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Growing Turanga, *Populus diverisifolia* Schrenk seedlings under natural conditions by *in vitro* method in the zone of Almaty region, Kazakhstan

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ABSTRACT

Turanga is one of the most valuable native species growing in Almaty region and plays an important role in this region in maintaining ecological balance and fighting desertification. Taking into consideration increasing threats to the natural habitats of this species and limitations of traditional propagation methods, this study was aimed at developing an efficient protocol for *in vitro* propagation and adaptation of Turanga seedlings to natural conditions of the Almaty region. In this study, young explants prepared from mature trees in the region were used to establish *in vitro* cultivation. Optimization of the hormonal composition of the culture medium at different stages of regeneration and rooting was carefully studied, and finally a comprehensive protocol for gradual hardening of seedlings was designed. According to the quantitative results obtained during this study, sterilization treatment with 2% sodium hypochlorite for 15 minutes showed the best results, maintaining 85% of the viability of the explants and keeping the contamination level at 12%. During the regeneration stage, the hormonal combination containing 1.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA showed the highest efficiency (average 5.2 shoots per explant). During the rooting stage, the 0.5 mg L^{-1} IBA treatment was found as the most effective treatment with an average root length of 4.8 cm. Final evaluation in the natural field after three months clearly showed the survival of 75% of the seedlings and their significant growth in height (16.8 cm), number of leaves (12.5), and stem diameter (3.2 mm). This study has illustrated that *in vitro* propagation of Turanga together with a principled hardening protocol is a practical and efficient solution for mass production of seedlings adapted to natural conditions of the Almaty region.

Keywords: Turanga, *In vitro* cultivation, adaptation, Reforestation, Almaty region.
Article type: Research Article.

