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ORIGINAL ARTICLE

Developing an improved protocol for micropropagation of sycamore maple (*Acer pseudoplatanus* L.)

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Abstract

Sycamore maple (*Acer pseudoplatanus* L.) is an ecologically and economically important hardwood in Central Europe. For high-value genotypes, like wavy grain maple, in vitro propagation offers a promising tool for targeted multiplication and conservation. This study contributed to an optimised micropropagation protocol by testing disinfection protocols, silver nitrate supplementation, cytokinin type, and light conditions. Winter buds from 36 donor trees were disinfected with either sodium hypochlorite (NaOCl) or sodium dichloroisocyanurate (NaDCC). NaDCC markedly reduced contamination (5.2% vs. 42.2% for NaOCl in crown buds) and simplified handling, recommending it for routine application. Across genotypes, 11.8 µM silver nitrate significantly enhanced multiplication (up to 3.4-fold), elongation and shoot quality, particularly in difficult-to-propagate genotypes. Trans-zeatin riboside showed minor, genotype-specific advantages over trans-zeatin, whereas sterilisation method (filter-sterilisation vs. autoclaving) had no effect. Light intensity was the main driver of multiplication and biomass accumulation, with optimal results at 50–60 µmol m^{-2} s^{-1}. Red-enriched spectra (RB ratios of 3:1 or 1:1) further promoted multiplication and elongation, but higher irradiance reduced SPAD values and induced anthocyanin pigmentation, suggesting potential light-induced stress. The combination of NaDCC disinfection, silver nitrate supplementation, trans-zeatin riboside, and tailored light regimes provided a reproducible framework for efficient sycamore maple micropropagation. These findings support genotype-specific protocol adjustments to balance morphological gains with physiological quality, enabling effective clonal production of elite timber genotypes.

Key message

Optimised in vitro conditions regarding light, cytokinin and silver nitrate improved shoot multiplication, elongation and quality in *Acer pseudoplatanus*, thus enabling efficient clonal propagation of valuable genotypes.

Keywords Cytokinin · Light quality · Silver nitrate · Surface disinfection · Sodium dichloroisocyanurate · Woody plant tissue culture · Clonal propagation · Hardwood species

Abbreviations

B	Blue light (445 nm)
FL	Fluorescent light
GLM	Generalised linear model
HSD	Honestly significant difference
IBA	Indole–3–butyric acid
LED	Light–emitting diode
MS	Murashige and Skoog (1962) medium
NaDCC	Sodium dichloroisocyanurate
NaOCl	Sodium hypochlorite
PGR	Plant growth regulator
R	Red light (660 nm)
RB 1:1	Red:blue light ratio 1:1

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RB 3:1	Red:blue light ratio 3:1
RoT	Repetition over time
SPAD	Single photon avalanche diode
TDZ	Thidiazuron
Z	Trans–zeatin
ZR	Trans–zeatin riboside

Introduction

Vegetative propagation enables the clonal reproduction of woody plants, which is of major importance in forestry, conservation biology, and horticulture. Unlike sexual reproduction via seeds, it allows for the preservation of specific genotypes and the production of uniform plant material (Bonga and von Aderkas 1992; Winkelmann 2013). In forestry, vegetative propagation is particularly important for cloning disease-resistant or fast-growing individuals, and for establishing stands with a high proportion of high-quality trees, typically consisting of clonal mixtures. Techniques such as rooting of cuttings, grafting, layering and micropropagation (tissue culture) are commonly employed, each offering specific advantages and limitations (George et al. 2008; Barthwal et al. 2025).

Despite its advantages, the practical application of vegetative propagation in woody plants faces significant challenges. These include propagation difficulties with many mature or adult woody plants, low rooting and regeneration rates, strong genotype dependency, and high contamination rates in in vitro cultures (Jain 2007; Monteuuis 2016). The success of vegetative propagation depends on the physiological age of the donor plant, the propagation technique, and the optimisation of environmental and hormonal conditions (Greenwood 1987; Debergh 1988; Jain 2007).

Acer pseudoplatanus L. (sycamore maple) is a key broadleaved tree species in Central Europe, valued for its ecological plasticity, vigorous juvenile growth, and the production of high-quality timber (Hein et al. 2009). Of particular interest are individuals that exhibit wavy grain (Richter 2015; Lewandrowski et al. 2024), a naturally occurring wood anomaly characterised by undulating fibre patterns. This feature markedly enhances the commercial value of the timber (Eisold et al. 2024). Consequently, there is growing interest in reliably propagating trees with this trait to ensure its availability in future timber resources. Despite the ecological and economic significance of sycamore maple, robust and reproducible protocols for in vitro propagation remain scarce. Traditional vegetative methods such as grafting or rooting of cuttings are technically possible but often inefficient, especially when elite genotypes with desirable traits like wavy grain are involved (Ewald and Naujoks 2015; Vurbeva et al. 2019). The establishment of in vitro cultures from bud material in sycamore maple presents several specific challenges. High contamination rates due to endophytic bacteria have been reported, often emerging only after multiple subcultures and leading to the loss of valuable explants (Ewald and Naujoks 2015). Furthermore, shoot formation rates from bud explants are typically low, with many explants producing only leaves or callus, and regeneration is strongly genotype-dependent (Vurbeva et al. 2019). Consequently, there is a clear need for optimised in vitro protocols for sycamore maple, with research focusing on explant selection, effective surface-disinfection methods, optimised media composition, and the targeted application of growth regulators alongside the control of environmental factors.

The ontogenetical age and origin of the explant are widely recognised as decisive factors for successful in vitro propagation, especially in woody species. Juvenile tissues, such as epicormic shoots, basal branches, or seedling-derived material, exhibit significantly higher morphogenic potential compared to mature tissues, largely due to differences in endogenous plant hormone levels, epigenetic status, and cell wall composition (Read and Bavougian 2012).

Efficient surface disinfection is essential for establishing aseptic in vitro cultures, especially in woody species, where explants are frequently colonised by persistent epiphytic or endophytic microorganisms. However, the choice and application of disinfecting agents must be carefully optimised, as excessive chemical exposure can lead to stress and tissue damage, and significantly reduce explant viability. In sycamore maple, dormant vegetative buds from mature trees have been disinfected successfully with ethanol and $HgCl_2$ (Gebhardt and Bohnens 2005; Vurbeva et al. 2019), but high levels of latent endophytic contamination and tissue damage remained problematic (Ewald and Naujoks 2015).

Silver nitrate ($AgNO_3$) is widely used in plant tissue culture as an effective ethylene inhibitor, improving explant performance and morphogenic responses across a range of species. Ethylene, a gaseous plant hormone, can accumulate in sealed culture vessels and negatively affect in vitro cultures by promoting leaf senescence and abscission, shoot tip necrosis, and browning of tissues (Park et al. 2016; Teixeira da Silva et al. 2020). The addition of silver nitrate to the culture medium inhibits ethylene action and has been shown to enhance shoot regeneration, somatic embryogenesis, and overall plantlet quality in several woody species (Qin et al. 2005; Martínez et al. 2017; Bashir et al. 2022). Besides ethylene, further plant growth regulators (PGRs) govern key physiological processes such as cell division, shoot and root formation, and the maintenance of meristematic identity, with cytokinins and auxins, in particular, playing a central role in regulating morphogenesis in vitro. Cytokinins are particularly important for inducing and promoting shoot

proliferation (Pierik 1997; George et al. 2008). Two commonly used cytokinins in woody plant tissue culture are zeatin and its riboside form. Among the different isomers, the trans-forms, trans-zeatin (Z) and trans-zeatin riboside (ZR), are particularly important because of their high biological activity (Gajdošová et al. 2011). The choice of the cytokinin type can significantly affect in vitro performance, as different compounds vary in their biological activity, uptake efficiency, susceptibility to degradation, and side effects such as shoot tip necrosis or hyperhydricity. An additional factor is the chemical stability of cytokinins during media preparation. While cytokinins are often considered heat-instable, studies have shown that several adenine-based cytokinins remain chemically stable during autoclaving when dissolved in mildly alkaline solutions such as 0.05 N KOH (Hart et al. 2016).

Light is not only the primary energy source for photosynthesis but also a vital environmental signal that orchestrates nearly all stages of plant growth and development (Mawphlang and Kharshiing 2017; Zheng et al. 2019). Under in vitro conditions, light quality (spectrum) and quantity (intensity) can strongly influence shoot proliferation, elongation, biomass accumulation and chlorophyll content (Jung et al. 2021). Traditionally, fluorescent lamps have been used to illuminate tissue cultures. However, they emit broad-spectrum light that may include wavelengths that are ineffective or even inhibitory for certain species or processes (Sæbø et al. 1995; Azmi et al. 2016). The introduction of light-emitting diode (LED) technology has enabled much more precise control over light in plant tissue culture because spectral composition, intensity, and photoperiod can be fine-tuned and combined as required (Manivannan et al. 2017; Zheng et al. 2019). Many in vitro studies have demonstrated that red light enhances stem elongation and biomass accumulation, while blue light promotes compact growth and higher chlorophyll content, although responses are highly species- and genotype-specific and depend on the developmental stage of the culture (McCree 1971; Li et al. 2013; Jung et al. 2021).

The aim of this study was to improve micropropagation of sycamore maple by systematically addressing key biological and environmental factors that influence in vitro culture success. The experimental approach focused on optimising shoot proliferation, elongation, and physiological quality by testing the effects of light spectrum and intensity, cytokinin type and sterilisation method, as well as silver nitrate supplementation. In parallel, explant source and disinfection protocols were evaluated for their impact on contamination and establishment success. Throughout all experiments, genotype-specific responses were analysed to identify reproducible and broadly applicable culture conditions.

Materials and methods

Establishment of shoot cultures from winter buds

Winter buds of sycamore maple were collected during the winters of 2022 to 2024 from 36 donor trees, located mainly in Germany and, to a lesser extent, in Switzerland. The material was obtained from three sources: trees with visible wavy grain, whose felled logs were offered at high-value timber auctions; grafted trees with either wavy or regular grain, derived from a seed orchard established between 1959 and 1964 in Reinhardshagen, Hesse, Germany; and offspring of the aforementioned grafted trees, felled for wood analysis from a progeny trial planted in 1986 in Hesse, Germany (Quambusch et al. 2021; Lewandrowski et al. 2024). Depending on material availability, buds were harvested either from crown branches or stump sprouts (i.e. epicormic shoots). Two surface disinfection protocols were tested:

(i) NaOCl treatment: Intact buds were first rinsed under running tap water for 3 min, then immersed in 70% ethanol for 3 min. This was followed by surface disinfection in 6% sodium hypochlorite (NaOCl) solution containing 0.05% Tween-20 for 25 min. Afterwards, buds were rinsed three times for 5 min each with sterile, autoclaved deionised water. Subsequently, bud scales were removed under sterile conditions, and the apical meristematic dome including the innermost leaf primordia was isolated and used as explant.
(ii) NaDCC treatment: Buds were first rinsed under running tap water for 3 min, placed in a sterile beaker and transferred to a laminar airflow cabinet, where all subsequent steps were carried out under aseptic conditions. Under the laminar flow, the bud scales were then removed with sterile forceps and a scalpel. The isolated apical meristematic dome including the innermost leaf primordia was immersed in a 0.5% sodium dichloroisocyanurate (NaDCC) solution containing 0.05% Tween-20 for 35 min, followed by direct placement onto the culture medium without further rinsing.

