GENETIC DIVERSITY AND PARENTAGE ANALYSIS FOR DNA MARKER-BASED FOREST REPRODUCTIVE MATERIAL TRACEABILITY SYSTEM IN LITHUANIA

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Introduction

Scots pine is economically and ecologically important forest tree species in Lithuania. According to the Regulation of Forest Planting and Restoration of Lithuania (2018) and the Regulations on Forest Reproductive Material (FRM) (2017) forests must be planted and replanted with the target tree species, using high quality seeds and seedlings from a given provenance. EU Council Directive (1999/105/EC) indicates that EU Member States are responsible for the regulation, requirements and implementation of FRM quality and traceability system. The existing FRM control system in Lithuania is based on the control actions of the supporting documents regarding the origin of FRM. However, this FRM traceability system is limited and, in some cases, cannot ensure that FRM used for reforestation/afforestation originates from the declared place of origin or belongs to a certain seed or plant lot. In this study we aimed to assess and compare genetic diversity of scots pine seed orchard clones with seeds, and to test DNA based parentage analysis methods with Jonava scots pine seed orchard clones and seeds. Up to now DNA markers based FRM traceability system in Lithuania has not been tested and implemented.

Methodology

Material (cones with seeds and needles) collected in Jonava scots pine seed orchard (21 clone and 10 to 20 seed per clone), in total 415 samples were used for DNA extraction and further analysis.

Total genomic DNA was extracted from frozen needles according to an adjusted ATMAB DNA extraction method (Dumolin et al., 1995). DNA analysis based on 12 nuclear microsatellite markers (nSSR: psyl2; psyl16; psyl18; psyl25; psyl42; psyl44; psyl57 (Sebastiani et al., 2012); SPAC7.14; SPAC11.4; SPAC12.5 (Soranzo et al., 1998); PtTX4001; PtTX4011 (Auckland et al., 2002)).

Genetic diversity parameters were calculated for the two groups (21 clone and 394 seeds): number of different alleles (Na), number of effective alleles (Ne), observed (Ho)/expected (He)/unbiased (uHe)expected heterozygosity, and fixation index (F)) based on 12 microsatellite loci using the GenAlEx 6.5 software (Peakall und Smouse, 2012). Allelic richness (Ar) was estimated with the FSTAT 2.9.3. software (Goudet, 2001), the lowest number of samples (21) was used for rarefaction.

Discriminant analysis of principal components (DAPC) was used to examine the clustering of individuals (R package adegent 2.0.0 (Jombart, 2015; Jombart and Collins, 2015).

Maternity assignments were estimated with program CERVUS (Kalinowski et al., 2007) CERVUS version 3.0.7 (Kalinowski et al., 2007). Each Cervus run consisted of completing an allele frequency analysis, followed by a simulation of maternity analysis where the number of potential mothers was set to 21, with a proportion of mothers sampled set to 95%. This parameter allows the software to consider the possibility that the actual mother was not genotyped, which was assumed to be rarely possible. Proportion of typed loci was set to 94% as the allele frequency analysis has estimated. A minimum of 10 typed loci were required for progeny to be analysed for maternity, and the number of progenies simulated was set to 100000. The proportion of loci mistyped allows for errors in genotyping and was tested with different thresholds: 0.001, 0.01, 0.05 and 0.1.

Results

Table 1: The genetic diversity parameters of the two scots pine generations: clones and seed from Jonava seed orchard estimated based on 12 nSSR loci.

Generation	N	Na	Ne	Ar	Np	Ho	He	uHe	F
JSP_Clones	21	7.08	4.28	7.08	0	0.560	0.518	0.531	-0.069
JSP_Seed	394	13.83	5.09	7.40	81	0.464	0.549	0.550	0.170
Mean		10.46	4.69	7.24	40.5	0.512	0.534	0.540	0.050

* N - sample size; Na - mean no. of different alleles; Ne - mean no. of effective alleles; Ar - allelic richness (based on min. sample size of 21 individual); Np – no. of private alleles; Ho - observed heterozygosity; He - expected heterozygosity; uHe - unbiased expected heterozygosity; F—fixation index.

