Container plant potential of thuja occidentalis for the introduction of explants into in vitro culture during growth attenuation

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Abstract. An audit of the results of the introduction of thuja occidentalis explants under in vitro conditions without sterilisation showed that the investigated mother conifers under the conditions of the Gelendzhik district were significantly infected. It was assumed that the plants were infected under in vivo conditions, i.e. in the mother plant. In the second half of 2023, in the conditions of the Gelendzhik district, the authors set the task to study the features of container maintenance of Thuja occidentalis (Thuja occidentalis) varieties "Columna", "Smaragd" during the fading of growth processes, i.e. in the autumn period.

1 Introduction

Conifers play a positive role in creating a habitat favourable for people. They are used in urban greening. The main physiological role of these green spaces in green building is sanitary and protective, which includes the processes of photosynthesis, respiration, transpiration. Plants are able to reduce noise levels, normalise the temperature regime, reduce the amount of impurities, reduce emissions of heavy metals. In conditions of variability of abiotic factors, plants form an adaptive potential, release phytoncides.

On the basis of literature data, it was established that at the present stage, due to changing climatic conditions, there is a dynamic growth in the number of coniferous plants with obvious signs of desiccation (more than 50%). The qualitative condition of such tree species is determined by the presence of the following signs: poorly developed crown, dryness, and a complex of disease signs.

In Russia, foci of coniferous plant desiccation are spreading rather quickly. The possible cause of this phenomenon may be the influence of increased doses of UV-B radiation. It has been established that the maximum biological response of plants to ultraviolet radiation is manifested in the UV-B region of the spectrum (wavelength range of 280–320 nm).

It is known that increased doses of *UV-B* radiation cause numerous negative reactions in plants, including deterioration of metabolism, photosynthesis and transpiration, growth, and morphogenesis. In the case of coniferous plants, the negative effects caused by *UV-B* radiation can accumulate in the green mass and manifest themselves during the subsequent years [4].

Conservation of the coniferous plant gene pool is of priority importance in conditions of increased pressure on the ecological environment in urbanised areas. Coniferous tree and shrub crops are a significant aspect of design solutions in the sphere of gardening and landscape construction.

Conservation of genetic resources using in vitro plant material deposit banks, genetic passporting and seed certification allows information to be stored in a unique database of planting material [2].

Due to the analysis of literature sources, it can be concluded that most studies recommend selecting shoots from donor conifers during the period of active growth (spring-summer). Explants of vegetative parts of plants are of the greatest interest when conducting research in the field of microclonal propagation of coniferous plants. A very important link in the technology of microclonal propagation is the development of methods of induced organogenesis. The buds of stem organs fully retain genetic stability, so the use of apices as explants of conifers is especially relevant.

Famous domestic scientists, such as V.N. Nenyukhina, O.A. Timofeeva, believe that the season of the year affects the grafting of explants under conditions of modified nutrient media. It is recommended to select microcircle cuttings from summer shoots because of intensive callusogenesis. The optimal period for selection of apical shoots is the spring period of active growth.

Almost all studies on microclonal propagation of conifers confirm a high percentage of infection of explants by fungi and bacteria [6, 8].

The main condition for in vitro cultivation of plant cells, tissues and organs is sterility. As a rule, the sterilisation regime is established experimentally for

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each object. Species resistance to fungal infections allows increasing the yield of healthy microsprouts, improving the quality of sterilisation in the process of microclonal propagation.

The peculiarities of the formation of the variety of ornamental coniferous plantings in the South of Russia have been studied. It was established that evergreen plants are in demand in landscape design, among them the varieties of western thuja Degroots Spire, Mini Smaragd, Smaragd Variegata. Based on the identified features of adaptive and ornamental traits, taking into account the growth characteristics, as well as on the results of practical implementation of landscape projects, the studied varieties were distributed on a 5-point scale according to the directions of their use. Highly decorative varieties of western thuja received a score of 5 points for the possibility of use in the creation of a large number of green building objects [9].

In the second half of 2023, in the conditions of the Gelendzhik district, the task was set to study the agrotechnics of growing container plants of western thuja (Thuja occidentalis) varieties "Columna", "Smaragd" in order to determine the potential for further introduction of explants into in vitro culture during the fading of growth processes, i.e. in autumn. The studied cultures were biologically characterised.

