INDUCING *IN VITRO* REGENERATION FOR OVERCOMING RECALCITRANCE IN WOODY PLANTS

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Summary

The induction of *in vitro* regenerative ability of *Quercus robur* L., *Tilia platyphyllos* Scop. and *Acer platanoides* L. nodal stem segments was studied. A two-step regeneration stabilization procedure can overcome resistance and provide actively growing microshoots on Woody Plant Medium for further studies.

Introduction

Recalcitrance is the biological resistance/inability of plant cells or tissues to regenerate *in vitro* is often related to genetic factors (McCown 2000). Woody plants with a strongly episodic type of growth have low proliferative activity, so it is very difficult to achieve uniform, continuous growth of microshoots *in vitro* (McCown 2000). With age, the regenerative ability decreases, so it is difficult to reproduce old plants (Chmielarz et al. 2023). According to Nelson et al. (2025), to overcome recalcitrance, it is necessary to activate regeneration genes. For this purpose, it is necessary to develop individual procedures for stabilizing the growth of *in vitro* plant tissues. In previous studies, microshoots were obtained from 120-year-old English oak (*Quercus robur* L.), 50-year-old Norway maple (*Acer platanoides* L.) and 30-year-old large-leaved linden (*Tilia platyphyllos* Scop.), which have a low regeneration ability. The aim of this study is to induce regeneration of *Q. robur, A. platanoides* and *T. platyphyllos in vitro* explants for micropropagation.

Figure 1. *In vitro Acer platanoides* in the laboratory plant bank

Figure 2. *In vitro Quercus robur* plants

Methodology

For the studies, 1.0–1.5 cm nodal stem segments from aseptic *Q. robur, A. platanoides, T. platyphyllos* plants were used. The studies were carried out in July. A two-stage stabilization procedure was used. At the first stage, the explants were cultivated on the basic hormone-free Woody Plant Medium (WPM) (McCown & Lloyd 1981) in Petri dishes (8–10 pcs.) in a horizontal position at a temperature of +4±1℃ in the dark for 3 days. At the second stage, they were cultivated in a vertical position on WPM with 6 consecutive subcultivations for 18 days. A medium supplemented with 2.0 g/l activated charcoal and 2.0 mg/l povidone was used (3 days); thereafter the explants were subcultured on medium supplemented with 0.25−0.50 mg/l kinetin (3 days); the medium was changed every 3 days for 18 days. All nutrient media were supplemented with 100 mg/l inositol, 30 g/l sucrose, and 7.0−7.3 g/l microbiological agar, the pH adjusted to the level of 5.7–5.9. The plant material was cultivated in a laboratory room at a temperature of 24±1 °C and illumination of 2.0−3.0 klx with a 16-hour photoperiod and a relative humidity of 70–75%.

Published scientific papers

1. Chornobrov, O. (2019). *Optimization of in vitro tissues growth of Quercus robur L. plants*. Conservation of Forest Genetic Resources: 6th International Conference, Shchuchinsk, Kazakhstan. P. 257-258.
2. Chornobrov, O. (2021). Optimisation of the protocol for sterilising explants of some deciduous woody plants in culture *in vitro*. *Ukrainian Journal of Forest and Wood Science, 12*(3), 80-86.
3. Patent of Ukraine (utility model) № 133406, IPC (2019.01), A01H 4/00. The method of obtaining aseptic culture of English Oak plants fragments shoots (*Quercus robur* L.). Inventors: Chornobrov O.Yu., Bilous S.Yu., Karpuk A.I., et al. Published 04/10/2019, Bul. № 7. Ukraine.

Results and conclusions

According to the specified cultivation procedure of nodal stem segments a survival rate was 90–100 % on day 6. The percentage of proliferated *Q. robur* explants was 80-100 %, *A. platanoides* – 70-80 %, T. *platyphyllos* – 60-70 % on day 15. The regeneration rate of *Q. robur* explants was 70-80 %, *A. platanoides* – 60-70 %, *T. platyphyllos* – 50-60 % (fig. 3) on day 30. The regeneration rate of explants was 10–20 % without the stabilization procedure (control) on the 30th day of cultivation. Microshoots had typical anatomy, morphology and pigmentation, no vitrification were detected. 60-80 % of *Q. robur* (fig. 2) and 60-70 % of *A. platanoides* (fig. 1) microshoots produced callus tissue of dense consistency and different pigmentation (light green/green/cream) on the 45th day of cultivation. Callus grew at the base of the microshoot. *T. platyphyllos* microshoots did not produce callus. Therefore, the two-stage stabilization procedure can overcome recalcitrance and provide actively growing microshoots for further studies.

Figure 3. *In vitro Tilia platyphyllos* plants

References

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3. Nelson, A., Ranney, T., Liu, W., Kelliher, T., Duan, H., & Da, K. (2025). Overcoming Recalcitrance: A Review of Regeneration Methods and Challenges in Roses. *Plants*, *14*(24), 3797. DOI: 10.3390/plants14243797