Tissue Culture and Rapid Propagation of Fast-Growing Elm (Ulmus pumila L.): Postprint

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Abstract

Using semi-lignified shoots of fast-growing white elm as explants, sterilization was performed with 75% ethanol and 0.1% HgCl . After initiation culture, the explants were induced to form cluster buds in proliferation medium. Subsequently, the cluster buds were separated into individual shoots for rooting induction, ultimately establishing a mature tissue culture rapid propagation system for fast-growing white elm. The results showed that: the optimal sterilization treatment combination for explants was 75% ethanol for 30 s + 0.1% HgCl for 8 min, with a contamination rate of 17.3% and a survival rate of 78%; the sterilized explants were inoculated onto initiation medium and cultured for 25 days, and the most suitable medium for white elm explant initiation was determined to be MS + 1.0 mg/L 6-BA + 0.1 mg/L IBA + 30 g/L sucrose + 6.5 g/L agar, with an initiation rate as high as 87.5%; the axillary buds from initiated explants were excised and inoculated onto proliferation medium for cluster bud induction, and the optimal proliferation medium was determined to be MS + 0.5 mg/L 6-BA + 0.1 mg/L + 0.1 mg/L IBA + 30 g/L sucrose + 6.5 g/L agar, with a subculture cycle of 25 days and a proliferation coefficient as high as 6.2; the cluster buds were separated into individual shoots and inoculated onto rooting induction medium, and the optimal rooting medium was determined to be 1/2 MS + 0.1 mg/L IBA + 0.1 mg/L IAA + 30 g/L sucrose + 6.5 g/L agar, with a rooting induction period of 30 days and a rooting rate of 97%. After hardening the rooted plantlets outdoors, they were transplanted into a mixed substrate with a volume ratio of perlite:vermiculite:peat of 1:1:1, achieving a survival rate of over 90%. The high proliferation coefficient, rooting rate, and transplant survival rate can reduce production costs and thus enable industrialized seedling production.

Full Text

Preamble

Tissue Culture and Rapid Propagation of Fast-Growing Ulmus pumila

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Abstract

Using semi-lignified branches of fast-growing Ulmus pumila as explants, we established a complete tissue culture and rapid propagation system through disinfection with 75% ethanol and 0.1% HgCl , initiation culture, cluster bud induction in proliferation medium, and subsequent rooting of individual shoots. The optimal disinfection protocol was 75% ethanol for 50 seconds followed by 0.1% HgCl for 8 minutes, resulting in a contamination rate of 17.3% and a survival rate of 78%. When sterilized explants were inoculated onto initiation medium for 25 days, the most suitable formulation was MS + 1.0 mg·L ^{1} 6-BA + 0.1 mg·L ^{1} IBA + 30 g·L ^{1} sucrose + 6.5 g·L ^{1} agar, achieving an initiation rate of 87.5%. Axillary buds from initiated explants were excised and transferred to proliferation medium, where the optimal composition was MS + 0.5 mg·L ^{1} 6-BA + 0.1 mg·L ^{1} IBA + 30 g·L ^{1} sucrose + 6.5 g·L ^{1} agar, yielding a proliferation coefficient of 6.2 over a 25-day subculture cycle. For rooting, individual shoots were inoculated onto rooting medium, with the best results obtained using 1/2 MS + 0.1 mg·L ^{1} IBA + 0.1 mg·L ^{1} IAA + 30 g·L ^{1} sucrose + 6.5 g·L ^{1} agar, which produced a 97% rooting rate after 30 days. Following acclimatization, plantlets were transplanted to a substrate mixture of perlite:vermiculite:peat (1:1:1 v/v/v), achieving survival rates exceeding 90%. The high proliferation coefficient, rooting rate, and transplant survival rate collectively reduce production costs and enable large-scale commercial propagation.

Keywords: fast-growing Ulmus pumila, tissue culture, optimal medium, rapid propagation

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Ulmus pumila L., a deciduous tree in the family Ulmaceae, is the most widely distributed elm species in China and holds significant ecological and economic value [?]. Characterized by straight trunks, tall stature, dense shade, strong adaptability, and rapid growth, it serves as a primary species for urban greening, windbreak establishment, soil and water conservation, and afforestation of salinealkali soils [?, ?]. While ordinary U. pumila seedlings grow approximately 1.5 m annually, fast-growing varieties can reach heights of about 3 meters in a single year [?]. In fast-growing U. pumila plantations, annual diameter growth can exceed 3.5 cm, with an average diameter of 22 cm at six years and maximum diameters reaching 28 cm [?].

