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RESTORATION OF PERSPECTIVE WOOD-BRUSH GENOTYPES

Abstract. This paper describes the method of microclonal reproduction of valuable genotypes of woody plants for the conservation and restoration of growing regions, and also for the use as a green belt of urban massifs. In this study, meristem was used as an explant, which was cultured on various media, supplemented by various concentrations and combinations of cytokinins and auxins. Where the use of 0.2 mg /l BAP and 0.1 mg /l IAA provided better shoot proliferation and shoot tips growth when cultured on WPM medium. As a result, there was an increase in callus and the number of regenerated shoots from 4 to 8 shoots in half the WPM medium. Root formation was observed on the hormone-free WPM medium. However, the results showed that proliferation also depends on the genotype of the plant.

Keywords: arboreal and shrubby plants, genotype, microclonal reproduction.

Introduction. Conservation of biological diversity occupies a special place among the global problems of our time. The basis of biological diversity is its genetic component [1-5]. Reducing specific and genetic diversity poses a real threat to the biosphere, since the sustainability of the reproduction of natural ecosystems and agro ecosystems is directly related to their genetically determined potential for adaptation to changing environmental conditions [6-8]. For the first time the concept of the necessity to control and mobilize world plant resources was developed by N. I. Vavilov, which formed the basis for systematic work on the creation of plant resources banks in different countries.

A serious problem with the natural existence of forests and their genetic resources in Kazakhstan is the growing trend in the ageing of plantations. According to the data of the scientific and production center of forestry, even in conditions of specially protected natural areas, middle-aged and ripening plantations are most widely represented.

Therefore, the development and implementation of a method for the long-term conservation of the woody plants gene pool in the Mangistau region and the technology of cultivation accelerated of quality planting material, as forest-forming species of Kazakhstan, valuable invasive plants and natural populations that are on the verge of extinction, are particularly topical and priority areas of genetic selection studies in forestry.

The selective genetic study of the effectiveness of alternative methods of reproduction of wood (by the method of microclonal reproduction) is very relevant, as it allows to expand the methods of reproduction and alternative methods of preserving genetically unique genotypes and economically valuable forms of tree cultures.

The development of a method for clonal micropropagation for the long-term preservation of tree species in order to preserve their gene pool, as well as reproduction after prolonged conservation, is a very topical genetic-physiological and selection-seed problems of forest growing, in particular in cases of rare yield and ageing of plants, which is exciting issue in forestry.

Currently, many forms and species of woody crops are on the verge of extinction due to the deteriorating environmental conditions and irrational economic activities of man.

The study of tree species in tissue culture *in vitro* currently combines both fundamental and applied research in this field. The most priority is the study of the biology of the trees cultivated tissue develop-

ment in culture in vitro, as well as the features of the morphogenesis process passing in hormonal and non-hormonal regulation.

Tissue culture, cell selection with trained staff and a suitable laboratory base, and a greenhouse allows replicating valuable genotypes of forest cultures in a short time. An excellent example of this is the experience of German scientists who created triploid clones of aspen. Within 18 months, under *in vitro* culture, 50,000 copies of the recovered material were obtained from one young initial.

There are two approaches of the long-term conservation of the genetic resources of tree species: *in situ* conservation - ensuring the conservation of genetic reserves in natural populations (habitats); *ex situ* conservation - uterine and forest seed plots, clone archives, germplasm bank in the form of seeds. Genetic collections in the form of tube cultures, their individual organs, tissues and even cells can be supported: a method of regular subcultivation under conditions of normal growth (transplant cultures); deposition at a lower temperature, the addition of preservatives (retardants or osmotically active substances) that limit growth; storage for many years at ultra-low temperatures (at liquid nitrogen temperature -196 °C), complete absence of growth in cryobanks [7, 8].

However, it should be noted that each method has its advantages and disadvantages. Thus, prolonged and frequent subcultivation (once every 1-2 months) on nutrient media enriched with phytohormones increases the content of the collection and may lead to a change in its genetic stability, viability and loss of valuable traits [7]. A high content of retardants or osmotically active substances (for example: sucrose) in a nutrient medium can be a mutagenic factor.

Cryo-conservation in liquid nitrogen is a rather expensive and time-consuming method. In addition, cryopreservation methods have not been sufficiently developed for universal application [9], and for most hardwood species it is at the research stage.

Explants on such media take the form of the small shoots bundles, each of which can be cloned and regenerated. Excessive increase in the concentration of cytokinins in the medium, in order to obtain the maximum reproduction coefficient, causes such undesirable effects as a change in plant morphology, suppression of the axillary meristems proliferation, and a decrease in the ability of shoots to root. The use of nutrient media with the minimum concentration of cytokinins that provides a sufficient rate of micropropagation, as well as the alternation of culture cycles on media with low and high levels of phytohormones, helps to avoid the toxic effect of cytokinins due to their constant presence in the nutrient medium.

This method, according to researchers, has a minimum degree of risk with respect to the production of heterogeneous progeny, and the frequency of mutant plants appearance does not exceed the frequency of their occurrence during normal reproduction [15-18].