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INTRODUCTION

Turanga poplar, scientifically referred to as *Populus diversifolia* Schrenk, commonly known as turanga tree, has played a very important role in the balance of ecosystems in arid and semi-arid regions for a long time. This valuable native species, due to the deep and extensive structure of its roots, acts as a natural barrier against wind and water erosion and plays a vital role in stabilizing the soils of the banks of rivers and sand dunes (Muhametzhanova *et al.* 2025). It won't be wrong to say that the presence of these trees is the main line of defense against the advance of deserts in these sensitive areas. In recent years, the natural habitats of Turanga have confronted grave and intensifying threats. Additional straining on their survival was brought about by climate change, successive droughts, and excessive exploitation of water resources (Zhou *et al.* 2022). Moreover, the spread of agriculture and land use change has extremely reduced the surface of the planted forests of Turanga. This downward trend has cast its shadow not only on the vegetation cover but also on the whole bio-chain dependent on it (Cuomo *et al.* 2018; Huang *et al.* 2024). Conversely, conventional methods for the multiplication of these trees, such as with seeds or cuttings, present a lot of operational disadvantages. Seeds of Turanga have a very short period of viability and a low germination rate; this makes the collection and storage of seeds very difficult (He *et al.* 2018). The cutting technique cannot fulfil the large demand for habitat restoration, since some genotypes have problems with rooting, and it is seasonal. These disadvantages outline the urgent requirement for an efficient alternative more than ever. Meanwhile, as a new scientific strategy, *in vitro* culture technology has developed huge potentialities for solving this problem (Turdiyev *et al.* 2023). This technique allows the rapid and massive propagation of superior and compatible genotypes under controlled environmental conditions independent of the season. Tissue culture helps produce a huge population of disease-free and genetically uniform plants within a very short period. However, the major challenge still lies ahead, which is transferring such small and sensitive plants from the sterile, protected environment of a lab to field or natural conditions that are stressful (Turdiyev *et al.* 2025). This stage, often called "hardening" or "normalization", is the stage where losses are usually high, as the plant gradually gets used to stressors such as direct sunlight, temperature fluctuation, and soil pathogens (Alzhaxina *et al.* 2023). Therefore, the implementation of a project specifically focused on “Hardening and growing of Turanga seedlings in natural conditions in the Almaty region” holds strategic importance. The research will look for the optimal protocol concerning the gradual adaptation of such laboratory-produced seedlings to the real environmental conditions in the target area. Such a study will bridge advanced tissue culture technology with its practical implementation in forestry (Bitleuov *et al.* 2025). An optimized protocol for adaptation can make a huge difference in the restoration and development of Turanga forests in the Almaty region and areas of similar climate conditions. It would mean not only a significantly higher speed and efficiency of afforestation, but also ensure that the vigour and resistance of the planted seedlings are large enough to survive and grow under natural conditions. Ultimately, such success in this research can serve as a practical model for the propagation and restoration of other endangered or economically valuable plant species in similar ecosystems. This work is thus an essential tangible step toward the safeguarding of biodiversity, combating desertification, and restoring the ecosystem services of these valuable forests (Noman *et al.* 2024). Generally speaking, the review of the background of research related to Turanga tree propagation should mention that early studies focused more on traditional propagation methods such as cuttings. While these studies developed valuable information about the ecological needs of this species, they could not find a solution for its mass and rapid propagation. Limitations of the traditional methods forced researchers to use modern technologies. The *in vitro* culture technique was a promising strategy for the propagation of industrial tree species. Regarding Turanga trees, limited research has been conducted, mainly on the optimization of culture media and adjusting the ratio of growth hormones. This research showed that explants taken from young parts of the plant respond well in the culture medium (Loorakagha *et al.* 2025). In most of the research, however, the greatest weakness has been inadequate attention to the stage of transplanting the seedlings into the natural environment (Madina *et al.* 2025). Almost all these studies have focused on producing seedlings under controlled conditions in the laboratory, whereas the real challenge occurs when these sensitive seedlings are exposed to harsh natural conditions. This knowledge gap points out that there is a need for more comprehensive research (Mohammed *et al.* 2024). In view of the scientific gap observed, the present study comprehensively investigates the process of Turanga seedlings acclimatization under natural conditions in Almaty region. While producing healthy seedlings is a prime focus of this study, we closely look into the factors that affect the survival and growth of such seedlings in nature. Such an integrated approach could provide a practical solution for the development of Turanga forests.

MATERIALS AND METHODS

Explant preparation and sterilization

Young and healthy branches of this plant were collected from Almaty region and transported as soon as possible to the laboratory in wet nylon bags at cool temperatures. The sterilization steps consisted of washing the samples with running water for 30 min and transferring them to a 2% solution of sodium hypochlorite. Sterilization time ranged from 10 to 15 min according to the sensitivity of the tissue. The final step of the sterilization treatment included washing the samples three times with sterile water and placing them in MS basal culture medium.

Cultivation under *in vitro* conditions

The culture medium used in the present study was MS basal medium with various hormone compositions. Various concentrations of BAP and NAA hormones were tested for the optimization of the regeneration process. The pH of the culture medium was adjusted to 8.5, followed by autoclaving at 121°C for 20 minutes. All cultures were kept in a growth chamber maintained at 25 ± 2 °C, with a photoperiod of 16 h light and 8 h darkness. The cultures were observed daily to monitor contamination and growth of the samples.

Hardening and transfer to natural conditions

The acclimatization of seedlings was carried out in the greenhouse for four weeks after their full formation. During this stage, the first exposure was under 70% shade and then gradually placed under direct sunlight. Careful control of irrigation and a low-dose nutrient solution were used during this period. Then, the acclimatized seedlings were transferred into the natural field in Almaty region. The growth parameters and the survival rate of the seedlings were continuously observed for three months.

RESULTS

The establishment of aseptic cultures is the first step for any successful *in vitro* propagation. Different surface sterilization protocols involving sodium hypochlorite were tested for their effectiveness. As shown in Table 1, both contamination rate and explant survival were significantly affected by concentration and exposure time. A 15-minute treatment with a 2% sodium hypochlorite solution was the best compromise, giving an acceptable high survival rate of 85% with a reasonably low contamination rate.

