distance was observed between 462D and 462I field trials. In the same group, the highest genetic distance was observed between 462D and 462V field trials. In other group of 479 family genetic distance is larger than in 462 family group. The smallest genetic distance was observed between 479D and 479I, the highest genetic distance was observed between 479D and 479V field trials. Thus, these data indicate the dominant role of the original genetic structure present in seed lots (especially the maternal genotype) on the formation of the genetic structure of offspring in the field trial. The formation of genotypic and phenotypic structure in the field trials in a certain way is influenced by environment through natural selection.

DISCUSSION

Heterozygosity is a property of the whole genome. Plasticity, however, is not, since a given genome can display remarkably distinct patterns of plasticity for the same trait exposed to different environments or for different traits measured within the same set of treatments (while total heterozygosity, of course, remains invariant) (Pigliucci 2001b). This could be one of the reasons why sometimes it is possible to find significant relationship and sometimes do not. Pigliucci (2001b) states that it is possible to find such a correlation when nonspecific plasticities are studied, that is, cases of allelic sensitivity in which the response may be affected by many genes in a nonspecific manner. Mating system and life history have major influences on the patterns of genetic variation (Hamrick & Godt 1996).

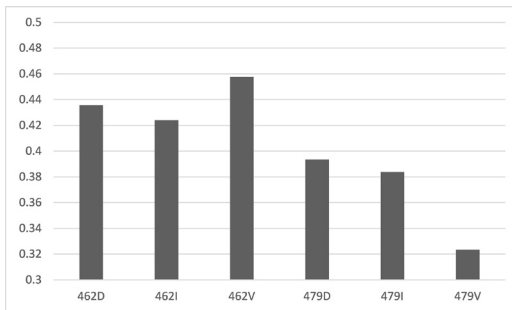


Fig. 6. Distribution of genetic diversity values by Nei at 5 loci among the investigated Scots pine trials.

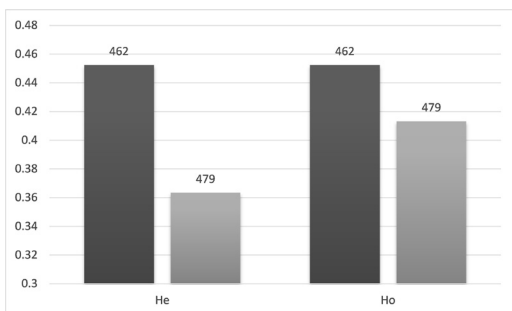


Fig. 7. Heterozygosity at 5 loci among the investigated Scots pine families.

Nucleotide polymorphism is influenced by several factors including mutation, migration, selection and random genetic drift. New things can be revealed about selection when the nucleotide polymorphism are correlated with phenotypic variation. Research on sequence variation, together with work on natural phenotypic variation would be significant for revealing the genetics of adaptations of tree populations (Savolainen & Pyhajarvi 2007). The assessed degree of polymorphism allows us to have

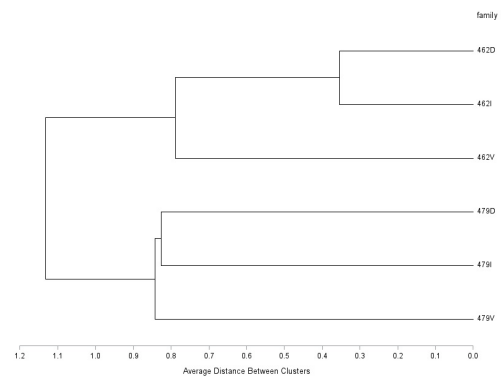


Fig. 8. Unweighted pair group method (UPGMA) dendrogram based on Nei's genetic distances among investigated half-sib open-pollinated families of one Lithuanian *Pinus sylvestris* L. population.

good relative estimates of the variation between populations to be used in breeding and genetic resource conservation programs (Lučić et al. 2011).

Molecular genetic markers, combined with population genetic principles and concepts, can greatly facilitate programs in conservation, restoration and sustainable management of forest genetic resources (Rajora & Mosseler 2001). Wang et al. (1991) compared differentiation of populations representing three different geographic varieties of *P. sylvestris* originating from Sweden and China. They found that expected and observed heterozygosities were high and ranged between 0.165 and 0.282, and between 0.164 and 0.278 respectively. Results of their study indicate that the patterns of genetic differentiation in *P. sylvestris* are more complex than previously thought. While some geographically distant populations appear little differentiated, unexpectedly large differences may occur among adjacent populations. Kosinska et al. (2007) investigated genetic variability of Scots pine in six populations originating from Poland representing two maternal populations and their natural and artificial progenies. Expected and observed heterozygosities values in maternal and progenies populations ranged from 0.254 to 0.256 and 0.234 to 0.237, respectively. Their results indicate that the level of genetic variation in all progeny populations is similar to maternal populations.

Karhu et al. (1996) examined patterns of variation of several kinds of molecular markers. Microsatellite markers show highest variability. Microsatellite polymorphism of *P. sylvestris* in two populations revealed 11 and 6 different alleles at the microsatellite loci. They also detected very high levels of expected heterozygosity ($H_E = 0.77$). The average of 7.8 alleles per locus across the five polymorphic loci characterized for Scots pine was comparable to or greater than microsatellite-based genetic studies of other pines. Nine alleles per locus was observed in *P. resinosa* (Boys et al. 2005), 6 alleles per locus observed in *P. radiata* (Smith & Devey 1994), and 6.7 alleles per locus in *P. sylvestris* (Soranzo

et al. 1998).