Recent research on U. pumila has focused on germplasm conservation, hybrid breeding, seedling selection, provenance variation, molecular genetics, and unconventional breeding methods. Regarding propagation, grafting techniques are well-established, with successful plant production using U. pumila scions grafted onto U. densa, U. pumila seedlings, and other elm species [?]. Cutting propagation using current-year softwood cuttings treated with different rooting agents has also been reported [?], though this remains experimental and has not been applied commercially. Tissue culture studies on U. pumila are limited; Wang et al. reported plant regeneration from leaf explants, but issues such as low bud induction rates, vitrification, and rooting difficulties remain unresolved [?]. Addressing these challenges, this study aimed to establish a robust tissue culture and rapid propagation system for fast-growing U. pumila with improved induction and rooting rates, providing a theoretical foundation for commercial-scale propagation.

1.1 Experimental Materials

Fast-growing Ulmus pumila was collected from the Dongying Nursery in Shandong Province. Healthy, disease-free plants were selected as mother stock, and branches with full, uniform, slender, semi-lignified buds were harvested as explant material and transported to the tissue culture laboratory.

1.2.1 Explant Disinfection

Branches bearing axillary buds were cut into 0.5–1.5 cm segments, with leaves and petioles removed. After washing under running water for 30 minutes and gentle surface cleaning with a soft brush, the explants were transferred to a laminar flow hood. They were immersed in 2% sodium hypochlorite solution for 2 minutes, then treated with 75% ethanol for either 30 or 50 seconds, and finally disinfected with 0.1% HgCl for 4, 8, or 12 minutes. Following four to five rinses with sterile water, surface moisture was absorbed with sterile filter paper, and the explants were placed in sterile containers for subsequent use. Each treatment consisted of 105 stem segments with three replicates. Different disinfection durations were compared by calculating contamination and survival rates.

1.2.2 Initiation Culture

Six media formulations were tested for axillary bud induction: (1) MS + 0.5 mg·L^{1} 6-BA + 0.1 mg·L^{1} IBA; (2) MS + 0.5 mg·L^{1} 6-BA + 0.2 mg·L^{1} IBA; (3) MS + 0.5 mg·L^{1} 6-BA + 0.3 mg·L^{1} IBA; (4) MS + 1.0 mg·L^{1} 6-BA + 0.1

mg · L^{1} IBA; (5) MS + 1.5 mg · L^{1} 6-BA + 0.1 mg · L^{1} IBA; and (6) MS + 2.0 mg · L^{1} 6-BA + 0.1 mg · L^{1} IBA. All media were supplemented with 30 g · L^{1} sucrose and 6 g · L^{1} agar, with pH adjusted to 5.8. One explant was inoculated per vessel, with 75 vessels per treatment and three replicates. Cultures were maintained at (25 ± 3)°C under a 12 h · d^{1} photoperiod with light intensity of 3,500 lux. Axillary bud growth was assessed after 25 days, and initiation rates were recorded.

1.2.3 Proliferation Culture

Using MS as the basal medium, an L9(3) orthogonal experimental design was employed with three factors: 6-BA (0.1, 0.5, 1.0 mg · L^{1}), IBA (0.1, 0.2, 0.3 mg · L^{1}), and IAA (0, 0.1, 0.2 mg · L^{1}) . The proliferation coefficient was calculated 25 days after inoculation.

1.2.4 Rooting Culture

An L9(3) orthogonal design was also used for rooting medium optimization , testing three basal media (1/2 MS, 3/4 MS, MS) and two auxins: IBA (0.1, 0.2, 0.3 mg · L^{1}) and IAA (0, 0.1, 0.2 mg · L^{1}). Healthy shoots exceeding 2 cm in height from subculture were selected and inoculated onto these media. Rooting was assessed after 30 days, and rooting percentages were calculated.

1.2.5 Acclimatization and Transplanting

After 30 days of rooting culture, plantlets were transferred to natural environmental conditions for 3–5 days. Culture vessel caps were then loosened for 2 days with regular misting to maintain leaf turgor and prevent desiccation, facilitating adaptation to ambient conditions. Plantlets were removed from vessels, rinsed free of culture medium, disinfected in 5‰ potassium permanganate solution for 3 minutes, and finally washed with tap water before transplanting into a substrate mixture of perlite:vermiculite:peat (1:1:1 v/v/v). Regular watering was provided, and transplant survival rates were calculated after 30 days using the formula: (surviving plantlets / total transplanted plantlets) × 100%.

1.3 Data Processing and Analysis

Contamination rate = (number of contaminated explants / total inoculated explants) × 100%; survival rate = (number of surviving explants / total inoculated explants) × 100%; initiation rate = (number of sprouted stem segments / total sterile segments after inoculation) × 100%; proliferation coefficient = (total number of adventitious buds induced / total original inoculated stem segments); rooting rate = (number of rooted explants / total inoculated explants) × 100%.

