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Adaptation of in vitro turanga-poplar plants to greenhouse conditions

The reproduction of turanga-poplar is better carried out by the method of micropropagation, which allows you to obtain high-quality material that is advisable to use in reforestation and landscaping of cities, especially with arid climates. Adaptation of *in vitro*plants was carried out in greenhouse conditions in containers with a volume of 450 ml. Different in terms of composition substrates were used. The substrate of option 3 turned out to be the most effective: peat and perlite in layers, peat in the lower part, perlite in the upper part; and option 4 — a mixture of peat and black soil in a ratio of 6/4 with a recess filled with perlite. The experiments were carried out in natural daylight in a frame heated greenhouse with a film coating at a temperature of +20...+27 °C. Plants were used in three stages of root system development. After transplantation, they were watered with an antifungal drug solution and water. To prevent evaporation of moisture from *in vitro* plants and from the surface of the substrate, a transparent cap with a screw-off lid was covered from above. Containers with turanga plants fully adapted to non-sterile conditions were transferred to open areas outside the greenhouse for hardening.

Keywords: adaptation, turanga, micropropagation, *invitro,* plant, non-sterile conditions, drug, substrate.

Introduction

Populusdiversifolia Schrenk, further *P. diversifolia*, is a tree belonging to the genus Poplar *Populus*, reaching a height of 11–16 meters and a diameter up to 90–100 cm in individual specimens. The tree has an openwork spreading crown, which acquires a beautiful golden color in the autumn period. It is a sand fixer, capable of growing in arid conditions and on saline soils of Kazakhstan, which makes this tree indispensable not only for reforestation, but also for landscaping cities with a harsh windy arid climate [1–3].

Unfortunately, due to the difficulty of reproduction and cultivation by artificial means, this crop is practically not bred in forest nurseries. But recently, great interest has been shown in this species for its uniqueness, both for reforestation on sandy soils susceptible to wind erosion in order to consolidate them, and in order to restore degraded populations of *P. diversifolia*.

The following ways of reproduction of *P. diversifolia*exist: they are seeded and vegetative. The following vegetative methods of propagation are distinguished: by cuttings, root layering and microclonal.

Propagation by cuttings and root layering is a laborious and not always successful method due to the low rooting ability of this type of poplar [1].

The most optimal method of reproduction for *P. diversifolia* is micropropagation (in vitro), which is widely used due to its high success rate and scientific justification [4, 5].

It is especially important to obtain turanga-poplar plants with a closed root system in containers as a result of micropropagation. The plants obtained have a high survival rate for the restoration of natural populations, whose range narrows over time due to natural causes or due to human activity [6–8].

However, this method has its difficulties, especially at the stage of *in vitro* transplantation of plants into containers with a soil substrate, as well as during the period of adaptation to non-sterile conditions. With micropropagation, large losses occur when transplanting plants into non-sterile conditions (50 percent or more of death). This is due to the fact that at this stage plants are experiencing a lot of stress, primarily water stress, leading to dehydration of tissues and destruction of plant membranes. It is complemented by poor adaptation to photoautotropy due to the weak activity of carbon-fixing enzymes, that is, the ability of plants to absorb CO^2 after transplantation into non-sterile conditions decreases 4-5 times. Another significant factor that creates problems during transplantation is the poorly developed root system of adaptable plants, which is unable to absorb nutrients and water in sufficient quantities [9, 10].

The purpose of this study was to select the optimal composition of the substrate, the need to use an antifungal drug, a suitable temperature regime, the season of transplanting adaptable plants into non-sterile con-

ditions for high survival and growth, the need for hardening on sites outside the greenhouse for planting in the open ground, for growing in nurseries to the required size in order to carry out forestry work or landscaping industrial cities with arid climate.

Experimental

The object of the study was *P. diversifolia*, propagated in culture *in vitro* in the laboratory of cryopreservation of germoplasm of the Institute of Plant Biology and Biotechnology. In order to determine the optimal composition of the nutrient medium for rooting, three options were tested: Murashige and Skuga medium (MS) with the addition of 0.5 mg/l indolylbutyric acid (IBA); MS½ + 0.5 mg/l IBA and ½MS without hormones.