The evolutionary history of *P. sylvestris* appears to have affected individual populations and localities in a heterogeneous way. The generally low level of genetic variation of *Pinus sylvestris* results from its evolutionary processes. Eriksson (1998) summarises papers concerning pine species studies and hypothesized that a historical genetic bottleneck during the glacial episodes of the Holocene was the main reason for low polymorphism in this species. A large difference between observed and expected heterozygosity can be caused by non-random mating (e.g., inbreeding) or selection acting against certain genotypes.

The estimates of H_O and H_E for family No.462 indicate the presence of an equilibrium (Hardy-Weinberg equilibrium) of genetic structure. At the same time family have a significant excess of heterozygotes. Thus, the elimination of extreme (alternative) homozygous genotypes are likely a reflection of the process of narrowing the genetic and phenotypic diversity, and as a result reduce the plasticity of morphological traits in the family. Locus heterozygosity is related to the polymorphic nature of each locus. A high level of average heterozygosity at a locus could be expected to correlate with high levels of genetic variation at loci with critical importance for adaptive response to environmental changes (Kotzé and Muller 1994).

CONCLUSIONS

It was found that genetic polymorphism of family No.462, which has a high ecological plasticity for majority of tested traits, is significantly higher than estimated for family No.479, which showed low ecological plasticity. This result was consistent through the trials.

ACKNOWLEDGEMENT

The paper presents research findings, which have been obtained through long-term research

programme „Genetics and purposeful change of genotypes of agricultural and forest plants“ implemented by Lithuanian Research Centre for Agriculture and Forestry.

REFERENCES

- Boys J., Cherry M., Dayanandan S. 2005. Microsatellite analysis reveals genetically distinct populations of Red pine. *American Journal of Botany*, 92(5): 833–841.
- Bradshaw A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*. 13: 115–155.
- Eriksson, G. 1998. Evolutionary forces influencing variation among populations of *Pinus sylvestris*. *Silva Fennica*, 32(2): 173-184.
- Hamrick J. L. 2004. Response of forest trees to global environmental changes. *Forest Ecology and Management*. 197: 323–335.
- Hamrick J. L., M. J. W. Godt. 1996. Effects of life history traits genetic diversity in plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 351: 1291–1298.
- Karhu A., Hurme P., Karjalainen M., Karvonen P., Kärkkäinen K., Neale D., Savolainen O. 1996 Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? *Theoretical and Applied Genetics*, 93(1-2): 215-221.
- Kosinska J., Lewandowski A., Chalupka W. 2007. Genetic variability of Scots pine maternal populations and their progenies. *Silva Fennica*, 41(1) : 5-12.
- Kotzé A., Muller, G. H. 1994. Genetic relationship in South African cattle breeds. In: *Proceedings of the 5th world congress on genetics applied to livestock production, Guelph, Canada*. University of Guelph, Guelph, Ontario, Canada. 21: 413–416.
- Lerner L. M. 1954. Genetic homeostasis. Edinburgh: Oliver and Boyd. 134.
- Lučić A., Isajev V., Rakonjac L., Ristić D., Kostadinović M., Babić V., Nikolić A. 2011. Genetic divergence of Scots pine (*Pinus sylvestris* L.) populations in Serbia revealed by RAPD. *Archives of Biological Sciences*, 63(2): 371-380.
- Matesanz S., Gianoli E., Valladares F. 2010. Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*. 1206: 35–55.
- Nei M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, USA. 512.
- Pigliucci M. 2001(b). Phenotypic Plasticity: Beyond Nature and Nurture. Baltimore: Johns Hopkins University Press. 328.
- Pigliucci M. 2001a. Environmental Heterogeneity: Temporal and Spatial. Published Online: 19 APR. Wiley online library. 1-7. URL: <http://onlinelibrary.wiley.com/doi/10.1038/npg.els.0001766/full>
- Rajora O. P., Mosseler A. 2001. Molecular markers in sustainable management, conservation, and restoration of forest genetic resources. In: Müller-Starck G., Schubert R. (eds.): Genetic Response of Forest Systems to Changing Environmental Conditions Forestry Sciences Vol. 70. London. Pp. 187-202.
- Savolainen O., Pyhajarvi T. 2007. Genomic diversity in forest trees. *Current Opinion in Plant Biology*. 10(2): 162–167.
- Schlichting C. D. 1986. The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, 17:667-693.

Shukla G. K. 1972. Some statistical aspects of partitioning genotype–environment components of variability. *Heredity*, 29: 237–245.

Received: 27.04.2015.

Accepted: 08.06.2015.

Smith D. N., Devey M. E. 1994. Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genome*, 37: 977–983.

Soranzo N., Provan J., Powell W. 1998. Characterization of microsatellite loci in *Pinus sylvestris* L. *Molecular Ecology*, 7: 1247–1263.

Sultan S. E. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science*, 5: 537–542.

Wang X. R., Szmidt A. E., Lindgren D. 1991. Allozyme differentiation among populations of *Pinus sylvestris* (L.) from Sweden and China. *Hereditus*, 114: 219-226.