Thuja occidentalis 'Smaragd' is a popular variety of coniferous plants. The crown is cone-shaped, which at ten years of age can reach an average height of 3 m with a crown diameter of 1 m. Plants are characterised by annual growth in height within 20 cm. The optimum distance in a row can be from 0.5 to 3 metres. The needles are shiny scaly emerald green, densely arranged vertically, which does not lose its colour during the year. However, browning may be observed during the period of relative dormancy. A decorative feature is the presence of oblong-ovate strobili of brown colour up to 0.7 cm [5].

Fig. 1. Thuja occidentalis "Smaragd"

Plants are resistant to drought, sunburn, light-loving, shade tolerant, able to adapt to room conditions, prefer fresh loam. In summer, they are responsive to regular rationed watering.

Thuja occidental "Columna" is an evergreen tree with an elegant narrow conical dense crown with a branched root system.

The plants of this variety are characterised by short shoots, tightly adhering to each other. At ten years of age, the crown height can be 3.0 m, the height of an adult plant can reach 10 m with an average crown diameter of 3 metres. Plants are fast-growing, with an average annual growth of 20 cm in height and 7 cm in width. The studied plants of Thuja occidentalis "Columna" are characterised by decorative features, among them red-brown rough bark; shiny dark-green scaly needles densely arranged on shoots. Small brown strobili up to 1 cm on the branches are rare. It is recommended to place plants of this variety at a distance of 0.7 m in a well-lit area or in conditions of semi-shade. The culture prefers loose fertile loams. Plants of the thuja western variety "Columna" are characterised by stress resistance, they can withstand both dry soil and its excessive moisture. At a young age, thuja western "Columna" is responsive to fertilisers, well tolerates decorative pruning. A varietal feature is resistance to sunburn. To protect against spring and summer sunburns, it is recommended to shade the plants under study.

In the natural conditions of the Gelendzhik district, thuja west is low-demanding for soil fertility, responsive to moderate irrigation, winter-hardy [7, 8, 5].

The Black Sea coast is characterised by the absence of sharp boundaries between the seasons of the year [1].

It is known that the climate of the Gelendzhik district is a moderately humid Mediterranean type with hot, dry summers and warm, wet winters.

The beginning of spring corresponds to the transition of mean daily temperature through 0°C to positive values. According to long-term data, this transition is observed in the last decade of February. The next stage of spring warming (transition through 5°C) causes the beginning of vegetation revival. This is most often the second half of March. The end of the autumn period and the transition to winter are usually considered to be the transition of the average daily temperature through 0°C (second decade of December). The winter period can last on average for 75 days. A stable winter is very rare on the Black Sea coast. The average air temperature during the fading of growth processes, i.e. the autumn period, averages 5 °C. Nordstorms may occur during this period. Northeastern winds can lead to a rapid drying of the assimilation apparatus [3].

In the conditions of the Black Sea coast, thuja occidentalis is adapted to anthropogenic load. In landscape design, it is presented in alley plantings, in group compositions, decorative as single plantings. It performs the most important functions associated with the release of oxygen, phytoncides, air ionisation [5, 7, 8].

In the second half of 2020, in the conditions of the Gelendzhik district, the task was to develop and determine favourable dates for introduction into in vitro culture during the fading of growth processes, i.e. in the autumn months. When developing methods of isolating explants from various organs of mother plants, the plants of western thuja (Thuja occidentalis), which grew in the open ground, were selected as research objects.

During material preparation, micro cuttings were isolated from isolated shoots, which after sterilisation, were introduced under in vitro conditions.

Explants represented by microcuttings were introduced into the culture. The method of E.N. Jigallo [10] was used.

A general procedure for the preparation of WPM nutrient medium is as follows.

1) in the process of work performance, initial solutions of inorganic compounds were prepared. These solutions included plant growth regulators, micro-, macroelements, vitamins;

2) agar-agar was added to a 0.5 litre measuring cylinder, dissolved in water, and the volume was brought to 1 litre. The solution was heated until it was completely dissolved.

