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Micropropagation of Populus euphratica Using BAP, ISK, NSK and IMK under Saline Conditions in Uzbekistan

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Annotation: Populus euphratica is a drought-resistant tree species indigenous to dry and saline areas of Central Asia. Its ecological function in stabilising damaged landscapes in Uzbekistan is well documented; yet, natural regeneration is ineffective due to low seed viability and inadequate field survival. This research sought to establish a dependable in vitro micropropagation methodology utilising explants from indigenous populations. Experiments were performed from 2023 to 2024 at the Biotechnology Laboratory of the National University of Uzbekistan. Nodal and apical segments were cultivated on MS medium augmented with BAP and ISK for shoot induction, subsequently rooted in White medium containing IMK. The optimal shoot induction (92%) and an average of 5.1 shoots per explant were achieved with 1.5 mg/L BAP and 0.5 mg/L ISK. Rooting was most effective with 1.0 mg/L IMK in

White medium, resulting in an 85% rooting rate and an average root length of 5.8 cm. Plantlets were acclimatised in a peat:sand:perlite mixture under greenhouse conditions, achieving an 82% survival rate. All phases of regeneration were subjected to statistical analysis via ANOVA and Duncan's test ($p < 0.05$). The established technique has significant reproducibility, morphogenic stability, and practical applicability for the restoration of saline and dry ecosystems in Uzbekistan. These findings provide a scalable approach for the extensive dissemination of P. euphratica, aiding national initiatives in ecological restoration and desertification mitigation.

Keywords: Populus euphratica, mikroklonal ko‘paytirish, in vitro regeneratsiya, fitogormonlar, o‘simlik to‘qima madaniyati, sho‘rlangan tuproqlar, bazal ozuqa muhiti, regeneratsiya darajasi, biodiversitetni saqlash, biotexnologik yondashuv.

INTRODUCTION

Populus euphratica, also referred to as Euphrates poplar, is a halophytic and xerophytic tree species native to Central Asia, North Africa, and certain regions of the Middle East. Its tolerance to strong environmental stressors, such as salt, drought, and elevated temperatures, renders it a crucial species for ecological stabilisation in damaged settings. P. euphratica is mostly found in the lower basins of the Amudarya River in Uzbekistan, where it is essential for alleviating soil erosion, decreasing salinisation, and enhancing microclimatic and soil conditions [1]. Nonetheless, despite its ecological importance, the natural regeneration of P. euphratica is constrained by inadequate seed viability, fast dormancy loss, and elevated seedling mortality in the field.

Consequently, conservation and extensive reforestation initiatives utilising this species are limited by the ineffectiveness of conventional propagation methods. In this context, in vitro micropropagation is a viable option, facilitating quick and uniform plant growth under regulated conditions. This is especially beneficial for endangered or ecologically significant species such as P. euphratica, which demonstrate restricted sexual reproduction and face escalating environmental stress from land degradation, water mismanagement, and climate change [2]. Prior research in China and Iran has established the viability of employing tissue culture for P. euphratica; nevertheless, the genotype-specific responses and sensitivities to medium composition require the formulation of region-specific methods [3]. Local ecotypes of Uzbekistan, adapted to salt soils and arid climates, necessitate tailored media formulations and hormone combinations to optimise regeneration success. This research seeks to establish and refine an effective micropropagation procedure specifically designed for Uzbek ecotypes of Populus euphratica. The objectives were: (1) to assess the impact of specific concentrations of cytokinins (BAP, ISK) and auxins (IMK, NSK) on shoot and root development; (2) to identify the optimal basal media for rooting and acclimatisation; and (3) to evaluate the overall viability of in vitro-derived plantlets

under greenhouse conditions. This research facilitates large-scale propagation through a reproducible and feasible method, thereby supporting national strategies for afforestation, biodiversity conservation, and the rehabilitation of saline and degraded soils in Uzbekistan.

