Effect of Explant Type and Inoculation Date on In Vitro Propagation of Ornamental Cherry Prunus subhirtella ‘Autumnalis'

Petra Kunc

petra.kunc@bf.uni-lj.si

University of Ljubljana

Gregor Osterc

University of Ljubljana

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Abstract

Micropropagation of woody plants is often limited by explant type, physiological maturity, and season of collection. This study evaluated the influence of explant type and collection date on the *in vitro* propagation of *Prunus subhirtella* 'Autumnalis', an ornamental cherry from a mature (~ 60-year-old) tree. Three explant types—dormant buds, breaking buds, and nodal segments—were collected at multiple dates and cultured through induction, multiplication, and rooting stages using MS-based media supplemented with auxins, gibberellins and cytokinins. Dormant buds failed to survive *in vitro*, whereas nodal explants showed the highest survival (46.7%), followed by breaking buds (16%). Explant collection date significantly affected culture establishment: breaking buds showed improved survival in later collection dates, while nodal explants exhibited decreasing survival across successive dates. During multiplication and rooting, no significant differences in survival or rooting success were observed among explant types, though contamination and necrosis remained challenges. The results highlight nodal explants and optimally timed bud collections as key factors for successful in vitro propagation, providing a foundation for clonal propagation and conservation of mature *Prunus subhirtella* 'Autumnalis' genotypes.

INTRODUCTION

Micropropagation is an *in vitro* plant propagation technique used for the rapid, large-scale production of genetically identical, physiologically uniform, and pathogen-free plantlets (Rathore et al., 2005, Grzelak et al., 2024). Compared to conventional vegetative propagation, it offers several advantages, including higher multiplication rates, reduced production time, improved plant quality, and the generation of disease-free planting material. Owing to these benefits, micropropagation is increasingly applied in horticulture, agriculture, forestry, and conservation programs (Rathore et al., 2005).

The success of micropropagation is based on the principles of cellular totipotency and cellular plasticity. Totipotency refers to the inherent ability of a single plant cell to divide, differentiate, and regenerate into a complete plant identical to the donor plant. Cellular plasticity enables cultured cells to modify their developmental pathways in response to *in vitro* conditions, allowing regeneration through organogenesis or somatic embryogenesis. These characteristics make tissue culture an effective tool for overcoming propagation barriers in difficult-to-propagate species (Jha et al., 2023).

Woody plants include trees and shrubs with lignified stems and comprise both angiosperms and gymnosperms. They occur in natural forests, plantations, and cultivated systems, including ornamental and fruit trees. Woody plants are of considerable economic, ecological, and environmental importance; therefore, maintaining their genetic diversity and elite genotypes is essential for productivity, conservation, and sustainable forestry (Thorpe & Harry, 1990, Pandey & Chaudhary, 2025). However, compared to herbaceous species, woody plants are more difficult to propagate asexually and *in vitro*. Consequently, the practical and commercial application of micropropagation in woody species remains

limited. Although successful protocols have been developed for certain species, they are often highly species-specific and difficult to scale up (Grzelak et al., 2024).

Micropropagation of woody plants is strongly influenced by ontogenetic age, physiological condition, and environmental history of the donor plant (Pandey & Chaudhary, 2025). *In vitro* propagation of woody plants faces multiple biological and technical challenges, including recalcitrance of mature tissues to *in vitro* establishment (Pandey & Chaudhary, 2025), excessive phenolic exudation and browning of the medium (Grzelak et al., 2024; Jha et al., 2023), basal callusing (Grzelak et al., 2024), shoot tip necrosis and vitrification (Giri et al., 2004; Timofeeva et al., 2014), high levels of endogenous microbial contamination (Jha et al., 2023), poor shoot multiplication and rooting (Grzelak et al., 2024; Jha et al., 2023) and low survival rates during acclimatization (Grzelak et al., 2024; Jha et al., 2023, Pandey & Chaudhary, 2025). These problems are often more pronounced in explants taken from mature trees and species from stressful or seasonally variable environments.

Maturation is one of the major limitations in woody plant micropropagation. As woody plants undergo a phase change from juvenility to maturity, their regenerative capacity declines. Maturation is associated with reduced growth rate, poor rooting ability, plagiotropism, and loss of morphogenetic competence *in vitro*. Juvenile tissues respond more favorably to tissue culture conditions, whereas physiologically mature tissues are often recalcitrant (Thorpe & Harry, 1990, Rathore et al., 2005, Pandey & Chaudhary, 2025). Successful micropropagation of woody plants depends on several critical factors such as type of explant, physiological age and maturation stage, season and time of explant collection, health and management of donor plant, surface sterilization and contamination control, culture medium and growth regulators, environmental conditions, rooting and acclimatization strategies (Feng et al., 2010; Girijashankar, 2012; Jha et al., 2023; Kumar et al., 2011; Lee et al., 2011).