In both protocols, no additional trimming of the explants was performed after disinfection. Each explant was placed individually in a sterile test tube containing 15 mL of solid Rugini Olive medium (Rugini 1984) (Duchefa Biochemie, Haarlem, The Netherlands), supplemented with 0.045 µM L^{-1} thidiazuron (TDZ), 0.025 µM indole-3-butyric acid (IBA), 20 g L^{-1} sucrose, and 6 g L^{-1} Phyto agar (Duchefa Biochemie, Haarlem, The Netherlands). The pH was adjusted to 5.7 prior to autoclaving. Cultivation took place under the conditions specified below for the micropropagation experiments. Buds that developed elongated shoots

were classified as successfully established. Contamination was defined as any visible microbial growth on the explant or in the culture medium.

Micropropagation experiments

Plant material, explant preparation, culture media and general culture conditions

Unless stated otherwise, four genotypes of sycamore maple (E87, Schussi, Gött47-38, and SFHb1, Supplementary Table S1) were used in all experiments. Explants consisted of shoot tips and nodal segments excised from both main and lateral shoots of pre-existing in vitro stock cultures of these genotypes (see Supplementary Table S1 for details on origin and establishment). Due to the opposite leaf arrangement, each nodal segment typically beared two axillary buds. However, in cases where internodes were particularly short and compressed, up to four visible axillary buds per segment could occasionally be observed.

Unless otherwise stated, cultures were maintained on a modified medium composed of half-strength MS salts (Murashige and Skoog 1962) (Duchefa Biochemie, Haarlem, The Netherlands) and half-strength Rugini Olive salts (Rugini 1984), supplemented with 20 g L^{-1} sucrose, 11.8 µM silver nitrate ($AgNO_3$), 0.025 µM IBA, and 13.69 µM trans-zeatin. Trans-zeatin was sterilised by filtration (0.22 µm) and added to the medium after autoclaving, once cooled to approximately 50 °C. The medium was solidified with 6 g L^{-1} Phyto agar and adjusted to pH 5.7 prior to autoclaving (121 °C, 15 min). This formulation was used in Experiments 3–5, with modified versions applied in Experiments 1 and 2 (as explained below). Explants were cultured in 370 mL glass jars (Sturz-Glas, J. WECK GmbH u. Co. KG, Wehr-Öflingen, Germany), each containing approximately 70 mL of medium. A nonwoven fabric ring was placed between the jar and the glass lid to allow for gas exchange. Cultures were incubated at 22 °C under a 16-hour photoperiod, with a light intensity of ~20 µmol m^{-2} s^{-1} provided by LED lamps (spectrum: AP67, Valoya, Helsinki, Finland). Shoots were subcultured every five weeks. Unless stated otherwise, data were recorded after one culture passage.

The uppermost shoot tips (up to 3 cm long) were taken from healthy, well-developed elongated shoots and rooted in vitro using a modified MS-based medium composed of one-third-strength MS macronutrients, full-strength MS micronutrients, vitamins, and iron, supplemented with 0.492 µM IBA, 10 g L^{-1} sucrose, and solidified with 6 g L^{-1} Phyto agar. The pH was adjusted to 5.7 prior to autoclaving. After three to four weeks on the rooting medium, when most shoots had developed roots, all shoots, including those without visible roots, were transferred to cell trays filled with fine-structured substrate (Stender ZB 20, Art. no. 111008256, Stender GmbH, Schermbeck, Germany) composed of 90% peat, wood fibres, clay, and a fertiliser containing nitrogen, phosphorus, potassium, and micronutrients. The substrate had a pH of 6.0 according to the manufacturer. Acclimatisation took place in a foil-covered tunnel inside a greenhouse under high humidity and reduced light conditions for four weeks.

Micropropagation experiment 1: effect of silver nitrate

To investigate the effects of silver nitrate ($AgNO_3$) on in vitro shoot development, media were prepared either with $AgNO_3$, as described above, or without its addition. For each treatment and genotype, five culture vessels containing 10 explants each were prepared ($n = 50$ per group). The experiment was initiated in August 2022.

Micropropagation experiment 2: type, source and sterilisation of zeatin

To assess the effects of zeatin origin, molecular form, and sterilisation method on in vitro shoot multiplication and elongation, explants of sycamore maple were cultured on media containing either trans-zeatin (Z) or trans-zeatin riboside (ZR). Trans-zeatin was obtained from two commercial suppliers (Duchefa Biochemie, Haarlem, The Netherlands; and Phygenera, Hannover, Germany), while ZR was tested only from one source (Duchefa Biochemie). Cytokinins were incorporated into the medium either before autoclaving or by sterile filtration (0.22 µm) followed by addition to the cooled, autoclaved medium. The basal medium composition, including 11.8 µM $AgNO_3$ and IBA, was as described above. Depending on the treatment, either 13.69 µM Z or 8.54 µM ZR was used. For each treatment (Table 1), four culture vessels containing 10 explants each were prepared ($n = 40$ per treatment), and the experiment was initiated in February 2023.

Micropropagation experiment 3: light spectrum and intensity

To investigate the effects of light spectra and intensity on in vitro shoot development, five culture vessels per treatment and genotype were prepared, each containing 10 explants (total $n = 50$ per group). The basal medium composition, including 11.8 µM $AgNO_3$, 13.69 µM trans-zeatin, and IBA, was as described above. The experiment was conducted in two repetitions, initiated in June and December 2022, using commercially available LED lamps with spectra AP67 and NS12 (Valoya, Helsinki, Finland), each tested at three intensities (20, 40, and 60 µmol m^{-2} s^{-1}). Light intensity

Table 1 Overview of cytokinin treatments used in the experiment

No.	Cytokinin type	Source	Sterilisation method	Concentration [µM]	Concentration [mg/L]
1	trans-zeatin (Z)	Duchefa Biochemie	Autoclaved	13.69	3.0
2	trans-zeatin (Z)	Duchefa Biochemie	Filter-sterilised	13.69	3.0
3	trans-zeatin (Z)	Phygenera	Autoclaved	13.69	3.0
4	trans-zeatin (Z)	Phygenera	Filter-sterilised	13.69	3.0
5	trans-zeatin riboside (ZR)	Duchefa Biochemie	Autoclaved	8.54	3.0
6	trans-zeatin riboside (ZR)	Duchefa Biochemie	Filter-sterilised	8.54	3.0

measurements and the determination of emission spectra were performed for each light treatment using a spectroradiometer (Mavospec Base, Gossen Metrawatt GmbH, Nuremberg, Germany; results are presented in Supplementary Fig. S1). The measured waveband composition corresponded well with the values reported by the manufacturer (Supplementary Table S2).

Micropropagation experiment 4: light quality

To enable more precise control over light quality, a dedicated light module was implemented using a custom-designed LED growth cabinet (Bethge 2018; Assou et al. 2023). Five light qualities were tested at a comparable photon flux density of 15 µmol m^{-2} s^{-1}: fluorescent light (FL, 6500 K), blue light (B, 445 nm), red light (R, 660 nm), and red:blue combinations in ratios of 1:1 (RB 1:1) and 3:1 (RB 3:1). The experiment was initiated in January 2023 with three genotypes. The cabinet featured independently programmable compartments with adjustable LED spectra, controlled via a web interface hosted locally by a Raspberry Pi single-board computer. Compartments were covered with black curtains to prevent light contamination, and shelf temperature was maintained at 20 ± 0.5 °C using an automated water-based cooling system. For each light treatment and genotype, eight culture vessels were prepared (seven in the case of Schussi), each containing six explants (total $n = 48$ per group for E87 and SFHb1; $n = 42$ for Schussi).

Micropropagation experiment 5: light intensity 2

In a further experiment, the effects of different light intensities on in vitro shoot development were investigated using the same programmable LED growth cabinet. The spectral composition was held constant using red and blue LEDs in a fixed ratio of 3:1, while the photon flux density varied between 20, 30, 40, 50, and 60 µmol m^{-2} s^{-1}. The experiment was started in July 2023. For each light intensity and genotype, eight culture vessels were prepared, each containing six explants (total $n = 48$ per group).

Evaluation parameters

Data collection was performed at the end of the five-week cultivation period. Depending on the experimental setup, different parameters were assessed: In Experiments 1 to 5, shoot multiplication and elongation were assessed. The multiplication factor was defined as the number of apical and nodal explants per source explant. Shoot length was measured using a standard ruler. In Experiments 4 and 5, SPAD values were recorded using a portable SPAD meter (SPAD-502Plus, Konica Minolta, Japan), which calculates a dimensionless index based on differential light absorption at 650 nm and 940 nm. The device was calibrated prior to use according to the manufacturer's instructions. Readings were taken from the youngest fully expanded leaf, measured three times adjacent to the central vein. The mean SPAD value was calculated from these three readings.

Furthermore, in Experiments 4 and 5, shoot biomass was expressed as shoot dry mass per explant (mg), obtained by dividing the total dry mass of all explants per vessel by the number of shoots. Shoots were dried in paper bags at 70 °C for 48 h.

Statistical analyses

Statistical analyses were performed in R (version 4.4.2; R Core Team 2024) using RStudio (version 2024.12.1 + 563; Posit team 2025). The packages *car* (Fox and Weisberg 2019), *dplyr* (Wickham et al. 2023), *readr* (Wickham et al. 2024), *ggplot2* (Wickham 2016), *emmeans* (Lenth 2025), *multcompView* (Graves et al. 2024), and *multcomp* (Hothorn et al. 2008) were used for data handling, statistical analyses, and visualisation. Model assumptions were evaluated using Shapiro–Wilk tests for normality and Levene's tests for homogeneity of variances. In Experiments 1–3, multiplication factor and shoot length were analysed with generalised linear models (GLMs) assuming a Gamma error distribution and log-link. In Experiment 2, multiplication factor was analysed with a model including genotype, variant, source, and sterilisation, plus the interactions genotype × variant and genotype × sterilisation, while shoot length was analysed with a model including genotype × variant × sterilisation and source. In Experiment 3, the model included genotype,

RoT (repetition over time), intensity, and spectrum together with all two-way interactions; higher-order terms were excluded. In Experiment 4, multiplication factor and shoot length were analysed using GLMs with a Tweedie distribution and log-link, while dry mass and SPAD values were analysed using GLMs with a Gamma distribution and log-link. In Experiment 5, multiplication factor and shoot length were analysed using GLMs with a Tweedie distribution and log-link, SPAD values with a Gamma distribution and log-link, and dry mass with a two-way linear model assuming normally distributed residuals and homoscedasticity. Model significance was assessed with Type II likelihood-ratio chi-squared tests (car package) for GLMs and F-tests for the linear model. When significant main effects were detected, post hoc pairwise comparisons were performed using Sidak's correction for multiple testing, except for dry mass in Experiment 5, where Tukey's honestly significant difference (HSD) test was applied. All statistical tests were conducted at a significance level of $\alpha = 0.05$. Group means represent averages of all individual measurements per genotype × treatment combination.

