



# Stable in vitro propagation of *Fagus sylvatica* using micro-cuttings established from seedlings

Franka N. Thiesen<sup>1</sup> · Paweł Chmielarz<sup>2</sup> · Tomasz A. Pawłowski<sup>2</sup> · Ben Bubner<sup>1</sup>

Received: 7 September 2025 / Accepted: 15 November 2025  
© The Author(s) 2025

## Abstract

The objective of this study was to establish a reliable protocol for the in vitro multiplication of *Fagus sylvatica* L. shoots. This species is increasingly threatened by pathogen pressure and drought stress, making in vitro propagation a potentially valuable tool for breeding and resistance research. Although protocols for in vitro culture of *F. sylvatica* have been reported, they do not ensure stable propagation, which is an essential prerequisite for studies on resistance to biotic and abiotic stress. In our study, mature seeds from seven provenances in western and southern Poland were germinated in soil, sterilized, and established in vitro on semi-solid Woody Plant Medium. During the establishment phase, cultures contained a mixture of genotypes from the different provenances, which were later identified by microsatellite analysis and assigned to single multilocus genotypes. For the multiplication phase, shoots were cultured with different phytohormones and carbon sources (sucrose or glucose), and shoot length and multiplication rates were assessed. A second experiment compared semi-solid medium in Erlenmeyer flasks with liquid medium in Temporary Immersion System bioreactors. Multiplication rates varied among provenances, with the highest rates achieved when shoots were cultured on Woody Plant Medium supplemented with zeatin and propagated in Temporary Immersion System bioreactors. These findings demonstrate that stable in vitro propagation of *F. sylvatica* is possible and provide a foundation for future breeding and resistance studies.

## Key message

This study documents successful *Fagus sylvatica* establishment and stable propagation in vitro. We maintained consistent multiplication rates in vitro, marking a significant advancement in the sustainable cultivation of *F. sylvatica*.

**Keywords** Clonal propagation · Temporary immersion system · Microsatellite · Beech

## Introduction

European beech, *Fagus sylvatica* L., is one of the ecologically and economically most important deciduous trees of Central Europe and together with oaks defines the climax vegetation of this region. Its natural range extends from the Iberian Peninsula to Eastern Europe, where the north-eastern limit of this species is in Poland (Szafer 1932; Latałowa 1992). Beech stands cover approximately 6% of the Polish forest area (Grabska-Szwagrzyk et al. 2024). Research on *F. sylvatica* has become increasingly important, as climate warming and reduced summer rainfall intensify drought stress. Severe droughts in 1976, 2003, and 2018/19 caused crown defoliation, dieback, and mortality, raising concerns about its drought tolerance and future forestry viability (Leuschner 2020). Beech growth in the easternmost marginal population is drought-limited (Roibu et al. 2022).

---

Communicated by José Carlos Lorenzo Feijoo

---

✉ Ben Bubner  
ben.bubner@thuenen.de  
Franka N. Thiesen  
franka.thiesen@thuenen.de  
Paweł Chmielarz  
pach@man.poznan.pl  
Tomasz A. Pawłowski  
tapawlow@man.poznan.pl

<sup>1</sup> Thünen Institute of Forest Genetics, Eberswalder Chaussee 3a, 15377 Waldsiedersdorf, Germany

<sup>2</sup> Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, Kórnik 62-035, Poland

Preserving existing diversity and local adaptations, including drought stress response, is crucial for the survival of *F. sylvatica* (Rukh et al. 2023). Understanding drought avoidance and tolerance mechanisms is therefore vital for future forestry planning and management in the face of climate change.

Additionally, *F. sylvatica* trees are threatened by biotic stress, such as attacks by fungal microorganisms and insects, resulting in severe crown dieback. In particular the recently described complex diseases called Vitality loss of beech and the beech bark disease affect *F. sylvatica* stands in Europe (Langer and Buskamp 2023). Further reports state the identification of fungi on woody tissue of *F. sylvatica*, such as *Asterosporium asterospermum* (Pers.) S. Hughes, *Apiognomonium errabunda* (Roberge ex Desm.) Höhn., *Biscogniauxia nummularia* (Bull.) Kuntze, *Hypoxylon fragiforme* (Pers.) J. Kickx f., and *Neonectria coccinea* (Pers.) Rossman and Samuels (Hendry et al. 2002; Langer and Buskamp 2021). Due to the insufficient knowledge of the pathogenicity of various pathogens, conducting additional and prolonged pathogenicity tests, is necessary (Langer and Buskamp 2023).

In order to understand and improve the trees mechanisms to cope with current and future abiotic and biotic threats, continuous research is required. Biotechnology, with micropropagation of plant tissue as a key method, enables the effective cloning of forest tree species with valuable traits by producing genetically identical plantlets through in vitro propagation. These techniques facilitate the selection and propagation of *F. sylvatica* genotypes with desirable characteristics, such as improved wood or seed production and enhanced potential for disease resistance (Reed et al. 2011). The long breeding cycles are a specific problem in tree breeding and could partly overcome by using in vitro culture (Fenning 2019).

Since the 1980s, *F. sylvatica* has posed challenges for in vitro micropropagation, resulting in scarce publications on its in vitro culture establishment, primarily in the 1980s and 1990s (Ahuja 1984; Meier and Reuther 1994; Chalupa 1996). The in vitro cultivation of *Fagus* has been described as difficult and recalcitrant, with recalcitrance referring to the inherent difficulties and resistance these plants exhibit in tissue culture, especially low initiation rates, poor growth and development in the propagation process (Benson 2000). Most work has focused on somatic embryogenesis, the proliferation of shoots by axillary branching, and the induction of adventitious buds. The first attempts to regenerate *F. sylvatica* by axillary branching were made using shoot tips and nodal segments cultured on nutrition media like Murashige and Skoog (MS), McCown Woody Plant (WPM), and Driver and Kuniyuki Walnut (DKW) with cytokinins 6-benzylaminopurine (BAP), thidiazuron (TDZ) (Chalupa 1979,

1985). The most successful medium was WPM with BAP and indole 3-butyric acid (IBA), inducing axillary shoots within 4–6 weeks, while low thidiazuron concentrations promoted shoot formation. Rooting of excised shoots was achieved on WPM with IBA and  $\alpha$ -naphthaleneacetic acid (NAA), with success rates between 22% and 76% depending on the genotype (Chalupa 1979, 1985).

Vieitez et al. (1993) developed an in vitro shoot multiplication system for *F. sylvatica* from embryonic axes excised from seeds, using WPM supplemented with BAP, zeatin, and NAA. Repeated subculture on DKW was detrimental, while WPM and a combination of cytokinin and auxin stimulated shoot growth.

Somatic embryogenesis, a method with high reproduction potential, was tested but showed limited success (5%) with *F. sylvatica*. Hazubska-Przybyl et al. (2015) obtained early-stage embryogenic calli using various explants, including embryonic axes. In a study in the early 1990s, somatic embryogenesis was also achieved in *F. sylvatica* from immature zygotic embryos, although the conversion of embryos into plantlets was low and improved only after cold storage of the embryos (Vieitez et al. 1992).

Explants from mature trees were generally difficult to grow in vitro. A study from 1984 failed to culture bud explants from 70-year-old trees, while successful micropropagation of adult beech was achieved using rejuvenated tissues like graftings, epicormic shoots, and root sprouts (Ahuja 1984).

Adventitious bud formation was observed by Vieitez et al. (1993) on hypocotyl segments. Further studies by Vieitez and San Jose (1996) using leaf explants, lead to higher productivity in proximal leaf halves. Cuenca et al. (2000) found TDZ more efficient than BAP for internode segments, although higher concentrations led to clusters of small buds. Transferring explants to fresh medium every two weeks promoted shoot elongation. A recent study on the in vitro establishment of *F. sylvatica* used vegetative material as explants from different tree provenances, achieving shoot-formation rates in vitro of 57% of the provenance (Zahn et al. 2025).