Prunus subhirtella 'Autumnalis,' a diploid ornamental cherry belonging to the family Rosaceae, is valued for its winter hardiness and ornamental characteristics and has potential for use in horticulture and breeding programs (Kunc et al., 2026). Despite its importance, information on its *in vitro* propagation remains limited, particularly for explants derived from mature donor plants. The absence of established micropropagation protocols represents a major constraint for the large-scale clonal propagation, rejuvenation, and conservation of elite genotypes. Therefore, the aim of this study was to establish an efficient micropropagation protocol for *Prunus subhirtella* 'Autumnalis' by evaluating the suitability of different explant types for *in vitro* culture establishment and determining the optimal season and time for explant collection, with particular emphasis on explants derived from mature plants.

MATERIAL AND METHODS

Plant material and sample collection

The study was conducted on ornamental cherry (*Prunus subhirtella* Miq. 'Autumnalis') (Fig. 1), a mature tree approximately 60 years old, growing in the experimental field of the Biotechnical Faculty, University

of Ljubljana (46°3'4" N, 14°30'18" E). The tree had not been pruned or rejuvenated and was grown without any special horticultural care.

For the initiation of *in vitro* cultures, three types of explants were inoculated onto the culture medium: dormant buds (winter buds), buds at the initial stage of opening (breaking buds), and nodal explants. All explant types were sampled on three different dates. Dormant leaf buds (hereafter referred to as dormant buds) were excised from one-year-old shoots on 23 January 2023, 1 March 2023, and 16 March 2023. Buds were cut at the base of the shoot using a scalpel. To obtain breaking leaf buds (hereafter referred to as breaking buds), one-year-old shoots were collected 10 days prior to each inoculation date (23 January 2023, 1 March 2023, and 16 March 2023), placed in water, and kept at room temperature to promote bud opening (Fig. 2). After bud break, the leaf buds were excised at the base of the shoot. Later in the growing season, when sufficient current-year growth had developed (10 May 2023, 12 June 2023, and 23 June 2023), plant material for nodal explants was collected. Current-year shoots were excised and cut into nodal segments. A total of 285 dormant buds, 412 breaking buds, and 107 nodal explants were included in the *in vitro* experiment. The number of plants used for the initiation of *in vitro* culture depended on the availability of plant material.

Sample preparation and growing conditions

All manipulations were performed under aseptic conditions in a laminar airflow cabinet. All instruments and working surfaces were regularly sterilized and disinfected throughout the procedure. For dormant buds, the outer bud scales were removed (Fig. 3a), after which the buds were surface-sterilized with 96% ethanol (EtOH) for 1 minute, followed by a 15-min treatment with 1.6% dichloroisocyanuric acid (DICA). The explants were then rinsed three times for 5 min with bidistilled water. For breaking buds (Fig. 3b), the bud scales were removed, and the explants were surface-sterilized with 70% ethanol (EtOH) for 30 seconds and for 15 min with 1.6% dichloroisocyanuric acid (DICA), followed by three rinses of 5 min each with bidistilled water. Nodal explants were surface-sterilized with 70% ethanol (EtOH) for 30 seconds and for 15 min with 1.6% dichloroisocyanuric acid (DICA) and subsequently rinsed three times for 5 min with bidistilled water.

The plants cultivated in the experiment were grown in a growth chamber at a temperature of 25 ± 1°C, under a 16/8 h photoperiod (light/dark), with a light intensity of 34 µmol m^{-2} s^{-1}.

Micropropagation of ornamental cherry

For the micropropagation of ornamental cherry (*Prunus subhirtella* ‘Autumnalis’), the process was carried out in three sequential stages: induction, multiplication, and rooting, each using a different

culture medium. Explants were initially cultured on an induction medium. After 21 days, the number of explants that survived and initiated growth (Fig. 4a), as well as the number of explants that became contaminated or deteriorated, was evaluated. The surviving explants were subcultured onto a multiplication medium, where they were grown for six weeks. Six weeks after transfer to the multiplication medium, explants were again assessed for possible contamination or tissue decay. Shoots measuring 1.5–2.0 cm in length were then subcultured onto a rooting medium, and rooting success was evaluated after five weeks (Fig. 4b).

Both types of bud explants were inoculated in Petri dishes (Ø 90 × 15 mm), with five explants per Petri dish. In cases of contamination, non-contaminated explants were transferred to fresh Petri dishes. Once shoot growth was established (21 days after inoculation), propagules that had initiated growth were transferred to separate culture tubes containing multiplication medium. During the rooting stage, shoots were also maintained in culture tubes.

Nodal explants were cultured in test tubes from the beginning of the experiment, with one nodal segment per tube, and were sequentially transferred to induction, multiplication, and rooting media.