Results

Establishment of shoot cultures from winter buds

To establish in vitro shoot cultures of selected valuable sycamore maple genotypes, winter buds were collected from 36 donor trees, originating either from crown branches or from stump sprouts. Successful establishment was defined as the formation of non-contaminated, elongating and multipliable shoots. In the initial phase of the work, NaOCl disinfection was applied mainly to crown-derived material, whereas in later years NaDCC was predominantly used, particularly for buds from stump sprouts. Under these respective conditions, contamination rates averaged 42.2% for NaOCl (crown) and 55.9% for NaOCl (stump sprouts), whereas NaDCC treatments yielded 5.2% (crown) and 15.7% (stump sprouts) contamination (Supplementary Table S3). Although disinfectant type, source material and collection year were not varied independently, the consistently lower contamination levels observed with NaDCC led to its adoption as the standard method. Explants from stump sprouts also tended to show higher establishment rates (6.7% in average) than those from crown branches (3.4%) (Supplementary Table S4). Given the non-independent variation of factors, these values are presented descriptively and should not be interpreted as statistically tested effects.

Micropropagation experiment 1: effect of silver nitrate

The effect of $AgNO_3$ on shoot elongation and multiplication was assessed under standard culture conditions. Both, $AgNO_3$ treatment and genotype, had a highly significant effect on multiplication factor (Supplementary Table S5) and shoot length (Supplementary Table S6), and a strong genotype × treatment interaction was observed. The addition of $AgNO_3$ increased the multiplication factor in all genotypes (Fig. 1a). The most pronounced effect was observed in genotype E87, where the multiplication factor rose from 1.3 without $AgNO_3$ to 4.4 with $AgNO_3$. In contrast, the smallest increase occurred in Götte47-38, where values increased from 1.1 to 1.5. For shoot length, the strongest response to $AgNO_3$ was again observed in E87, where mean values increased significantly from 0.9 cm to 4.7 cm. Smaller but still significant increases occurred in Götte47-38 (from 1.1 cm to 1.5 cm) and Schussi (from 0.6 cm to 1.3 cm), whereas SFHb1 showed no significant change, with mean shoot length changing from 2.9 cm without $AgNO_3$ to 3.5 cm with $AgNO_3$ (Fig. 1b).

$AgNO_3$ treatment also led to notable improvements in shoot appearance (Fig. 1c). The proportion of high-quality shoots increased in all genotypes, most markedly in E87, where over 90% were rated as high quality with $AgNO_3$, compared to only approximately 10% without. Moreover, the proportion of pale green shoots decreased, while medium green became predominant. Dark green shoots remained rare across treatments.

Micropropagation experiment 2: type, source and sterilisationof zeatin

As no significant differences were detected between the two sources of zeatin (Duchefa Biochemie and Phygenera; Supplementary Tables S7, S8), the data were pooled for further analyses.

Shoot multiplication was significantly affected by genotype and cytokinin type, whereas sterilisation and the tested interactions had no significant effects (Supplementary Table S7). On average across genotypes, multiplication rates were significantly higher under ZR (2.2) than under Z (2.0). Within genotypes, a significant difference between Z and ZR was found only for Götte47-38 (1.9 vs. 2.1). For the other genotypes (E87, Schussi, SFHb1), multiplication rates were also slightly higher under ZR compared with Z, although these differences were not statistically significant (Fig. 2a).

Shoot elongation was significantly affected by genotype, cytokinin type, and the cytokinin sterilisation method (Supplementary Table S8). On average, shoots were longer under ZR than under Z. Across genotypes, SFHb1 produced

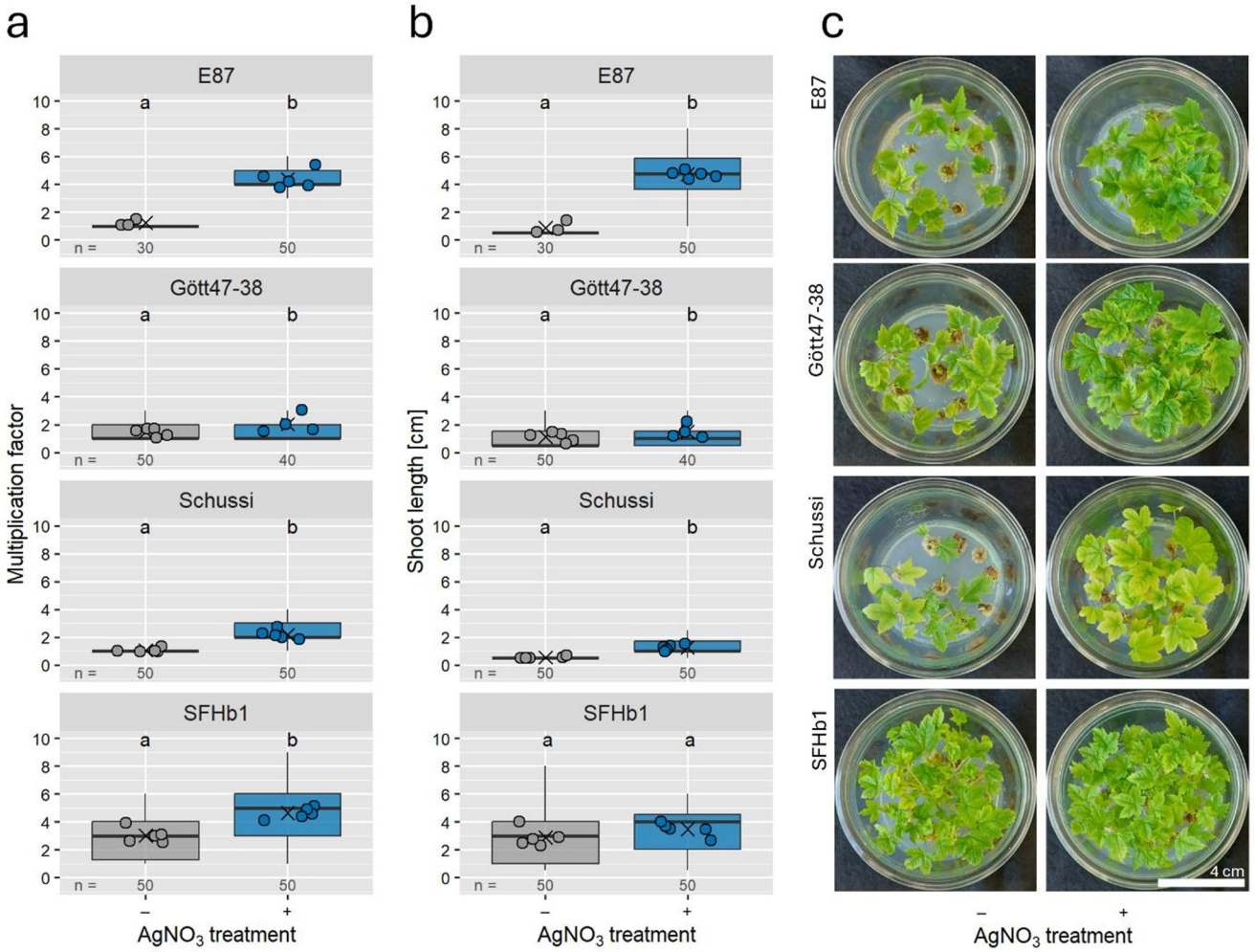


Fig. 1 Effects of silver nitrate ($AgNO_3$) on in vitro shoot development in four sycamore maple (*Acer pseudoplatanus* L.) genotypes (E87, Götti47-38, Schussi and SFHb1). (**a**) Multiplication factor and (**b**) shoot length with (+) and without (–) $AgNO_3$ supplementation (11.8 µM). Each dot represents the mean value per culture vessel; crosses indicate group means. Sample sizes (n) refer to the number of shoots measured for multiplication factor and shoot length. Different letters indicate statistically significant differences within genotypes (generalised linear model followed by Sidak-adjusted pairwise comparisons, $\alpha = 0.05$). (**c**) Representative culture vessels under $-AgNO_3$ and $+AgNO_3$ conditions. Scale bar: 4 cm

the longest shoots (2.4–3.9 cm), followed by Götti47-38 (1.3–2.1 cm) and Schussi (0.9–1.1 cm), whereas E87 exhibited the least elongation (0.7–0.9 cm). Although significant effects of sterilisation and a genotype × variant interaction were detected, pairwise comparisons did not reveal significant differences between sterilisation methods within genotypes. In contrast, clear differences among genotypes were evident within individual treatments (Supplementary Fig. S2). Detailed mean values for each treatment combination are provided in Supplementary Table S9.

Micropropagation experiment 3: light spectrum and intensity

The effects of light spectra on shoot development were investigated using commercially available LED lamps with spectra AP67 and NS12, each tested at three intensities, i.e. 20, 40 and 60 µmol m^{-2} s^{-1}. All cultures were grown on the same basal medium containing 11.8 µM $AgNO_3$, 13.69 µM trans-zeatin, and IBA, as described above.

Shoot multiplication was significantly affected by genotype and light intensity, whereas the GLM did not indicate a significant effect of the light spectrum (Supplementary Table S10). Significant genotype × repetition and genotype × intensity interactions, however, revealed that responses to repetition and intensity differed among genotypes. In repetition 1, multiplication rates ranged from about 2.0–2.3 in Schussi to around 3.0 in SFHb1, with intermediate values for E87 and Götti47-38. E87 increased from 2.7 at 20 µmol m^{-2} s^{-1} to 3.2 at 40 µmol m^{-2} s^{-1}, with little further change at 60 µmol m^{-2} s^{-1} (3.3). Götti47-38 showed a similar trend, rising from 3.0 at 20 µmol m^{-2} s^{-1} to 3.4 at 40 µmol m^{-2}