Turanga (*P. diversifolia*) plants at different stages of root system development were used for transplantation from an *in vitro* culture into non-sterile conditions of the greenhouse. The first stage was characterized by the beginning of formation and active growth of the root system (root length from 0.5 to 1.0 cm). At the second stage, the roots began to branch (the appearance of second-order roots), the formation of hairs occurred; the length of the roots was 1.5-2.5 cm. The third stage was characterized by the presence of branches with the formation of root hairs and the length of the roots from 2.5 to 3.5 cm.

At the same time, when transferring to non-sterile conditions, the optimal composition of the soil substrate was selected, for which five options were tested. In the first option, peat (brown and dark sphagnum) of the Kekilla Professional brand (pH — 5.5) was used. For the second option, a mixture of peat and expanded perlite was used in a ratio of 8/2, respectively. In the third option, peat and perlite were not mixed together but filled the container with layers: peat in the lower part, perlite in the upper part. In the fourth option, the container was filled with a mixture of peat and black soil in a ratio of 6/4, respectively, and then a recess of 1.0–4.0 cm was made and filled with perlite. For the fifth option, a mixture of peat and sifted river sand with fraction sizes of 0.1-0.3 mm in a ratio of 1/1 was taken. Polypropylene containers with a volume of 450 ml, a height of 9.5 cm and a diameter of 9 cm were used as a container for transplantation.

The plant was carefully removed from the culture jar, while the remains of the nutrient medium were not removed; the transplant was carried out together with it. This was done in order not to damage the delicate root system of the adaptable plant. Next, a small recess was made in the experimental substrate, the root system was placed there, evenly without bends upwards, the plant was sprinkled and leveled.

When transferred from an *in vitro* culture to non-sterile conditions, the root system of plants can be affected by pathogenic soil-dwelling fungi, which subsequently lead to plant death. To avoid this, the effect of the drug "Maxim Dachnik" (active ingredient fludioxonyl 25 g /l) on the survival rate of *P. diversifolia* was studied. For this purpose, in the first option, the planted plants were watered with a solution of the drug in a concentration of 2 ml / 1 liter of water, 100–150 ml for each plant, and in the second option the drug was not used.

In order to avoid evaporation of moisture, both from the surface of the substrate and from the tissues of transplanted plants, the containers were covered from above with a transparent cap with a screw-off lid (diameter 2.0 cm) and placed on racks.

The plants were transplanted under natural daylight in a frame heated greenhouse with a film coating at a temperature of +20...+27 °C from mid-March to the end of September. The plant survival rate was taken into account weekly. 5–7 days after transplanting plants into containers, the caps were unscrewed to allow air to enter. After *P. diversifolia* showed signs of active growth and development, the caps were gradually lifted and completely removed with full adaptation to non-sterile conditions, the entire stage took from 3 to 4 weeks.

After the plants transplanted from the *in vitro* culture were fully adapted to the non-sterile conditions of the closed ground, they were placed on sites outside the greenhouse in order to harden for planting in the open ground.

Results and Discussion

To determine the optimal composition of the nutrient medium for rooting, three options were tested: MS, IBA — 0.5 mg/l; ½MS + IBA — 0.5 mg/l and ½MS without hormones.

The results of optimizing the nutrient medium for obtaining the root system of *P.diversifolia* showed that a decrease in the concentration of macro and microelements by MS and the introduction of phytohormone IBA into the medium at a concentration of 0.5 mg/l leads to the formation of roots in 95–97 % of plants in *in vitro* culture. In this case, the first roots began to appear after 14 days, followed by

intensive growth, development, and branching accompanied by the formation of root hairs. After 30 days, they reached a length of 1.0-1.5 cm and had a white color with a beige tint.