Culture medium composition

Induction, multiplication, and rooting media were prepared based on the macro- and microelements and organic components of MS (Murashige and Skoog, 1962) medium (Table 1). Sucrose, serving as a carbon source, was added at 30 g/L in the induction and multiplication media and at 20 g/L in the rooting medium. The media mainly differed in their plant hormone composition and concentration. The induction medium contained 0.02 mg/L indole-3-butyric acid (IBA), 1 mg/L benzylaminopurine (BAP), and 0.1 mg/L gibberellic acid (GA3). The multiplication medium contained the same concentration of IBA, 0.5 mg/L BAP, and 0.1 mg/L GA3. The rooting medium contained only 2 mg/L IBA. All media were adjusted to pH 5.7 and solidified with 8 g/L agar (Table 1).

Table 1
Composition of culture medium for induction, multiplication, and rooting of *P. subhirtella* 'Autumnalis' ornamental cherry.

	Medium for		
	Induction	**Multiplication**	**Rooting**
Macroelements (mg/L)			
KH2PO4	170	170	170
NH4NO3	1650	1650	1650
MgSO4 x 7H2O	370	370	370
KNO3	1900	1900	1900
CaCl2 x 2H2O	440	440	440
Microelements (mg/L)			
MnSO4 x 4H2O	22.3	22.3	22.3
H3BO3	6.2	6.2	6.2
ZnSO4 x 7H2O	8.6	8.6	8.6
KJ	0.83	0.83	0.83
CuSO4 x 5H2O	0.025	0.025	0.025
CoCl2 x 6H2O	0.025	0.025	0.025
Na2MoO4 x 2H2O	0.25	0.25	0.25
Na2Fe- EDTA	37.2	37.2	37.2
FeSO4 x 7H2O	27.8	27.8	27.8
Carbohydrates (g/L)			
Sucrose	30	30	20

Organic substances, vitamins, hormones (mg/L)			
Inositol	100.0	100.0	100.0
Thiamine	0.1	0.1	0.1
Nicotinic acid	0.5	0.5	0.5
Pyridoxine	0.5	0.5	0.5
Glycine	2.0	2.0	2.0
IBA	0.02	0.02	2.0
BAP	1.0	0.5	—
GA3	0.1	0.1	—
Gelling agent (g/L) and pH value			
Agar	8	8	8
pH	5.7	5.7	5.7

IBA (Indole-3-butyric acid); BAP (6-Benzylaminopurine); GA3 (Gibberellic acid)

Culture medium preparation

The media were prepared by weighing commercial Murashige and Skoog with vitamins (MS) medium, sucrose, and hormones (IBA, BAP, GA3) into a beaker containing bidistilled water (Table 1). Once the components dissolved on a magnetic stirrer, the final volume was adjusted in a volumetric flask, and the pH was set to 5.7 by adding sodium chloride (NaCl) or hydrochloric acid (HCl), while continuously stirring. The subsequent steps included adding agar and autoclaving, depending on the type of culture vessels used:

For media preparation in test tubes: Agar was added to the prepared medium and dissolved using a microwave oven. After the solution boiled three times and all agar was dissolved, 10 mL of medium was dispensed into each test tube using an automatic media dispenser. The tubes were then capped and autoclaved for 20 minutes at 121°C and 1.1 bar.

For pouring media into Petri dishes: The solution was transferred to a 1 L Schott bottle, and 8 g of agar was added. The bottles were labeled and autoclaved for 20 minutes at 121°C and 1.1 bar. For pouring into Petri dishes, the agar must be completely dissolved, and the medium should be clear. If the medium is not poured immediately after autoclaving, the solidified medium must first be re-melted. A microwave oven was used to re-dissolve the medium. Heating produced a clear solution without visible undissolved agar. The medium was then poured into sterile round Petri dishes. All media were handled and poured in a laminar flow (dust-free) cabinet.

Chemicals

The following chemicals were used in this study: Murashige and Skoog (MS) basal medium including vitamins, sucrose, indole-3-butyric acid (IBA), benzylaminopurine (BAP), gibberellic acid (GA), sodium chloride (NaCl), hydrochloric acid (HCl), and ethanol were obtained from Duchefa Biochemie (Haarlem, The Netherlands). Agar was used as the gelling agent, and dichloroisocyanuric acid (DICA), used for surface sterilization, was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). All chemicals were of analytical grade unless stated otherwise.

Statistical analysis

Data was collected and initially processed using Microsoft Excel. Statistical analyses and data visualization were performed in R (R Commander interface). Associations between categorical variables were assessed using the Chi-square test of independence. In cases where expected cell frequencies were low or zero, Fisher's exact test was applied instead of the Chi-square test.

P-values were calculated based on the test statistics, and a significance level of $\alpha = 0.05$ was used. Results with $p \leq 0.05$ were considered statistically significant, indicating a likely association between the analyzed variables.