Overall, it can be stated that in the past, several attempts have conducted experiments on the establishment of *F. sylvatica* in vitro culture. Most published studies describe the establishment of *F. sylvatica* in vitro cultures, but none report the successful long-term maintenance and clonal propagation of stable cultures, indicating that stable micropropagation across multiple genotypes has not yet been achieved. To our knowledge, currently there is no in vitro culture available, suitable for clonal propagation. For the implementation of research and breeding programs this would be an important prerequisite. In our study, the initial material consisted of a mixture of genotypes, and because genotype has been shown to strongly influence the

establishment and maintenance of *F. sylvatica* *in vitro* cultures (Meier and Reuther 1994), it was important to identify and separate individual genotypes before attempting long-term clonal propagation.

Genotyping through microsatellite analysis serves as a powerful tool for distinguishing *in vitro* clones. Microsatellites, or simple sequence repeats (SSRs), are short, repetitive DNA sequences scattered throughout the genome, providing unique genetic fingerprints to distinguish individuals and have long been used in forest tree studies (Barrett et al. 1997). When applied to *in vitro* clones, microsatellite genotyping allows to detect genetic variations among these clones with high precision (Wilhelm et al. 2005). This is particularly important when using *in vitro* clones for the *ex vitro* transfer. Therefore, microsatellite analysis served as tool to sort distinct clones out of a clonal mixture of *in vitro* shoots.

The objective of the present study was (i) to develop an efficient *in vitro* micropropagation protocol, focusing on stable shoot growth and propagation, and (ii) to test the use of microsatellite technology to evaluate the genetic purity of the selected clones. This genotyping step was necessary to identify distinct clones within the mixed cultures and ensure stable and reliable clonal propagation.

## Materials and methods

### Sample material and preparation

Seeds were collected in autumn 2021 from seven provenances in Poland: Gryfino (Gr), Świebodzin (Sw), Jugów (Ju), Prudnik (Pr), Wisła (Wi), Jamy (Ja) and Tomaszów Lubelski (To) (Fig. 1). Seeds were collected from the ground and represent a pool of different trees at each site, serving as a repository of the local genetic diversity.

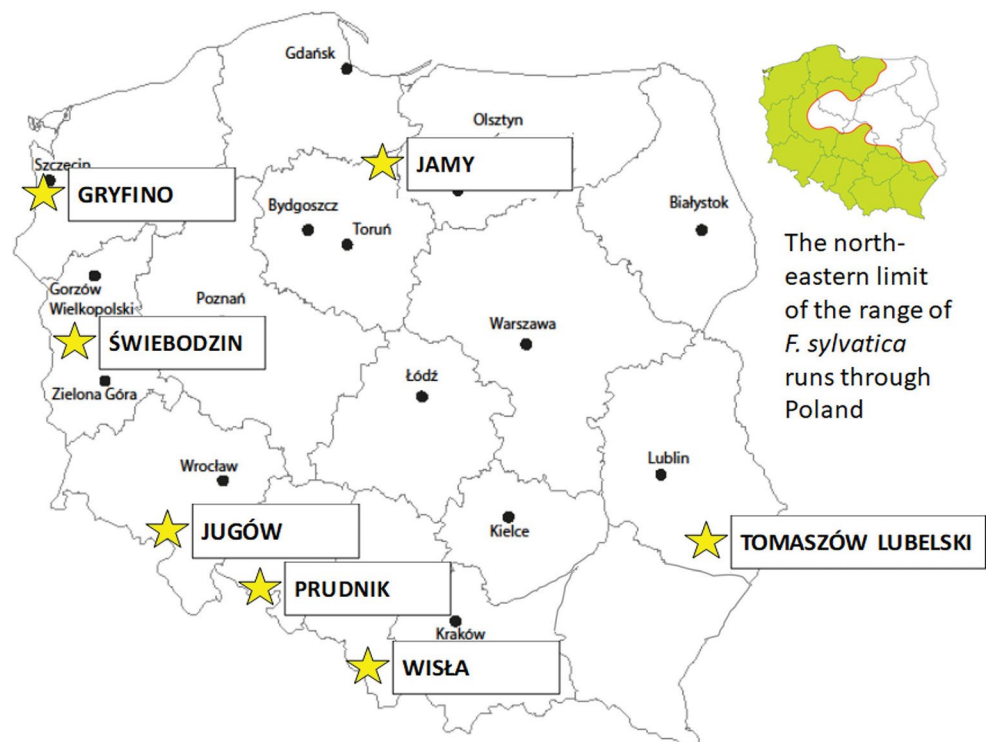
### Stratification of seeds and germination

To break seed dormancy, beech nuts were stratified in medium (peat and sand 1:1) at 3°C using the method described by Suzska et al. (1996). A seed was scored as germinated when the radicle protruded 2 mm. When 5% of the seeds had germinated, the stratification process was defined as completed. Stratified seeds were sown in the same medium in plastic containers with a translucent cover (30 seeds per container) and placed in a climate chamber under illumination with light intensity of 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (day-night cycle of 12/12 h), at 20 °C for 3 months.

### Establishment of *in vitro* culture from 3-month-old seedlings

Three months after germination, the shoot tips of the seedlings were used for the *in vitro* culture establishment

**Fig. 1** Seeds of *F. sylvatica* collected in Poland from seven provenances: Gryfino, Świebodzin, Jugów, Prudnik, Wisła, Jamy and Tomaszów Lubelski. Adopted from Pawlowski et al. (2024)



(Fig. 2a). The leaves were excised from the seedlings and an apical bud accompanied by a small shoot fragment served as explants (Fig. 2b). Explants underwent sterilization in 0.1% mercury chloride ( $\text{HgCl}_2$ ) for 2.5 min, followed by triple rinsing in sterile water. Subsequently, sterilized explants were placed onto WPM medium (McCown Woody Plant Basalt Salt Mixture, Sigma Aldrich) containing BAP, 30 g/L sucrose and 7.0 g/L phytoagar (Fig. 2c). The culture medium was supplemented with 0.4 ml/L BAP, 100 mg/L myo-inositol, 2000  $\mu\text{L/L}$  glycine, 20 ml/L  $\text{K}_2\text{SO}_4$ , 500  $\mu\text{L/L}$  pyridoxine, 500  $\mu\text{L/L}$  nicotinic acid, and 1000  $\mu\text{L/L}$  thiamine. Adjustment of the medium pH to 5.7 preceded autoclaving. During establishment process, cultures were routinely subcultured onto fresh WPM-BAP medium every five weeks (Fig. 2e). The standard growth chamber conditions consisted of a 16-hour photoperiod with light intensity of 60  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , at 23 °C. The establishment of the in vitro cultures was conducted in Kórnik, Poland, where shoots were cultivated according to their provenance rather than individual genotype. All in vitro material was subsequently transferred together to Waldsiefersdorf, Germany. As the transferred material represented a mixture of genotypes within each provenance, individual in vitro shoots were genotyped by microsatellite analysis to identify and assign distinct clones for further propagation.