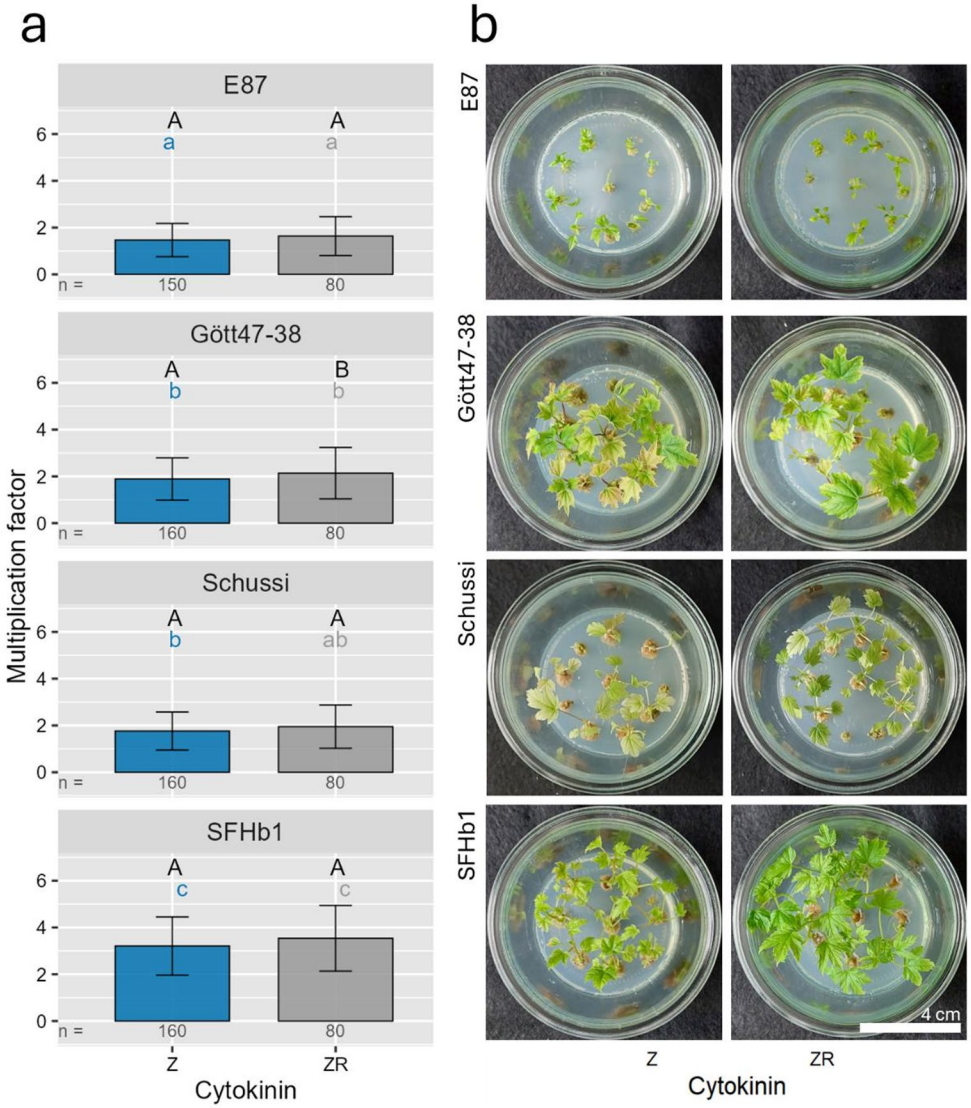


Fig. 2 Effects of cytokinin type on shoot multiplication in four sycamore maple (*Acer pseudoplatanus* L.) genotypes (E87, Götт47-38, Schussi and SFHb1). (**a**) Multiplication factor under trans-zeatin (Z, blue) and trans-zeatin riboside (ZR, grey) across the four genotypes. Bars represent means, and error bars indicate standard deviations (SD). Uppercase letters indicate statistically significant differences between cytokinin treatments within each genotype, while lowercase letters indicate differences between genotypes within each treatment (generalized linear model followed by Sidak-adjusted pairwise comparisons, $\alpha = 0.05$). Sample sizes (n) refer to the number of shoots measured. (**b**) Representative culture vessels of each genotype under trans-zeatin (left) and trans-zeatin riboside (right). Scale bar = 4 cm

s^{-1} and reaching 3.6 at 60 µmol m^{-2} s^{-1}. SFHb1 remained stable at around 3.0 across all intensities. In repetition 2, the overall response to light intensity was comparable for E87, Götт47-38 and Schussi, with increases from lower values at 20 µmol m^{-2} s^{-1} to higher rates at 40 µmol m^{-2} s^{-1}, followed by a plateau at 60 µmol m^{-2} s^{-1}. SFHb1, however, reached consistently higher multiplication rates across all intensities (4.3–4.4), compared with about 3.0 in repetition 1, reflecting the significant genotype × repetition interaction (Fig. 3a).

Shoot length was significantly affected by genotype, light intensity and repetition (RoT), while the spectrum had no detectable effect (Supplementary Table S11). In repetition 1, shoot length ranged from about 2.0–2.2 cm in SFHb1 to 0.9–1.1 cm in Schussi, with intermediate values for E87 and Götт47-38. E87 increased from 1.6 cm at 20 µmol m^{-2} s^{-1} to 2.5 cm at 40 µmol m^{-2} s^{-1}, with no further change at 60 µmol m^{-2} s^{-1}. Götт47-38 showed a similar trend, rising from 2.0 cm at 20 µmol m^{-2} s^{-1} to 2.4 cm at 40 µmol m^{-2} s^{-1} and remaining at 2.4 cm at 60 µmol m^{-2} s^{-1}. In repetition 2, shoot length was generally higher. E87 increased from 1.9 cm at 20 µmol m^{-2} s^{-1} to 2.8–2.9 cm at 40 and 60 µmol m^{-2} s^{-1}. Götт47-38 rose moderately from 1.6 cm at 20 µmol m^{-2} s^{-1} to 1.8 cm at 60 µmol m^{-2} s^{-1}. Schussi remained shortest, with values of 1.1 cm at 20 and 40 µmol m^{-2} s^{-1} and 1.2 cm at 60 µmol m^{-2} s^{-1}. SFHb1 consistently produced the longest shoots, measuring 3.5 cm at 20 µmol m^{-2} s^{-1}, 3.3 cm at 40 µmol m^{-2} s^{-1} and 3.7 cm at 60 µmol m^{-2} s^{-1}, without significant differences among intensities (Supplementary Fig. S3a).

Shoot colour was visually affected by light intensity and differed notably between repetitions. In repetition 1, higher intensities led to yellowing, especially in Schussi and Götт47-38. In repetition 2, shoots remained predominantly green across all treatments (Fig. 3b, Supplementary Fig. S3b). In both experimental repetitions and all genotypes,

higher light intensities induced anthocyanin production, leading to reddish leaf colour.

Micropropagation experiment 4: light quality

The effects of five defined light qualities were tested at a fixed intensity of 15 µmol m^{-2} s^{-1} using a programmable LED cabinet and employing only three genotypes due to shortage of material for Gött47-38. Multiplication was significantly affected by genotype, light quality, and their interaction (Supplementary Table S12). SFHb1 showed consistently high multiplication rates across all light treatments (range: 3.7–4.5), with no significant differences between treatments. In contrast, genotypes E87 and Schussi responded differently. For E87, multiplication increased from 1.4 under FL to 2.4 under B and 2.3 under RB 3:1. A similar trend was observed in Schussi, with factors from 1.2 (FL) to 2.7 (RB 3:1). For both genotypes, multiplication under FL was significantly lower compared to other light treatments (Fig. 4a).

Shoot length was significantly influenced by genotype, light treatment, and their interaction (Supplementary Table S13). SFHb1 developed the longest shoots among all genotypes, ranging from 2.0 cm under RB 3:1 to 3.9 cm under red light (R). E87 and Schussi exhibited shorter shoots overall. E87 shoot lengths varied between 0.6 cm (RB 1:1) and 1.0 cm (RB 3:1), with significantly shorter values under RB 1:1. Schussi showed no statistically significant differences between treatments, with lengths ranging from 0.6 cm (FL) to 0.9 cm (RB 3:1) (Fig. 4b).

SPAD measurements revealed significant effects of genotype, light quality, and their interaction (Supplementary Table S14). SFHb1 exhibited the highest SPAD values across all treatments, ranging from 10.6 (B) to 16.7 (R), with R and FL yielding the highest values (16.7 and 16.6), while B resulted in significantly lower values. In Schussi, SPAD values varied significantly among light treatments, with the lowest values under B (9.9) and FL (12.4), and higher values under R, RB 1:1, and RB 3:1 (13.1–14.6). E87 showed minimal variation, with values between 13.7 (B) and 16.5 (RB 3:1), and no statistically significant differences among treatments (Fig. 4d, Supplementary Fig. S4a, b).

Destructive harvesting enabled biomass quantification as dry mass per explant (Supplementary Table S15). SFHb1 accumulated the highest dry masses under all light treatments, ranging from 15.7 mg (FL) to 25.5 mg (B), with similarly high values under R, RB 1:1, and RB 3:1 (22.3–24.9 mg). In contrast, Schussi showed much lower shoot dry masses, with values ranging from 5.7 mg under FL to 9.7 mg under RB 3:1. Across light treatments, all means remained below 10 mg. E87 had the lowest biomass overall, with means between 2.5 mg under FL and 4.8 mg under RB 3:1 (Fig. 4c).

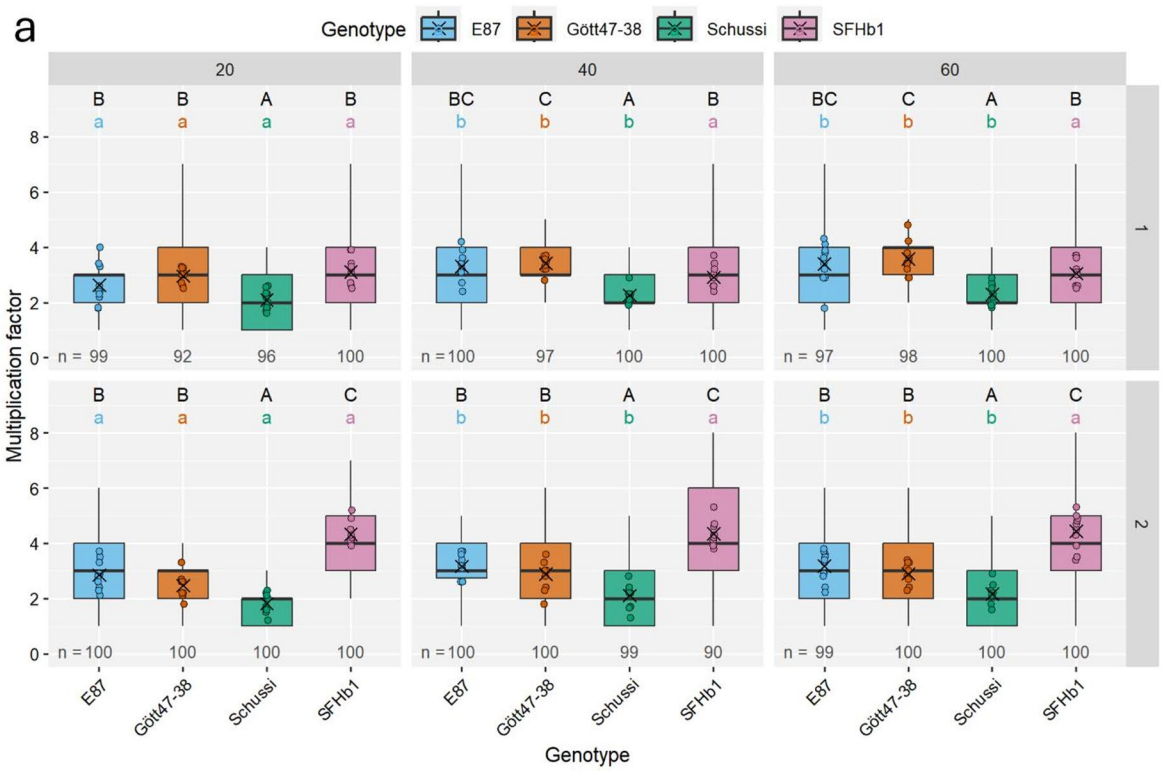
Micropropagation experiment 5: light intensity 2

The last experiment investigated light intensity again, but this time for a fixed red:blue ratio of 3:1 and in smaller increments. Multiplication was significantly affected by genotype, light intensity, and their interaction (Supplementary Table S16). SFHb1 displayed the strongest light response, with a steady increase in multiplication rate from 3.0 at 20 µmol m^{-2} s^{-1} to 4.0 at 60 µmol m^{-2} s^{-1}. In contrast, Gött47-38 showed consistently low values across all intensities (1.8–2.4), with no significant differences observed. E87 exhibited a moderate response, increasing from 2.9 (20 µmol m^{-2} s^{-1}) and 2.4 (40 µmol m^{-2} s^{-1}) to a peak of 3.5 at 50 µmol m^{-2} s^{-1}, followed by a slight decline at 60 µmol m^{-2} s^{-1}. A similar trend was observed in Schussi, with multiplication rising from 2.0 to 2.9 at 50 µmol m^{-2} s^{-1}, then slightly decreasing to 2.8 at 60 µmol m^{-2} s^{-1} (Supplementary Fig. S5a).