RESULTS

Induction of in vitro cultures

A frequency analysis was performed to compare explant survival between different explant types. Nodal explants showed a higher survival rate than breaking buds. Out of 107 inoculated nodal explants, 50 survived (46.7%), whereas only 66 out of 412 breaking buds survived (16.0%).

Dormant buds exhibited 0% survival at all tested time points, so a Chi-squared test could not be applied for this group due to zero variability in the response variable. Therefore, the statistical comparison was restricted to nodal and breaking buds (Table 2, Fig. 5).

Table 2
Inoculated explants and survival/failure (%) after 21 days by explant type and inoculation timing.

Type of explant Date	Number of inoculated explants	Number of infected/dead explants	Number of surviving explants	Infected/dead explants (%)	Surviving explants (%)
Nodal explants	107	57	50	53.3	46.7
10.may	35	15	20	42.9	57.1
12.jun	42	20	22	47.6	52.4
23.jun	30	22	8	73.3	26.7
Breaking buds	412	346	66	84.0	16.0
23.jan	190	171	19	90.0	10.0
1.mar	162	144	18	88.9	11.1
16.mar	60	30	30	50.0	50.0
Dormant buds	285	285	0	100.0	0.0
23.jan	125	125	0	100.0	0.0
1.mar	130	130	0	100.0	0.0
16.mar	30	30	0	100.0	0.0

A Pearson's Chi-squared test revealed a highly significant difference in survival between nodal and breaking buds ($X^2 = 45.153$, df = 1, $p < 0.001$), confirming that nodal explants survived at a significantly higher rate. Expected counts and Chi-square components further supported this result, with substantial contributions from all deviations to the overall statistics (Fig. 5).

We also investigated whether, in addition to explant type, the date of explant collection affects induction success. In both types of explants, survival differed significantly according to inoculation date. When breaking buds were analyzed across the three dates, survival varied highly significantly ($X^2 = 58.78$, df = 2, $p < 0.001$), with the highest survival observed on March 16 (Date 3) (Table 2, Fig. 6). For nodal explants alone, survival also differed across the three dates ($X^2 = 6.91$, df = 2, $p = 0.032$), with the lowest survival observed on 16th March (Date 3). (Table 2, Fig. 7).

Multiplication of in vitro cultures

A total of 115 propagules were subcultured from the induction medium onto the multiplication medium, comprising 65 propagules regenerated from breaking buds and 50 from nodal explants. After six weeks, only 13.8% of propagules derived from breaking buds survived, whereas nodal explants showed survival

rate of 20.0%, indicating an overall low survival percentage on the multiplication medium (Table 3). Although nodal explants showed a slightly higher survival (20%) than breaking buds (13.8%), the difference was not statistically significant ($X^2 = 0.776$, df = 1, $p = 0.378$). It was observed that contamination or bud necrosis continued to occur even after nine weeks following inoculation.

Table 3
Survival and percentage survival of propagules derived from different explant types during multiplication

Type of explant Date	Number of propagules	Number of surviving propagules (After 6 weeks)	Percentage of surviving propagules (After 6 weeks) (%)
Breaking buds	65	9	13.8
23 January 2023	18	1	5.6
1 March 2023	17	3	17.6
16 March 2023	30	5	16.7
Nodal explants	50	10	20.0
10 May 2023	20	3	15.0
12 June 2023	22	0	0.0
23 June 2023	8	7	87.5

The survival of propagules derived from breaking buds did not differ significantly among the three collection dates after 6 weeks of multiplication ($X^2 = 1.44$, df = 2, $p = 0.486$). The survival of propagules derived from nodal explants varied significantly among the three collection dates after 6 weeks of multiplication. Survival was highest on June 23, 2023 (87.5%), very low on May 10, 2023 (15%), and absent on June 12, 2023 (0%). The results indicate that the date of explant collection has a strong effect on the success of multiplication for nodal explants. Due to small expected counts in some cells, Fisher's exact test was used instead of the Chi-squared test. The test indicated a highly significant difference in survival across collection dates in nodal explants ($p < 0.001$).

Rooting of in vitro cultures

Rooting success did not differ significantly between propagules derived from breaking buds and nodal explants. Rooting was observed in 55.6% of propagules derived from breaking buds and in 50.0% of those derived from nodal explants ($\chi^2 = 0.05$, df = 1, p = 0.819) (Table 4).

