Quercus robur L. (Fagaceae) is a species that occurs in most of Europe and is economically important due to its high-quality timber, which some individuals can live for more than 1000 years. These older specimens are of particular interest, as they managed to survive for so long under environmental conditions that varied over the centuries. This highlights their adaptive abilities and makes them valuable genetic material. However, despite their genetic and historical importance, some ancient individuals over 400 years old have been killed in Poland in recent years as a result of vandalism (e.g., Napoleon oak, Chrobry oak, Mieszko I oak). For this reason, methodologies that allow the cloning and conservation of those forest individuals must be applied.

The conventional methods for propagating clonally *Q. robur* are inefficient. Such response is related to the rooting ability of shoot cuttings, and the rhizogenesis capacity can decrease as donors age (Aumond et al. 2017). Thus, tissue culture techniques can be an alternative to cloning these ancient individuals. However, after *in vitro* establishment, other factors must also be considered, including minimizing plant material manipulation through frequent transfers to a new medium and losing viability during this process. For *Q. robur*, it is reported that transfer to a fresh medium takes between 4 and 6 weeks. Subcultures in a fresh medium are necessary to avoid phenolic oxidation and death of plant material. This high handling demand can also increase the chances of plant material contamination *in vitro* and maintenance costs. Therefore, changes in environmental cultivation conditions that induce a lower growth rate and increase permanence in the same cultivation medium, also known as slow-growth storage, should be explored. This approach has a primary goal of diminishing the intervals between the periodic transfer of explants to new containers and fresh medium (subculturing) and can last from a few weeks to over a year (Benelli et al. 2022).

Slow-growth storage has shown promising results; however, it can lead *in vitro* shoots to exhibit signs of stress, such as necrosis and low regrowth ability. The 6-benzylaminopurine (BAP) supplementation into the culture medium has been adopted for most of *Quercus* (Capuana and Lonardo 2013). This cytokinin may help preserve chlorophyll content and hormonal balance. Nevertheless, BAP can induce callus in the explants' base, which produces an enormous quantity of phenolic compound, leading to a phenol oxidation into the medium (Jaiswal et al. 2021). Adding activated charcoal (AC) to the medium can reduce phenolic oxidation (Oliveira et al. 2022). However, its use may decrease the BAP's effectiveness or alter the hormonal balance of explants.

By implementing this scientific activity, I plan to verify the following hypotheses: **high BAP concentration can induce higher leaf longevity and better hormonal balance during slow-growth conditions (i); AC can prolong slow-growth storage (ii); The viability of explants subjected to long periods of slow-growth storage conditions is only possible when BAP and AC act synergistically (iii)**.

In this study, I plan to use clones of an 800-year-old tree (Rogalin Landscape Park, Poland) already established and maintained *in vitro* in a multiplication medium (3.5 µM BAP + 30 g L^{-1} sucrose + 7 g L^{-1} agar). The segments will be cultured in a culture medium supplemented with 40 g L^{-1} sucrose and 7 g L^{-1} agar. Treatments will consist of two concentrations of BAP (3.5 and 7.0 µM) combined with two levels of AC (0 and 2 g L^{-1}). After transfer to the culture media, the plant material will be kept in a growth room at 21ºC for seven days and then transferred for 48 hours to 15ºC. Finally, the plant material will be cultivated in the dark and under 3ºC or 10ºC in growth chambers. To identify slow-growth conditions for as long as possible, part of the plant material from each treatment will be collected every 45 days of cultivation and over 135 days. Time 0 (zero) will be considered the moment of cultivation for 48 hours under 15ºC. After each slow-growth storage interval (0, 45, 90, and 135 days), part of the explants from each treatment will be transferred to the multiplication culture medium to quantify whether the organogenic capacity and regrowth were maintained. To identify the effects of treatments on the plant material, physiological analyses will be performed immediately after the intervals of slow-growth conditions and after 45 days of regrowth (as shown in Fig. 1). The physiological status of the plant material in each treatment and at each interval will be determined by the content of proline, H_2O_2, and photosynthetic pigments, as well as the hormonal profile and performance of the photosynthetic apparatus. Explants from the multiplication medium will be used as a control. **Aumond Jr, M.L., et al. 2017**. Front. Plant Sci. 8:1734. **Benelli, C., et al. 2022**. Plants, 11:3188. **Capuana, M., Lonardo, S. 2013**. In Vitro Cell. Dev. Biol-Plant, 49:605-610**. Jaiswal, N., at al. 2021**. Vegetos, 34:495-504. **Oliveira, L. Et al. 2022**. J. For. Res. 33:1573-1584.

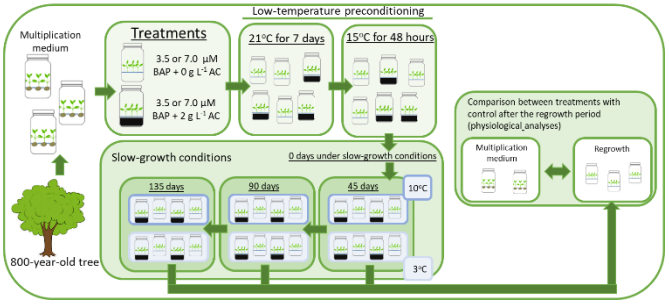

Figure 1. Planned experiments.