The conducted studies have shown that the stage of development of the root system and its length had a significant impact on the survival and adaptation of plants in non-sterile conditions. As can be seen from Figure 1, plants at the first stage of root system development had the lowest survival rate of 43 %. This was due to the fact that plants at this stage completely lacked second-order roots and root hairs responsible for the absorption of water and nutrients. The highest rate was in plants at the second stage — 92 %. The reason was the active growth of second-order roots with the formation of root hairs on them, and a length not exceeding 2.5 cm. Survival rate at the third stage was 79 %. The root system, which was more than 3.0 cm long, bent and broke during transplantation, which prevented the supply of nutrients and contributed to damage by pathogenic microflora. This led to a decrease in the survival rate of turanga in the third stage of root development.

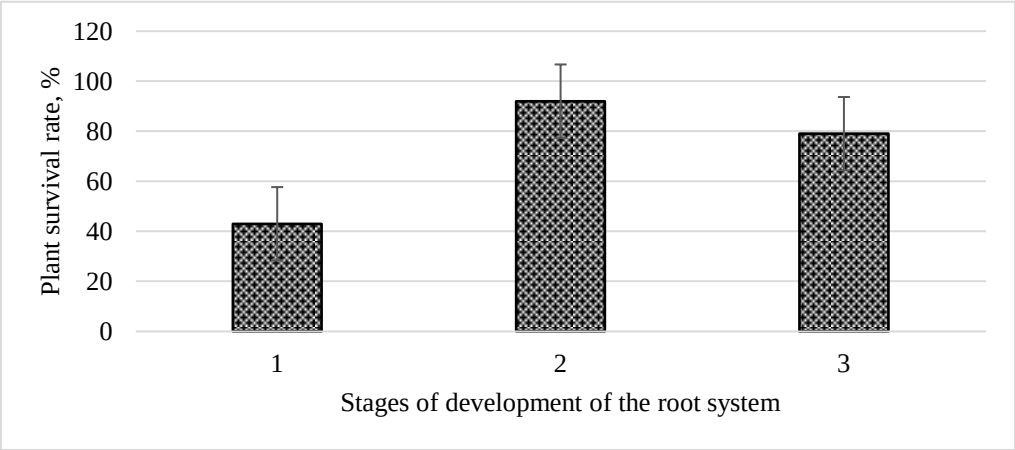
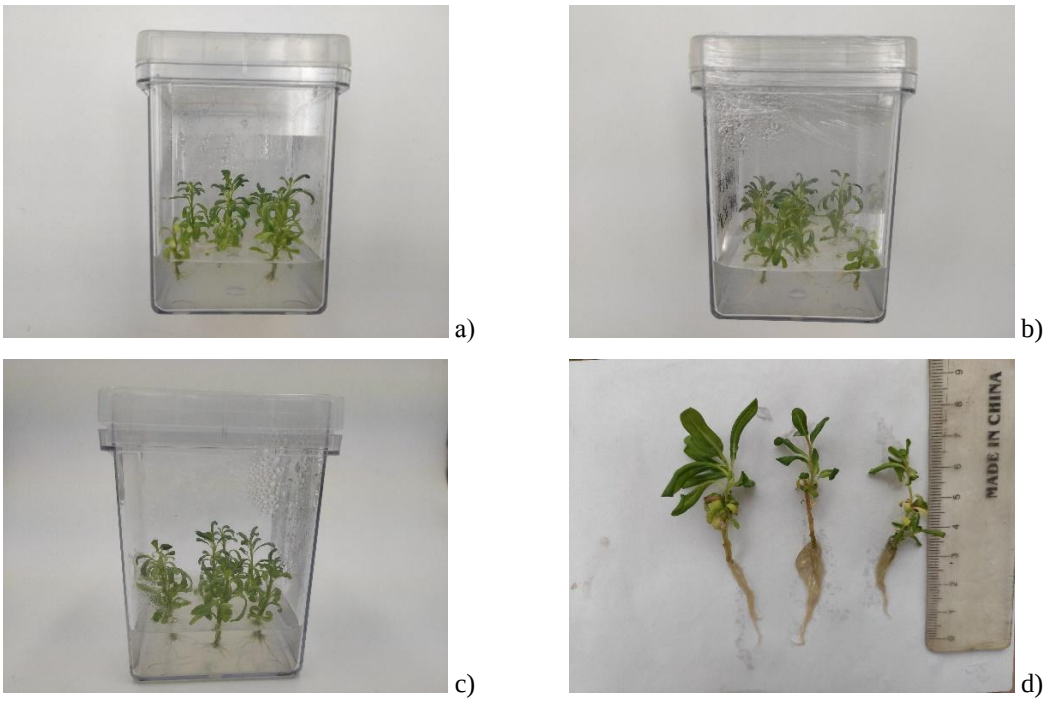


