• objectives, description of the problem to be solved, ■ description of tools and/or research/didactic methods, • justification of choosing the host institution, adequate research and mentoring support, the research achievements of the host institution in the field of the planned research)

• expected impact of the planned research on the development of science, civilization and society, etc.

[Quercus robur L., known as pedunculate or English oak is a large, rugged, deciduous broadleaved tree, native to most of Europe. This plant species is economically and ecologically important as a hardwood tree species in European forests and has been greatly used by industrialization and the expansion of cropland and urban areas. In addition, there are age-old historic oaks growing in Poland, ranging from 500 to 800 years. These trees are actually biological monuments to Polish history. The propagation of

\ oaks by seeds may be challenged due to their short period of viability. This is because the seeds have embryonic axes with sensitivity to dehydration (recalcitrant seeds), j Plant tissue culture techniques offer an alternative for large-scale propagation of economic interest plants. Furthermore, these techniques allow the ex situ conservation of i specific species or genotypes, such as for Quercus species. In the literature, there are some reports of in vitro propagation of Quercus. These studies with in vitro culture of

Quercus were focused on multiplication and rooting rates during micropropagation and in vitro conservation, so the researchers did not pay attention to anatomical changes of roots and leaves of in vitro plants as well as their physiological status in a deeper level, e.g., how the in vitro conditions can affect the performance of photosynthetic apparatus : of plants. These changes can interfere significantly during the acclimatization phase. Although tissue culture is a useful tool for conservation and multiplication of plant species : but it has been a challenge with Quercus species, often resulting in low growth and survival. It could be related to anatomical and physiological disorders induced in function of in vitro conditions.

j The environmental conditions of conventional in vitro culture consist of sealed containers, with high relative humidity, reduced gas exchange, and artificial temperature and luminosity conditions. In addition, inside the containers, high variations in CO2 and ethylene accumulation may occur during the day. In general, these conventional in vitro propagation techniques are carried out by adding sugars to the medium as the main carbon source. Such in vitro condition can result in low photosynthetic activity and chlorosis of plants, and it is considered to be one of the major limiting factors of micropropagation efficiency. This may also induce other physiological alterations and anatomical disorders in plants cultivated in vitro, such as decreased photosynthetic pigment content and poor leaf tissue function. The main exogenous carbohydrate source employed in vitro culture is sucrose, but its effects on plant development and growth remain highly varied. Exogenous sucrose at moderate concentration may decrease photosynthesis during in vitro culture, but it can increase the survival rate after the transfer to ex vitro conditions. In fact, a critical aspect of in vitro propagation is related to the survival capacity of micropropagated plants outside the culture containers.

The supplementation of plant growth regulators (PGRs), such as cytokinins and auxins, can also influence the plant in vitro morphogenesis. The optimal concentrations and combinations of PGRs differ by species and among tissues used as explants. Physiological disorders, as well as anatomical changes in the plants' leaves induced by exogenous cytokinins, have already been reported. Likewise, use of synthetic cytokinins during in vitro propagation induced long-term physiological defects, even after 120 days without its direct exposure. In contrast, direct exposure of a synthetic auxin, applied

as an isolated PGR during in vitro rooting, may improve the anatomical and physiological quality of plants, as well as improving their growth rate after the ex vitro transfer. However, the physiological and anatomical responses induced by PGRs during in vitro culture remain still not well clear.

Therefore, taking into account that in vitro culture conditions can interfere significantly with the regulation of in vitro growth and development, including the quality and physiological status, as well as anatomical traits of micropropagated plants. The aim of this project will be to analyze the impacts of in vitro conditions on the anatomy and photosynthetic performance of in vitro-propagated Q. robur plants, with the purpose of elucidating and solving the problems related to losses in the acclimatization phase.