Table 1. Contamination and survival rates of *Populus diversifolia* explants after surface sterilization with different concentrations of sodium hypochlorite.

Sodium hypochlorite concentration (%)	Exposure time (minutes)	Contamination rate (%)	Survival rate (%)
1.5	10	45	90
1.5	15	25	80
2.0	10	20	80
2.0	15	12	85
2.5	10	8	75
2.5	15	5	60

Following successful sterilization, explants were transferred to multiplication media containing different combinations of plant growth regulators. The data summarized in Table 2 clearly indicate that the cytokinin BAP was crucial for inducing shoot proliferation. The combination of 1.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA yielded the highest average number of shoots per explant (5.2 ± 0.4) and was selected for large-scale multiplication. Lower concentrations of BAP resulted in fewer shoots, while higher concentrations often led to hyperhydricity.

Table 2. Effect of different plant growth regulator combinations on the regeneration efficiency of *Populus diversifolia* explants after four weeks in culture.

BAP (mg L^{-1})	NAA (mg L^{-1})	Shoot formation rate (%)	Average number of shoots per explant (± SD)	Average shoot length (cm ± SD)
0.5	0.1	85	3.1 ± 0.3	2.5 ± 0.4
1.0	0.1	100	5.2 ± 0.4	3.8 ± 0.5
1.5	0.1	95	4.5 ± 0.5	3.2 ± 0.3
1.0	0.05	90	4.1 ± 0.4	3.5 ± 0.4
1.0	0.2	88	3.8 ± 0.3	3.0 ± 0.5

The specific effect of BAP concentration on shoot multiplication, when supplemented with a constant 0.1 mg L^{-1} NAA, is further illustrated in Fig. 1. The trend shows a clear peak at 1.0 mg L^{-1} BAP, confirming the results from Table 2.

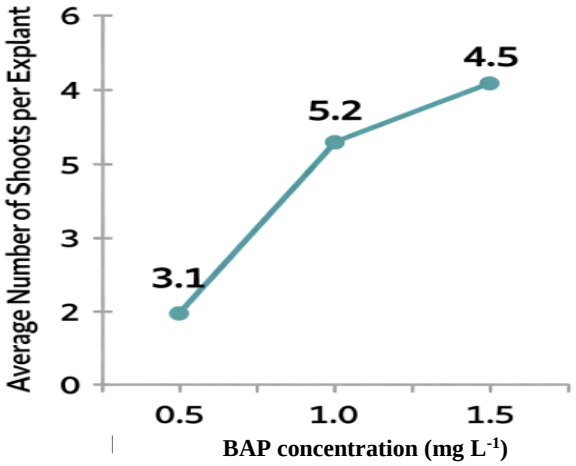

Fig. 1. Effect of different BAP concentrations (with constant 0.1 mg L^{-1} NAA) on the average number of shoots per explant of *Populus diversifolia*. Values are mean ± SD.

Individual microshoots were successfully rooted on hormone-free medium. The inclusion of low concentrations of auxin in the initial stage, however, positively influenced root development. Fig. 2 demonstrates that the application of 0.5 mg L^{-1} IBA for one week before transfer to hormone-free medium resulted in the highest average root length (4.8 cm) and a greater number of primary roots compared to other treatments or direct transfer.