Table 4
Number and Percent (%) of rooted plantles by explant type

Explant type	Rooted	Not rooted	Rooting (%)
Breaking buds	5	4	55.6
Nodal explants	4	4	50.0
Pearson's Chi-squared test, $X^2 = 0.05$, df = 1, p = 0.819			

Discussion

In our study, the establishment of *in vitro* culture and propagation of woody plants also proved to be challenging, which is consistent with numerous previous reports (Singh et al., 2014; Sota et al., 2025; Thorpe & Harry, 1990; Timofeeva et al., 2014). It is well established that not all explants are equal in terms of their regenerative capacity, and the source and type of explant represent critical factors influencing *in vitro* culture success, especially in woody plants (Jha et al., 2023). Our results show significant difference in *in vitro* culture success. Dormant buds consistently exhibited 100% contamination and explant necrosis across all sampling dates, resulting in complete failure of regeneration. The poor performance of the *in vitro* cultures can likely be attributed to the presence of endophytic microorganisms, particularly given that the donor mother tree was very old and unmanaged. As reported by Jha et al., (2023), epiphytic microbes can be removed by surface sterilization, whereas endophytic microbes persist within the explant and lead to contamination in culture. Explants collected from the external environment are therefore prone to microbial exposure and subsequent mortality, emphasizing the need for healthy, disease-free donor plants (Jha et al., 2023). Although surface sterilization is an essential step in plant tissue culture, sterilant must be applied at optimal concentrations and exposure times, as higher concentrations or prolonged treatments can rapidly kill explants (Jha et al., 2023). Traore et al., (2005) reported that in winter buds with thick scales, the shielded and enclosed immature shoot and inner tissues are isolated from the external environment and are generally free of contaminants; however, this was not observed in our study. Consequently, dormant buds of *Prunus subhirtella* 'Autumnalis' are unsuitable explants for *in vitro* culture initiation under the applied sterilization and culture conditions.

Traore et al., (2005), reported that winter buds exhibited lower contamination rates than spring buds, which is not consistent with our study, in which breaking buds with the remaining bud scales removed regenerated at a rate of 16.0%. Traore et al., (2005) also reported that Douglas fir bud sterilization was most effective when the sterilization treatment was followed by dissection to remove both the outer and inner bud scales. This indicates that most contaminants are located on the outer scales exposed to the external environment, and that some microorganisms may persist and contaminate cultures unless the scales are physically removed from the buds.

In our experiment, higher regeneration success was achieved using nodal explants and open buds; nevertheless, a high proportion of explants still became contaminated. We also confirmed that the

timing of explant collection significantly influenced propagation success. In open buds, inoculation and regeneration success increased across evaluation dates, whereas in nodal explants the opposite trend was observed, with earlier collection dates yielding better results. High rates of fungal and bacterial contamination in initial teak cultures have been reported to depend strongly on the season of explant collection (Jha et al., 2023). In a previous study, Bergmann et al., (1997) identified April as the most suitable time for bud collection, as summer buds exhibited high contamination frequencies, while fall and winter buds showed poor development; this is consistent with our results. Seasonal variation in microbial contamination has also been reported in other studies (Hohtola, 1988). In the present study, *in vitro* cultures were initiated from nodal sections collected from adult, field-grown plants. Seasonal differences in contamination may be attributed to increased tissue resistance to microbial infection during periods of active growth and/or increased resistance of microorganisms to decontaminants during winter (Hohtola, 1988). In addition, year-to-year variation in weather conditions may influence disease pressure and microbial development.

Based on our results, regeneration success increased over successive evaluation dates in open buds, whereas it decreased in nodal explants. Therefore, initiating *in vitro* cultures during intermediate periods, particularly in April and May, appears most promising. Given the advanced age of the donor tree, the findings further suggest that hard pruning of mature mother trees should be applied to induce rejuvenated shoots for explant collection, and that donor plants should be maintained under conditions that minimize exposure to pathogenic organisms to reduce explant contamination.

During the multiplication phase, no significant differences were observed among explant types, indicating that once an *in vitro* culture of *Prunus subhirtella* 'Autumnalis' was successfully established, multiplication efficiency was independent of explant type. The same pattern was observed for rooting. However, substantial losses due to contamination and explant necrosis occurred up to six weeks after transfer to the multiplication medium. Such delayed contamination is often of endogenous origin, as the microorganisms are not in direct contact with the culture medium. Endogenous bacterial infections are considered largely unavoidable during the *in vitro* culture of conifers and may persist through multiple subcultures (Sarmast, 2018). In addition, controlling fungal and bacterial contamination in woody plant material remains particularly challenging (Niedz & Bausher, 2002).

Regarding the timing of explant collection, survival on the multiplication medium did not differ among open buds, whereas nodal explants showed marked variation: none of the propagules from the second collection date survived, while the highest survival was observed from the third collection date. After six weeks, all nine propagules derived from open buds and eight of ten nodal explants produced shoots measuring 1.5–2.0 cm in length. These shoots were subsequently transferred to rooting medium, where no differences in rooting success were observed among explant types. These results suggest that explant type primarily influences the induction phase—the successful establishment of *in vitro* culture—rather than subsequent multiplication or rooting.

To date, there are no reports on the micropropagation of *Prunus subhirtella* 'Autumnalis' from mature trees. In the present study, *in vitro* cultures were initiated from explants collected from adult, field-grown plants. Establishing a reliable culture system from mature plants is essential, as it provides a source of explants capable of efficient regeneration and enables the propagation of elite trees selected based on their adult-stage phenotypes.