! Methods

Experiment 1 - The first experiment will involve the verification of physiological and anatomical changes as a function of concentrations of synthetic cytokinin. Plants previously established in vitro and with similar morphology will be used as explants. The plants will be transferred to containers containing woody plant medium (WPM) supplemented with 6-benzylaminopurine (BAP) at different concentrations (pM). The experiment will be conducted with five plants per glass container. After inoculation in the laminar flow cabinet, the plant material will be placed in a growth room with controlled photoperiod and temperature. Analyses of enzymatic activity of the antioxidant systems (Elisa Spectrophotometer), photosynthetic apparatus performance (chlorophyll fluorimeter) and, the anatomy of leaves and stem (light microscopy) will be performed at the end of the experiment. From the results, we will be able to understand the possible disorders of plants under the most import phase of in vitro culture (multiplication).

Experiment 2 - The best treatment obtained from the first experiment will be performed over again. Then the side shoots formed in the explants' base will be cultured in WPM supplemented with indole-3-butyric acid (IBA) or 1-naphthaleneacetic acid (NAA), at different concentrations (pM). The experiment will be conducted with five plants per glass container. The plant material will also be placed in a growth room with controlled photoperiod and temperature. Analyses of photosynthetic apparatus performance (chlorophyll fluorimeter), leaf photosynthetic gas exchange (IRGA) and, the anatomy of leaves, stem and, roots (light microscopy) will be performed at the end of the experiment. From the results, we will be able to understand if auxin supplementation in the medium can partially reverse the possible negative effects induced by BAP during the in vitro multiplication phase as well as to verify the exogenous-auxin dependence to induce the formation of a functional root system.

Experiment 3 - The third experiment will be performed with the purpose of produce plants with higher quality by improving their photosynthetic rate as well as their functional anatomy of in vitro plants. Therefore, the best auxin concentration verified from the second experiment's results will be taken into account. The side shoots obtained from the best treatment of the first experiment will be used as explants sources. The side shoots will be transferred to 1000 mL polypropylene containers (Microbox Combiness®) and containing WPM with different sucrose concentrations. Two different sealing systems will be tested: lids with an XL yellow filter (permitted gas exchange system), and lids with an XL yellow filter covered with three layers of polyvinylchloride (PVC) transparent film (blocking fluent gas exchange). In addition, the culture containers containing the plants will be placed into acrylic boxes with forced ventilation: one with the atmospheric concentration of CO2 and the other with external input of CO2. Analyses of photosynthetic apparatus performance (chlorophyll fluorimeter), leaf photosynthetic gas exchange (IRGA), the anatomy of leaves, stem and, roots (light microscopy) and, CO2 and ethylene content inside of culture containers (gas chromatography) will be

performed at the end of the experiment. To evaluate growth after the acclimatization period, plants from each treatment will be transferred to transplanted to plastic pots containing a potting medium and kept covered with transparent polyethylene plastic lids in order to maintain high relative humidity for 1 week. After this period lids will be removed.

Statistical analyses - All experiments will be properly delineated, containing repetitions and respecting statistical predispositions. The obtained data will be submitted to analysis of variance (ANOVA) and the averages will be compared by the test of Tukey to 5% of probability.

After all the experiments were done, we might talk about new possibilities of the additional epigenetic research in collaboration with the Institute.

The Institute of Dendrology of the Polish Academy of Sciences is a Polish center of multidisciplinary research on trees species and has offered great contributions on plant sciences. In the Laboratory of Reproduction Biology and Population Genetics has been working successfully on the improvement of in vitro propagation and conservation of many woody plant species. Paweł Chmielarz works as an associate professor at the same research institution and he is the head of the Laboratory of Reproduction Biology and Population Genetics. So far, he has approved several projects with financial sources, which allowed the development of research at various levels in the area of woody plants as well as the publication of articles in scientific journals with high impact factor. Recently, this his lab successfully cloned, by in vitro techniques, some age-old historic oaks. It was a great step for this species conservation. However, researches focused on the factors that modulate the physiological and anatomical responses of in vitro plants have not yet been performed. Such studies can have an optic as the practical use, developing research actually applied to the present days. Moreover, the production of Q. robur plants with high quality by in vitro culture can offer a safe way of germplasm conservation of this plant species so important in both economic, ecological and historical levels in Poland.