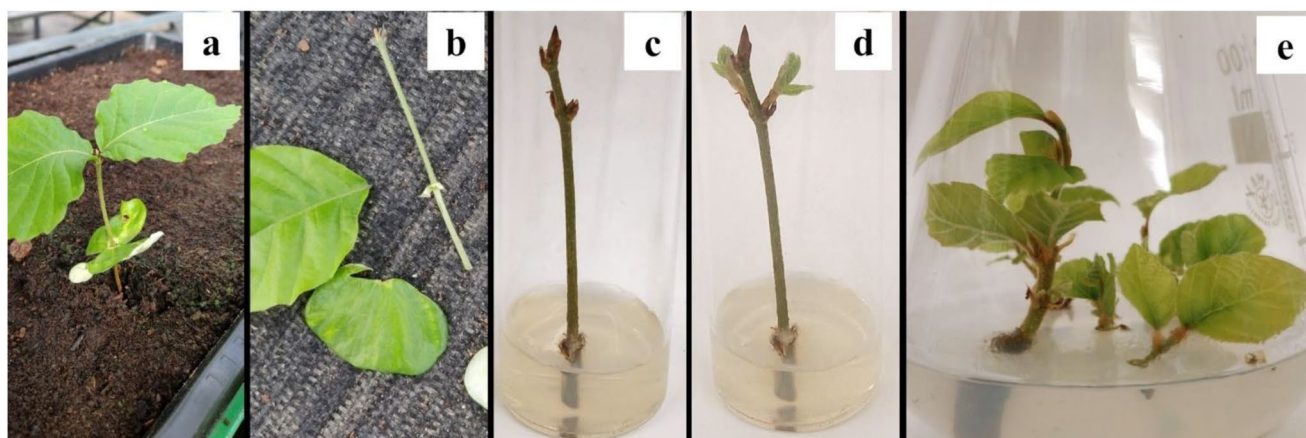
### Genotyping of in vitro shoots

For genotyping of in vitro shoots, leaf samples were collected from 109 in vitro shoots and dried. Subsequent sample homogenization and DNA isolation followed a standard operating procedure outlined in Bruegmann et al. (2022). PCR amplifications targeting 17 microsatellite loci were performed using two multiplex primer mixes. The first mix (Set1) included primers *csolfagus\_05*, *csolfagus\_06*,

*csolfagus\_19*, *csolfagus\_29*, *FS1-15a*, *mfc5b*, *sfc0036*, and *sfc1143*, while the second mix (Set2) comprised *concat14\_A\_0*, *DE576\_A\_0*, *DUKCT\_A\_0*, *DZ447\_A\_0*, *EEU75\_A\_0*, *EJV8T\_A\_0*, *EMILY\_A\_0*, *ERHBI\_A\_0*, and *FS1\_03a* (Lefèvre et al. 2012; Eusemann et al. 2017). PCRs were conducted using HOT FIREPol Multiplex Mix (SolisBiodyne, Kisker Biotech Steinfurt, Germany) in a 13  $\mu\text{l}$  reaction volume containing 5–10 ng template DNA in Multiplex PCR Master Mix, which included HOT FIREPol DNA Polymerase (SolisBiodyne, Kisker Biotech Steinfurt, Germany), Multiplex buffer, dNTP mix, BSA, and 10 mM  $\text{MgCl}_2$ . Primer concentrations ranged from 0.01 to 0.03  $\mu\text{M}$ . PCR cycling conditions involved an initial activation step at 95 °C for 15 min, followed by 28 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 90 s, and extension at 72 °C for 60 s, with a final extension step at 60 °C for 30 min. PCR products were analysed on a Beckman Coulter CEQ 8000 capillary sequencer. Allele calling was performed using GeneMarker software (SoftGenetics, GeneMarker V3.0.0.), followed by manual verification. Allele tables were generated for Set1 and Set2. A clone list was created based on multilocus genotypes by using the Excel AddIn GenAIEx (Peakall and Smouse 2012). After assigning shoots to different genotypes by microsatellite analyses, 31 clones corresponding to the seven provenances were identified and used for subsequent multiplication experiments.

### Propagation of established clones

For propagation, all in vitro clones were cultured in Waldsiefersdorf, Germany on propagation medium, Z-Gluc, previously documented as Wz Gluc for larch propagation (Ewald 2007). Z-Gluc comprised 1x WPM (Woody Plant Medium, McCown formulation including vitamins, Duchefa – Haarlem Netherlands), 0.18 M glucose (equivalent to



**Fig. 2** Establishment of *F. sylvatica* in vitro culture: (a) 3-month-old seedling (b) the apical bud with fragment of a shoot was taken as explant (leaves were removed) (c) shoot after disinfection and transfer

into establishment medium (d) shoots starts to develop in vitro from an apical bud, (e) multiplication of in vitro growing shoots

**Table 1** Comparison of propagation efficiency on four different propagation media. McCown Woody plant (WPM) served as basic medium and the different carbon sources (sucrose or glucose) and phytohormones were added (6-Benzylaminopurine (BAP), 1-Naphthalene-acetic acid (NAA) or Zeatin)

medium name	basic medium	carbon source	Phytohormones
BN-Suc	WPM	sucrose (30 g/l)	BAP (2.2 $\mu\text{M}$ ) NAA (0.108 $\mu\text{M}$ )
B-Suc	WPM	sucrose (30 g/l)	BAP (0.89 $\mu\text{M}$ )
B-Gluc	WPM	glucose (32.87 g/l)	BAP (0.89 $\mu\text{M}$ )
Z-Gluc	WPM	glucose (32.87 g/l)	Zeatin (7 $\mu\text{M}$ )

32.87 g/L), 8 g/L phytoagar (Duchefa – Haarlem Netherlands), and 7 $\mu\text{M}$  (equivalent to 1.535 mg/L) of the phytohormone Zeatin (Duchefa – Haarlem Netherlands). Cultivation conditions involved a photoperiod of 16/8 hours (day/night) at an intensity of 30  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and a temperature range of 18–22 °C. For propagation, in vitro cultures were maintained and sub cultured over a period of two years. Cultures were transferred to fresh medium every six weeks. Propagation procedures involved the separation of in vitro shoots from newly developed shoots. Assessment of propagation success was conducted by calculating the shoot multiplication rate, determined as the ratio in shoot numbers before and after each subculture.

## Propagation optimization

### Propagation in semi-solid medium

To improve shoot multiplication, we compared the effect of four culture media: (i) BN-Suc medium, (ii) B-Suc medium, (iii) B-Gluc medium, and (iv) Z-Gluc medium (Table 1).

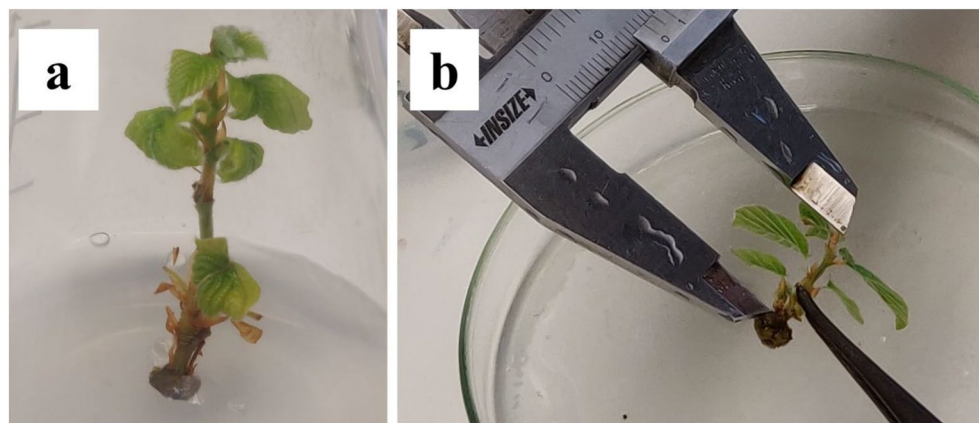
For this experiment, we used 67 single shoots of the Świebodzin provenance (from three different clones) and 94 single shoots of the Gryfino provenance (from four different clones) for the propagation on four different propagation media. Shoots were cultured individually in 100-ml Erlenmeyer flasks containing 25 ml of fresh medium. The length of the longest shoot of each explant was measured before

each of the three successive six-week subculture intervals (Fig. 3a, b).