Shoot length was significantly influenced by both, genotype and light intensity, although no significant interaction was found (Supplementary Table S17). SFHb1 produced the longest shoots overall, increasing from 1.8 cm at 20 µmol m^{-2} s^{-1} to a maximum of 2.3 cm at 50 and 60 µmol m^{-2} s^{-1}. Gött47-38 and Schussi exhibited the shortest shoots (0.9–1.4 cm), with no statistically significant differences among light intensities, although a general upward trend with increasing light intensity was apparent. E87 showed a clearer response to light, with shoot length increasing from 1.2 cm to 1.8 cm across the light gradient. Overall, shoot elongation tended to increase with light intensity across genotypes, with SFHb1 responding most strongly (Supplementary Fig. S5b).

SPAD values were significantly influenced by genotype, light intensity, and their interaction (Supplementary Table S18). SFHb1 recorded the highest SPAD values at all light levels, with values declining significantly from 20.6 (20 µmol m^{-2} s^{-1}) to 14.3 (60 µmol m^{-2} s^{-1}). E87 showed a comparable pattern, with a significant reduction from 20.2 to 13.1. The most pronounced decline occurred in Gött47-38, where values decreased from 17.9 to 8.1, also with significant differences across intensities. Schussi experienced a similar decrease from 15.5 to 9.7, becoming significant from 40 µmol m^{-2} s^{-1} onwards (Fig. 5a). Clear patterns were also observed in leaf pigmentation (Fig. 5a), where anthocyanin accumulation became more apparent under higher light intensities.

Shoot dry mass was significantly affected by genotype and light intensity (Supplementary Table S19). E87 consistently produced the lowest dry masses, ranging from


a
Genotype
E87
Götti47-38
Schussi
SFHb1
20
40
60
1
2
Multiplication factor
B B A B
a a a a
BC C A B
b b b a
BC C A B
b b b a
B B A C
a a a a
B B A C
b b b a
B B A C
b b b a
n = 99 92 96 100
n = 100 97 100 100
n = 97 98 100 100
n = 100 100 100 100
n = 100 100 99 90
n = 99 100 100 100
0
2
4
6
8
E87
Götti47-38
Schussi
SFHb1
Genotype

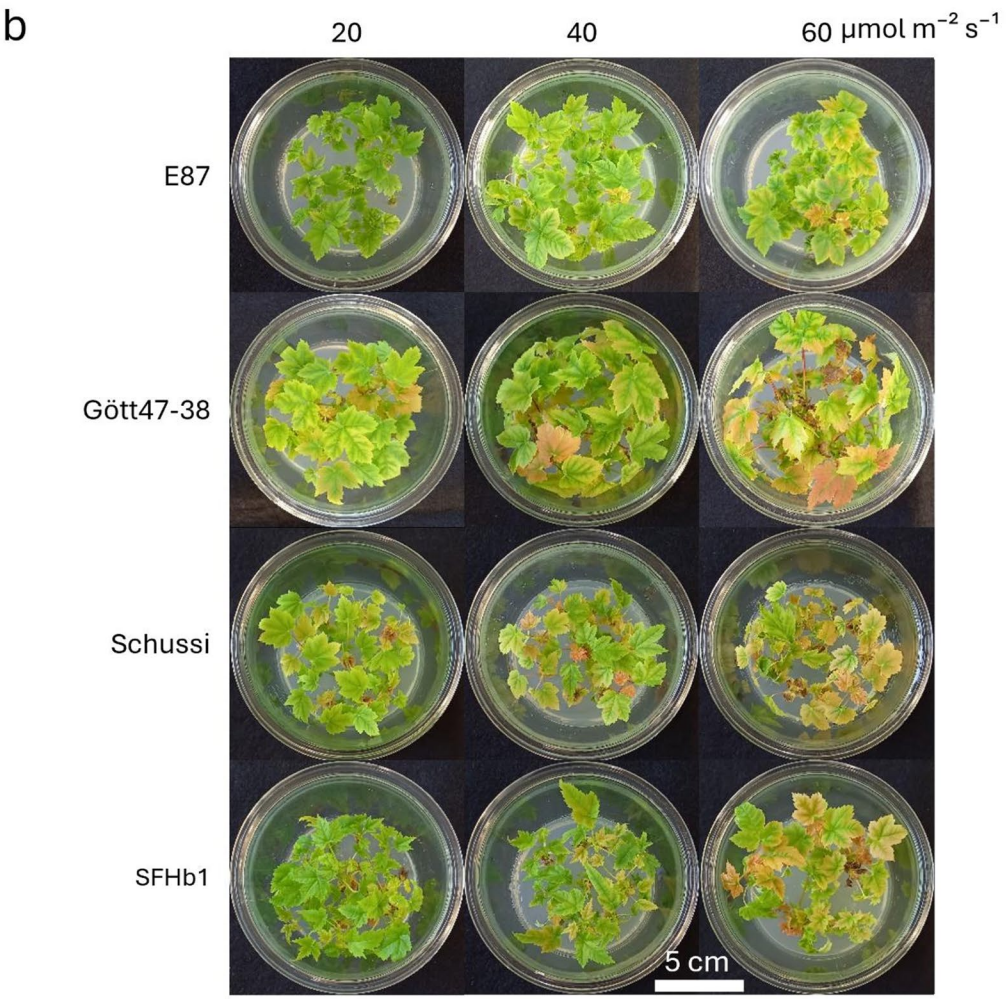

b
20
40
60 µmol m⁻² s⁻¹
E87
Götti47-38
Schussi
SFHb1
5 cm

Fig. 3 Effects of light intensity on shoot multiplication and phenotype in four sycamore maple (*Acer pseudoplatanus* L.) genotypes (E87, Gött47-38, Schussi and SFHb1). (**a**) Multiplication factor at three light intensities (20, 40 and 60 µmol m^{-2} s^{-1}). Each dot represents the mean per culture vessel; crosses indicate group means. Sample sizes (n) refer to the number of shoots measured for the multiplication factor. Uppercase letters indicate significant differences between genotypes within a light intensity and repetition, lowercase letters compare light intensities within a genotype and repetition (generalised linear model followed by Sidak-adjusted pairwise comparisons, $\alpha = 0.05$). (**b**) Representative shoot cultures under the tested light intensities in repetition 1, spectrum AP67. Scale bar = 5 cm

19.8 mg at 20 and 30 µmol m^{-2} s^{-1} to 33.7 mg at 60 µmol m^{-2} s^{-1}, with no significant differences between light intensities. In contrast, SFHb1 showed the strongest response to increasing irradiance, reaching 73.9 mg at 60 µmol m^{-2} s^{-1}, significantly higher than at lower intensities (e.g. 40.5 mg at 20 µmol m^{-2} s^{-1}). Dry masses of Gött47-38 and Schussi shoots also responded positively to light intensity, increasing from approximately 41–44 mg at 20 µmol m^{-2} s^{-1} to over 64 mg at 60 µmol m^{-2} s^{-1}; however, these increases were less consistent and not always statistically significant (Fig. 6).

Rooting and acclimatisation

Rooting and acclimatisation were evaluated as proof of concept only and for a relatively low number of shoots. Clear genotype-dependent differences in rooting success were observed (Supplementary Fig. S6): While some genotypes, such as E87 exhibited maximal in vitro rooting rates of 100%, others like Schussi showed poor root formation with only 21% of rooted shoots. Interestingly, it was occasionally observed that shoots, which had not developed roots in vitro, subsequently rooted after transfer to substrate. The successful acclimatisation of plantlets in substrate under controlled conditions generally resulted in stable plant development.

Discussion

Sycamore maple (*Acer pseudoplatanus*) is an ecologically and economically important hardwood species in Central Europe. For the wavy grain phenotype, a wood anomaly that significantly increases timber value (Eisold et al. 2024), strong evidence of a genetic basis was reported recently (Bäucker et al. 2020). In vitro propagation of selected valuable trees offers a promising strategy for the targeted multiplication and conservation. In this context, we evaluated in vitro establishment, multiplication and shoot quality of sycamore maple across genotypes and culture conditions, including explant sources, disinfectants, silver nitrate, cytokinin type and light. Although rooting and acclimatisation were not systematically evaluated, preliminary trials indicated in vitro rooting rates ranging from 20 to 100%, with successful acclimatisation under controlled conditions in most cases. The fact that unrooted shoots could also be acclimatised suggests that ex vitro rooting may represent a viable alternative for sycamore maple genotypes, potentially reducing the need for resource-intensive in vitro rooting.

Establishment of sycamore maple shoot cultures

Dormant winter buds of sycamore maple proved to be suitable for in vitro culture establishment and represent a feasible explant source during the dormant season. Previous reports indicate that both, dormant winter buds (Gebhardt and Bohnens 2005) and sprouting vegetative buds (Rohr and Hanus 1987) can be used successfully, highlighting their general feasibility as explant sources under appropriate conditions. The present study shows for the first time, that NaDCC is an effective disinfectant for sycamore maple in vitro establishment. It led to consistently lower contamination rates than NaOCl and maintained tissue viability, presumably due to the physiologically compatible pH value of 5.8 (Bartsch et al. 2014). As it does not require rinsing steps, handling time and labour are reduced, making it highly suitable for routine or commercial applications. Compared to earlier protocols using $HgCl_2$ (Ewald and Naujoks 2015; Vurbeva et al. 2019), which initially reduced contamination but failed to prevent latent endophyte growth and tissue damage, stable shoot cultures were established using NaDCC and NaOCl. In addition, the advantage of low toxicity of NaDCC, which is also used for disinfection of swimming pools, is important. The importance of selecting an appropriate disinfectant, treatment protocol and explant type is also evident from work on *Acer macrophyllum*, where Zhou and Mattsson (2021) reported markedly higher survival after disinfection for greenhouse-grown shoots than for field-collected sprouts, likely due to lower microbial load.

The ethylene antagonist silver nitrate ($AgNO_3$) significantly improved propagation and shoot quality

The inclusion of silver nitrate ($AgNO_3$) at 11.8 µM substantially enhanced shoot multiplication, elongation, and quality across all genotypes, with the most pronounced effects observed in the otherwise recalcitrant genotype E87. These improvements support the established role of silver ions as competitive inhibitors of ethylene perception (Martínez et al. 2017) and are consistent with reports from other species where $AgNO_3$ improved shoot proliferation and reduced leaf drop (Qin et al. 2005; Drisya Ravi et al. 2019; Mani et al. 2024). This confirms $AgNO_3$'s effectiveness as an ethylene antagonist, especially in ethylene-sensitive genotypes.