At the final stage, vitamins were added. The grafted and outgrown explants were passaged onto nutrient propagation media. At the stage of nutrient medium preparation, different groups of phytohormones, such as auxins, cytokinins, gibberellins, and steroid hormones, were introduced [11–14].

The prepared medium was transferred to the penicillins. The penicillins were sealed with foil and then autoclaved for 15 minutes at 1 atmosphere (112^0C). The sterile medium was used for a week.

Fig. 2. Cultivation of explants

In 2023, it was possible to germinate seeds of the western thuja cultivar Woodwardii in the in vitro culture under home laboratory module conditions.

The sterilising agent Oka-tab was studied to reduce planting infection and increase the yield of healthy coniferous plant explants when introduced into in vitro culture [15–18].

The proportion of clean, infection-free explants that regenerated microshoots served as the efficiency evaluation criterion. At the same time, the proportion of explants killed due to the suppressive effect of chemical agents and the proportion of explants infected with pathogenic microflora were taken into account.

It was found that various sterilising agents could not completely solve the problem of health improvement of explants represented by microcuttings. As a result of treatment with chlorine tablets "Oka-tab", necrosis was observed in soft tissues of Thuja occidentalis.

An audit of the results of the introduction of explants under in vitro conditions without sterilisation showed that all mother plants tested were significantly infected. It was assumed that the mother plants were infected under in vivo conditions [19, 20].

The issues of recovery of mother plants were becoming more urgent. In order to solve the issues related to the recovery of thuja occidentalis plants, it was decided to develop a plant protection system.

In the second half of 2023, in conditions of the Gelendzhik district, the task was set to study the agrotechnics of growing container plants of western thuja (Thuja occidentalis) varieties "Columna", "Smaragd" to determine the potential for further introduction of explants into in vitro culture during the fading of growth processes, i.e. in autumn.

On the basis of data analysis, it was established that the technology of growing mother coniferous plants depends on the peculiarities of soil-climatic factors, the timing of phenological phases, varietal characteristics, agrotechnical methods [21].

We have concluded that the mild climate of the Gelendzhik district may contribute to the expansion of the range of diseases and pests. Weakened plants as a result of stress factors may die due to the influence of pathogens.

It was found that the container volume has a direct effect on the physiological characteristics of the studied plants.

In addition, the container plants under study are not wind resistant. This factor can provoke mechanical damage to the crown.

We found out that some coniferous plants on the experimental plot had a poorly developed closed root system, it led to slow growth processes. In some cases, death of the studied crops was observed [22].

The work states that the conifers under study may be exposed to excessive UV radiation due to exposure to bright sunlight. Whitened needles should be removed manually from the plants.

In the process of observations, the authors established that during the growing season of 2023, in some container plants of western thuja (Thúja occidentális) varieties "Columna", "Smaragd" in the conditions of the Gelendzhik district, a very dangerous pest, spider mite were revealed, which forms a thin web on the conifers.

In order to further commercialise agro-technologies, issues are being considered to increase the investment attractiveness of project proposals [23].

It has been found that coniferous feedstock can be waste-free and is recommended for the production of

biologics of high commercial interest in reducing pesticide load in agriculture.

In 2023, verbal agreements were reached with I.T. Trubilin Federal State Budgetary Educational Institution of Higher Professional Education "KubGAU named after I.T. Trubilin" in Krasnodar, with the Union of Organic Agriculture, and with leading landscape centres in the Krasnodar region to promote projects related to the study of biopreparations based on conifer extracts. The results of the research are planned to be scaled up in landscaping neighbourhoods, fruit growing and viticulture [24]. At the moment, there is a verbal agreement with the Gelendzhik branch of the State Budgetary Educational Institution "Novorossiysk Medical College" in terms of development of a municipal innovation platform on the basis of MBDOE kindergarten No. 8 "Buratino" in the village of Arkhipo-Osipovka. Its goals and objectives are focused on the development of healthy nutrition programmes for preschool children. In the planning of the programme, fruit products grown by organic technologies are presented. The results achieved formed the basis for the organisation of the Ecological Action "Kind Garden" within the framework of the Network Educational Project "School Agrarian".

On the basis of data analysis, it was established that the technology of growing mother coniferous plants depends on the peculiarities of soil-climatic factors, the timing of phenological phases, varietal characteristics, agrotechnical methods.

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