### Propagation in liquid medium

To enhance shoot multiplication rates, we explored the use of Temporary Immersion System (TIS) bioreactors to cultivate three in vitro clones of *F. sylvatica* from the Gr provenance: Gr-K, Gr-N, and Gr-13. This approach was based on preliminary laboratory tests conducted with various forest tree species, which indicated its potential efficacy. The experimental setup was adapted from Welander et al. (2014). In this study, 50 shoots per clone were cultured individually in PlantForm™ TIS bioreactors (purchased at Plant Form AB, Hjärup, Sweden), each containing 500 ml of liquid propagation medium (Z-Gluc without plant agar). The bioreactors were assembled and autoclaved according to the manufacturer's instructions. During the in vitro shoot transfer process, shoots were placed on the surface of the TIS bioreactor, with clone Gr-K divided into two bioreactors, each containing 50 shoots. The TIS bioreactors were connected to a pump system, with an immersion interval of 15 min every 6 h. After each immersion, air was pumped into the upper part of the reactor for 5 min to facilitate aeration and push the medium down, ensuring adequate oxygenation and nutrient distribution. The bioreactors were maintained under the same light and temperature conditions as described above: a 16-hour photoperiod with a light intensity of 60  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at 23 °C. Explants consisted of apical shoot sections with leaves, similar to those used for semi-solid medium cultures. The experiment was conducted over a single subculture interval of 6 weeks, as preliminary experiments and literature reviews indicated that this duration was optimal for shoot multiplication in TIS bioreactors. As a reference, for each clone, seven in vitro shoots were placed on semi-solid Z-Gluc propagation medium in standard Erlenmeyer flasks. After six weeks, shoot multiplication was assessed by calculating the multiplication rate

**Fig. 3** For propagation optimization, single shoots were cultured on different propagation media and shoot length was assessed. (a) single shoot of Gr provenance on Z-Gluc medium (b) shoot length measurement of shoot from Sw provenance using a caliper



**Table 2** Number of in vitro shoots established for each of the seven provenances with geographical coordinates

Provenance	Geographical coordinates of the provenances	number of shoots established
Gryfino	Gr 53.33, 14.65	24
Jamy	Ja 53.59, 18.93	13
Jugów	Ju 50.60, 16.58	15
Prudnik	Pr 50.29, 17.46	5
Świebodzin	Sw 52.28, 15.27	38
Tomaszów	To 50.28, 23.55	5
Lubelski		
Wisła	Wi 49.57, 18.84	9

and comparing the data obtained from TIS bioreactors and semi-solid media cultures.

### Statistical analysis

The normal distribution of the data was first assessed using the Shapiro-Wilk test. Data that followed a normal distribution were analyzed using one-way ANOVA, with pairwise comparisons performed using the `pairwise.t.test` function and Bonferroni correction. For data that was not normally distributed, the Kruskal-Wallis test was applied, with pairwise comparisons conducted using the `pairwise.wilcox.test` function and appropriate corrections for multiple testing. Differences between two independent samples were assessed using the Welch Two Sample t-test. All results are presented as means  $\pm$  standard error (SE). Graphical representations were produced using the `ggplot2` and `ggpubr` packages in R version 4.3.1, R Core Team, 2023, integrated with RStudio version 2024.04.1.748. Microsatellite data were analysed using population genetics calculations implemented in the `adegenet` R package.

## Results

### Establishment

The disinfection of explants in mercuric chloride resulted in 90% sterile shoots, with 80% of shoot development. Observations of these proliferated shoots showed that the optimal time of a subculture interval on WPM-BAP medium was 5 weeks. In each subculture interval, shoots showed normal growth which was characterised by green leaves and viable apical buds. During successive subculture the number of proliferated shoots gradually increased, resulting in five to 38 shoots per provenance (Table 2) that were available for genotyping using microsatellites and further propagation.

**Table 3** Clones based on microsatellite multilocus. Analysis of the microsatellite allele table resulted in identification of identical allele pattern and thus assignment to clones, as indicated with labels A – O. Samples labelled with numbers are individual clones

provenance	Clone	number of shoots genotyped
Gr	K	12
	B	4
	N	4
Ja	1, 11, 12, 13	each 1
	O	4
	G	3
	F	2
Ju	6, 7, 15, 17	each 1
	A	8
	L	4
	M	2
Pr	16	1
	C	5
Sw	I	27
	H	3
	J	2
	3, 4, 5, 8, 9, 10	each 1
To 1	D	5
Wi 2	E	8
	2	1

### Genotyping using microsatellites

DNA extraction from the 109 leaf samples of in vitro shoots was successful and subsequent PCR amplification and capillary electrophoresis resulted in clear and distinct peaks for each microsatellite locus. Allele calling in the GeneMarker software was manually verified and an allelic table containing each analysed shoot was generated (supplementary material: OSM Table 1).

Based on the allele tables, we identified 15 recurring allele patterns, each of which was observed in 2 to 27 samples. These recurring multilocus allele patterns are denoted by letters A through O, with each letter representing a specific clone (supplementary material: OSM Table 2). Labels 1 through 17 indicate unique clone characterized by allele patterns. In summary, microsatellite analysis revealed a total of 31 different clones among the 109 in vitro samples analysed. The identified in vitro clones were subsequently propagated as distinct individual clones. (Table 3).

### Propagation of in vitro clones

The propagation of in vitro clones on Z-Gluc medium was assessed through monitoring the multiplication rate during each subculture interval over a period of two years. Most in vitro shoots formed axillary shoots that have been used for propagation (Fig. 4).



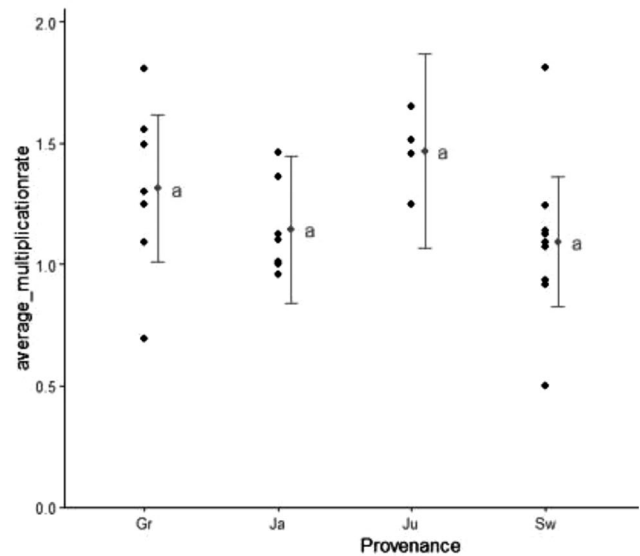
**Fig. 4** In vitro propagation of a clone from Gr provenance on propagation medium Z-Gluc. Propagation occurred by separating the newly formed shoots

**Table 4** Propagation of *F. sylvatica* in vitro clones was documented over four subculture intervals (from July to November 2023). Average multiplication rates were calculated for each clone and summarised to average multiplication rate ± SE per provenance

provenance	number of clones	average multiplication rate
Gr	7	1.31 ± 0.359
Ja	7	1.15 ± 0.111
Ju	4	1.47 ± 0.167
Pr	1	1.48 ± 0.294
Sw	9	1.09 ± 0.098
To	1	1.20 ± 0.294
Wi	2	1.33 ± 0.207

For all 31 clones, multiplication rates were compared depending on their provenance. Multiplication rates of each clone ranged from 0.7 to 1.81, with values above 1.0 indicating an increase in shoot numbers and values below 1.0 indicating a reduction in shoots due to necrosis or contamination. 25 out of 31 clones showed an average multiplication rate above 1.0 and 10 clones above 1.5. Across different provenances, average multiplication rates varied, with the highest rates observed in the Ju provenance (1.47 ± 0.167), Pr provenance (1.48 ± 0.294) and lowest for Sw provenance (1.09 ± 0.098) (Table 4).

