Preservation of Ancient and Old Value Trees of Ukraine with Using Biotechnology Methods

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Abstract – the practical significance of the work is to search 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 and development of ornamental horticulture. In this work was shown peculiarities of getting aseptic culture and first stages of micropropagation of ancient trees..

Keywords – Ancient trees, explant, regeneration, in vitro

I. Introduction

In connection with the natural aging process, the condition of centuries-old trees is characterized by a high risk of death due to the action of biotic, abiotic and anthropogenic factors. The death or destruction of centuries-old trees will lead to a permanent loss of their historical and biological value associated with outstanding events and personalities, the processes of development of society, culture and art. Furthemore, such trees are having unique importance in terms of studying their ecosystem role and the history of ecosystem functioning. Most of the centuries-old trees are a natural heritage and heritage, the importance of which can not be overestimated for cultural and spiritual development [2, 10].

The progress of modern biotechnologies can solve the problem of preserving the unique gene pool of such plants, but such development is very difficult to create in connection with the biological characteristics of objects. One of such problems is obtaining aseptic culture of centuries-old trees.

In Ukraine, the experience of the study of centuries-old trees by biotechnological methods is rather limited [1, 2], there is practically no data on microclonal reproduction of centuries-old trees, there are no attempts of DNA-certification unique historical-valuable trees to analyze the possibilities of establishing their evolutionary-ecological features.

A comparatively large contribution to the development of research of ancient trees in Ukraine was carried out by the scientists of the Institute of Evolutionary Ecology of the National Academy of Sciences of Ukraine who conducted an inventory of age-old oak trees on the territory of the memorial-park of national significance "Feofaniya" and created a map of their location [3], and investigated the features of their growth [7].

The group of researchers led by A. I. Kushnir [6], whose efforts are directed to the development of technical solutions and means for healing centuries-old trees in Ukraine, deals with the preservation and treatment of centuries-old trees.

An important experience of preserving centuries-old trees and historical plantations was formed in the dendrological park "Olexandria" of the NAS of Ukraine [4]. Scientists have shown the richness of the centuries-old trees of the dendrological park and their role in the formation of the landscape.

Most of scientific works devoted to centuries-old trees are the results of dendrochronological studies of the history of climate and the evolution of ecosystems [8, 9].

According to the data of the re-calculation of territories and objects of the natural reserve fund of national and local significance [5] in Ukraine more than 600 centuries-old, historically valuable and unique trees have been identified. Such trees and groups of trees have, generally, the status of a botanical nature monument. Almost 70% of the total number of valuable centuries-old trees and their groups are represented by common oak (Quercus robur L.), and the third part of the total number of centuries-old trees is the heart-shaped lime (Tilia cordata Mill.), Pinus sylvestris L., (Fagus sylvatica L.), Platanus Orientalis (Platanus orientalis L.), Spruce (Picea abies (L.) H.Karst.), European Larix (Larix decidua Mill.), Acer platanoides L., Walnut (Juglans regia L.) and other species. There are single specimens of centuries-old apple trees (Malus P. Mill.) And pears (Pyrus L.). Obviously, the list does not include all existing centuries-old, historically valuable and unique trees of Ukraine and will gradually be supplemented.

II. Methods and research objects

As the source of explants were used ancient trees from natural conditions such as: Linden of P. Mogulu, (Kyiv) age over 400 years, Linden of T. Shevchenko, (Chernihiv region) age over 600 years, Oak of T. Shevchenko, (Kyiv) age over 300 years, Oak of M. Zalizniak, (Cherkasy region) age over 1000 years.

In researches, optimal explants for introduction to the culture in vitro of centuries-old trees were as winter shoots and awakening shoots had been getting from deferred shoot in control laboratory condition.

III. Results and discussion

As a result, for winter shoots the most effective is sterilization with using shoots (3.0–5.0 cm) which have washed under soapy water 20 min, and then immersed in 75% (v/v) alcohol for 30 s, before surface sterilization in 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for 8–10 min.

