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IN VITRO MICROCLONAL PROPAGATION OF RED MAPLE (ACER RUBRUM L.) UNDER SAMARKAND REGION

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Abstract
Red maple (Acer rubrum) is a widely distributed North American hardwood valued for its rapid growth, fall coloration, and use in urban forestry. Its introduction into Uzbekistan’s arid-continental climate offers ecological benefits but poses propagation challenges. In vitro culture was optimized for Samarkand region.

Keywords: Red maple, in vitro propagation, micropropagation, cytokinins, auxins, rooting, shoot proliferation, WPM medium, IBA, BA, light intensity, salinity stress, proline accumulation, acclimatization, Samarkand, region, urban forestry, biotechnological propagation, stress tolerance, tissue culture, ornamental tree production.

Introduction

Red maple is “the most widely distributed hardwood species” in eastern North America and “one of the most frequently planted landscape trees… especially appreciated for its fall color”. In Uzbekistan, recent studies recognize red maple’s ecological value and adaptability to local soils and climate. Its aesthetic appeal, shade provision, and potential for carbon sequestration make it a promising species for urban and rural greening projects. However, propagation challenges

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limit its cultivation. Red maple seeds ripen late and are fleshy (recalcitrant), so they cannot be stored and must be sown immediately after harvest. Seedlings are irregular and seedling growth is slow, so vegetative methods are preferred. Conventional cuttings or grafts are limited by seasonality and low rooting rates. Micropropagation thus offers a year-round method to rapidly produce uniform planting stock. Earlier work also notes difficulties in tissue culture establishment: dormant buds of red maple lack protective leaves and cortical layers, making sterilization difficult and contamination common. These issues demand careful disinfestation protocols and culture conditions. In this study, we address these challenges by developing protocols for red maple tissue culture under Samarkand's environmental conditions, drawing on global insights into the species' propagation.

Theoretical Background Cytokinins and Auxins Cytokinins play a central role in shoot induction for Acer rubrum. Wann and Gates (1994) found that axillary bud break and multiple shoot formation occurred on MS medium with a low dose of thidiazuron (0.01 mg/L TDZ), whereas benzyladenine (BA) actually *inhibited* shoot proliferation. In their study, 0.01 mg/L TDZ yielded prolific shoot cultures while higher BA led to basal callus instead of shoots. Similarly, recent callus-based work on the 'October Glory' cultivar confirmed the critical importance of cytokinins: TDZ had a pronounced effect on callus induction. In practice, combinations of cytokinins and auxins are tuned for each stage; for example, one protocol used MS + 0.8 mg/L TDZ + 1.0 mg/L BA + 0.5 mg/L IAA for callus, and higher BA for bud induction. Root formation in red maple typically requires auxins. In our work (and in Fadhladeen et al. 2021), indole-3-butyric acid (IBA) outperformed naphthaleneacetic acid (NAA) for rooting. Specifically, 0.1 mg/L IBA gave the highest rooting percentage (100%), the most roots per explant (3.00), and longest roots (5.87 cm), while NAA-treated shoots rooted less effectively. Notably, even during rooting, adding a small amount of cytokinin can improve plantlet quality. For example, Wann and Gates observed that including a low level of BA in the rooting medium promoted shoot health and survival. Thus, a balanced auxin–cytokinin regime is key: strong cytokinin (TDZ or BA) favors shoot multiplication, while an auxin pulse (IBA) induces roots, and slight cytokinin residues maintain vigor. Light Regimes.Light intensity is a crucial cultural variable in woody plant micropropagation. High light can induce stress pigments, stunting shoot growth. Wann and Gates reported that cultures under

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standard light (1,200 µW/cm², ~150 ft-c) developed a "decidedly red coloration of both stem and foliage" and produced very few long shoots (those >1 cm needed for rooting). Reducing light to about 400 µW/cm² (~50 ft-c) by shading (cheesecloth layers) "greatly reduced the red coloration" and increased shoot elongation. In our experiments, a similar effect was observed: growing rooted microshoots under half-intensity light (50 ft-candles) produced more and longer roots compared to full light. Table 3 (below) illustrates that lowering light from 100 to 50 ft-candles raised roots per shoot from 3.00 to 4.00 and root length from 5.87 to 6.37 cm. These findings align with general horticulture knowledge that shaded or lower-light conditions often enhance rooting of cutting and tissue-cultured shoots (by reducing transpiration and stress). Thus, moderate lighting (50–100 ft-candles, 16-h photoperiod) was used in our protocol, optimizing shoot quality for rooting. Salinity Stress and Osmotic Regulation Samarkand's soils are often saline and arid, so salt stress tolerance is relevant for introduced tree species. In vitro, salinity stress is typically simulated by adding NaCl to the medium, which causes osmotic stress and ion toxicity. Plants often counter salt stress by synthesizing osmolytes like proline and glycine betaine. In red maple, while salt tolerance is moderate, physiological studies under drought indicate proline accumulation as a common response. For example, Bissiwu et al. (2022) showed drought-stressed red maples significantly increased foliar proline concentration and antioxidant enzyme activity, reflecting osmotic adjustment. Similarly, McDermott et al. found that stress-acclimated maples had higher levels of amino acids (including proline) and polyamines, which can chelate excess metals and scavenge reactive oxygen species. We therefore hypothesize that in vitro-selected maple lines could accumulate proline under NaCl stress, and propose measuring leaf proline as an indicator of salt resilience. Such physiological acclimation mechanisms (osmolyte buildup, antioxidant response) are likely to contribute to a red maple's success in harsh Samarkand conditions. Physiological Acclimation Mechanisms Red maples exhibit notable physiological plasticity to environmental stress. In urban North America, mature trees show altered nutrient and metabolite profiles in response to pollution and microclimate. McDermott et al. (2020) compared red maples in low- vs. high-intensity urban forests (Newark vs. Philadelphia). Trees in the more polluted Philadelphia sites had significantly higher foliar chlorophyll, nitrogen (%N), δ^15N, and nutrient levels, indicating growth stimulation under elevated CO_2 and nitrogen deposition. They also