A statistical analysis was conducted for the provenances Gr, Ja, Ju and Sw, leaving out Pr, To and Wi as they comprise less than three clones. There was no significant difference in the propagation rate of the 4 analysed provenances (Fig. 5).



**Fig. 5** Multiplication of all clones from the four provenances with more than two clones, recorded over four subculture intervals. Gr, Sw, Ja, Ju; The average multiplication rate was computed based on four multiplication rates recorded from July 2023 to November 2023. Each Raw data point (black dots) represents the average multiplication rate of one clone. Adjusted means along with 95% confidence intervals are depicted using grey dots and error bars. Statistical analysis: one-way ANOVA ( $\alpha=0.05$ )

**Table 5** In vitro shoot-length development on the four different propagation media after of 3 subculture intervals; table showing mean in vitro shoot length ± SE for provenance Sw (clones Sw-H, Sw-I, Sw-J) and Gr (clones Gr-11, Gr-12, Gr-13, Gr-N). Statistical differences among media were analysed using one-way ANOVA followed by bonferroni's post hoc test

propagation media	provenance	number of shoots	average shoot length (mm)
BN-Suc	Sw	9	9.21 ± 6.61 (a)
B-Suc	Sw	35	10.20 ± 2.82 (a)
B-Gluc	Sw	14	9.13 ± 3.00 (a)
Z-Gluc	Sw	9	14.8 ± 5.45 (b)
ANOVA			0.0006051 ***
BN-Suc	Gr	23	12.3 ± 3.61 (a)
B-Suc	Gr	34	12.7 ± 3.41 (a)
B-Gluc	Gr	14	11.7 ± 4.98 (a)
Z-Gluc	Gr	23	18.0 ± 5.17 (b)
ANOVA			3.808e-06***

### Optimization of propagation

#### Shoot length

Four different propagation media (BN-Suc, B-Suc, B-Gluc, Z-Gluc) were tested. We measured differences in shoot-lengths of shoots from the provenances Sw (clones Sw-H, Sw-I, Sw-J) and Gr (clones Gr-11, Gr-12, Gr-13, Gr-N) as average shoot length over 3 measurements (Table 5). Notably, shoots on the Z-Gluc medium yielded the highest mean

**Table 6** Table of average multiplication rate, standard deviation and ANOVA of in vitro shoots of the provenances Sw (clones Sw-H, Sw-I, Sw-J) and Gr (clones Gr-11, Gr-12, Gr-13, Gr-N) on the four different propagation media, evaluated over 3 subculture intervals. Statistical differences among media were analysed using one-way ANOVA followed by bonferroni's post hoc test

propagation media	provenance	name of clones	multiplication rate
BN-Suc	Sw	Sw-H, Sw-I, Sw-J	0.90±0.224 (a)
B-Suc	Sw	Sw-H, Sw-I, Sw-J	1.05±0.0809 (a)
B-Gluc	Sw	Sw-H, Sw-I, Sw-J	1.00±0.0786 (a)
Z-Gluc	Sw	Sw-H, Sw-I, Sw-J	1.13±0.213 (a)
ANOVA			0.2498
BN-Suc	Gr	Gr-11, Gr-12, Gr-13, Gr-N	0.986±0.24 (a)
B-Suc	Gr	Gr-11, Gr-12, Gr-13, Gr-N	1.26±0.114 (ab)
B-Gluc	Gr	Gr-11, Gr-12, Gr-13, Gr-N	1.24±0.246 (ab)
Z-Gluc	Gr	Gr-11, Gr-12, Gr-13, Gr-N	1,41±0.168 (b)
ANOVA			0.03436**



**Fig. 6** Physiological state of the shoots of the clone Sw, six weeks after the last subculture on the different propagation media BN-Suc, B-Suc, B-Gluc, Z-Gluc (from left to right). In vitro shoots on Z-Gluc showed

better leave development and growth. Statistical analysis: one-way ANOVA followed by Bonferroni post hoc test ( $\alpha=0.05$ )

shoot length, with shoots from Gr provenance recording the highest value of  $18.0 \pm 5.17$  mm and shoots from Sw provenance with  $14.8 \pm 5.45$  mm. Conversely, shoots on BN-Suc medium exhibited the lowest mean shoot length in both locations, with shoots from Gr provenance at  $12.3 \pm 3.61$  mm and shoots from Sw provenance at  $9.21 \pm 6.61$  mm. These trends were confirmed by one-way ANOVA, followed by pairwise comparisons with a Bonferroni post hoc test, demonstrating significant differences of shoot lengths on the four media for shoots from both provenances, Sw ( $p < 0.0006051$ ) and Gr ( $p < 3.808e-06$ ).

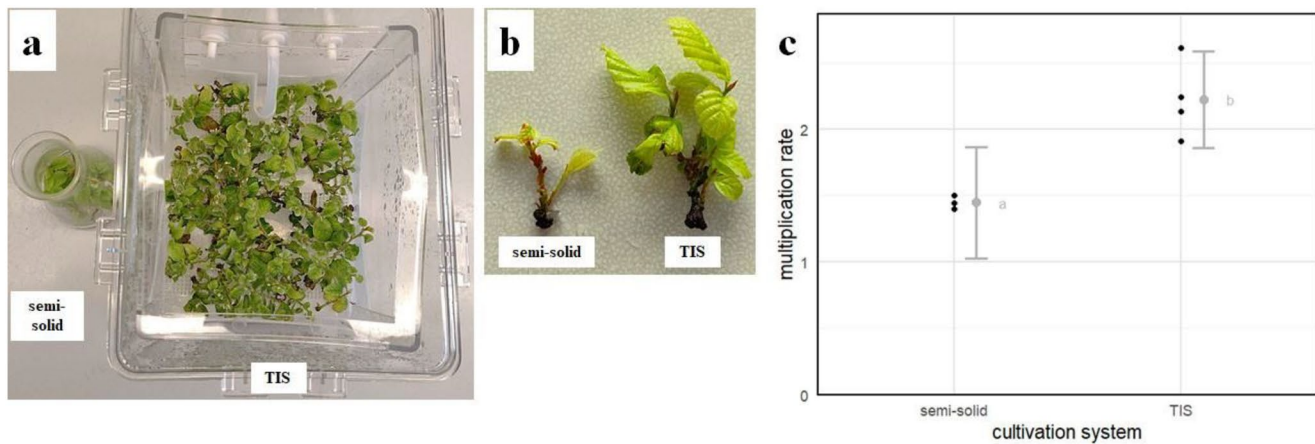
### Multiplication rates

The multiplication rates of the four Gr clones Gr-11, Gr-12, Gr-13, Gr-N and the three Sw clones Sw-H, Sw-I, Sw-J exhibited variability (Table 6). Across all three subculture intervals, the average multiplication rates for Gr clones were higher than multiplication rates for Sw clones. Among the four media tested, BN-Suc medium containing NAA and BAP did not support shoot propagation. For clones from the Gr provenance, significantly higher average multiplication rates were reached on Z-Gluc medium, whereas for clones from the Sw provenance no significant differences between

the four propagation media have been observed. But still average multiplication rates were higher when using Z-Gluc medium. Utilisation of different sugars in BAP-containing medium did not show significant differences in multiplication rates (Table 6). When visually comparing the physiological state of the in vitro shoots, we observed better leaf development and shoot vitality on Z-Gluc medium than on the other three media (Fig. 6).