The results of studies with productive triploid forms of aspen, turangi, nitraria, taxus baccata, paulownia, birch, berberis showed that the microclonal propagation method is effective not only for mass replicating of valuable genotypes, but also for the manifestation at an earlier age of economically valuable traits - accelerated passage of individual stages of ontogeny [9].

For most cultures, the induction of morphogenesis and the regeneration of plants do not present great difficulties. However, the significant dependence of these processes on the characteristics of the genotype, plants age and growing conditions, require special developments for individual forms, clones and genotypes, and other species.

Modifications of the methods of the foreign researches majority can be used in works on conservation and reproduction of rare and valuable tree species, as well as conservation of biological diversity of the plant species under study, which grow in Kazakhstan.

The analysis of literature data shows that the development of a reproduction method for each particular object requires further experiments and complicated manipulations with the material being studied, since the technology and type of reproduction may be specific not only for individual species but also for individual forest tree genotypes [7-20].

Methods. The objects of research are tree-shrubby plants that are on the verge of extinction in the collection of the Mangyshlak experimental botanical garden: *Crataegus ambigua, Nitraria schoberi, Pópulus trémula, turanga, paulownia tomentoza, Quércus castaneifólia, Paliurus spina-christi, Cotoneaster microphyllus Wall. ex Lindl., Bétula péndula, Berberis integerrima Bunge.* The starting materials were lignified branches cut from adult trees (ages are 20-30 years) in February and late March - early

April. Apical and axillary buds were used as explants for *in vitro* cultivation. Branches with kidneys were put in a glass with water until the appearance of a green cone.

Whiteness and hydrogen peroxide served as a sterilizing agent to produce an aseptic culture. The segments were washed in a soap solution for 5 minutes, kept under running water for 2 minutes, and this procedure was repeated 3 times.

The subsequent stages of sterilization were carried out in a laminar box, the explants were placed in a sterile boiling flask and filled with a sterilizing solution. The following sterilization options were used: 10% hydrogen peroxide with a temporary exposure of 5, 10 and 15 minutes. Whiteness solution - 1%, 20% or 50%, treatment time 4 and 8 min. It was dried on a filter paper and transferred to a pet container with a nutrient medium.

The method of introduction and cultivation of explants was carried out according to R.G. Butenko [12-18]. Also, the selection of the nutrient medium by modification of the composition and concentration was carried out experimentally.

After analyzing the literature works that were carried out similar studies, we decided on approbation of the nutrient medium, which is the most optimal for induction of kidney growth - the WPM environment (Woody Plant Medium) и MS (Murashigi and Scoog).

Environment variants used in the work: 1) MS 0.1-2.5mg/l 2.4Д + 0.1-1.5 mg/l BAP; 2) WPM 0.1-1 mg/l BAP + 0.1-1.5 mg/l Kinetin; 3) ½ WPM IBA 0.2 mg/l + μ WK 0.1 mg/l; 4) ¼ WPM IBA 0.01-5 mg/l; 5) ½WPM 0.01-11 mg/l BAP; 6) MS 0.01-1.5 mg/l BAP; 7) MS 0.01-1.2 mg/l 2.4-D; 8) ½ WPM 0.05-5 mg/l 2.4-D + 0.01-2 mg/l BAP; 9) ½ MS 0.05-2 mg/l 2.4-D; 10) ½ MS 2 mg/l BAP; 11) ½ MS 1 mg/l BAP + 3 mg/l IAA; 12) ½ WPM without hormonal; 13) WPM without hormonal. The nutrient medium is poured over the pet containers and autoclaved at 1 atm, at a temperature of 120°C for 15 minutes. Then, the explants are planted, the cultivation is carried out under luminescent illumination of 10.000 lux, a 16-hour photoperiod, at a temperature of 19-21°C. There were assessed the viability, growth and development of explants every week.

The experiments were carried out in 8-10 replicates. The number of explants in one replication was 30 pieces.

After approbation of all disinfecting solutions and selection of nutrient media, it was found that the most effective for the preparation of an aseptic culture as a sterilizing agent was chosen a 10% solution of hydrogen peroxide - 15 minutes, with subsequent washing in three portions of sterile distilled water, in aseptic conditions, the interstices of the shoots were cut into segments of 1-1.2 cm and transferred to a nutrient medium in sterile culture containers. Sterilization of tissues introduced into the culture ensures the improvement of plant material.

The most suitable method of cultivation was the use of apical meristems on a nutrient medium containing phytohormones: 1/2 WPM + BAP in concentration 0.2 mg/l and 0.1 mg/l IAA. After 4 weeks, a morphogenic callus is formed with the kidneys, which then form a set of shoots. For the mass formation of shoots callus must be transplanted to a fresh initial nutrient medium.