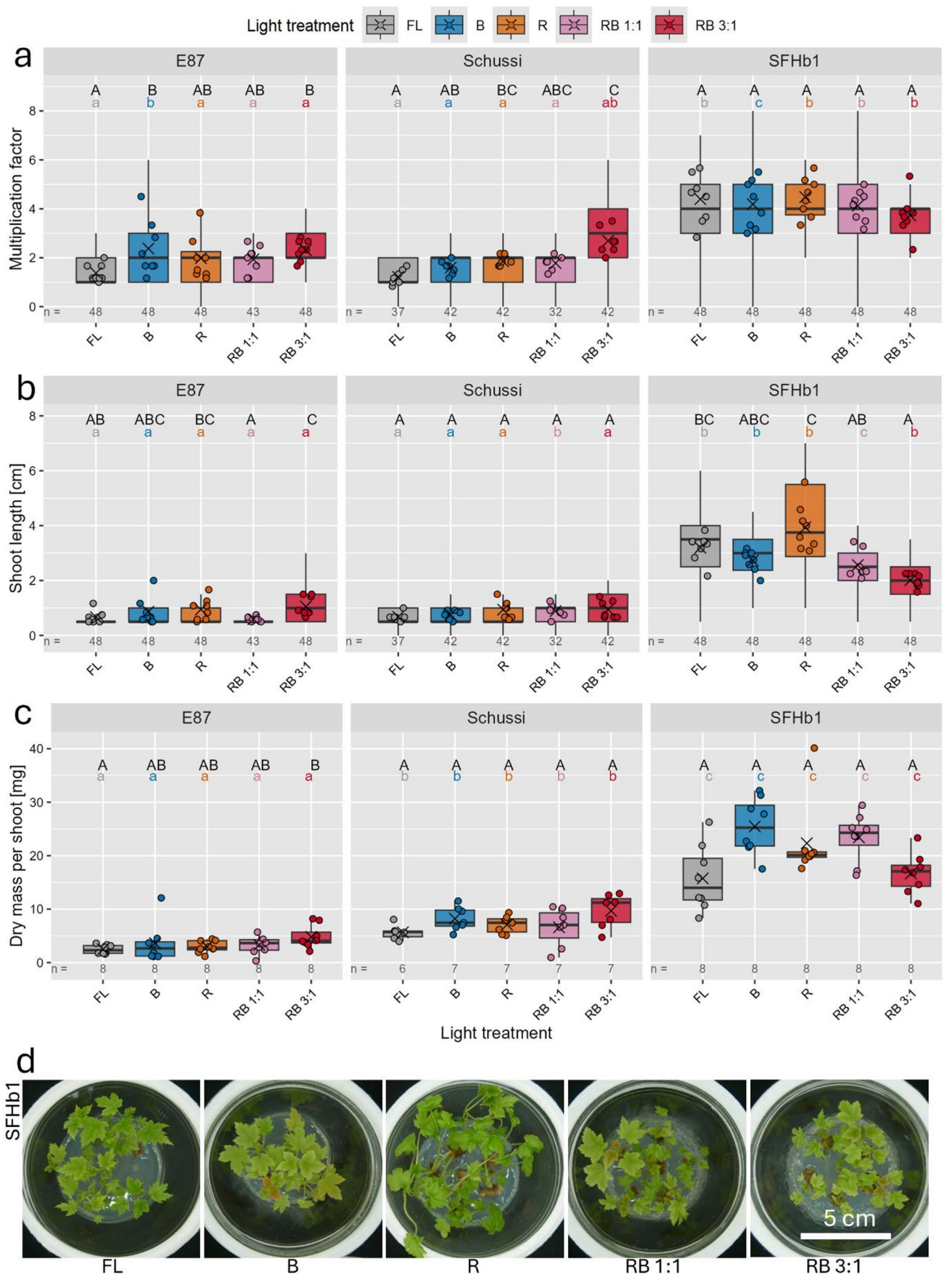

Light treatment
FL
B
R
RB 1:1
RB 3:1
a
E87
Schussi
SFHb1
Multiplication factor
b
Shoot length [cm]
c
Dry mass per shoot [mg]
Light treatment
d
SFHb1
5 cm
FL
B
R
RB 1:1
RB 3:1

Fig. 4 Effects of light quality on shoot development in the three sycamore maple (*Acer pseudoplatanus* L.) genotypes (E87, Schussi and SFHb1). (**a**) Multiplication factor, (**b**) shoot length, and (**c**) dry mass under five light treatments: fluorescent light (FL, 6500 K), blue light (B, 445 nm), red light (R, 660 nm) red:blue 1:1 (RB: 1:1) and red:blue 3:1 (RB 3:1). Each dot represents the mean per culture vessel; crosses indicate group means. Sample sizes (n) refer to the number of shoots (multiplication factor, shoot length); for dry mass, n indicates the number of vessels. Uppercase letters indicate differences between light treatments within each genotype; lowercase letters compare genotypes within each treatment (generalised linear model followed by Sidak-adjusted pairwise comparisons, $\alpha = 0.05$). (**d**) Representative shoot cultures of genotype SFHb1 under the tested light treatments. Scale bar = 5 cm

The significant genotype × treatment interaction observed here suggests varying levels of ethylene sensitivity, indicating that ethylene inhibition may be particularly beneficial in genotypes that are prone to ethylene-induced morphogenic inhibition. In addition to quantitative gains, silver nitrate also led to marked improvements in qualitative traits, particularly shoot morphology and pigmentation: a near-complete shift towards high-quality shoots in genotype E87, along with the stabilisation of shoot quality in SFHb1. These improvements not only enhanced the visual quality of the cultures, but may also contribute to better rooting performance and acclimatisation success in subsequent stages.

Effects of zeatin type and sterilisation on in vitro shoot development

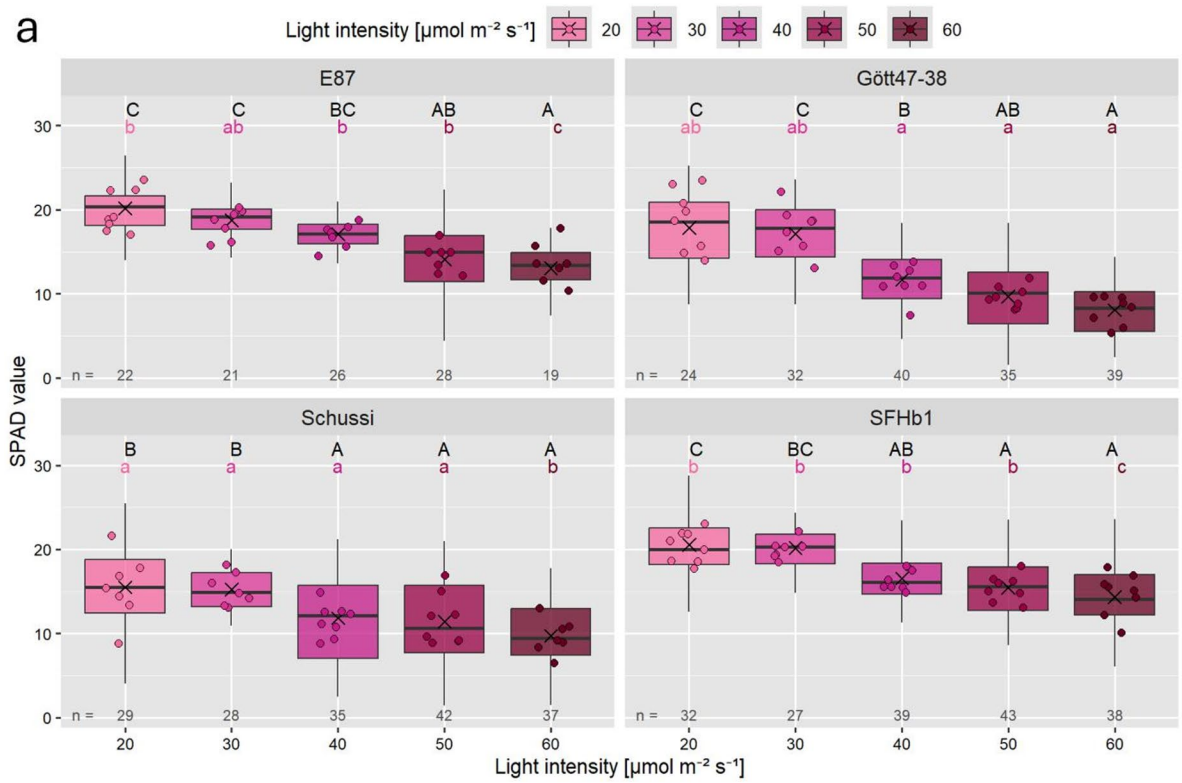
In the present study, 8.54 µM trans-zeatin riboside (ZR) generally promoted higher shoot proliferation and elongation compared with an equimolar concentration of trans-zeatin (Z, 13.69 µM) (Fig. 2, Supplementary Fig. S2). Although mean values were slightly higher under ZR across all genotypes, a statistically significant difference in multiplication rate was observed only in Gött47-38, whereas the other genotypes exhibited non-significant trends in the same direction. The fact that ZR performed equally well, and in many cases better, despite its lower concentration underlines its biological effectiveness relative to Z. Similar advantages of ZR over other cytokinins have been reported in *Vaccinium corymbosum* and hybrid *Castanea* spp., where ZR strongly promoted shoot multiplication without inducing shoot tip necrosis (Rowland and Ogden 1992; Song et al. 2021). The superior performance of ZR across diverse plant species supports the hypothesis that cytokinin conjugates promote more sustained morphogenic responses due to their gradual release of the active hormone (Hangarter et al. 1980; Rowland and Ogden 1992), which may be particularly relevant in genotypes with inherently low cytokinin responsiveness, as illustrated by genotype E87 in the current study.

No significant differences were observed between the zeatin products from two commercial suppliers, suggesting comparable biological activity under standard culture conditions. The method of cytokinin sterilisation significantly affected shoot elongation, although pairwise comparisons did not reveal significant differences between sterilisation methods within genotypes. Nevertheless, there was a tendency towards slightly longer shoots when cytokinins were autoclaved compared with filter-sterilised (Supplementary Fig. S2). These observations challenge earlier assumptions about the thermal sensitivity of zeatin. Hart et al. (2016) demonstrated that adenine-based cytokinins, including trans-zeatin, remain chemically stable during autoclaving when dissolved in mildly alkaline solutions, and in the present study multiplication factors were not influenced by the method of sterilisation.

Light effects on shoot multiplication

Light intensity emerged as the most consistent driver of shoot multiplication in sycamore maple, with significantly higher multiplication rates observed at 50 and 60 µmol m^{-2} s^{-1} across both, commercially available broad-spectrum (AP67 and NS12) LEDs and red-blue LED setups (Fig. 3a, Supplementary Fig. S5a). These results are consistent with findings indicating that increasing light intensity up to approximately 60 µmol m^{-2} s^{-1} can enhance shoot proliferation in many species, often followed by a stagnation (Cavallaro et al. 2022). In contrast, shade-adapted species such as *A. saccharum* exhibit stress responses at similar intensities, emphasising the species-specific nature of light sensitivity in vitro (Singh et al. 2017).