Materials and Methods

This research was performed from 2023 to 2024 in the plant biotechnology laboratory at the National University of Uzbekistan. Nodal and apical explants of Populus euphratica were obtained from naturally occurring trees in the saline-affected areas of Karakalpakstan, situated along the lower basin of the Amudarya River. These places exemplify the characteristic arid and salinised soils in which P. euphratica flourishes naturally, rendering them optimal for the selection of genotypes exhibiting environmental resistance. Recently harvested explants were promptly wrapped in damp paper towels, placed in sterile containers, and transferred to the laboratory under cold circumstances to maintain vitality. Upon arrival, explants were meticulously rinsed under running tap water with a few drops of laboratory detergent to eliminate surface dirt and dust. Surface sterilisation was performed under laminar airflow conditions utilising 70% ethanol for 30 seconds, followed by immersion in a 0.1% sodium hypochlorite solution for 5 minutes. The explants were thereafter cleaned thrice in sterile distilled water to eliminate all remnants of disinfectant prior to inoculation onto culture media. The Murashige and Skoog (MS) basal media served as the basis for all shoot induction tests. The media was formulated with different amounts of the cytokinin 6-benzylaminopurine (BAP) at 1.0 and 1.5 mg/L, and the auxin indoleacetic acid (IAA) at 0.2 and 0.5 mg/L. All media were solidified with 0.8% agar and comprised 3% sucrose. The pH was modified to 5.8 before to autoclaving at 121°C for 20 minutes. Explants were positioned vertically on the medium and incubated under regulated environmental conditions at 25 ± 2°C, with a photoperiod of 16 hours of light and 8 hours of darkness under fluorescent illumination (40 μmol m^{-2} s^{-1}). The initial three-day dark incubation enhanced the responsiveness of explants by promoting endogenous hormonal equilibrium. Following shot emergence, cultures were subcultured every three weeks using the same medium composition to promote multiplication. Each treatment comprised at least 20 explants, and all studies were conducted in triplicate.

Root induction was evaluated using two distinct hormonal administration methods. The initial method involved pulse therapy by immersing the bases of regenerated shoots in sterile aqueous solutions of indolebutyric acid (IMK) or naphthaleneacetic acid (NSK) at concentrations ranging from 20 to 50 mg/L for a duration of 2 to 4 hours. Subsequent to the soaking, the shoots were relocated to hormone-free MS medium for the purpose of rooting. In the second method, shoots were directly grown on modified White medium with low sucrose (1%) and treated with 1.0 or 1.5 mg/L of IMK or NSK. White medium was favoured over MS at this stage because of its reduced salt concentration, which promotes root elongation and mitigates hyperhydricity. Root development was assessed by quantifying the number of roots, calculating the average root length, and determining the rooting percentage after 21 days of culture.

Successfully rooted plantlets were carefully extracted from the culture jars, rinsed to eliminate remaining agar, and transferred into plastic pots filled with a sterilised soil mixture of peat, sand, and perlite in a 1:1:1 ratio. To sustain elevated humidity throughout the initial hardening period, the pots were encased in transparent plastic domes or cups and situated in a greenhouse with regulated temperature (22°C) and relative humidity (80%). During the subsequent four weeks, the plants were systematically acclimatised to ambient conditions by intermittently removing the covers to facilitate gradual exposure to external humidity and light. The overall survival rate during acclimatisation was assessed through visual evaluation of foliar health, root stability, and ongoing

shoot development.

All gathered data, encompassing shoot induction %, average number of shoots per explant, rooting percentage, and root length, were subjected to statistical analysis by one-way analysis of variance (ANOVA). Duncan's multiple range test was utilised for post-hoc comparisons to identify significant differences among treatments. The threshold for statistical significance was established at $p < 0.05$. The analyses were conducted utilising SPSS software version 25.

Results

The in vitro propagation experiments on Populus euphratica produced high-efficiency regeneration and rooting outcomes through the selection of plant growth regulators and their appropriate dosages. The judicious application of cytokinins (BAP and ISK) and auxins (IBA and NAA) was pivotal in influencing the success rate of each micropropagation phase.