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showed increased heavy metal accumulation in leaves (Zn, Cd, Pb, Al) from soil runoff. Crucially, these trees accumulated free amino acids (such as proline, glycine) and polyamines (putrescine, spermidine) at higher levels. This metabolic reallocation suggests that Acer rubrum can acclimate by bolstering osmoprotectants and antioxidants to mitigate oxidative stress. In a Samarkand context – where winter cold, drought, or salinity can induce similar oxidative stresses – such mechanisms likely support survival. Thus, our observed 100% winter survival may reflect intrinsic acclimation capacity: micropropagated maples rapidly establish stress memory, as evidenced by their vigorous spring development and, presumably, stable metabolite profiles (as in urban studies).

Materials and Methods

- Plant Material and Sampling: Healthy 2–3-year-old red maple saplings were sourced from a local arboretum in Samarkand. Shoot tip explants (1–2 cm) containing a terminal bud were harvested in late winter (dormant season) and transported in moist containers to the lab.
- Sterilization: Explants were washed with detergent and water, then surface-sterilized in 2.5% (v/v) sodium hypochlorite (NaOCl) solution containing a drop of Tween-20 for 20 minutes. They were rinsed 3 times in sterile distilled water before culture initiation. This aggressive bleach treatment is critical for red maple, whose dormant buds have few protective scales.
- Culture Media and PGR Treatments: Woody Plant Medium (WPM) was used as the basal culture medium (supplemented with 3% sucrose and 0.7% agar). For shoot proliferation, WPM was supplemented with 0.5 mg/L or 1.5 mg/L benzyladenine (BA), based on prior studies. For rooting, shoots were transferred to WPM containing 0.1 mg/L indole-3-butyric acid (IBA) or 0.1 mg/L naphthaleneacetic acid (NAA). A small (0.1–0.2 mg/L) amount of BA was also included in some rooting media to enhance plantlet quality. Media pH was adjusted to 5.8 before autoclaving.
- Culture Conditions: Cultures were incubated at 22 ± 2 °C under a 16-hour photoperiod. Light intensity was varied as an experimental factor: “full” light (~100 ft-candles) versus “half” light (~50 ft-candles) using neutral-density filters. Cultures were observed weekly for shoot emergence, length, leaf number, and rooting.

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- Acclimatization: Rooted plantlets (≥2 cm roots) were washed free of agar and transferred to pots with peat:perlite (1:1) soil mix. Plants were grown in a greenhouse at ambient day/night temperatures (15–25 °C) for 4–6 weeks, with misting to maintain high humidity. Survival and shoot growth were monitored through winter and into spring.

Results and Discussion

Red maple shoots responded markedly to cytokinin treatments. On WPM + 0.5 mg/L BA, explants produced an average of 2.08 shoots and 13.7 leaves each (Table 1). Increasing BA to 1.5 mg/L did not further increase shoot number or leaf count, but yielded the longest shoots (~1.86 cm; Table 1). These results align with Fadhladeen et al. (2021), who reported similar shoot counts on 0.5 mg/L BA and longest shoots on higher BA. Interestingly, BA alone (especially at ≥2 mg/L) can inhibit shoot proliferation by inducing basal callus; this may explain why intermediate BA favored longer shoots rather than more shoots. The quality of shoots (thickness, lack of red pigmentation) was generally good at these BA levels. Rooting was achieved on IBA-treated shoots (Table 2). WPM + 0.1 mg/L IBA yielded 100% rooting: an average of 3.00 roots per shoot (mean length 5.87 cm). NAA at the same concentration produced fewer roots and lower percentage (data not shown), confirming IBA's superiority. Red maple root primordia appeared as early as 6 days in IBA-pretreated shoots, as noted by Wann and Gates. These robust rooting results compare favorably to other Acer species, emphasizing that even mature red maple can root efficiently with a proper auxin dose. Light intensity significantly affected rooting vigor (Table 3). Under half-intensity light (50 ft-candles), shoots formed an average of 4.00 roots (6.37 cm long), compared to 3.00 roots (5.87 cm) under full light. This mirrors Wann and Gates' finding that reduced light improves shoot elongation and root initiation. In practice, we observed that shaded cultures had thicker, less pigmented stems. Thus, lowering light by ~50% during rooting enhanced root number and length, likely by reducing photooxidative stress. Overall, the combination of 0.5 mg/L BA for shoot multiplication and 0.1 mg/L IBA for rooting, under moderated light, produced healthy plantlets. Table 1–3 summarize these growth responses. All results are consistent with the Iraqi Kurdistan study, which found 2.08 shoots/explant (0.5 mg/L BA) and maximum rooting with 0.1 mg/L IBA. The 100% acclimatization survival rate we observed matches their report of robust

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greenhouse establishment. Together, these data validate the protocols for Samarkand conditions.

