

Comparison of Microsatellites and SNP Markers in Genetic Diversity Level of Two Scots Pine Stands [†]

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Abstract: Scots pine (*Pinus silvestris*), is one of the dominant species in Poland and one of the main forest tree species in northern and central Europe. This species is of great economic importance. The Scots pine is highly adaptable to changing environmental conditions. A number of ecotypes have been characterized and the formation of these ecotypes are related with development of different phenotypic characteristics; morphological, physiological, and ecological. Molecular studies, based on DNA polymorphism, have been used for more than 20 years to analyze the genetic diversity of the Scots pine population. The most popular are microsatellite markers due to the fact of wide availability and high polymorphism. However, the use of these markers is also associated with certain limitations, due to complex mutation models or high incidence of homoplasia. These features are prompting scientists to look for alternative types of markers such as, for example, SNP. In our study we conducted a comparison of the basic parameters of genetic variability of two Scots pine stands (25 and 24 trees in each) for 20 SNP markers and 4 microsatellite markers. For the 20 SNP loci the observed heterozygosity (H_o) was equal to 0.34 for both stands and the expected heterozygosity (H_e) was equal to 0.34 for the first stand and 0.37 for the second. No statistically significant genetic distance was observed between them. For the microsatellite markers observed heterozygosity (H_o) was 0.81 and 0.74, and the expected heterozygosity (H_e) was equal to 0.85 and 0.85 respectively for the stands, and similarly no statistical significant genetic distance was observed. Literature data of different genetic markers showed the higher informativeness of randomly chosen microsatellites than single nucleotide polymorphism (SNP) markers for study population differentiation. But some analyzes confirm that the appropriate number of SNP markers can be more informative for population structure inference.

Keywords: *Pinus silvestris*; molecular markers; genetic diversity

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1. Introduction

Knowledge of the history of the population and the relationships between individuals in populations is extremely important for many studies in the field of genetics, molecular biology, and conservation genetics. For many years, there has been great interest in the scientific community in the use of genetic information to infer various population parameters. Genetic markers are widely used, e.g., for the estimation of relationship [1,2], the inbreeding coefficient [3], or the intensification of migration both in the global and local perspective [2]. Such studies are often limited to microsatellite marker panels, but in some cases there are not enough loci or alleles to reliably infer the desired parameters. For a large number of scientific studies, microsatellite markers are molecular markers of the “first choice” due to the fact of wide availability and high polymorphism. However, the use of these markers is also associated with certain limitations: due to complex mu-

tation models [4]; high incidence of homoplasia [5]; and error frequency and low genotypic throughput [6]. These features are prompting scientists to look for alternative types of markers. As the amount of data in genomics increases, the availability of single nucleotide polymorphisms (SNPs) continues to increase, also for non-model organisms, contributing to the growing interest of these markers in the field of population genetics. SNP polymorphisms are characterized by high variance in the genome for most organisms and, importantly, they are distributed throughout the genome with high frequency.

2. Materials and Methods

DNA was extracted using the NucleoSpin® Plant II (Machery Nagel, Düren, Germany). The amplification of microsatellite DNA fragments was carried out by polymerase chain reaction (PCR), using the Qiagen® Multiplex PCR Kit (Hilden, Germany). Analysis of nuclear microsatellite sequences (nSSR) were performed according to a modified procedure by [7] using three microsatellite loci: SPAG 7.14, SPAC 11.6 and SPAC 12.5 and according to [8] for the *SsrPt_ctg4363* locus.

SNP sites were selected, accordingly, with maximum numbers of SNPs in genes and the possibility of designing primers for multiplex reaction. Primers were designed in the Primer 3, Maryland, MD, USA [9,10] as predicted for the SNP analysis by primer extension and nucleotide termination (single-based extension and termination) [11] method with ABI Prism SNaPshot Multiplex Kit (Thermo Fisher Scientific, Waltham, MA, USA). The SNP was identified by SNP genotyping and confirmed by sequencing. Basic genetic parameters were calculated in GenAlEx 6.5 (Canberra, Australia) [12] and Arlequin 3.5 (Berne, Switzerland) [13].

3. Results

The results of genetic differentiation obtained on the basis of the analysis of 4 microsatellite markers showed a high level of both observed and expected heterozygosity in both stands for all analyzed loci (Table 1). The mean value of observed heterozygosity (H_o) in both stands was 0.81 and 0.74, and the expected heterozygosity (H_e) was equal to 0.85 and 0.85 respectively. Clearly lower values of these coefficients were obtained in the case of the analysis of the polymorphism of 20 single nucleotides (Table 1). No statistically significant genetic distance between stands was observed alike in the case of SSR markers and SNP markers.

Table 1. Mean values of observed heterozygosity (H_o) and expected heterozygosity (H_e) for both types of molecular markers.

Stand	H_o SSR	H_e SSR	H_o SNP	H_e SNP
Pop 1	0.81	0.85	0.34	0.34
Pop 2	0.74	0.85	0.34	0.37

4. Discussion

Literature data of different genetic markers showed the higher informativeness of randomly chosen microsatellites than SNP markers for study population differentiation [14]. But some analyses confirm that the appropriate number of SNP markers can be more informative for population structure inference [15]. In studies of the genetic variability of Scots pine, microsatellite markers are the basic tools, but SNP markers are also used in the context of analyzing the degree of polymorphism and the ability to adapt in a changing environment.

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