Statistical analysis was performed using SPSS 16.0 and Excel software.

2.1 Explant Disinfection

As shown in Table 3 , the combination of 75% ethanol and 0.1% HgCl effectively reduced contamination rates with increasing disinfection duration ($P < 0.05$). Treatment 1 (shortest duration) and Treatment 6 (longest duration) showed significant differences in contamination rates ($P < 0.05$). Although Treatment 6 exhibited the lowest contamination rate, the extended disinfection time caused substantial damage to explants, resulting in only 65.7% survival. Considering both contamination and survival rates, Treatment 5 (75% ethanol for 50 seconds + 0.1% HgCl for 8 minutes) was identified as the optimal protocol, providing low contamination with high explant survival (78%).

2.2 Initiation Culture

Table 4 demonstrates that media (1), (2), and (3) induced slow, weak growth unsuitable for U. pumila initiation. Media (5) and (6) promoted rapid growth but caused severe vitrification, leaf curling, and excessive basal callus formation, making them unsuitable for axillary bud induction. Medium (4) yielded the highest initiation rate (87.5%) with robust, rapidly growing buds. Therefore, the optimal initiation medium was identified as (4) MS + 1.0 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} IBA.

2.3 Proliferation Culture

Axillary buds from initiation cultures were excised and transferred to proliferation media for cluster bud induction. Table 5 shows that the nine treatments affected proliferation differently, with coefficients ranging from 1.5 to 6.5. R-value analysis indicated that 6-BA had the greatest effect ($R = 3.5$), followed by KT and IBA. Variance analysis in Table 6 confirmed that the cytokinin 6-BA exerted the most significant influence on proliferation coefficient ($F = 171.438$, $P < 0.01$), while kinetin (KT) was also significant ($F = 24.813$, $P < 0.05$). Auxin IBA showed no significant effect. Among all treatments, medium #4 produced the highest proliferation coefficient of 6.2. Thus, the optimal subculture medium was MS + 0.5 mg · L ^{1} 6-BA + 0.1 mg · L ^{1} IBA + 0.1 mg · L ^{1} IAA.

2.4 Rooting Culture

Range analysis from Table 7 revealed that basal medium type was the most critical factor affecting rooting, followed by IBA, with IAA having minimal effect. Variance analysis in Table 8 confirmed that medium type was highly significant ($F = 847.459$, $P < 0.01$), IBA was significant ($F = 41.703$, $P < 0.05$), and IAA had no significant effect. Based on these results, the optimal rooting medium was (7) 1/2 MS + 0.1 mg · L ^{1} IBA + 0.1 mg · L ^{1} IAA, achieving a 97% rooting rate.

2.5 Acclimatization and Transplanting

After 30 days, transplant survival rates exceeded 90%. Approximately 50 days post-transplantation, U. pumila plantlets exhibited expanded, dark green leaves with new leaf emergence from apical buds.

Contamination represents the foremost challenge in tissue culture, and successful acquisition of adequate sterile material is the critical first step [?]. While single disinfectants cause less explant damage, they are less effective; combining multiple disinfectants significantly reduces contamination rates [?]. This study achieved effective disinfection using 75% ethanol for 50 seconds + 0.1% HgCl for 8 minutes. Future research should explore orthogonal combinations of two or more disinfectants to identify superior protocols.

Plant growth regulators are essential additives in tissue culture media, with cytokinins and auxins playing crucial roles in inducing differentiation [?]. This investigation examined various concentrations and combinations of 6-BA, KT, and IBA on U. pumila micropropagation. Cytokinin 6-BA most strongly influenced cluster bud induction; low concentrations induced slow growth with low proliferation coefficients, while higher concentrations increased bud numbers but caused severe vitrification. The optimal 6-BA concentration was determined to be 0.5 $\text{mg} \cdot \text{L}^{1}$, consistent with findings by Wang et al. (2009) [?]. These results further confirm the dual nature of plant hormones: promotion at low concentrations and inhibition at high concentrations.

In plant micropropagation, appropriate auxin concentrations facilitate rooting induction [?]. Auxin IBA significantly affected U. pumila rooting, with the optimal combination of 1/2 MS + 0.1 $\text{mg} \cdot \text{L}^{1}$ IBA + 0.1 $\text{mg} \cdot \text{L}^{1}$ IAA yielding a 97% rooting rate. While this study optimized the rooting formula using MS-based media, future research comparing different basal medium types could potentially achieve even higher rooting rates and further optimize the protocol.

In summary, this study established a mature in vitro rapid propagation system for fast-growing U. pumila, providing both theoretical foundations and technical support for industrial-scale seedling production. This system offers an efficient propagation method for U. pumila afforestation and supplies high-quality planting stock for the nursery market.

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