Conclusion

The success of *in vitro* propagation of *Prunus subhirtella* 'Autumnalis' from mature trees is strongly influenced by explant type and collection timing. Dormant buds were unsuitable due to complete contamination and necrosis, whereas nodal explants and breaking buds were more successful for culture initiation. Nodal explants showed the highest overall survival, although survival decreased with later collection dates, while breaking buds performed better when collected later in the season. Once established, multiplication and rooting were largely independent of explant type, but contamination and delayed necrosis remained challenges throughout the process. These findings emphasize the importance of selecting appropriate explants and optimal collection periods, providing a foundation for the clonal propagation and conservation of elite mature genotypes of *Prunus subhirtella* 'Autumnalis'.

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics, Consent to Participate, and Consent to Publish:

Not applicable.

Clinical Trial Registration:

This study is not a clinical trial; therefore, trial registration is not applicable.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

Conceptualization, P.K. and G.O.; Data curation, P.K. and G.O.; Formal analysis, P.K. Funding acquisition, G.O. Investigation, P.K.; Methodology, P.K.; Project administration, G.O.; Resources, G.O. Visualization, P.K.; Writing—original draft, P.K.; Writing—review & editing, G.O. All authors have read and agreed to the published version of the manuscript.

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Data Availability

Access to the data is required during the review process; the datasets can be requested directly from the corresponding author

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Figures

Figure 1

Prunus subhirtella ‘Autumnalis’ flowers

Figure 2

One-year-old shoots placed in water, and kept at room temperature to promote bud opening

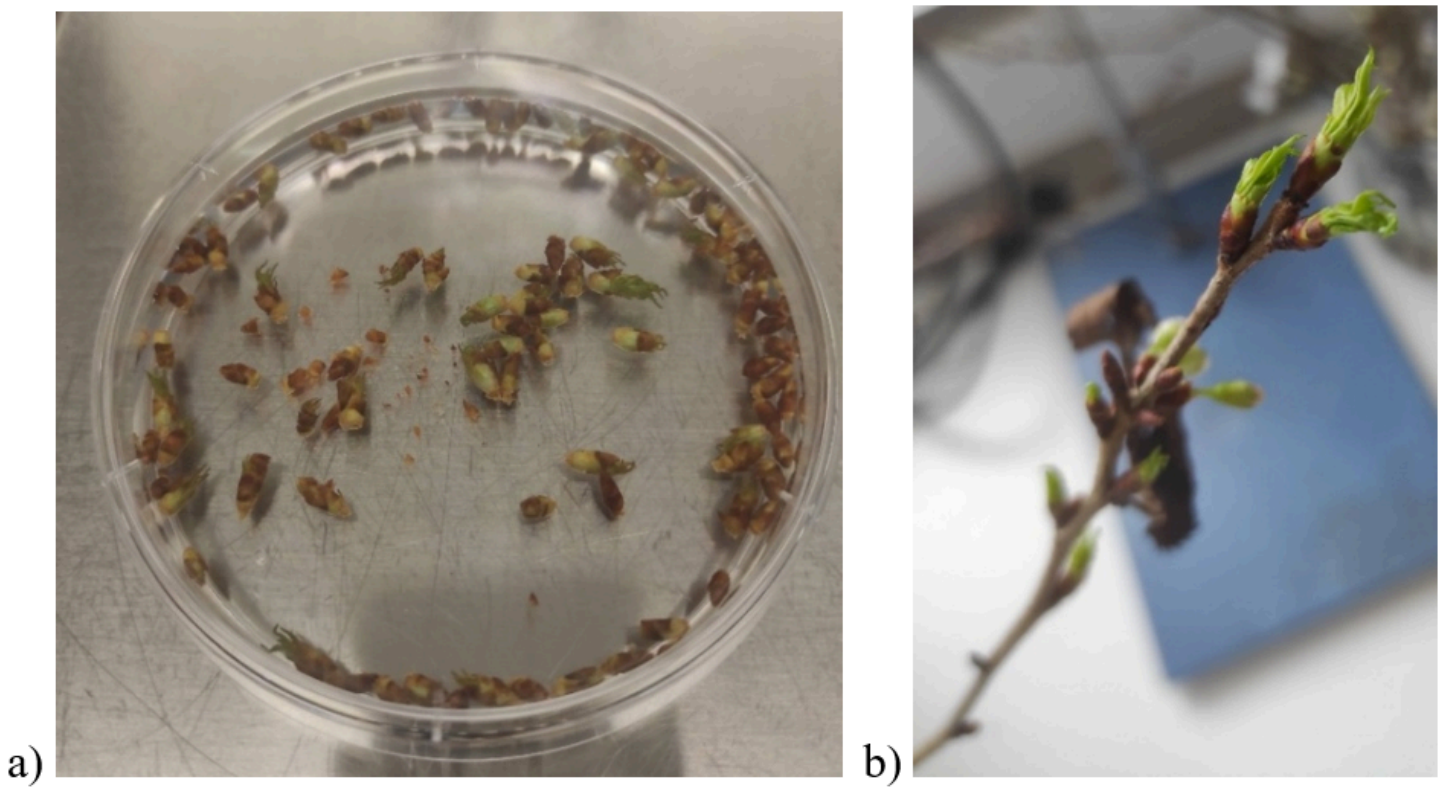

Figure 3

a) Dormant buds with the outer bud scales were removed b) Breaking buds.