On another way for shoots of centuries-old trees, which have been awakening were used sterilization solution of 25% (w/v) perhydrol (H_2O_2) for 7-10 min. After being washed three times with sterile distilled water (5 min) all shoot tips (0.5–1.5 cm) and nodal segments (0.5–1.0 cm) were excised and implanted to MS medium [12] for culture initiation. All nutrition medium have contained 30 g/l sucrose, 1 g/l glytation and 6.5 g/l agar. The pH of medium was adjusted to 5.7-5.8. All cultures have maintained in a growth chamber at 24–25 °C under cool, white fluorescent lamps with 16 h photoperiod.

After 3-4 weeks have recieved 70-80% of aseptic explants developed into 2.0–3.0 cm shoots, which were used for the following studies.

Conclusion

The centuries-old 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 such a way introduction of biotechnologies methods in practice of preservation of ancient trees have significant role and important for future research.

References

[1] S. Bilous, “Biotekhnolohichni aspekty rozmnozhennia velykovikovoho dereva duba Maksyma Zalizniaka v umovakh in vitro”, NUBiP, Forestry and gardening, No. 2., 2012. Mode of access http://nbuv.gov.ua/j-pdf/licgoc_2012_2_3.pdf.

[2] S. Bilous, “Osoblyvosti otrymannia aseptychnoi kultury bahatovikovoho dereva Tilia cordata Mill. “Lypy T.H. Shevchenka”, Naukovyi visnyk NUBiP Ukrainy. Ser.: Lisivnytstvo ta dekoratyvne sadivnytstvo. Vyp. 187, ch. 3., pp. 213-217, 2013.

[3] R. K. Matiashuk, V. B. Nebesnyi, S. M. Koniakin, “Vikovi duby «Feofanii» – pam’iatky zhyvoi pryrody kraiu”, Naukovi dopovidi Natsionalnoho universytetu bioresursiv – pryrodokorystuvannia Ukrainy, 2014. № 6. Rezhym dostupu: http://nbuv.gov.ua/UJRN/Nd_2014_6_18

[4] S.I. Halkin, N. V. Drahan, N. M. Doiko, “Dosvid zberezhennia vikovykh derev ta istorychnykh nasadzhen u dendrolohichnomu parku «Oleksandriia»” NAN Ukrainy, Introduktsiia roslyn, № 4. – pp. 42–50, 2013.

[5] Zvitni materialy Derzhavnoho kadastru pryrodno-zapovidnoho fondu Ukrainy stanom na 01.01.2013 [Elektronnyi resurs], Rezhym dostupu do resursu: http://pzf.menr.gov.ua, 2017.

[6] A.I. Kushnir, O. A. Sukhanova, I.L. Kushnir “Naukove zakliuchennia stanu unikalnoi pam’iatky yevropeiskoi i svitovoi pryrody bahatovikovoho istorychnoho dereva – «Duba Maksyma Zalizniaka» i neobkhidni zakhody shchodo yoho likuvannia”, Kyiv: NUBiP Ukrainy, 25 p., 2010.

[7] M. V. Netsvetov, Iu. S. Prokopuk, “Vik i radialnyi pryrist starovikovykh derev Quercus robur parku “Feofaniia”, Ukrainskyi botanichnyi zhurnal, T. 73, № 2., pp. 126–133, 2016.

[8] K. F. Kipfmueller, T. W. Swetnam et al., “Using dendrochronology to reconstruct the history of forest and woodland ecosystems”, The historical ecology handbook: a restorationist's guide to reference ecosystems. Island Press, Washington, DC, P. 199-228, 2001.

[9] M. Netsvetov, M. Sergeyev, V. Nikulina et al., “The climate to growth relationships of pedunculate oak in steppe”, Dendrochronologia, P. 31–38, 2017.

[10] Schnaider S., Boreiko V., Stecenko N. 500 outstanding trees of Ukraine, Kyiv Ecological and Cultural Center, State Service for Protected Areas of Ukraine, 204 P., 2011.

[11] A. Traore, Z Xing., A., Bonser J. Carlson Optimizing a protocol for sterilization and in vitro establishment of vegetative buds from mature douglas fir trees, Hort Science 40 (5), 1464-1468, 2005.

[12] T. Murashige, F. Scoog, “A revised medium for rapid, grow than dbioassays with tobacco tissue cultures”, Physiol. plant. 15, 473–497, 1962.