### TIS bioreactor

In the experiment using four PLANTFORM™ TIS bioreactors, three clones from the Gr provenance (Gr-K, Gr-N, Gr-13) were used. The in vitro shoot development showed differences in the two treatments (Fig. 7a, b). In vitro shoots had a tendency to develop longer shoots with more nodal segments and larger leaves when grown in liquid propagation media in the TIS bioreactor when compared to the cultivation on semi-solid conditions. The majority of in vitro shoots in the TIS bioreactor formed one up to 4 new axillary shoots. This is in contrast to the tendency of forming primarily one new shoot of the in vitro clones growing on semi-solid medium. After one subculture interval of six weeks shoot multiplication rate of the clones in the TIS bioreactor



**Fig. 7** Comparison of *in vitro* shoot propagation using liquid medium in TIS bioreactor and semi-solid propagation media; **(a)** *In vitro* shoots growing in Erlenmeyer flasks with semi-solid medium (left) and liquid medium in TIS bioreactor (right) **(b)** *in vitro* shoot development after 8 weeks, when grown on semi-solid medium (left) and grown in liquid

**Table 7** Multiplication rates of three different clones (Gr-K, Gr-N, Gr-13) of *in vitro* shoots after 8 weeks of subculture in semi-solid and TIS bioreactor. Mean multiplication rate  $\pm$  SE is indicated for each cultivation system

clone	cultivation system	multiplication rate
Gr-K	TIS	2.62
Gr-K	TIS	2.13
Gr-N	TIS	1.91
Gr13	TIS	2.24
average		2.23 $\pm$ 0.295
Gr-K	semi-solid	1.50
Gr-N	semi-solid	1.40
Gr13	semi-solid	1.44
average		1.45 $\pm$ 0.05

averaged  $2.23 \pm 0.295$  and was significantly higher than in the semi-solid media ( $p=0.007^{**}$ ) (Table 7; Fig. 7c).

## Discussion

### Establishment

Earlier studies (Vieitez and San Jose 1996) demonstrated that the age and type of explant material is crucial for successful *in vitro* culture establishment, with axillary buds of 3-month-old seedlings showing good establishment. In a recent publication Zahn et al. (2025) also used young plant material, but shoot tips of seedlings were cut already after 3 weeks. In our experiments, shoot tips from 3-month-old seedlings showed high success rates. The disinfection method used significantly impacts contamination rates. Chalupa (1979) used ethanol and chlorine, while we found that using mercuric chloride effectively disinfected shoot

medium in TIS bioreactor; d) graph of all multiplication rates, black dots represent raw data, grey dots and error bars represent adjusted means with 95% confidence, with a  $p$ -value of ( $p=0.007^{**}$ ). Statistical analysis: Welch Two Sample t-test ( $\alpha=0.05$ )

tips with a low contamination rate. For medium composition during establishment, WPM supplemented with BAP (0.2–0.4 mg/L) was effective for shoot growth, aligning with past findings that WPM with various cytokinins supports shoot proliferation (Chalupa 1985). Lastly, longer subculture intervals in the establishment stage, specifically five weeks, were found to be optimal for shoot elongation and multiplication, contrasting with earlier findings that recommended more frequent transfers (Vieitez et al. 1993; Cuenca et al. 2000).

### Genotyping using microsatellites

Continuous exposure to high concentrations of growth regulators and nutrients during *in vitro* culture of forest tree shoots can induce soma clonal variation and morphological alterations. (Cuesta et al. 2010). Thus *in vitro* propagation of valuable genotypes requires genetic stability evaluation. In addition, genotyping can help to ensure the identity of clones in *in vitro* culture when handling many clones and an exchange of clonal material between laboratories or laboratory staff takes place. Generally, the microsatellite approach for genotyping is a more reliable technique than for example the random amplified polymorphic DNA (RAPD) technique because it is gene specific and gives better resolution of amplified fragments (Barrett et al. 1997). The selected set of 17 microsatellite loci have been used in previous studies and demonstrated a low probability of identity (PID) with a value of  $1.8 \times 10^{-17}$  (Eusemann et al. 2017). This means that each multilocus genotype represents a distinct individual that can be traced back to a single seed. In our study, we employed microsatellite analysis to identify *in vitro* clones and were able to assign each of the 109 established shoot to

the 31 distinct clones. Across replicates of the same clone, allele patterns were identical (supplementary material: OSM Table 1), confirming phenotypic and genetic stability within clones. Genotyping using methods such as microsatellite analysis, serves as a crucial quality control for working with clonal plant material. Defined clones can thus be propagated, transferred to *ex vitro* conditions and the plants could be used for breeding programs, as grafting material, for resistance testing in the greenhouse or as material for trial plots.

## Propagation

(Vieitez et al. 1993; Meier and Reuther 1994). Most studies on *F. sylvatica* propagation have reported difficulties in long-term in vitro cultivation due to limited shoot growth and low multiplication rates (Meier and Reuther 1994); (Vieitez et al. 1993). Establishing a large number of clones is particularly important when working with a recalcitrant tree species like *F. sylvatica*, as it increases the probability of identifying suitable genotypes for successful in vitro culture. In our study, we used material from seven provenances, comprising 31 genotypes, which allowed us to screen for clones with high multiplication ability. We found that multiplication rates varied depending on the provenance of the original explant material and over a period of two years, we identified 10 out of 31 clones with stable multiplication rates above 1.5. This represents a substantial improvement over previous reports, where only single clones showed high multiplication potential. These 10 clones now provide a stable basis for long-term in vitro propagation of *F. sylvatica*.

Commonly, in vitro propagation is conducted by axillary shoot proliferation with the separation of in vitro shoots into several nodal fragments that each develop new shoot growth, for example for *Quercus suber* (Martínez et al. 2023) or for *Fraxinus excelsior* (Schönweiß 2005). A prerequisite for this method is that the culture shows sufficient shoot elongation for separation of nodal segments. Compressed in vitro shoot growth is less suitable for separation into nodal segments, as is the case for *F. sylvatica*. Previous studies have shown that *F. sylvatica* can be propagated through axillary shoot proliferation using in vitro plantlets derived from embryonic axes (Vieitez et al. 1993). In our study, new shoot formation occurred primarily via activation of axillary buds. For the multiplication stage, we focused on achieving a high rate of axillary shoot formation to compensate for the limited shoot elongation. Once the in vitro clones are to be used for ex vitro transfer, sufficient shoot elongation remains critical for successful acclimatisation (Vieitez et al. 1993).

The effect of the culture medium is primarily determined by the addition of externally supplied phytohormones, which act as plant growth regulators and influence

the development of the plant cells. (Phillips and Garda 2019). Adding cytokinin promotes cell division, leading to increased growth, improved nutrient uptake and enhances the formation of axillary shoots. Benzyladenine (BAP) is the most commonly used synthetic cytokinin in in vitro culture (Schönweiß 2005; San José et al. 2020) and a compound of our propagation media BN-Suc, B-Suc and B-Gluc. Zeatin on the other hand, is a natural cytokinin, that has been compared with BAP in various studies (Schönweiß 2005; Phillips and Garda 2019). In our research we applied the propagation medium Z-Gluc enriched with Zeatin and found higher multiplication rates and shoot length compared to using propagation media containing BAP. Hence, using BAP enriched medium for in vitro establishment should be switched to Zeatin enriched propagation medium for in vitro propagation.

Another key factor in improving multiplication rates and shoot development can be the carbon source in the propagation medium. While glucose and fructose enhance in vitro shoot multiplication compared to sucrose (Cuenca and Vieitez 2000), our findings indicate that using glucose instead of sucrose does not generally increase shoot length and multiplication rates. However, when comparing propagation media B-Gluc and Z-Gluc, we observed longer shoot growth and significantly higher multiplication rates in Z-Gluc, suggesting that the phytohormone is the driving factor for enhanced axillary shoot formation and multiplication.