The resulting shoots are transplanted to the hormone free medium WPM, where the rhizogenesis occurs (root formation occurs within 1-1.5 months). The last and one of the important stages of obtaining a planting material by the method of microclonal propagation is the transfer of regenerative plants (clones) under non-sterile conditions. Every plant in nature, in a room, office for its existence requires certain conditions. The main of these conditions are moisture, batteries, air, light heat. Since the regenerative plants obtained under sterile conditions experience considerable stress, falling into non-sterile environmental conditions.

For this, plants with rootlets 1.5-2 cm long carefully extracted with tweezers from the agar medium and planted in the soil substrate are covered with agrospanbond (non-woven material) or with a polyethylene film to maintain the high humidity necessary for preserving the turgor.

Depending on the survival of the clones, after 1-2 weeks, the cover is removed and transferred to the daylight of the laboratory. Landing in the open ground is carried out in the spring. When grown in the laboratory for 6 months, the growth of clones in height is 1-1.5 meters.

Results and their discussion. Analysis of literature data shows that the development of a method of reproduction for each particular object requires further experiments and complex manipulations with the

material being studied, since the technology and type of reproduction can be specific not only for individual species but also for individual forest tree genotypes.

It is established that for microbial reproduction of birch as a base, the usage of nutrient medium WPM, ½ WPM + BAP in a concentration of 0.01 mg/l for explants (hawthorn dubious, nitraria shober, aspen, turanga) marked by active morphogenesis, the formation of a bush with numerous shoots. The most optimal environment for rhizogenesis and suitable for all studied species is the non-hormonal WPM medium, which provides the formation of roots (80-85%) from the first passage.

Due to the fact that the wood is cut every year, the use of the fuel material as a building material and fuel material depletes the forest. To this end, it is necessary to create a collection of biomaterials preserving its genetic structure.

In order to realize the maximum genetic effect obtained in breeding programs, and to shorten the time for obtaining improved planting material by 2-3 times, it is necessary to use the microclonal propagation method.

The populations of arboreal and shrubby plants studied by us made it possible to identify model subpopulations with good growth and development parameters. The clones obtained in vitro are mainly represented by forms that have a normal phenotype and development, preserving the specific features of the initial genotypes for growth and development.

The method of long subcultivation on the hormone-free nutrient medium (the transplant frequency once every 4-6 months) will enable mass replication of valuable genetic material at the right time, regardless of the season. The tissue culture, cell selection, in the presence of trained personnel and a suitable laboratory base and greenhouse, will allow replicating valuable genotypes of forest cultures in a short time.

Conclusions. The method of clonal micropropagation developed by us is recommended to be used in selection and genetic improvement and creation of a collection of valuable clones for the formation of plantation plantations. The increase of the forest cover of the Republic's territory can be solved only in a complex way, including through the active introduction of the plantation method of forest management.

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ДАМЫТУШЫЛЫҚ АҒАШ-БҰТА ГЕНОТИПТЕРДІҢ ҚАЛПЫНА КЕЛТІРУ

Аннотация. Жұмыста ағаш өсімдіктердің бағалы генотиптерді сақтау және қалпына келтіру үшін және оларды қалалық алқаптардың жасыл белдеуде қолдану мақсатында микроклоналдық әдісімен көбейтуін сипаттайды. Бұл зерттеуде бастапқы өсімдік, яғни эксплант ретінде әр түрлі қоректік орталарда өскен меристемаларды пайдаланды. Қоректік ортаны ауксин мен цитокин гормондардың түрлі концентрациясын және комбинациясын өзгерткен. WPM қоректік ортада 0,2 мг/л БАП және 0,1мг/л ИУКты қолданған кезде, өсімдіктің өркенің үздік пролиферациясын және төбе бүршіктің өсуін бақыладық. Осының нәтижесінде каллус көбейді және қалпына келген өркеннің саны 4-тен 8-ге деійн өсті. Тамырлардың құруы WPM гармонсыз қоректік ортада бақыланды. Бірақ нәтижелерге сүйінсек, пролиферация өсімдіктің генотипінан да байланысты.

Түйін сөздер: ағаш-бұта өсімдіктер, генотип, микроклоналдық көбейту.

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ВОССТАНОВЛЕНИЕ ПЕРСПЕКТИВНЫХ ДРЕВЕСНО-КУСТАРНИКОВЫХ ГЕНОТИПОВ

Аннотация. В работе описывается метод микроклонального размножения ценных генотипов древесных растений для сохранения и восстановления регионов произрастания, а также для использования в качестве зеленого пояса городских массивов. В этом исследовании в качестве эксплантов использовали меристему, которую культивировали на различных средах, дополняя различными концентрациями и комбинациями

цитокининов и ауксинов. Где, использование 0,2 мг/л БАП и 0,1 мг/л ИУК обеспечило лучшую пролиферацию побегов и рост кончиков побегов при культивировании на среде WPM. В результате происходило увеличение каллуса и количество регенерированных побегов с 4 до 8 побегов на половинной среде WPM. Образование корней наблюдалось на безгормональной среде WPM. Однако результаты показали, что пролиферация также зависит и от генотипа растения.

Ключевые слова: древесно-кустарниковые растения, генотип, микроклональное размножение.

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