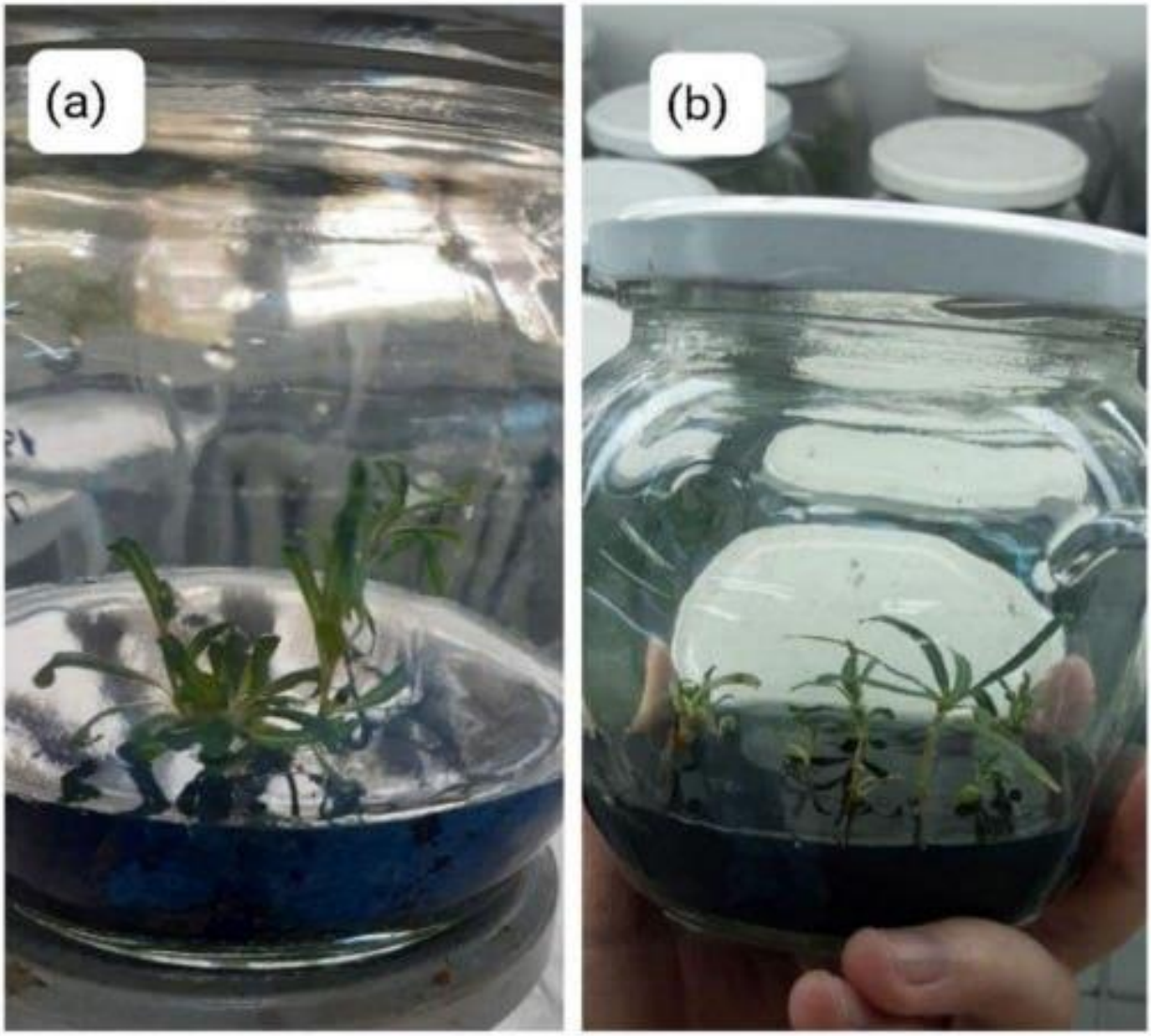
During the shoot proliferation phase, MS (Murashige and Skoog) media was augmented with varying doses of BAP and ISK. The experimental results indicated that the optimal combination was 1.5 mg/L BAP and 0.5 mg/L ISK, with a 92% regeneration rate, an average of 5.1 microshoots per explant, and a shoot length of 4.2 cm. Concentrations below or above this ideal ratio resulted in diminished multiplication efficiency and inferior morphological traits.