This study reports, for the first time, the application of Temporary Immersion System (TIS) bioreactors for the micropropagation of *F. sylvatica*. Cultivation in TIS bioreactors resulted in significantly higher multiplication rates compared to semi-solid medium, demonstrating a positive response of beech shoots to this system. Additionally, we observed more newly developed axillary shoots per initial shoot in the propagation in TIS. The reason for this could be that in TIS, in vitro shoots absorb nutrients more effectively due to intermittent immersion in liquid media (San José et al. 2020). Generally, this nutrient availability, along with the presence of phytohormones and a gaseous environment, results in better growth, propagation, and preparation for rooting and acclimatization in several woody plant species (San José et al. 2020). PLANTFORM™ TIS bioreactors have been used for various forest tree species. Studies on *Alnus* and *Castanea* have shown an improved multiplication rate when comparing TIS and semi-solid media (Vidal et al. 2015; San José et al. 2020). One challenge in using large-scale TIS bioreactors, such as the PLANTFORM™ system, is that they require a substantial number of in vitro shoots as starting material, to make full use of the vessel capacity. Consequently, these systems are less suitable for recalcitrant species with very limited shoot availability. However, for the species *F. sylvatica*, we present the first approach to in

in vitro propagation using a PLANTFORM™ bioreactor system, which offers a promising, labour-efficient method for propagation, with potential for successful in vitro rooting and future *ex vitro* transfer. We note that replication in this experiment was limited ( $n=1$  per clone, except Gr-K with  $n=2$ ), and therefore, while the results are very promising, they should be considered preliminary.

## Outlook for the utilisation of in vitro shoots

In vitro rooting, *ex vitro* transfer and acclimatization of *F. sylvatica* shoots are crucial steps as only few studies have reported successful rooting and acclimatization for a limited number of genotypes (Zahn et al. 2025). The subculture conditions shown in this study are suitable for a reliable multiplication but need to be modified when aiming for rooting and *ex vitro* transfer. The culture medium would then need to be adjusted to promote plant features such as shoot elongation and leaf number and enable root formation. Upon transfer of in vitro shoots to soil, the microclimate changes and plant physiology has to transition to autotrophic growth (Benson 2000). A study on *Fagus grandifolia* showed the genera's ability to develop roots in vitro, despite low rooting percentages and no sustained *ex vitro* growth (Ramirez et al. 2007). Recently, the potential of in vitro rooted shoots to survive the *ex vitro* transfer was shown in a proof of principle (Zahn et al. 2025). Achieving lasting *ex vitro* growth of acclimatized plants remains an important subject of research.

Repeated in vitro rooting experiments with a high number of clones can help establish effective methods for rooting and soil transfer. As a prerequisite for carrying out these experiments, we are able to provide a large number of in vitro shoots from different clones with the results of the present study. In particular, the use of the optimized propagation medium Z-Gluc and propagation in the TIS bioreactor leads to in vitro shoots with well-developed leaves and shoot growth. Developing in vitro rooting protocols would be the next important step in order to provide clonally propagated *F. sylvatica* plants in the future. These plants can in turn be used for resistance or pathogenicity tests to investigate the species' capacity to cope with drought and biotic stress factors.

## Conclusion

Our study presents a comprehensive system for the establishment, genotyping, and propagation of *F. sylvatica*. We provide a practical protocol for efficient in vitro micropropagation, which includes the selection of multiple genotypes from different provenances to identify clones with high

multiplication ability, propagation through axillary shoot proliferation on Zeatin-enriched medium (Z-Gluc), and cultivation in PLANTFORM™ Temporary Immersion System (TIS) bioreactors to enhance shoot multiplication and axillary shoot formation.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11240-025-03304-y>.

**Acknowledgements** We are grateful to Diana Morgenroth (Thünen Institute of Forest Genetics) for carrying out the in vitro and genotyping laboratory work. We thank Pascal Eusemann (Thünen Institute of Forest Genetics) for leading the project BucheTIG and him and Khira Deecke (Thünen Institute of Forest Genetics) for supporting with microsatellite analysis. Thank you to Magdalena Sobczak (Institute of Dendrology PAS) for her technical support concerning in vitro culture. We also thank Virginia Zahn and Anne-Mareen Eisold (Thünen Institute of Forest Genetics) for critical reading of the manuscript.

**Author contributions** Conceptualization: Ben Bubner, Paweł Chmielarz; Methodology: Paweł Chmielarz and Tomasz A. Pawłowski performed establishment in Kórnik – Poland, Franka N. Thiesen performed propagation and further analyses in Waldsiefersdorf – Germany; Formal analysis and investigation: Franka N. Thiesen; Writing - original draft preparation: Franka N. Thiesen, Paweł Chmielarz, Ben Bubner; Writing - review and editing: Paweł Chmielarz, Franka N. Thiesen, Ben Bubner, Tomasz A. Pawłowski; acquisition: Ben Bubner. The authors read and approved the final manuscript.

**Funding** Open Access funding enabled and organized by Projekt DEAL. This work was part of the project the project BucheTIG\_In-Vitro (project funding number 2219WK60A4) which is financially supported by the German Federal Ministry of Food and Agriculture, and the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety via the Management of Fach-Agentur Nachwachsende Rohstoffe e.V. The work was further supported and inspired by the COST Action CA21157 “European Network for Innovative Woody Plant Cloning”, [www.copyright.eu](http://www.copyright.eu), supported by COST (European Cooperation in Science and Technology) and Institute of Dendrology of the Polish Academy of Sciences.

**Data availability** The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

**Code availability** The custom code and software application generated and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical approval** Not applicable.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate

