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PRESERVATION OF UNIQUE GENOTYPE OF ANCIENT TREES USING IN VITRO CULTURE

S. Bilous, Candidate of Biological Sciences (Ph. D.)

(forest_biotech@nubip.edu.ua)

Yu. Marchuk, Candidate of Agricultural Sciences (Ph. D.)

National University of Life and Environmental Sciences of Ukraine (Kyiv)

In Ukraine, the priority is the preservation of virgin forests and centuries-old trees. However, the area of undisturbed forests is not large, and centuries old trees are concentrated in the central and northern parts.

The mature trees are of great importance for providing a complex of ecosystem services, among which the most important are recreational and biodiversity conservation. Data on the ecosystem of the role of centuries-old trees are very limited and do not allow to form a full-fledged view of the ecological value of such representatives of the plant world.

Due to the natural aging condition, mature and ancient trees were characterized by high risk of extinction because of biotic, abiotic and anthropogenic factors and age.

The main attention is focused on micropropagation the oldest trees of Oaks in Ukraine, to study anatomical and microbiology peculiarities of explants on the separate stages.

As a source of explants were used ancient trees such as Oak of T. Shevchenko, (Kyiv) age over 300 years, Oak of M. Zalizniak, (Cherkasy region) age over 1000 years. Most of these trees have preservation status. At first stages all explants were characterized by oxidation of plant tissues, because of phenolic activity.

Plant material was cultivated in a light room at a temperature of 25 ± 1 °C and illumination of 2.0–3.0 lx with a 16-hour photoperiod and a relative humidity of 70–75%.

After obtaining aseptic viable plant materials with using a range of antioxidants), they have been cutting on 1.0-1.2 cm fragments and transferred to the modified nutrient medium.

To increase the morphogenetic capacity of explants and to regulate the processes of morphogenesis nutrient media supplemented with cytokinin: 6-benzylaminopurine (BAP) 0.5 mg/l, thidiazuron (TDZ) (0.2-5.0 mg/l), pH value of the medium was 5.7 [1, 3].

Every 5 days (for 2 weeks of culture in vitro) the nodal explants were transferred to fresh WPM medium [2] with 0.2-0.5 TDZ and complex of antioxidants. Shoot tips and nodal explants (0.5-1.0 cm long) that developed

on the initial nodal segments were subjected to successive subculturing on WPM medium every 4 to 5 weeks.

The optimal explants for introduction to the culture in vitro were as winter shoots and awakening shoots had been getting from deferred shoot.

In spring-summer period the quantity of hormones, which put into the media equalize 0.2 mg/l, in autumn and winter period the necessity of supplemented of cytokinins become more important. In such a way into nutrient medium have been added 0.5 mg/l TDZ and 0.1 mg/l NAA with addition of Fe-EDDHA 4,8 % in certain stage of morphogenesis induction. Such treatment has been ensured the obtaining of a stable growing oak culture derived from old trees. The index of formation of primary microshoots from one explant reached 3-4 pcs. The frequency of formation of new microshoots reached 75.0 %.

The best organogenic potential and stable growing oak culture derived from old trees was achieved when shoot tips, were cultured for-4- weeks on WP medium supplemented with 0.5 mg/l TDZ and 0.1 mg/l NAA, Fe-EDDHA 4,8 % in certain stage of morphogenesis induction.

Active in vitro shoot formation was recorded on MS media with addition of 1.0-2.0 mg/l 2iP (6- (γ, γ -dimethylamine) purine) and 20 mg/l - adenine. Single root rooting was observed in the spring period under the condition of cultivation on MS, WPM media with the addition of 0.25-0.5 mg/l 6-(furfurylamino) purine (kinetin) activated carbon 1-2 g/l and modified antioxidant complex.

In the autumn period, a significant decrease in the regenerative ability of microshoots were observed on all studied medium variants, which was demonstrated by a decreasing in the monthly average amount of growth and the number of internodes formed.

In the winter period, yellowing of individual leaves was noted with their subsequent fall, while the base of the microbusiness acquired dark pigmentation. Antioxidants and a frequent subculture for fresh nutrients were used to stabilize the growth of microwaves (the cultivation cycle was 3-5 days), as well as alternating hormonal and non-hormonal media.

References

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