Table 1. Shoot regeneration efficiency in Populus euphratica using different BAP and ISK concentrations

Treatment Variant	Regeneration (%)	Avg. Shoots/Explant	Avg. Shoot Length (cm)
MS + 0.5 mg/L BAP + 0.2 mg/L ISK	67.4	2.6	2.1
MS + 1.0 mg/L BAP + 0.5 mg/L ISK	84.2	4.3	3.5
MS + 1.5 mg/L BAP + 0.5 mg/L ISK	92.0	5.1	4.2
MS + 3.0 mg/L BAP + 0.5 mg/L ISK	75.3	3.7	3.0

The results indicate that 1.5 mg/L BAP combined with 0.5 mg/L ISK provides the optimal hormonal equilibrium for shoot proliferation. Variations from this ideal ratio either diminished shoot quantity or resulted in inhibited growth. The morphological alterations observed under treatments with 3.0 mg/L Kin and 3.0 mg/L BAP are illustrated below to visually corroborate the data.

Picture **1. Shoot proliferation in Populus euphratica**

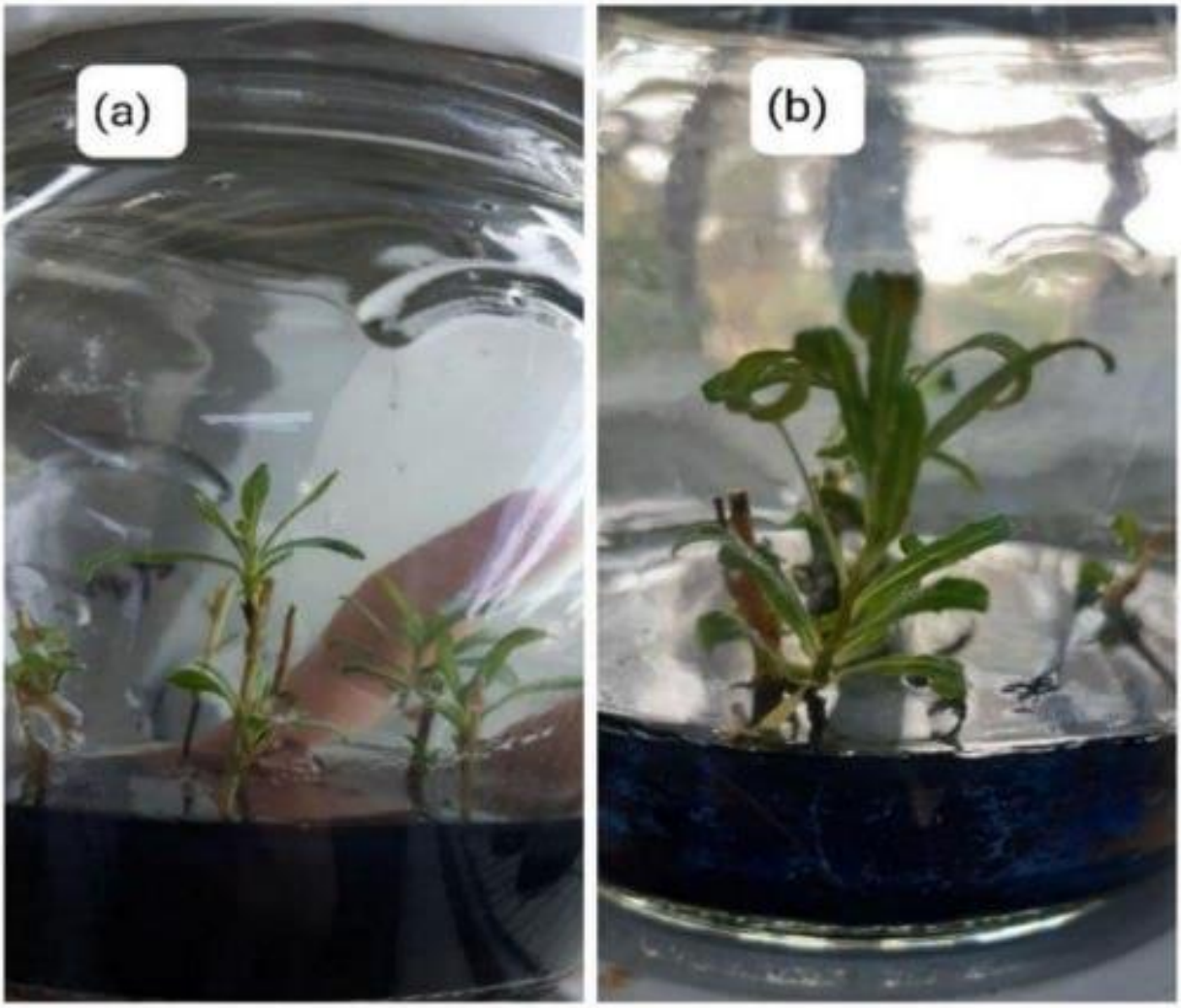


(a) MS + 3.0 mg/L Kin (b) MS + 3.0 mg/L BAP

(b) The image illustrates that BAP induced denser, healthier, and more compact microshoots, while Kin produced fewer and thinner shoots.

Further morphological variations were observed with combinations of Kin + NAA and BAP + NAA, as shown in the next picture.

Picture 2. Shoot development under Kin + NAA and BAP + NAA combinations



(a) MS + 3.0 mg/L Kin + 0.5 mg/L NAA (b) MS + 3.0 mg/L BAP + 0.5 mg/L NAA Picture 2 reveals that BAP + NAA supported apically dominant and morphologically stable shoot clusters, whereas Kin + NAA led to elongated and less vigorous shoots.

The rooting phase (rhizogenesis) was conducted by culturing shoots in MS medium supplemented with IBA and NAA in various concentrations. Four treatment variants were evaluated with 30 shoots per group. The best results were obtained with MS + 1.0 mg/L IBA + 0.1 mg/L NAA, which resulted in 64.5% rooting, an average of 3.7 roots per shoot, and a root length of 3.9 cm.

Table 2. Rooting performance of Populus euphratica using IBA and NAA combinations

Treatment Variant	Rhizogenesis (%)	Avg. Roots/Explant	Avg. Root Length (cm)
MS + 0.5 mg/L IBA	35.7	1.6	2.5
MS + 1.0 mg/L IBA	53.0	2.9	3.1
MS + 1.0 mg/L IBA + 0.05 mg/L NAA	58.6	3.2	3.5
MS + 1.0 mg/L IBA + 0.1 mg/L NAA	64.5	3.7	3.9