if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

- Ahuja MR (1984) In vitro induction of organogenesis in juvenile and mature Beech. *Silvae Genet* 33:241–242
- Barrett C, Lefort F, Douglas GC (1997) Genetic characterization of oak seedlings, epicormic, crown and micropropagated shoots from mature trees by RAPD and microsatellite PCR. *Sci Hort* 70:319–330. [https://doi.org/10.1016/s0304-4238\(97\)00065-4](https://doi.org/10.1016/s0304-4238(97)00065-4)
- Benson E (2000) Plant recalcitrance: an introduction. *Vitro Cell Dev Biol-Plant* 36:141–148
- Bruegmann T, Fladung M, Schroeder H (2022) Flexible DNA isolation procedure for different tree species as a convenient lab routine. *Silvae Genet* 71:20–30. <https://doi.org/10.2478/sg-2022-0003>
- Chalupa V (1979) In vitro propagation of some broad-leaved forest trees. *Communicationes Inst Forestalis Českosloveniae* 11:159–170
- Chalupa V (1985) In vitro propagation of *Larix*, *Picea*, *Pinus*, *Quercus*, *Fagus* and other species using adenine type cytokinins and Thidiazuron. *Communicationes Inst Forestalis Českosloveniae* 14:65–90
- Chalupa V (1996) *Fagus sylvatica* L. (European beech). In: Bajaj YPS (ed) *Trees IV, biotechnology and forestry* 35. Springer, Berlin, Heidelberg, New York, pp 138–154
- Cuenca B, Vieitez AM (2000) Influence of carbon source on shoot multiplication and adventitious bud regeneration in in vitro Beech cultures. *Plant Growth Regul* 32:1–12. <https://doi.org/10.1023/a:1006329510280>
- Cuenca B, Ballester A, Vieitez AM (2000) In vitro adventitious bud regeneration from internode segments of Beech. *Plant Cell Tiss Org Cult* 60:213–220. <https://doi.org/10.1023/a:1006428717309>
- Cuesta C, Ordás RJ, Rodríguez A, Fernández B (2010) PCR-based molecular markers for assessment of Somaclonal variation in *Pinus Pineae* clones micropropagated in vitro. *Biol Plant* 54:435–442. <https://doi.org/10.1007/s10535-010-0079-y>
- Eusemann P, Preuss A, Liesebach M, Liesebach H (2017) Optimierte Saatgutqualität durch einzelbaumweise Beerntung - eine untersuchung an Buche (*Fagus sylvatica* L.). *Forstarchiv* 88:17–23
- Ewald D (2007) Micropropagation of *Larix species* via organogenesis. In: Jain S, H H (eds) *Protocols for micropropagation of Woody trees and fruits*. Springer, Dordrecht, Heidelberg, London, pp 125–136
- Fenning TM (2019) The use of tissue culture and in-vitro approaches for the study of tree diseases. *Plant Cell Tiss Org Cult* 136:415–430. <https://doi.org/10.1007/s11240-018-01531-0>
- Grabska-Szwagrzyk E, Tiede D, Sudmanns M, Kozak J (2024) Map of forest tree species for Poland based on Sentinel-2 data. *Earth Syst Sci Data* 16:2877–2891. <https://doi.org/10.5194/essd-16-2877-2024>
- Hazubaska-Przybyl T, Chmielarz P, Bojarczuk K (2015) In vitro responses of various explants of *Fagus sylvatica*. *Dendrobiology* 73:135–144. <https://doi.org/10.12657/denbio.073.014>
- Hendry SJ, Boddy L, Lonsdale D (2002) Abiotic variables effect differential expression of latent infections in Beech (*Fagus sylvatica*). *New Phytol* 155:449–460. <https://doi.org/10.1046/j.1469-8137.2002.00473.x>
- Langer GJ, Busskamp J (2021) Fungi associated with Woody tissues of European Beech and their impact on tree health. *Front Microbiol* 12. <https://doi.org/10.3389/fmicb.2021.702467>
- Langer GJ, Busskamp J (2023) Vitality loss of beech: a serious threat to *Fagus sylvatica* in Germany in the context of global warming. *J Plant Dis Prot* 130:1101–1115. <https://doi.org/10.1007/s41348-023-00743-7>
- Latalowa M (1992) Man and vegetation in the pollen diagrams from Wolin Island (NW Poland). *Acta Paleobotanica* 32:123–249
- Lefèvre S, Wagner S, Petit RJ, de Lafontaine G (2012) Multiplexed microsatellite markers for genetic studies of Beech. *Mol Ecol Resour* 12:484–491. <https://doi.org/10.1111/j.1755-0998.2011.03094.x>
- Leuschner C (2020) Drought response of European beech (*Fagus sylvatica* L.)—A review. *Perspect Plant Ecol Evol Syst* 47. <https://doi.org/10.1016/j.ppees.2020.125576>
- Martínez MT, Cuenca B, Mosteiro F, Piñeiro P, Pérez F, Solla A, Corredoira E (2023) Screening of Cork oak for resistance to *Phytophthora cinnamomi* and micropropagation of tolerant seedlings. *Horticulturae* 9. <https://doi.org/10.3390/horticulturae9060692>
- Meier K, Reuther G (1994) Factors controlling micropropagation of mature *Fagus sylvatica*. *Plant Cell Tiss Org Cult* 39:231–238. <https://doi.org/10.1007/bf00035975>
- Pawlowski TA, Suszka J, Mucha J, Zadworny M, Alipour S, Kurpisz B, Chmielarz P, Jagodzinski AM, Chmura DJ (2024) Climate legacy in seed and seedling traits of European beech populations. *Front Plant Sci* 15. <https://doi.org/10.3389/fpls.2024.1355328>
- Peakall R, Smouse PE (2012) GenAEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* 28:2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Phillips GC, Garda M (2019) Plant tissue culture media and practices: an overview. *Vitro Cell Dev Biol-Plant* 55:242–257. <https://doi.org/10.1007/s11627-019-09983-5>
- Ramirez M, Krasowski MJ, Loo JA (2007) Vegetative propagation of American Beech resistant to Beech bark disease. *HortScience* 42:320–324. <https://doi.org/10.21273/hortsci.42.2.320>
- Reed BM, Sarasan V, Kane M, Bunn E, Pence VC (2011) Biodiversity conservation and conservation biotechnology tools. *Vitro Cell Dev Biol-Plant* 47:1–4. <https://doi.org/10.1007/s11627-010-9337-0>
- Roibu CC, Palaghianu C, Nagavciuc V, Ionita M, Sfecla V, Mursa A, Crivellaro A, Stirbu MI, Cotos MG, Popa A, Sfecla I, Popa I (2022) The response of Beech (*Fagus sylvatica* L.) populations to climate in the Easternmost sites of its European distribution. *Plants* 11:3310. <https://doi.org/10.3390/plants11233310>
- Rukh S, Sanders TGM, Krüger I, Schad T, Bolte A (2023) Distinct responses of European Beech (*Fagus sylvatica* L.) to drought intensity and length - a review of the impacts of the 2003 and 2018–2019 drought events in Central Europe. *Forests* 14. <https://doi.org/10.3390/f14020248>
- San José MC, Blázquez N, Cernadas MJ, Janeiro LV, Cuenca B, Sánchez C, Vidal N (2020) Temporary immersion systems to improve alder micropropagation. *Plant Cell Tiss Org Cult* 143:265–275. <https://doi.org/10.1007/s11240-020-01937-9>
- Schönweiß (2005) Etablierung neuer Methoden zur in vitro Kultivierung der Baumart *Fraxinus excelsior* L. und Entwicklung von Verfahren zur Kryokonservierung von in vitro Sprossspitzen. Dissertation, Kassel
- Suszka B, Muller C, Bonnet-Masimbert M (1996) Seeds of forest broadleaves - from harvest to sowing. INRA edition, Paris
- Szafer W (1932) The Beech and the Beechforest in Poland. Veröffentlichungen Des Geobotanischen Institutes Rübel Zürich 8:168–181
- Vidal N, Blanco B, Cuenca B (2015) A temporary immersion system for micropropagation of axillary shoots of hybrid chestnut. *Plant*

- Cell Tiss Org Cult 123:229–243. <https://doi.org/10.1007/s11240-015-0827-y>
- Vieitez AM, San Jose MC (1996) Adventitious shoot regeneration from *Fagus sylvatica* leaf explants in vitro. *Vitro Cell Dev Biol-Plant* 32:140–147
- Vieitez FJ, Ballester A, Vieitez AM (1992) Somatic embryogenesis and plantlet regeneration from cell suspension cultures of *fagus sylvatica* L. *Plant Cell Rep* 11:609–613. <https://doi.org/10.1007/bf00236383>
- Vieitez AM, Ferro EM, Ballester A (1993) Micropogagation of *Fagus sylvatica* L. *Vitro Cell Dev Biol-Plant* 29:183–188
- Welander M, Persson J, Asp H, Zhu LH (2014) Evaluation of a new vessel system based on temporary immersion system for micro-propagation. *Sci Hort* 179:227–232. <https://doi.org/10.1016/j.scienta.2014.09.035>
- Wilhelm E, Hristoforoglu K, Fluch S, Burg K (2005) Detection of microsatellite instability during somatic embryogenesis of oak (*Quercus Robur* L). *Plant Cell Rep* 23:790–795. <https://doi.org/10.1007/s00299-004-0891-y>
- Zahn V, Fendel A, Sievers AJ, Fladung M, Bruegmann T (2025) Benefiting from the past: Establishing in vitro culture of European Beech (*Fagus sylvatica* L.) from provenance trial trees and seedlings. *Plant Methods* 21:31. <https://doi.org/10.1186/s13007-025-01350-3>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.