Spectral composition also played a decisive role at low light intensity (15 µmol m^{-2} s^{-1}). Red-enriched spectra, particularly RB 3:1, significantly increased multiplication rates compared to fluorescent tube light or blue light (Fig. 4a). The RB 3:1 condition proved optimal for the genotypes E87 and Schussi, while SFHb1 responded best to monochromatic red light, suggesting phytochrome-mediated stimulation of organogenic pathways and/or cell elongation. Similar promotive effects were reported in *Camellia oleifera*, where red:blue (4:1) light resulted in the highest shoot multiplication rates and improved morphological and physiological traits (He et al. 2020). A superiority of the red-enriched AP67 spectrum (Valoya, Helsinki, Finland) was not observed at any of the tested light intensities in Experiment 3, indicating an additional effect of other parts of the spectrum. Compared to NS12, AP67 contains higher proportions of red and far-red light, whereas NS12 is more enriched in blue and green wavelengths (Supplementary Table S2).


a
Light intensity [µmol m⁻² s⁻¹] 20 30 40 50 60
E87
Götti47-38
Schussi
SFHb1
SPAD value
Light intensity [µmol m⁻² s⁻¹]

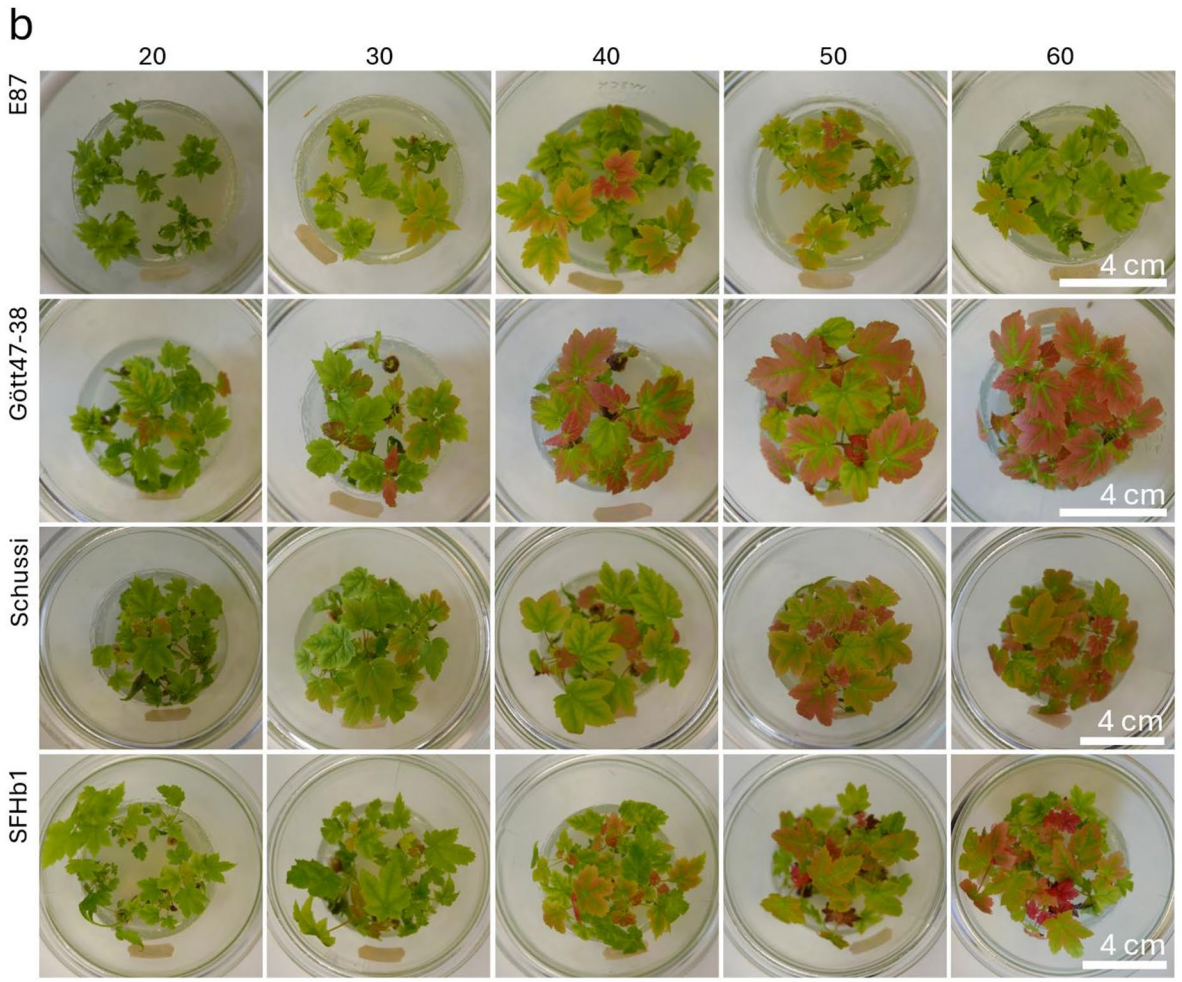

b
20 30 40 50 60
E87
Götti47-38
Schussi
SFHb1
4 cm

Fig. 5 Effects of light intensity under a red:blue light ratio of 3:1 on SPAD values and shoot appearance in four sycamore maple (*Acer pseudoplatanus* L.) genotypes (E87, Gött47-38, Schussi and SFHb1). (**a**) SPAD values across four genotypes and five light intensities (20–60 µmol m^{-2} s^{-1}). Each dot represents the mean per culture vessel; crosses indicate group means. Sample sizes (n) refer to the number of shoots measured. Uppercase letters indicate differences between light intensities within genotypes; lowercase letters compare genotypes within each intensity (generalised linear model followed by Sidak-adjusted pairwise comparisons, $\alpha = 0.05$). (**b**) Representative shoot cultures at the end of the experiment. Columns correspond to light intensities (left to right); rows show genotypes. Scale bar = 4 cm

Light effects on shoot elongation

A clear linear relationship ($R^2 = 0.498$, $r = 0.706$, $p < 0.001$) was observed between multiplication factor and shoot length across all genotypes, indicating that proliferation rate increased proportionally with shoot elongation. Since multiplication involved nodal and apical explants, this relationship was not unexpected and highlights the importance of shoot length as a parameter. Elongated shoots enable an easier handling during subculturing and are preferred. Elongation was stimulated by both increased light intensity and red-light-enriched spectra. Across the experiments, shoot length increased steadily from 20 to 60 µmol m^{-2} s^{-1}, with SFHb1 reaching up to 3.9 cm under red light and 2.3 cm under high-intensity RB 3:1 conditions. These findings align with observations in *Eucommia ulmoides*, where an optimal light intensity of 80 µmol m^{-2} s^{-1} significantly promoted shoot elongation compared to both lower and higher intensities (Wang et al. 2024). However, the degree of elongation remained highly genotype-dependent with Schussi and Gött47-38 showing little elongation, whereas SFHb1 responded positively under nearly all light regimes.

Light conditions with a high red-light proportion at elevated intensities promoted shoot elongation, however, in highly responsive genotypes such as SFHb1, excessive elongation occasionally led to spatial constraints in standard culture vessels, with shoot tips reaching the lid and showing necrosis. For such genotypes, larger vessels or modified culture passage durations may be necessary to obtain shoots of good quality.

Light effects on dry mass accumulation

Dry mass accumulation increased significantly under both, elevated light intensities and specific spectral treatments. The highest values (~60–74 mg per explant) were observed at 60 µmol m^{-2} s^{-1} in SFHb1 and Gött47-38. Substantial gains were also recorded under blue light and RB 1:1 spectral conditions, particularly in SFHb1. These results suggest that both light quantity and quality influence dry mass accumulation. Future investigations should focus on the physiological explanations for these observations, especially, if photosynthetic carbon assimilation which is normally considered to be of minor importance under in vitro culture conditions is the driver of an increased uptake of carbon and nutrients from the culture medium.

Blue light, despite its comparatively weaker effects on elongation and multiplication, led to a significantly increased shoot dry mass in SFHb1 and showed a similar trend in Schussi (not statistically significant). This response may be related to enhanced chloroplast development and increased leaf thickness, as reported for *Gossypium hirsutum* (Li et al. 2010) and *Betula pendula* (Sæbø et al. 1995). In these species, blue light stimulated palisade tissue formation, chlorophyll accumulation, and increased functional chloroplast surface area, all contributing to possibly enhanced photosynthetic capacity.

However, plant responses to blue light are highly species-specific under in vitro conditions, as demonstrated by contrasting reports in *Ficus benjamina*, *Vitis vinifera* hybrids, *Malus domestica* and *Pyrus communis* (Chée 1986; Chée and Pool 1989; Muleo and Morini 2008; Lotfi et al. 2019). Some of these contradicting findings may be due to differences in the wavelengths selected for applying the blue light, among other experimental factors.

SPAD value representing relative chlorophyll content decreased with increasing light intensity

SPAD values decreased with light intensity, particularly beyond 40 µmol m^{-2} s^{-1}, indicating a decline in relative chlorophyll content. A significant correlation between SPAD readings and directly measured chlorophyll content was confirmed in a complementary trial (Supplementary Fig. S7, $r = 0.632$, $p < 0.001$), based on chlorophyll quantification using modified Lichtenthaler (1987) equations, supporting the use of SPAD as a reliable proxy in this context. This pattern was consistent across all genotypes and stands in contrast to biomass and elongation trends, suggesting that higher irradiance may induce photooxidative stress or inhibit chlorophyll biosynthesis (Takahashi and Badger 2011). Under low-intensity conditions (15 µmol m^{-2} s^{-1}), fluorescent light resulted in the highest leaf SPAD values, particularly in SFHb1, while blue light resulted in the lowest values in Schussi and SFHb1, even though it promoted dry biomass. This decoupling of physiological and morphological responses was also noted by Singh et al. (2017) in *A. saccharum*, where biomass increased with light intensity, but chlorophyll content and stress parameters worsened.