The combination of IBA and low quantities of NAA markedly improved rooting vs to IBA alone. The root number and length were enhanced, resulting in plantlets exhibiting healthier root

architecture.

Rooted plantlets were relocated to a peat:sand:perlite (1:1:1) substrate and acclimatised under greenhouse conditions (22°C, 80% humidity). After four weeks, 82% of the plantlets persisted. The regenerants displayed fibrous roots, robust shoot development, and a generally healthy appearance. The results validate that the established procedure for micropropagation of Populus euphratica is both effective and appropriate for implementation in ecological restoration initiatives aimed at saline and degraded areas.

Discussion

This study's findings indicate that Populus euphratica, a halophytic tree indigenous to arid and saline habitats, may be effectively propagated in vitro using particular combinations of cytokinins and auxins. The findings are significantly pertinent to initiatives aimed at rehabilitating ecologically damaged lands in Central Asia, especially in Uzbekistan, where soil salinisation and desertification present substantial challenges to indigenous flora. The maximum shoot proliferation occurred on MS medium augmented with 1.5 mg/L BAP and 0.5 mg/L ISK, yielding a 92% regeneration rate. This outcome is consistent with prior research on other Populus species, including P. alba and P. deltoides, which identified BAP as the most efficacious cytokinin for promoting axillary bud activation and shoot proliferation. The advantage of BAP compared to kinetin, as demonstrated in this study, may be ascribed to its enhanced bioactivity in facilitating cell division and the creation of meristematic tissue. Furthermore, the addition of ISK (indoleacetic acid) at moderate concentrations established a balanced hormonal milieu that promoted both shoot induction and elongation, as well as apical dominance. Morphological findings provide additional corroboration for the data. BAP treatments produced healthier, thicker shoots with vigorous nodal growth, whereas Kin treatments resulted in thinner and elongated microshoots. The incorporation of NAA with BAP enhanced shoot compactness and symmetry, indicating a synergistic interaction between cytokinin and auxin pathways in early organogenesis.