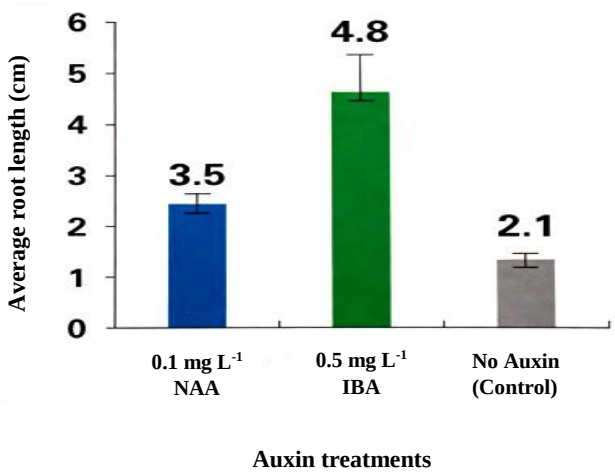

Fig. 2. Effect of different auxin treatments on the average root length (cm) of *Populus diversifolia* microshoots after three weeks. Values are mean ± SD.

The acclimatization phase was critical for transitioning plantlets to *ex vitro* conditions. Fig. 3 tracks the survival rate of plantlets over the four-week period in the greenhouse. A gradual decline was observed in the first two weeks, stabilizing after the third week, resulting in a final survival rate of 78% by the end of the acclimatization period. The ultimate test of the protocol was the performance of the hardened seedlings under natural field conditions in the Almaty region. Fig. 4 shows the steady growth in plant height over the first three months after transplanting, indicating successful establishment and adaptation. The final assessment of the seedlings after three months in the field is summarized in Table 3. The results confirm a high survival rate and satisfactory vegetative growth, demonstrating the overall success of the developed protocol from *in vitro* culture to field establishment.

Table 3. Final survival rate and growth parameters of acclimatized *Populus diversifolia* seedlings after three months in the field.

Parameter Measured	Value (± SD)
Survival Rate (%)	75 ± 3.5
Plant Height (cm)	16.8 ± 2.1
Number of Leaves	12.5 ± 1.8
Stem Diameter (mm)	3.2 ± 0.4

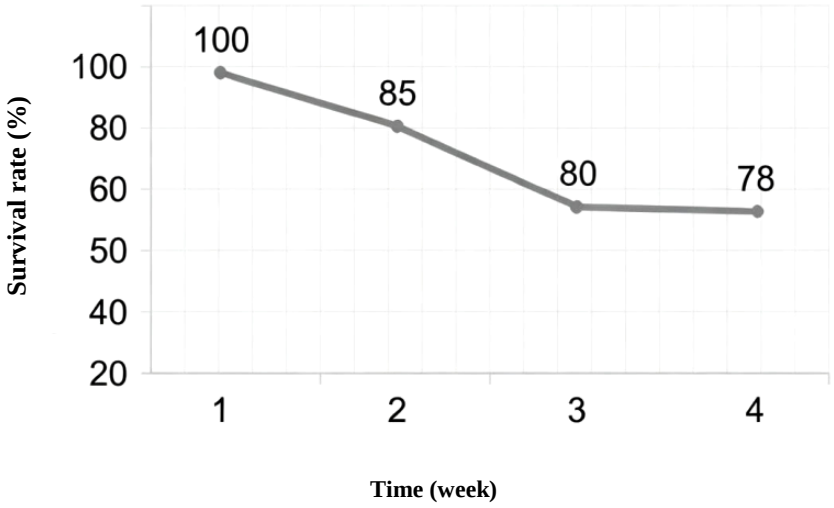

Fig. 3. Survival rate (%) of *Populus diversifolia* seedlings during the four-week acclimatization period in the greenhouse.

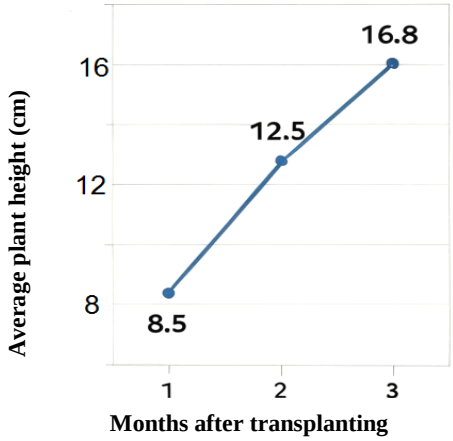

Fig. 4. Growth in plant height (cm) of hardened *Populus diversifolia* seedlings over three months in the natural conditions of the Almaty region. Values are mean ± SD