Figure 1. Survival rates of *P. diversifolia* at different stages of root system development

Figure 2 shows *P. diversifolia* plants at various stages of root system formation in *in vitro* culture.



a) the first stage of development*; b)* the second stage of development*;*
c) the third stage*; d)* the development of the root system at different stages

Figure 2. Development of the root system of *P. diversifolia* in *in vitro* culture.

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As a result of the conducted studies, it was found that the highest percentage of plant survival during adaptation from *in vitro* culture was when using substrate options 3 and 4 and amounted to 50.7 % and 65.7 %, respectively. Planting in pure perlite resulted in active root system growth. This is due to the good aeration and hygroscopicity of the material, the absence of pathogenic microflora in it, which causes rotting of the roots and the base of the stem. When using options 1, 2 and 5, the survival rate of adaptable plants did not exceed 35 %, 31 % and 22.3 %, respectively. This was due to the close contact of roots and shoots with pathogenic microorganisms living in a non-sterile substrate. At the same time, the aeration of these substrate options was less intense than when using pure perlite. Data on the survival rate of plants are presented in the Table.

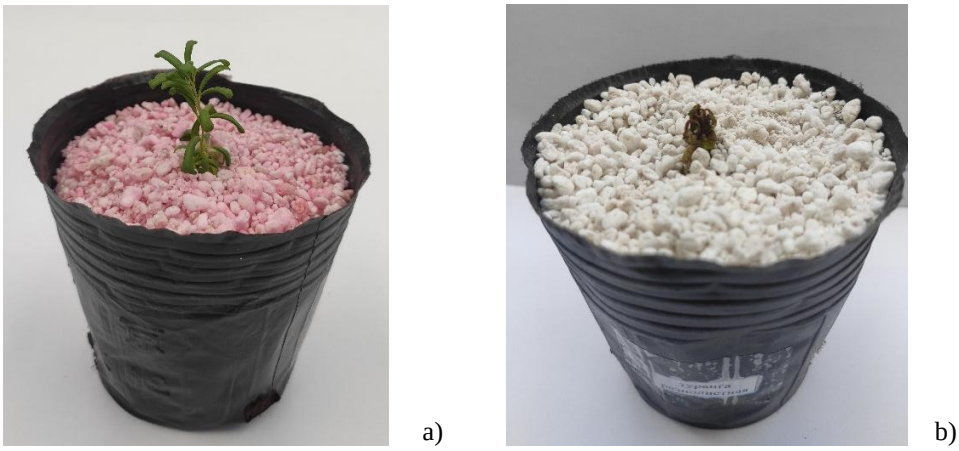
T a b l e

Survival results of *P. diversifolia* on different substrates (average)

The composition of the substrate	The number of plants that took root, %
peat (option 1)	35±2,0
peat + perlite mixture 8:2 (option 2)	31±1,7
peat bottom layer + perlite top layer (option 3)	50,7±1,03
peat + black soil mixture 6:4 + recess with perlite (option 4)	65,7±5,1
peat + sand mixture 1:1 (option 5)	22,3±3,3

The results achieved were compared with data from previous studies on the use of substrates using the example of various crops. For example, the number of plants that took root when transplanted into non-sterile conditions of strawberries of the Redgontlit variety with the use of perlite was 80 %; when using the peat: sand substrate 1:1, the number of plants that took root was 58.6 % [9]. The survival rate of triploid aspen test tube plants planted in peat tablets averaged 46.6 % [10]. Thus, our studies have allowed us to obtain sufficiently high rates of *P. diversifolia* survival to non-sterile conditions.

