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Development of DNA marker-based traceability system for forest reproductive material in Lithuania

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INTRODUCTION

Scots pine, Norway spruce and Pedunculate oak are among (European beech will be) the most economically and ecologically important forest trees species in Lithuania. According to the Regulation of the Republic of Lithuania on Forest Planting and Restoration (2018) and the Regulations on Forest Reproductive Material (FRM) (2017) forests must be planted and replanted with the target tree species, which seeds and seedlings meets quality and origin requirements. 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. Existing FRM control system in Lithuania is based on the control actions and supporting documents/certificates of origin. However, this FRM traceability system is limited and cannot ensure that FRM used for reforestation/afforestation originates from documented/declared place of origin or belongs to the same seed or plant lot. We seek to build a two level DNA based control FRM traceability control system based (1) on DNA markers reference seed lot samples from each seed collection and (2) for seed orchards only based on DNA "fingerprints" of seed orchard clones.

Existing forest genetic research infrastructure in Lithuania and Good Practice Examples in Europe (e.g. Germany and Slovenia) provide opportunities to implement FRM traceability system based on DNA markers. The main aim of the project is to perform a pilot FRM traceability study based on microsatellite markers and to prepare a methodology for FRM traceability system implementation in Lithuania.

Research idea

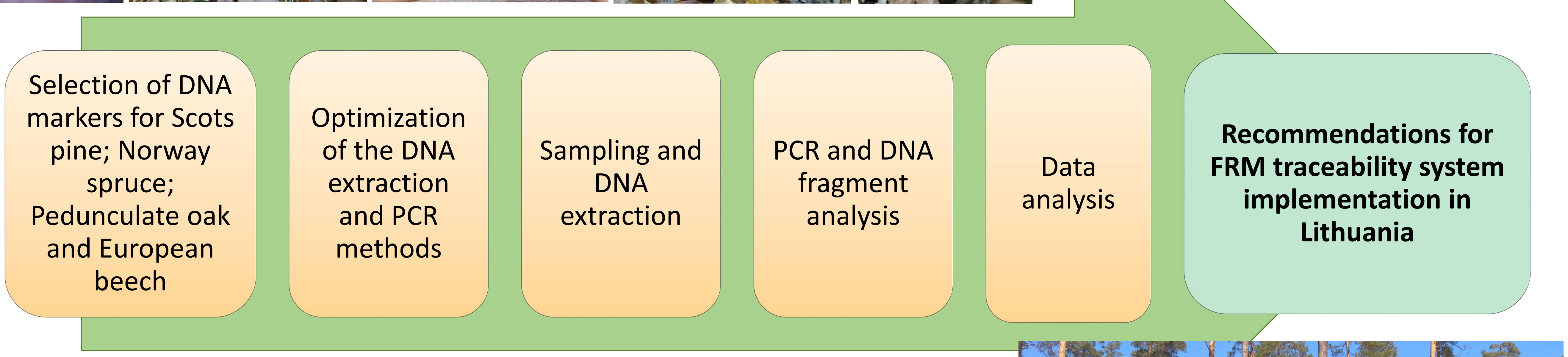
The main research idea is to create DNA testing methods for DNA based FRM traceability system in Lithuania, which is particularly important for adaptation and productive growth of future forest under changing environment and climate. The main objectives of the project are:

- To prepare an overview of existing FRM traceability control systems in Europe and to pre-select species specific sets of DNA markers (SSR microsatellites) for Scots pine, Norway spruce, Pedunculate oak and European beech, which could be used for DNA based traceability control study
- To develop species-specific DNA genotyping methods for origin traceability: to optimize DNA extraction and PCR multiplex protocols and to test the pre-selected DNA markers on selected tree species
- To carry out genotyping of sets of individuals for each species to select optimum sample sizes given particular DNA markers sets for reliable DNA based assignment of samples to particular seed lots
- For each of the above mentioned tree species, to test the newly developed method in practice by choosing samples from known populations/seed lots to estimate the reliability of seed lot/population identification based on the sampled sets
- Based on the DNA results to prepare FRM traceability control system methodology and recommendations for selected tree species.

Each forest stand is considered as different seed collection object / three stands in different regions will serve as replicates.

Sampling is planned according to each species specifically.

Species	No of stands	No of samples per stand	No of samples in total
Scots pine	3	100	300
	10 far distant populations	10-20 trees per population	100 - 200
	1 seed orchard	20 clones x 20 seed	400
Norway spruce	3	100	300
Pedunculate oak	3	100	300
European beech	3	100	300



Selection of DNA markers for Scots pine; Norway spruce; Pedunculate oak and European beech

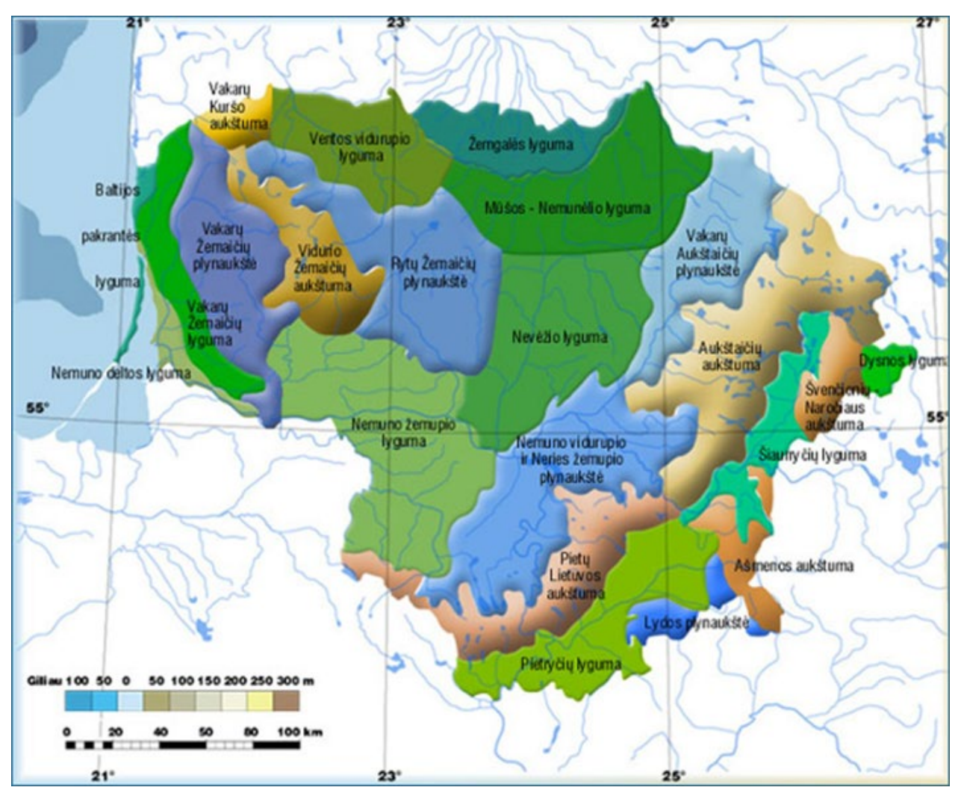
Optimization of the DNA extraction and PCR methods

Sampling and DNA extraction

PCR and DNA fragment analysis

Data analysis

Recommendations for FRM traceability system implementation in Lithuania



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