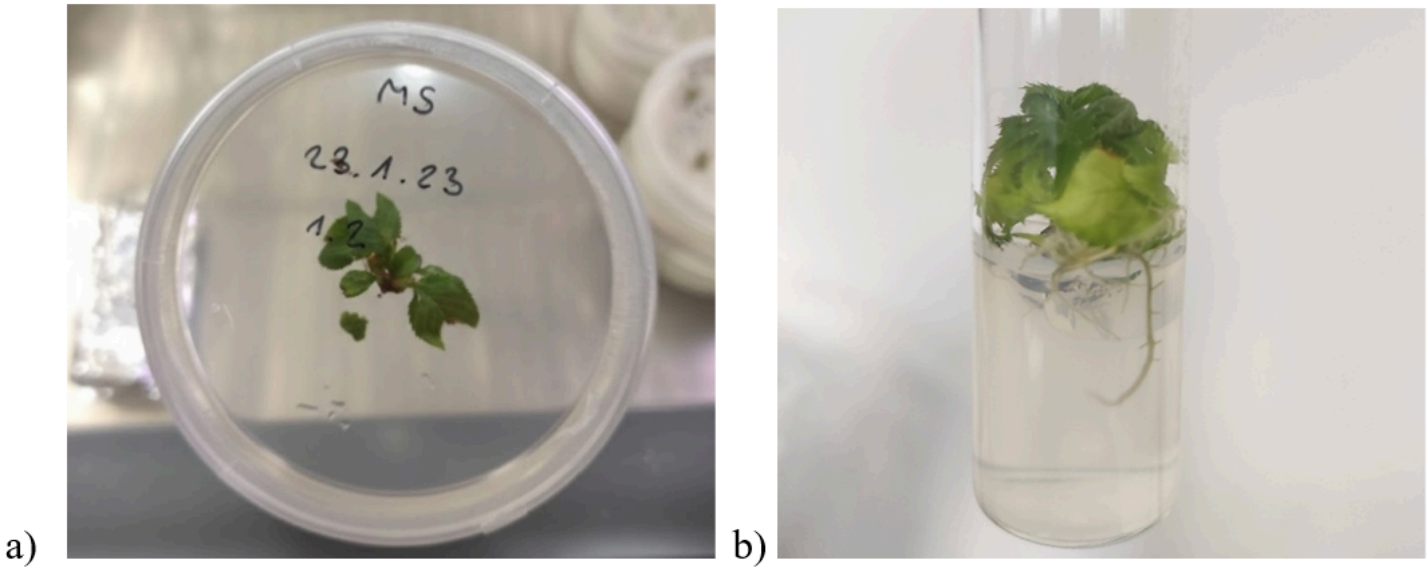

Figure 4

a) Successful regeneration and b) rooting of *Prunus subhirtella* 'Autumnalis' explants