The use of an antifungal drug to treat the substrate directly during transplantation gave a positive result compared to watering with just water. The bases of the shoots of some plants, the soil under which was watered without the use of a fungicide, began to darken a week after transplantation, then wilting and complete rotting were observed. In specimens planted with the use of a fungicide, such symptoms were observed much less frequently or were absent altogether (Fig. 3).



a) with the use of a fungicide; *b)* without the use of a fungicide

Figure 3. Plants of *P. diversifolia*, a week after transplantation

The results of the effect of the fungicide “Maxim Dachnik” on the *in vitro* survival of plants after transplantation into non-sterile conditions are presented in Figure 4.

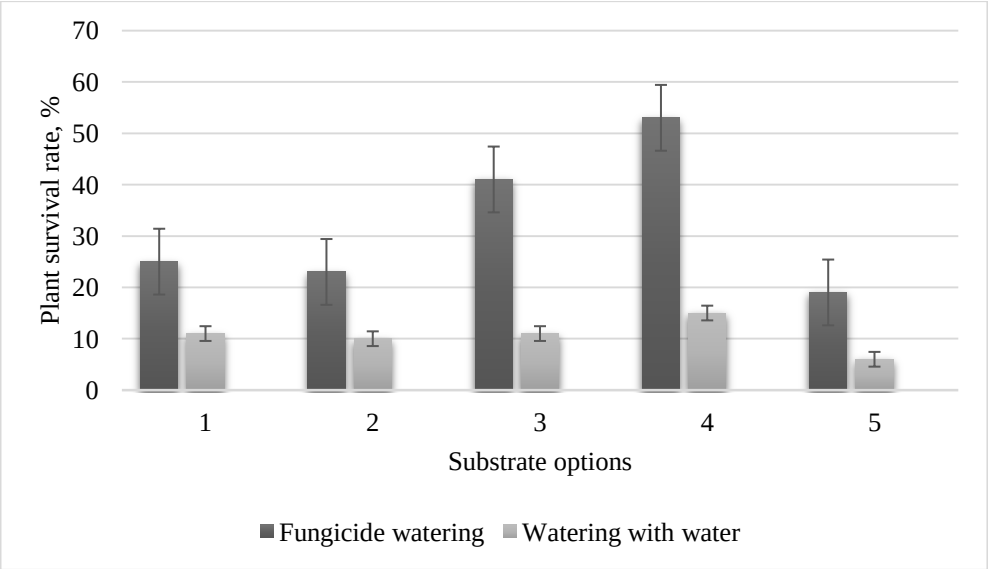


Figure 4. The effect of a fungicide on the survival rate of *P. diversifolia* in non-sterile conditions

As can be seen from Figure 4, the survival rate of turanga when watered with an antifungal drug after transplantation into non-sterile conditions was on average 53 %, without the use of the drug on average did not exceed 15 %.

During the experiments, it was noted that the optimal temperature for adaptation to non-sterile conditions was 20...23 °C and natural daylight in the greenhouse conditions. The optimal time for the transfer of *P. diversifolia* from an *in vitro* culture to a soil substrate is the period from mid-March to the end of September. During this period, the transplanted plants did not lack sunlight for active growth and development.

Hardening of *P. diversifolia* for cultivation in the open ground was carried out on a site outside the greenhouse and seedlings with a height of 20–30 cm with a closed root system in containers were obtained (Fig. 5).

Figure 5. Hardening in the open area of *P. diversifolia*

Conclusions

The best results for the rooting of *P.diversifolia* under *in vitro* conditions were obtained using the medium MS½ + IBA — 0.5 mg/l.

When transferring to non-sterile conditions, it is recommended to use *in vitro* plants with a root system having second-order roots with root hairs and a length of 1.5-2.5 cm.

As a result of the experiments conducted on the adaptation of *P.diversifolia* plants to non-sterile indoor conditions, the highest percentage of survival was shown by substrate 3, consisting of a mixture of brown and dark sphagnum peat with expanded perlite, placed into a container in layers in the lower part peat, in the

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upper part perlite; and substrate 4 — a mixture of peat and black soil in a ratio of 6/4, respectively, with a recess filled with perlite.

