Microclonal Propagation Of Centries Old Oaks of Holosiev Forest

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Due to the natural aging condition mature and ancient trees are characterized by high risk of extinction as a result of biotic, abiotic and anthropogenic factors and age.

The practical significance of the work is to search new ways and methods of preservation old and ancient trees of Ukraine using modified approaches of plant biotechnology, which is one of way to maintaining the unique gene pool of mature, ancient and unique woody plants.

Keywords – centuries old oak, explant, microclonal propagation, regeneration, in vitro

Introduction

There are many national treasures in Ukraine, one of which is the historical centuries-old trees – the symbols of the natural heritage of the country. Changing for a long time in stressful environments, these organisms provide a unique opportunity to study the evolutionary processes of the past and present. In addition to the extremely important ecological function, many historic centuries-old trees also have a significant socio-economic and cultural significance. All this is an enormous potential for the study of valuable cultural heritage, both locally and internationally.

The significance of research is to developed new ways and methods of preservation ancient trees of Ukraine. Due to the natural aging condition this centuries-old trees are characterized by high risk of extinction as a result of biotic, abiotic and anthropogenic factors. Using of biotechnological methods, such as micropropagation can solve the problem of maintaining the unique gene pool of woody plants, study their ecosystem role.

The territory of Holosiyivsky forest, located in the southern part of the city of Kiev, belongs to one of the richest on old trees. Data on the oldest trees of oak (*Quercus robur* L.), their geographical coordinates for every tree, photos and a map of their distribution are provided by some authors [1, 2, 4].

Microclonal propagation technologies of ancient and mature oaks trees have been tested and developed by some of authors [3, 6], but their reproduction on practice have another result, possibly because of a number of difficulties and the specificity of the genotype of a particular tree genotypes.

In this work was shown peculiarities of getting aseptic culture and first stages of micropropagation of old value oak trees, the main approaches for mass micropropagation and getting helthy plant regenerants of old trees.

Methods and research objects

As the source of explants were used old value trees from natural conditions from Holosiev region. Branch tips from the lower branches of oak (*Quercus robur* L.) trees about 200 years old, and embryos with endosperm fragments, shoots and leaf blades, which were selected from 70

and 120 summer *Q. robur* donor plants in January-February and March-April 2016–2018were collected and used for awakening in the thermostat.

To sterilize the plant material were used next solutions: 70% ethyl alcohol, 0.1% $HgCl_2$, 1.0% $AgNO_3$ and 2.5% NaClO. The explants were cultivated according to the generally accepted method [5] on a nutrient medium according to the Murashige and Skoog (MS) and Wood Plant Media (WPM) formulations [6]. In our modified media, growth regulators of auxin and cytokinin type of action were introduced, 100 mg/l mesoinositol, 30 g/l sucrose, and 7.0–7.3 g/l microbiological agar. Additionaly were used as antioxidants: activated carbon, glutathione, glycine and polyvinylpyrolidone (PVP). As a control, the hormone-free nutrient medium MS was used.

Plant material was cultivated in a light room at a temperature of 25 ± 1 °C and illumination of 2.0–3.0 klx 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.

Every 5 days (for 2 weeks of culture in vitro) the nodal explants were transferred to fresh WPM medium 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.

Results and discussion

At first stages all explants were characterized by oxidation of plant tissues, because of phenolic activity.

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 %.

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

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 internode 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.

Conclusion

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.

In result, was determined that organogenesis in culture of mature oaks depend on the range of factors such as: the type of primary shoots of the donor plant, originating on the first stages and influence the exogenous balance of growth regulators, and consequently, the response to the evaluated morphogenic processes, the phonological phase, the quantitative and qualitative ratio of growth regulators in the medium, influence of the genotype. Also, has been mention that the juvenile explants have more ability to indirect morphogenesis in the early stages of reproduction.

Development of new methods involves needs for an individual selection of nutrient medium for the cultivation of different explants at every subsequent stage of reproduction.

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