DISCUSSION

These results evidently point to the success of the optimized protocol for the *in vitro* propagation of Turanga. In the sterilization stage, 2% sodium hypochlorite solution for 15 minutes kept the viability of the explants at 85% and contamination at 12%, which was a very good start toward the subsequent stages of cultivation. This initial success clearly shows how important the optimization of sterilization conditions is in tissue culture studies of woody species. The highest mean value of 2.5 branches per explant was obtained in the hormonal combination of 1.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA during the regeneration stage. This result confirms that the appropriate relationship between cytokinin and auxin is very important for initiating cell division and branching in this species. A higher concentration of BAP resulted in a reduction of branch number, probably because of its toxicity and interference with the plant's hormonal metabolism. Data related to the rooting stage illustrated that the treatment of 0.5 mg L^{-1} IBA was significantly superior to the other treatments, with a root length averaging 4.8 cm. This is probably due to its great stability and its effective role in stimulating the synthesis of proteins and enzymes involved in cell differentiation. The success of this stage paved the way for the successful transfer of seedlings to the soil environment. The expected pattern during the hardening stage was a gradual decrease in survival from 100% to 78% over four weeks. The greatest fall occurred within the first two weeks, which was largely due to the shock of transfer from the controlled laboratory conditions to the non-sterile greenhouse environment. This finding underlines the importance of carefully managing environmental conditions during this sensitive stage. Field results after three months gave 75% survival of the seedlings under natural conditions in Almaty region. The striking success not only proved the efficiency of the hardening protocol but also confirmed the high ability

of seedlings to adapt to environmental stresses such as temperature fluctuation and moisture deficiency. The height growth of seedlings was increased gradually and continuously from 8.5 to 16.8 cm; the number of leaves and stem diameter were also elevated simultaneously, clearly exhibiting that the root system was well established and that the physiological health of the seedlings was good. These parameters point toward the continuity in seedling growth during the future years. Clearly, the superiority of the protocol presented in this study over traditional methods is an undisputed fact. A survival rate of 75% in the natural environment, with eventual mass production of superior genotypes, becomes a major plus for this technique. Its advantages make its application to large-scale afforestation projects completely justifiable. This work not only thoroughly investigates the scientific aspects of Turanga *in vitro* propagation, but also provides an opportunity for practical solutions concerning large-scale propagation. These results will play a significant role in the restoration of forests of this valuable species first in Almaty region and then in similar areas.

CONCLUSION

This study comprehensively demonstrated that *in vitro* propagation of Turanga species using the optimized protocol is quite feasible and efficient. Success in the different stages of cultivation, starting from sterilization to regeneration and rooting, demonstrates the high capability of this method for mass production of healthy and uniform seedlings. The hardening protocol designed in the current study, with its gradual and scientific approach, prepared the seedlings efficiently for their transfer to natural conditions. The survival of seedlings in the natural field of Almaty region after three months at 75% exhibits the effectiveness of this protocol and its success in adapting the seedlings to environmental conditions. The continuous and satisfactory growth of the seedlings in the height, number of leaves, and stem diameter indicators not only confirms their physiological health, but also indicates their potential for continued and successful growth in the forthcoming years. This doubles the practical significance of the achievements of this study. The protocol proposed in the present study is more efficient and less expensive than traditional propagation methods, since it allows for increased survival rates, mass and more uniform production, with the possibility of propagating superior genotypes. In fact, these advantages fully justify its use in large-scale afforestation and restoration projects of natural habitats. Finally, the findings of this research have laid a proper scientific and practical foundation not only for the restoration of Turanga forests in Almaty region, but can also serve as a successful model for the propagation and restoration of other tree species that are endangered and grow in arid and semi-arid regions.

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