Also, shoot colour varied visibly in response to light intensity and spectrum, with a higher proportion of yellowing and red pigmentation observed under elevated irradiance. The occurrence of red pigmentation, likely associated with anthocyanin accumulation, may reflect a protective

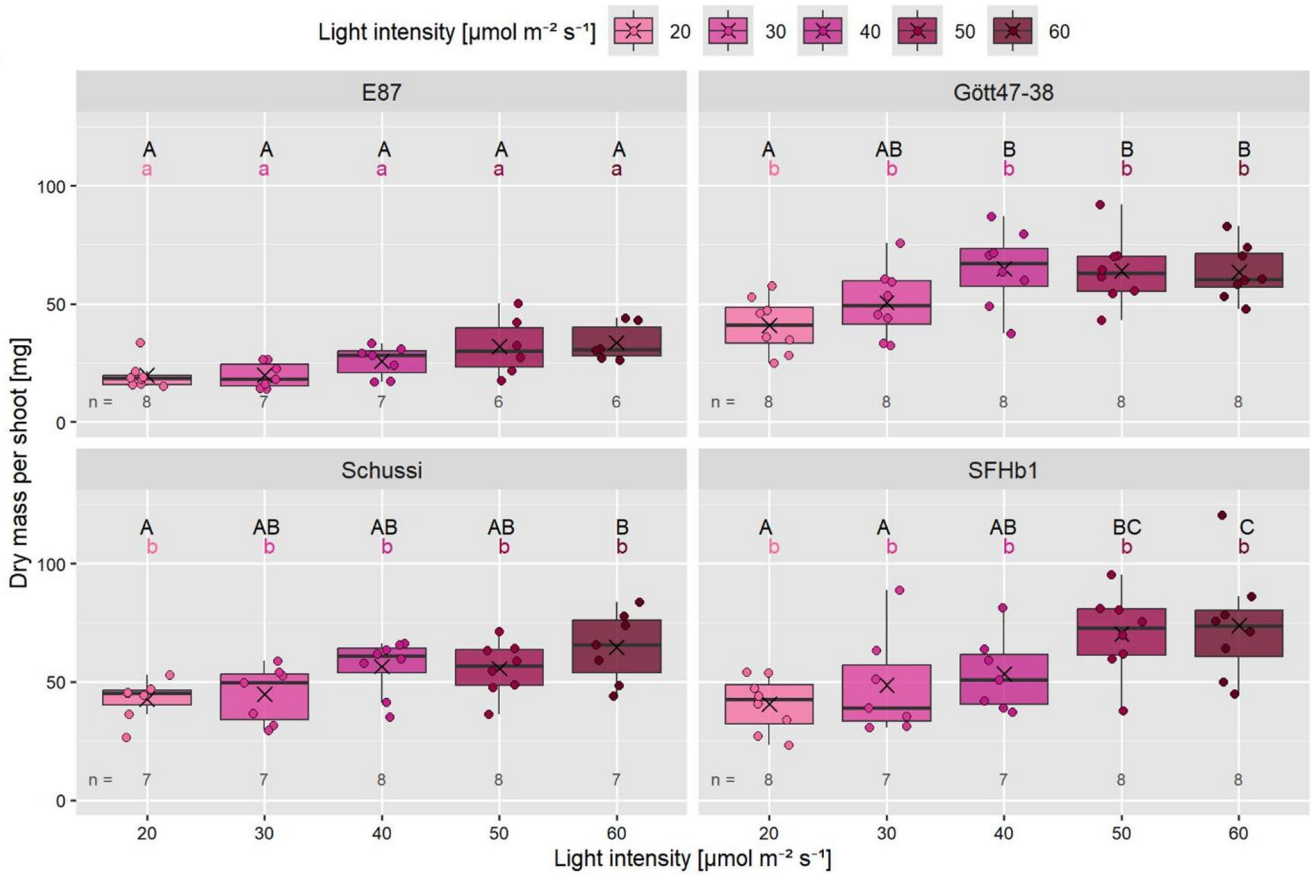


Fig. 6 Shoot dry masses under different light intensities in four sycamore maple (*Acer pseudoplatanus* L.) genotypes (E87, Götti47-38, Schussi and SFHb1). Dry mass is given per explant at the five tested light intensities under a red:blue light ratio of 3:1 (20–60 µmol m^{-2} s^{-1}). Each dot represents the mean per culture vessel; crosses indicate group means and n denotes the number of vessels. Uppercase letters indicate significant differences between light intensities within each genotype; lowercase letters compare genotypes within a light intensity (Tukey HSD, $\alpha = 0.05$)

response to light-induced stress and mirrors findings in *A. saccharum*, where Singh et al. (2017) reported strong anthocyanin accumulation under 40 µmol m^{-2} s^{-1}. Although moderate SPAD value declines may not impair shoot growth, persistent reductions during acclimatisation or rooting can indicate latent stress. Monitoring such changes, alongside maintaining appropriate light intensities, is essential for minimising stress and optimising all stages of micropropagation from culture initiation through rooting to ex vitro acclimatisation.

Current limitations for commercial adoption of the optimised protocol

Despite the discussed promising implications of the optimised protocol, the following limitations should be considered when applying the protocol in commercial practice. Given the strong genotype-specific responses observed, a one-size-fits-all approach should be avoided where feasible. While implementing fully individualised light regimes may not be practical in commercial labs, grouping genotypes by performance type and tailoring protocols accordingly offers a pragmatic compromise. LED lighting systems provide additional flexibility, enabling stage-specific lighting strategies (e.g. lower intensity during rooting, red-rich spectra during elongation) that can be used not only to maximise biomass production, but also to ensure physiological stability and uniformity across culture phases. A further limitation concerns the temporal scope of the experiments. All experiments were conducted over a single culture passage, so long-term effects such as cumulative stress, altered regeneration potential, or epigenetic changes remain to be investigated.

The routine incorporation of trans-zeatin riboside (ZR) and silver nitrate ($AgNO_3$) into *A. pseudoplatanus* culture media offers tangible benefits in multiplication efficiency, shoot elongation, and overall shoot quality. However, economic considerations must be taken into account, particularly for large-scale propagation systems. Based on current supplier prices (net, excl. VAT), ZR (Duchefa Biochemie) costs approximately € 640 per 250 mg (and € 770 at

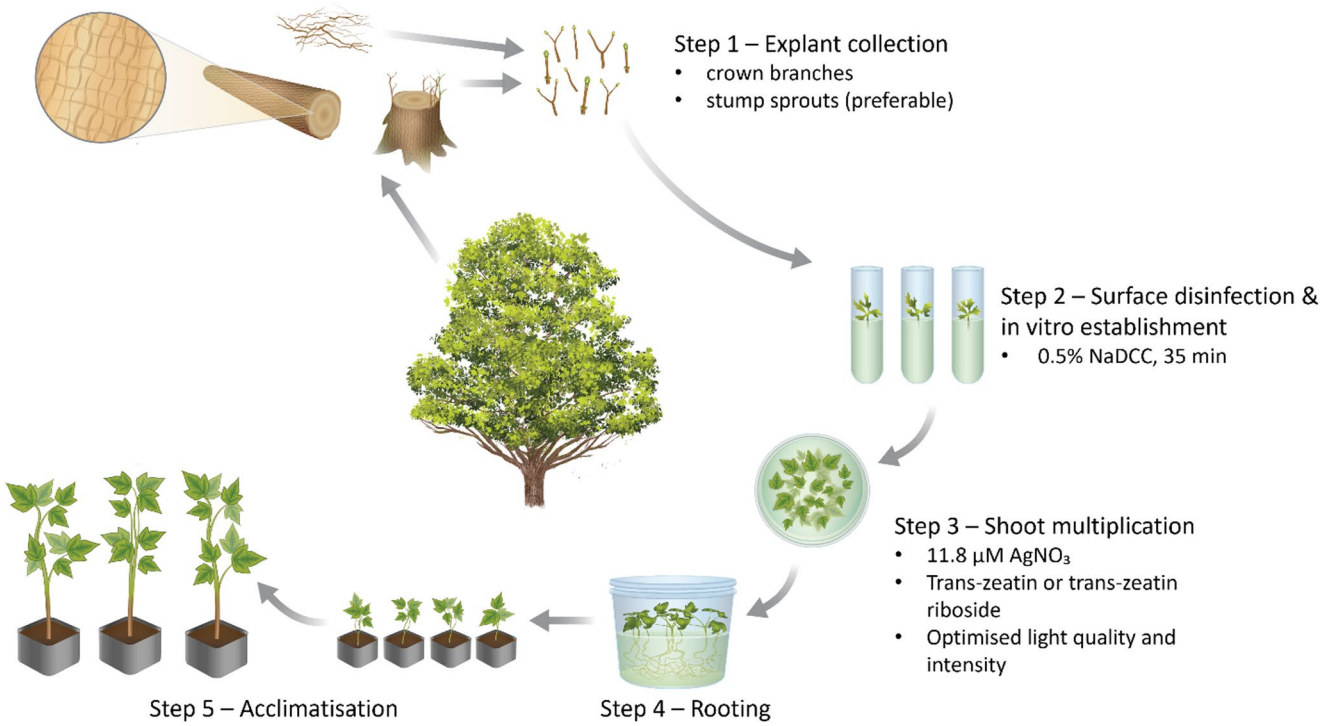


Fig. 7 Schematic workflow of the optimised micropropagation protocol for sycamore maple (*Acer pseudoplatanus L.*) developed in this study. The illustration summarises the main stages of the protocol from explant collection to acclimatisation: (1) collection of explants from crown branches and stump sprouts of selected donor trees, (2) surface disinfection using sodium dichloroisocyanurate (NaDCC) and in vitro establishment of shoot cultures, (3) shoot multiplication on media supplemented with trans-zeatin riboside (ZR) or trans-zeatin (Z) in combination with silver nitrate ($AgNO_3$) under optimised light regimes, (4) rooting, and (5) acclimatisation of plantlets to ex vitro conditions. Graphic: Kara Perilli, adapted for this study

Phygenera), whereas trans-zeatin ranges from € 362 (Duchefa Biochemie) to € 122 (Phygenera) for the same quantity. Although ZR showed genotype-dependent advantages over Z, its comparatively high cost may limit its routine use in commercial propagation systems.

Conclusions and recommendations for micropropagation of sycamore maple

This study provides comprehensive evidence that optimised light regimes, cytokinin composition, and ethylene inhibition through silver nitrate collectively enabled substantial improvements in shoot multiplication, elongation, and physiological quality of *Acer pseudoplatanus*.

The demonstrated effectiveness of NaDCC as a disinfectant offers a simplified and robust alternative to traditional disinfection protocols. Its use reduced contamination rates without requiring a rinsing step, which simplifies handling and increases suitability for commercial-scale operations.

In detail, silver nitrate ($AgNO_3$), applied at 11.8 µM, emerged as a low-cost and robust additive that enhanced shoot multiplication, elongation, and morphological quality, particularly in ethylene-sensitive or recalcitrant genotypes. Its routine inclusion in culture media is therefore strongly advised. Zeatin solutions also proved stable when autoclaved, providing an economical alternative. Light intensities of 50 and 60 µmol m^{-2} s^{-1}, combined with red:blue ratios of 3:1 or 1:1, were most effective in promoting shoot proliferation and elongation across genotypes. However, high irradiance levels also led to reductions in SPAD values and visible pigmentation, indicating light-induced physiological stress. These responses underline the need to balance morphological gains with physiological stability when designing lighting protocols. Notably, light intensity can also be used to regulate culture passage duration. Higher intensities may accelerate shoot development and shorten subculturing intervals, whereas lower intensities slow growth and extend passage duration. This relationship allows for operational flexibility, particularly when synchronising different genotypes or adjusting to capacity constraints.

While the present findings are based on a single culture passage, the demonstrated genotype-dependent responses and physiological trade-offs highlight the need for long-term studies on cumulative stress and (epi)genetic stability.

Taken together, the optimised in vitro protocol developed in this study (Fig. 7) provides a practical foundation for the

efficient clonal propagation of sycamore maple, particularly relevant for high-value phenotypes such as wavy grain.

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Author contributions TW, VK, and AMD conceived and designed the study. VK, HLB, and MM developed the methodology. VK and MM conducted the experiments and collected the data under the supervision of HLB, VK and TW. VK and MM performed the data analysis and interpretation with contributions from TW and AMD. VK drafted the first version of the manuscript. TW, AMD, MM, HLB, and AMH critically revised the manuscript. AMH also acquired funding. All authors read and approved the final manuscript.

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Data availability All raw data and statistical outputs generated during this study are provided in the supplementary material files associated with this article.

Declarations

Ethical approval Not applicable.

Conflicts of interest Traud Winkelmann is one of the Associate Editors of the journal at the time of submission. This had no impact on the peer review process and the final decision. The authors declare that they have no competing interests.

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