Root induction, or rhizogenesis, was another essential stage of the propagation process. The amalgamation of 1.0 mg/L IBA and 0.1 mg/L NAA elicited the most effective rooting response, resulting in 64.5% rooting, an average of 3.7 roots per explant, and a mean root length of 3.9 cm. These results align with other studies on Populus tremula and Populus nigra, wherein IBA combined with a low concentration of NAA promoted vigorous adventitious root development. The synergistic interaction between IBA and NAA is likely attributable to increased auxin absorption and translocation to the root initiation zone, hence enhancing the incidence and quality of rhizogenesis. In contrast, IBA alone, particularly at lower concentrations, resulted in much fewer roots, highlighting the importance of using mixed auxins for optimal root formation.

The 82% survival rate during the acclimatisation phase validates the physiological stability of the regenerated plantlets. These plants demonstrated robust roots systems and typical morphologies in greenhouse settings, confirming the efficacy of the in vitro technique for producing viable field-ready seedlings. This is especially significant for P. euphratica, which generally experiences inadequate seed germination and limited seedling establishment in natural environments. Despite the positive outcomes, specific restrictions must be recognised. This study examined nodal and apical explants from wild populations in Karakalpakstan; genotypic heterogeneity among other ecotypes may affect regeneration capacity. Furthermore, extensive field trials are essential to assess if in vitro-derived plants sustain their salt tolerance and ecological efficacy in extreme environmental circumstances. This study presents a dependable and scalable micropropagation methodology for the mass propagation of Populus euphratica. The combination of BAP and ISK for shoot induction, along with IBA and NAA for rooting, demonstrated the

highest efficacy. These findings endorse the utilisation of biotechnological techniques in afforestation initiatives and biodiversity preservation in salinised and dry regions of Uzbekistan and beyond.

Conclusion

This study effectively constructed a consistent and efficient in vitro micropropagation procedure for Populus euphratica, a keystone species in dry and saline habitats of Central Asia. Through systematic experimentation with different plant growth regulators, it was found that shoot proliferation is best induced using Murashige and Skoog (MS) medium supplemented with 1.5 mg/L benzylaminopurine (BAP) and 0.5 mg/L indoleacetic acid (IAA or ISK), resulting in a high regeneration rate of 92%. Root induction was most effective when utilising a combination of 1.0 mg/L indolebutyric acid (IBA) and 0.1 mg/L naphthaleneacetic acid (NAA), which resulted to enhanced rhizogenesis in terms of both frequency and root growth quality. The regenerated plantlets displayed healthy morphology, robust roots systems, and elevated survival rates following acclimatisation under greenhouse settings, affirming the physiological integrity and resilience of the micropropagated plants. The application of a White medium with diminished sugar levels during the rooting phase effectively mitigated vitrification and improved root quality, facilitating the successful transition of in vitro plants to ex vitro conditions. The findings have significant significance for the ecological restoration of degraded lands in Uzbekistan, where P. euphratica is essential for soil stabilisation, desertification mitigation, and biodiversity enhancement. The capacity for large production of this species through tissue culture offers a sustainable remedy to the issues of inadequate seed viability and restricted natural regeneration. In conclusion, the protocol established herein provides a scalable and reproducible approach appropriate for nursery production and reforestation initiatives aimed at salinised and arid environments. Subsequent research should concentrate on the field validation of these micropropagated plants and the examination of their long-term adaptation to various environmental conditions. This discovery represents a crucial advancement in utilising biotechnological techniques for conservation and environmental management in Central Asia.

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