Table 2: Cervus maternity analysis: Mother alone (all offspring*)

Minimum typed loci	Prop. Loci mistyped	Total no. of offspring	Total no. of offspring tested	Assignments observed (Delta 95%)	Assignment Rate % (Delta 95%)	Assignments observed (Delta 80%)	Assignment Rate % (Delta 80%)
10	0.001	394	370	341	92	341	92
10	0.01	394	370	352	95	352	95
10	0.05	394	370	339	92	361	98
10	0.1	394	370	301	81	363	98

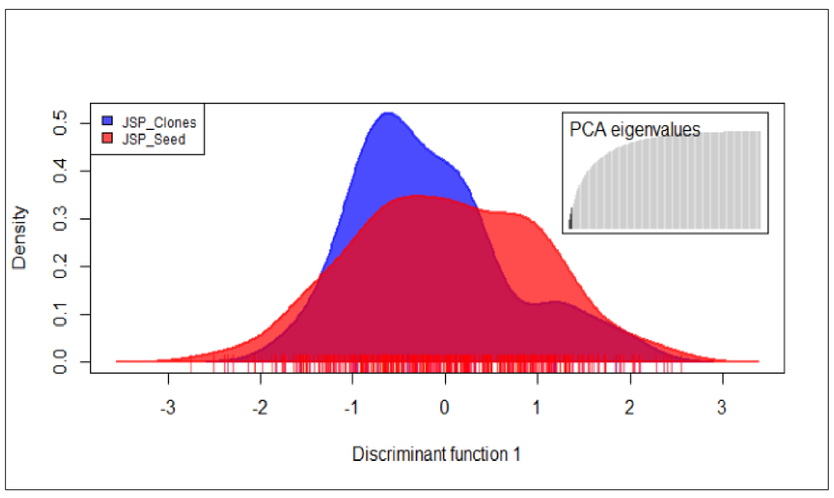

Figure 1: Discriminant analysis of Principal Components (DAPC) (R package *adegent*).

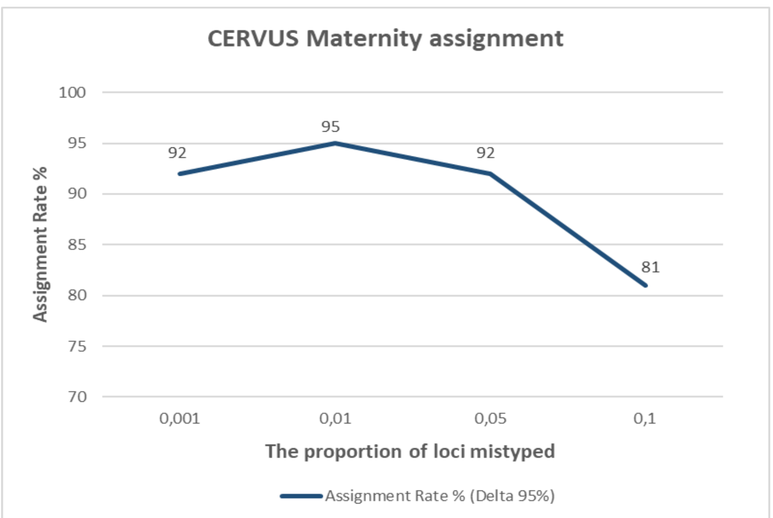

Figure 2 (right): The percentage of Jonava seed orchard progenies statistically significantly assigned to Jonava seed orchard clones depending on the proportion of the mistyped loci (X axis) based on the the Maximum Likelihood analysis in CERVUS maternity assignment. The solid line indicates an analysis scenario that allows progeny genotypes to have unknown alleles at up to two loci. The threshold of 70% of correctly assigned individuals may be considered as a safe lower margin for positive assignment of a seed lot to a particular seed orchard.

Main conclusions

Our first results based on 12 nuclear microsatellite markers indicated that genetic diversity among Jonava seed orchard clones and collected seeds were moderately high and comparable with other studies on scots pine in Europe. The test of the maternity analysis software CERVUS has showed very positive results in maternity assignments. Therefore, selected microsatellite markers in combination with accurate sampling design and specific DNA analysis methods should be improved and can be used for FRM traceability system based on DNA “fingerprints”.

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