



EUROPEAN UNION  
CO-FINANSED PROJECT

## **„Importance of Genetic Factors on Formation of Forest Stands with High Adaptability and Qualitative Wood Properties”**

**Activity: “Exploration of the molecular genetics methods for determination of the natural forest genetic diversity and assessing of forest trees for commercial properties”**  
**goals, objectives, expected final results**

### **Objectives of the research activity:**

- 1) characterize structure of genetic diversity in natural forests stands;
- 2) determine the genetic structure and gene expressions involved in formation of wood density;
- 3) evaluate structural changes of pine genome during adaptation to stress factors.

### **Goals and plans of the research activity**

To achieve first objective of the activity: “Characterize structure of genetic diversity in natural forests stands” following tasks are scheduled:

- a) extract DNA of sample trees of naturally regenerated aspen and spruce stands and carry out amplification micro satellite locus;
- b) analyze acquired micro satellite loci, determining the genetic diversity of stands, kinship structure, for aspens – also number of clones in each stand;
- c) develop recommendations for effective conservation of genetic diversity in reserves, as well as for the required number of clones of aspen plantations and spruce seed orchards in order to ensure stability of forests, regenerated with selected material.

To achieve second objective of the activity: “Determine the genetic structure and gene expressions involved in formation of wood density” following tasks are scheduled:

- a) perform the analysis of existing information considering genes, involved in formation of pine wood density, select target gene, based on evaluation of their importance, structure and availability of sequence in public databases;
- b) establish molecular markers for the target gene sequences, collect samples from individuals with different wood density (determined in Activity 2), perform RNS extraction, reverse transcription, RT-PCR with established specific markers;
- c) analyze expression level of each gene in trees with a certain wood density, drawing conclusions for the impact in formation of desired genotype;
- d) carry out structural analysis of genes for genotypes (trees) with the desired

wood density and specific gene-expression levels, seeking opportunities for the creation of molecular markers for identification, thus making possible the direct selection of desired wood properties and increasing the breeding efficiency.

To achieve third objective of the activity: “Evaluate structural changes of pine genome during adaptation to stress factors” following tasks are scheduled:

- a) extract mRNAs from the pine seedlings that have been exposed to stress initiating conditions previously;
- b) identify transcripts of retrotransposons, using reaction of reverse transcription and further amplification using complementary primers of conservative sequences among the different classes of retrotransposons;
- c) create markers of gene sequences for control which the number of transcript copies increase as a result of stress conditions;
- d) sequence isolated retrotransposon sequences, to explore the prevalence of conventional pine genome, creating specific markers for the selection of desirable properties and increasing efficiency of breeding work, improving adaptation capacity of selected material.

### **Expected results of activity**

Expected outcomes:

- developed recommendations for the required number of clones and their kinship to ensure sufficient levels of genetic diversity in hybrid aspen plantations and spruce seed orchards, as well as in genetic reserves;
- molecular markers and methodology for identification of the genotype with higher wood density and increase of tree breeding;
- protocol for identification of active retrotransposons in pine genome to recognize stress tolerant genotypes;
- obtained information for the stability of pine genome in stress conditions, environmental conditions, influencing tree genome and adaptation mechanism;
- results of project covered in scientific publications and reports in scientific conferences as well as in master thesis and undergraduate works.