Comparison of microsatellites and SNP markers in genetic diversity level of two Scots pine stands

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Introduction

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 has a great economic importance. The Scots pine is highly adaptable to changing environmental conditions. A number of ecotypes have been characterized and 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 analyzed genetic diversity of Scots pine population.

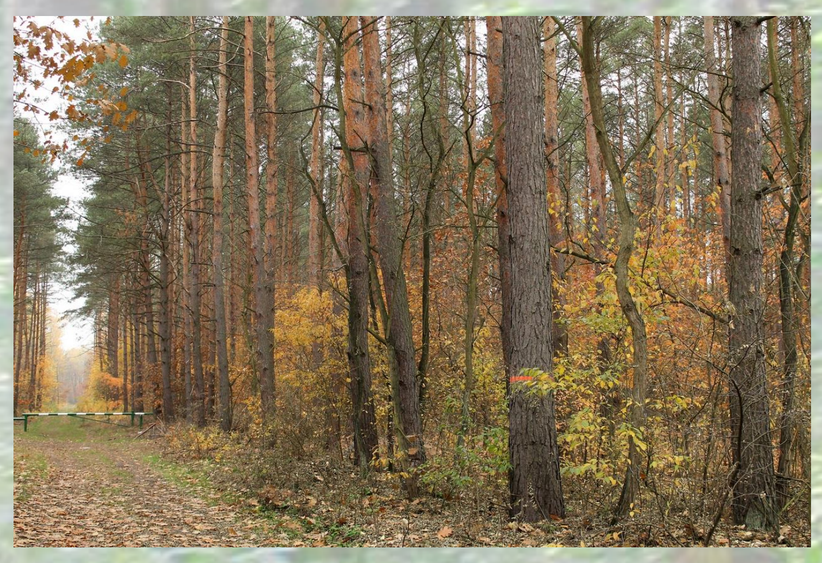
Materials and methods

SSR

DNA was extracted using the NucleoSpin® Plant II (Machery Nagel). The amplification of microsatellite DNA fragments was carried out by polymerase chain reaction (PCR), using the Qiagen® Multiplex PCR Kit. Analysis of nuclear microsatellite sequences (nSSR) were performed according to a modified procedure by Soranzo et al. [1998] using three microsatellite loci: SPAG 7.14, SPAC 11.6 and SPAC 12.5 and according to Chagné et al. [2004] for the SsrPt_ctg4363 locus.

SNP

SNP sites were selected, accordingly with maximum numbers of SNP's in genes and the possibility of designing primers for multiplex reaction. Primers were designed in the Primer 3 program (Untergasser et al. 2012), (Koressaar and Remm 2007), as predicted for the SNP analysis by primer extension and nucleotide termination (single-based extension and termination) (Pastinen et al. 1997) method with ABI Prism SNaPshot Multiplex Kit. The SNP was identified by SNP genotyping and confirmed by sequencing.



Photography by A. Pacia

Pop	Locus	N	No allelese	Ho	He
Pop1	SPAG 7.14	24	16,00	0,71	0,93
	SPAC 11.6	25	20,00	0,92	0,91
	SPAC 12.5	21	17,00	0,86	0,91
	SsrPt_ctg4363	18	5,00	0,78	0,66
Mean		22	14,50	0,81	0,85
Pop2	SPAG 7.14	23	18,00	0,96	0,92
	SPAC 11.6	22	20,00	0,86	0,93
	SPAC 12.5	18	13,00	0,67	0,89
	SsrPt_ctg4363	24	6,00	0,50	0,69
Mean		21,75	14,25	0,74	0,85

Tab. 1. Basic molecular diversity parameters. N- number of samples, No alleles – number of alleles, Ho- heterozygosity observed, He- heterozygosity expected

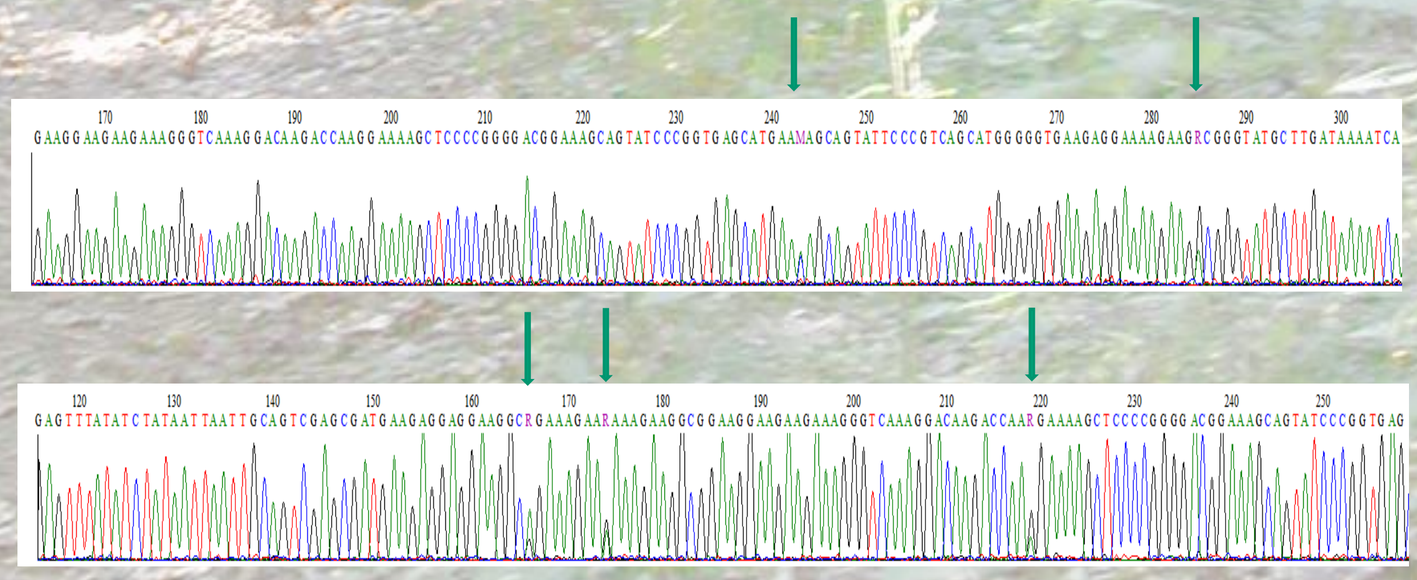


Fig.1. Examples of SNP polymorphism in two different genes in stand of scots pine. Arrows indicate sites of polymorphism.

Lp SNP	Pop1	Pop2
1	0,46	0,45
2	0,15	0,36
3	0,39	0,47
4	0,22	0,34
5	0,16	0,31
6	0,28	0,28
7	0,28	0,31
8	0,48	0,41
9	0,49	0,50
10	0,12	0,25
11	0,33	0,19
12	0,22	0,38
13	0,42	0,42
14	0,22	0,38
15	0,57	0,65
16	0,49	0,51
17	0,50	0,51
18	0,46	0,49
19	0,00	0,00
20	0,50	0,50
Mean	0,34	0,37

Tab. 2. Values of heterozygosity observed in twenty SNP markers for both stands (Pop 1 and Pop 2).

Conclusion

Literature data of different genetic markers showed higher informativeness of random chosen microsatellite than 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.

In a changing environment with the risk of occurring phenomena of drought in some areas, understanding species and ecotype variability in genes associated with different traits is important in comprehension the adaptability of important forest species.

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