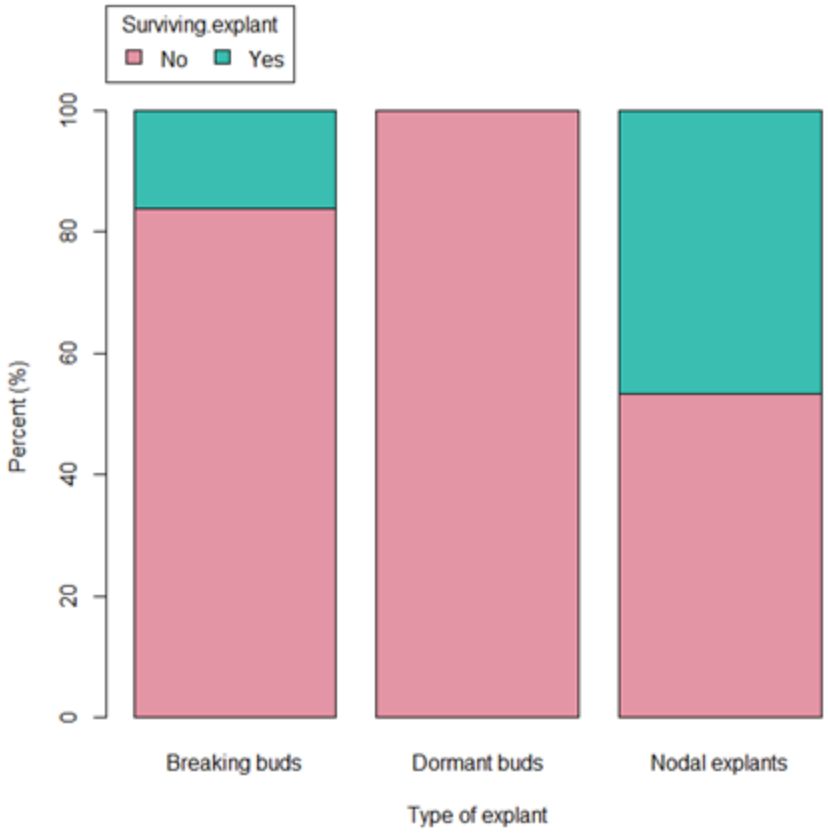

Figure 5

Explant survival (%) after 21 days by explant type (Chi-squared test between nodal and breaking buds only; dormant buds excluded. $p < 0.001$).

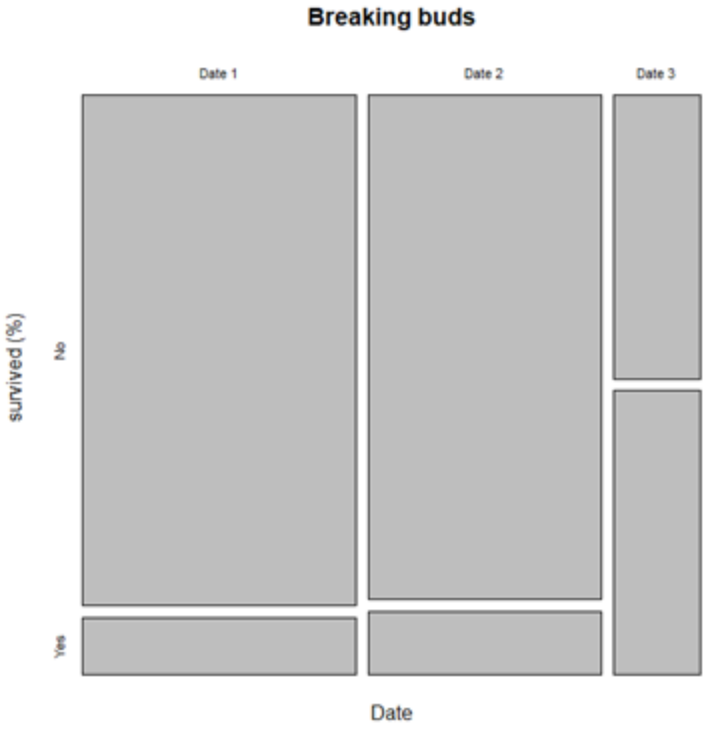

Figure 6

Survival of breaking buds (%) by inoculation date ($X^2 = 58.78$, df = 2, $p < 0.001$) (January 23, 2023 (Date 1), March 1, 2023 (Date 2), March 16, 2023 (Date 3).

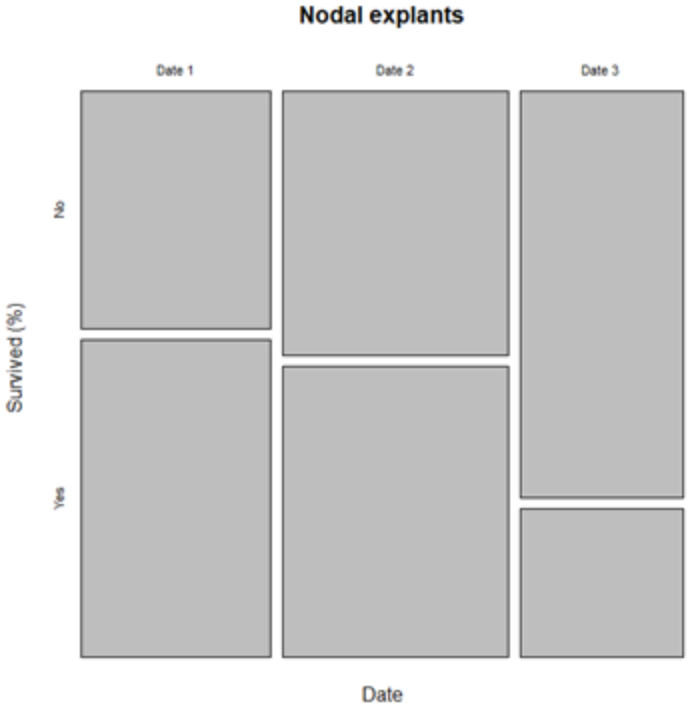

Figure 7

Survival of nodal explants (%) by inoculation date ($X^2 = 6.91$, df = 2, $p = 0.032$) (May 10, 2023 (Date 1), June 12, 2023 (Date 2), June 23, 2023 (Date 3).