After transplantation, it is recommended to abundantly spill the substrate with a solution of the fungicide “Maxim Dachnik” (active ingredient fludioxonyl 25 g/ l) in a concentration of 2 ml / 1 liter of water to reduce the negative effect of pathogenic microflora on the immature and vulnerable root system of plants *in vitro*.

In order to avoid evaporation of moisture from the tissues of the adapted plant, it is necessary to cover the top with a transparent cap with a screw-off lid (diameter 2.0 cm) and remove the lid after 5–7 days. After the appearance of signs of active growth of *P.diversifolia*, it is advisable to gradually open the caps until the plants fully adapt to non-sterile conditions. The whole stage takes from 3 to 4 weeks.

Transplantation of *in vitroP. diversifolia* plants is best performed at a temperature of 20...23 °C in natural daylight from mid-March to the end of September.

To prevent additional stress from plants adapted to greenhouse conditions when planting in open ground conditions, it is effective to carry out hardening in open areas outside the greenhouse.

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Тораңғы-терек өсімдіктерінің *in vitro* жабық топырақ жағдайларына бейімделуі

Тораңғы-теректі көбейту үшін микроклональды әдісті қолдану тиімді, себебі бұл сапалы материал алуға мүмкіндік береді, оны орманды қалпына келтіру мен қалаларды көгалдандыруда, әсіресе құрғақ климатта пайдалануға болады. *In vitro* өсімдіктерін жабық топырақта 450 мл көлеміндегі контейнерлерде бейімдеу жүргізілді. Әртүрлі субстрат құрамдары қолданылды. Ең тиімді нәтиже 3-нұсқа бойынша алынды: төменгі қабатта шымтезек, жоғарғы қабатта перлит; сондай-ақ 4-нұсқа

перлитпен толтырылған шымтезек және қара топырақтың 6/4 қатынасындағы қоспасы. Эксперименттер табиғи күн сәулесінде пленкамен жабылған жылыжайда +20…+27°С температурада өтті. Өсімдіктер тамыр жүйесінің дамуының үш кезеңінде қолданылды. Қайта отырғызылғаннан кейін олар саңырауқұлаққа қарсы препарат ерітіндісімен және сумен суарылды. *In vitro* өсімдіктерінің және субстрат бетінен ылғалдың буланууын болдырмау үшін үстіңгі жағы бұралмалы қақпағы бар түссіз қалпақпен жабылды. Толығымен стерильді емес жағдайға бейімделген тораңғы-терек өсімдіктері бар контейнерлер қатаю үшін жылыжайдан тыс ашық жерлерге ауыстырылды.

Кілт сөздер: бейімделу, тораңғы-терек, микроклональды көбейту, *in vitro*, өсімдік, стерильді емес жағдай, препарат, субстрат.

Н.В. Михайленко, Т.Т. Турдиев, Б.А. Кентбаева, З.Т. Туйгунов, С.В. Кушнаренко

Адаптация растений *in vitro* туранга-тополя к условиям закрытого грунта

Размножение туранга-тополя лучше проводить способом микроклонального размножения, что позволяет получать качественный материал, который целесообразно использовать в лесовосстановлении и в озеленении городов, особенно с засушливым климатом. Адаптацию *in vitro* растений проводили в условиях закрытого грунта в контейнерах объемом 450 мл. При этом использовали различные по составу субстраты. Наиболее эффективным оказался состав варианта 3: торф и перлит слоями, в нижней части торф, в верхней — перлит; и вариант 4 — смесь торфа и чернозема в соотношении 6/4 с углублением, заполненным перлитом. Эксперименты проводили при естественном дневном освещении в каркасной отапливаемой теплице с пленочным покрытием при температуре +20 –+27°С. Использовали растения в трех стадиях развития корневой системы. После пересадки поливали раствором противогрибкового препарата и водой. Для предотвращения испарения влаги из *in vitro* растений и с поверхности субстрата сверху накрывали прозрачным колпаком с откручивающейся крышкой. Контейнеры с полностью адаптированнными к нестерильным условиям растениями туранги переносили на открытые площадки вне теплицы для закаливания.

Ключевые слова: адаптация, туранга, микроклональное размножение, *in vitro*, растение, нестерильные условия, препарат, субстрат.

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