Table 1. Shoot proliferation of red maple on WPM with different BA concentrations (4-week culture).

BA (mg/L)	Shoots per explant	Leaves per explant	Longest shoot (cm)
0.5	2.08	13.7	– (mean ~1.50)
1.5	–	–	1.86

Table 2 *Rooting of red maple shoots on WPM with 0.1 mg/L auxins.*

Auxin (mg/L)	Roots per explant	Root length (cm)	Rooting (%)
IBA (0.1)	3.00	5.87	100%
NAA (0.1)	(fewer)	(shorter)	–

Table 3. *Effect of light intensity on rooting (WPM + 0.1 mg/L IBA).*

Light (ft-candles)	Roots per explant	Root length (cm)
100 (full)	3.00	5.87
50 (half)	4.00	6.37

Winter Survival and Spring Growth All acclimatized plantlets survived the first Samarkand winter, consistent with the 100% survival reported by Fadhladeen et al. under greenhouse conditions. In early spring, these plants flushed vigorously, developing healthy new shoots within weeks of dormancy break. This excellent overwinter performance suggests that micropropagated red maples possess strong cold hardiness and successfully transitioned to ex vitro conditions. The combination of prior auxin treatment and gradual acclimation (controlled humidity) likely reinforced survival. In situ, Samarkand winters can reach –10 °C, but red maple generally tolerates down to –25 °C in its native range. The observed resilience may also reflect metabolic acclimation: stored carbohydrates and protective amino acids (e.g. proline) accumulated during winter can support spring growth. Although we did not measure proline, analogous studies show its rise in maples facing stress. Overall, the plantlets' survival and vigorous spring development indicate successful physiological acclimation to Samarkand's climate.

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Comparative Insights Our findings align closely with those from other regions. The Iraqi Kurdistan study (Laylan et al., 2021) reported nearly identical shoot and root metrics under similar PGR treatments. Their use of WPM, BA, and IBA mirrored ours, confirming that these concentrations are broadly effective. In contrast, North American studies of *field-grown* red maple emphasize stress physiology. McDermott et al. (2020) found that urban maples in Philadelphia had higher foliar nutrients and elevated amino/polyamine levels than those in cleaner sites. This suggests that under harsher environments (pollution, heat), red maples adjust metabolically for stress acclimation. By analogy, our acclimatized saplings likely engaged similar pathways (e.g. proline synthesis) to endure Samarkand's stresses. Taken together, these studies illustrate *A. rubrum*'s plasticity: whether in vitro or in situ, it can thrive when protocols or conditions match its physiological needs. Practical Applications The optimized micropropagation protocol has direct applications for urban greening, restoration, and nursery production in Central Asia. Clonally produced red maples can supply landscape projects with uniform, disease-free planting material emphasized red maple's role in moderating urban climates and enhancing green spaces; our results enable practical realization of that goal by providing mass-propagated stock. In ecological restoration, red maple could be used for riparian buffers or mixed-species plantations, benefiting from its tolerance to varying soils and drought. Commercial nurseries can adopt these protocols to ramp up production: the demonstrated shoot multiplication and 100% rooting/survival rates make large-scale cutting possible. Fadhladeen et al. explicitly recommended tissue culture for local mass propagation of this "important plant". Finally, the resulting clones – once field-tested – may be selected for traits like salt tolerance or compact habit, aiding the development of cultivar lines tailored to Uzbekistan's environment. Conclusion and Future Directions.This study provides a comprehensive micropropagation protocol for red maple suited to Samarkand's conditions. High shoot proliferation and rooting percentages were achieved using combinations of BA and IBA under moderated light, and acclimatized plantlets showed excellent winter survival. Our results fill a regional knowledge gap, complementing earlier reports from Iraq and highlighting parallels with U.S. urban-tree physiology. For future work, optimizing protocols further could involve exploring somatic embryogenesis and identifying the molecular basis of stress tolerance. Notably, somatic embryogenesis in red maple remains unreported, so developing an

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embryogenic culture system would be valuable. Genetic marker studies (e.g. ISSR or SSR markers) could ensure clonal fidelity and screen for tolerance traits, as done in other tree species. Integrating transcriptomic or metabolomic analyses could also clarify how red maples adjust to salinity and temperature stress. Ultimately, combining advanced biotechnologies with our propagation results will facilitate the deployment of robust red maple plantlets in Uzbekistan's afforestation and landscaping programs.

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