

## The triad of tree biotechnology

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Tree breeding is slow due to the special characteristics of forest trees, such as slow growth and long generation cycles. The use of biotechnological methods for gene characterization could significantly accelerate tree breeding, which could become necessary for climate change adaptation. However, biotechnological methods are not available for the vast majority of forest tree species.

The junior research group “Genetic Technologies” established at the Thuenen Institute of Forest Genetics in 2021 focuses on a thematic triad and seeks to transfer biotechnological methods such as tissue culture, genetic transformation, and genome editing approaches to recalcitrant tree species. Based on the working technologies in the tree genus *Populus* (poplar), the currently severely impaired European beech (*Fagus sylvatica*) in particular is in focus for expanding the molecular toolbox. In addition, work is ongoing on pedunculate oak (*Quercus robur*), common ash (*Fraxinus excelsior*), and the invasive tree species *Ailanthus altissima*.

The goals of tissue culture include establishing sterile cultures from wild populations and testing protoplast isolation and regeneration. Genome editing involves testing different transformation methods such as *Agrobacterium*-mediated transformation, protoplast transformation, and ballistic transformation. If one of these methods is successful in the recalcitrant tree species, as indicated by the expression of optical markers such as RUBY or GFP, vectors for genome editing will be used to knock out selected candidate genes. In addition to simple CRISPR/Cas9 genome editing, other techniques such as the use of Cas12a or D10A nickase are carried out.

In regard to poplar and beech, the aim is to knock out selected candidate genes using CRISPR/Cas editing vectors in order to characterize the involvement of individual genes in drought stress tolerance in trees. Greenhouse experiments, including genome-edited poplars, are being conducted and analyzed under stress conditions using both invasive and non-invasive methods. The results can contribute to breeding research and accelerate the adaptation of trees to climate change.

More information: [www.thuenen.de/genetic-technologies](http://www.thuenen.de/genetic-technologies)