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IN VITRO PROPAGATION OF *EUONYMUS FORTUNEI* CULTIVARS FOR SUSTAINABLE URBAN GREENING AND DEGRADED LAND REHABILITATION

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Abstract

Euonymus fortunei (Turcz.) Hand.-Mazz. *(Fortune's spindle) is an evergreen ornamental species widely used in landscape design due to its adaptability to urban stress conditions such as pollution, drought, high insolation, low temperatures, and degraded soils. This study aimed to improve in vitro establishment and shoot multiplication of the cultivars 'Emerald 'n' Gold' and 'Emerald Gaiety'. Nodal segments and shoot tips from mature plants were surface sterilized and established on Murashige and Skoog (MS) medium supplemented with 1 mg/L 6-benzylaminopurine (BAP), 0.5 mg/L indole-3-acetic acid (IAA), 0.5 mg/L gibberellic acid (GA_3), with or without 0.2% (v/v) Plant Preservative Mixture (PPM™). PPM increased contamination-free explants from 55.56% to 88.89% in 'Emerald 'n' Gold' and from 48.39% to 95.71% in 'Emerald Gaiety'. During multiplication, MS medium containing 2 mg/L meta-topolin (mT) and 1 mg/L IAA produced the highest shoot numbers, with an average of 4.71 and 4.40 shoots per explant, respectively, compared with 1.30 and 1.45 on medium with 2 mg/L BAP alone. Meta-Topolin media also reduced basal callus formation, whereas shoot tip necrosis and callus browning were more frequent of media with BAP. These findings identify mT, particularly combined with IAA, as a promising alternative to BAP for in vitro multiplication of these E. fortunei cultivars and provide a basis for further protocol optimization and large-scale propagation and results demonstrate that meta-topolin-based media provide an efficient system for rapid in vitro multiplication of E. fortunei cultivars for use in urban landscaping and ecological restoration of degraded environments.*

Key words*: landscape, meta-topolin, micropropagation, plant growth regulators, shoot proliferation.*

INTRODUCTION

The genus *Euonymus* L (commonly known as spindle or spindle tree) comprises ornamental shrub species with both deciduous and evergreen foliage (creeping, climbing or upright forms) belonging to the Celastraceae family. These plants are highly valued for their adaptability, attractive foliage (sometimes variegated), and their spectacular autumn coloration. The genus is widely distributed and includes around 200 species, native to temperate and subtropical regions of Asia, Europe (including the native flora of Romania and the Republic of Moldova) and North America (Savinov, 2022).

Euonymus fortunei (Turcz.) Hand.-Mazz (Fortune's spindle) is an evergreen ornamental species widely used in landscape design due to its adaptability to urban stress conditions such as pollution, drought, high insolation, low temperatures, and degraded soils (Percival & Sheriffs, 2002). Its use in urban greening and land rehabilitation makes it relevant for sustainable environmental engineering applications. However, due to its adaptability to various conditions, *Euonymus fortunei* can naturalize in seminatural and synanthropic habitats, outside the area of the original cultivation (Bauer & Reynolds, 2016; Savinov, 2022). Native to East and Southeast Asia, it was reported to be naturalized in the Caucasus region and fragmentary on the Black Sea coast and other places (Savinov, 2022). Occasionally, problems with pests and diseases such as euonymus scale (*Unaspis euonymi*) (Cockfield & Potter, 1986; Gutue et al., 2015) or anthracnose (*Colletotrichum gloeosporioides*)

(Ningen et al., 2005) may occur. However, anthracnose mostly occurs in nurseries, and the production of new plantlets can be significantly decreased due to this disease (Ningen et al., 2004; Boyer et al., 2007; Cole et al., 2009). Heavier infestations with *Unaspis euonymi* can lead to massive defoliation and eventual loss of plants (Jefferson & Schultz, 1995). Plant tissue culture and micropropagation provide effective techniques for the mass multiplication of genetically consistent, pathogen-free plant material, regardless of seasonal variations or environmental limitations. *In vitro* propagation has been done on several species of the genus, as *Euonymus alatus* (Smith & Jernstedt, 1989; Chen et al., 2006; Thammina et al., 2011; Woo et al., 2021), *Euonymus europaeus* (Biahoua & Bonneau, 1999), *Euonymus bungeanus* (Ning et al., 2021; Cui et al., 2025) *Euonymus verrucosus* (Kirillov et al., 2022), *Euonymus japonicus* (Shang et al., 2006) *Euonymus koopmannii* (Gubaidullin et al., 2024; Kali et al., 2024)**,** *Euonymus fortunei* (Wang et al., 2004; Shang et al., 2009) or cell cultures in *Euonymus maximowiczianus* for anthocyanin and VLCFA (very-long-chain fatty acids) accumulation (Nosov et al., 2024)**.**

Despite these advances, microbial contamination remains a major limitation in the micropropagation of woody plants. In particular, endophytic contamination can be difficult to control because microorganisms residing within plant tissues may not be eliminated by conventional surface sterilization procedures. To date, no standardized protocol specifically addressing endophytic contamination during the in vitro establishment of the *E. fortunei* cultivars 'Emerald 'n' Gold' and 'Emerald Gaiety' has been reported. Contamination by endophytic bacteria is a common problem in the micropropagation of woody species (Permadi et al., 2025). Endophytic microorganisms inhabit internal plant tissues without necessarily causing visible symptoms of disease or harm to their hosts. They may colonize intercellular spaces, cell walls, and xylem vessels. Endophytes have also been detected in reproductive and storage organs, including flowers, fruits, pollen grains, and seeds, demonstrating their widespread distribution within plants (Chebotar et al., 2016; Boiu-Sicuia & Cornea, 2019).

Surface sterilization of explants does not guarantee complete elimination of microorganisms because endophytic bacteria and fungi may reside within internal tissues and therefore remain protected from surface disinfectants (Marino et al., 1996; Quambusch & Winkelmann, 2018). Newly inoculated explants may initially appear free of contamination because some endophytic microorganisms can remain dormant. Changes in culture conditions may subsequently stimulate their growth, resulting in visible contamination at the explant base and in the culture medium (Leifert & Cassells, 2001; Izarra et al., 2020). Endophytic bacteria belonging to genera such as *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Erwinia*, and *Burkholderia* have been reported in various woody plant tissues (Chebotar et al., 2016) The cultivars 'Emerald 'n' Gold' and 'Emerald Gaiety' were selected because they are widely recognized ornamental forms of *E. fortunei*, characterized by distinctive variegated foliage and desirable growth characteristics that contribute to their value in landscape applications. Their attractive foliage and ornamental performance make the development of reliable propagation protocols particularly relevant for the production of uniform planting material. However, the establishment of mature *E. fortunei* tissues *in vitro* may be constrained by microbial contamination, highlighting the need for improved disinfection strategies.

Therefore, this study aimed to develop an efficient *in vitro* propagation protocol for the cultivars 'Emerald 'n' Gold' and 'Emerald Gaiety', with particular emphasis on improving explant establishment and shoot multiplication to support the production of uniform planting material.

MATERIALS AND METHODS

Inoculation

For inoculation, nodal segments and shoot tips of two cultivars of *Euonymus fortunei* – 'Emerald 'n' Gold' and 'Emerald Gaiety' - were used. They were collected from green shoots that were harvested in August 2025 from mature plants grown in Chișinău, Republic of Moldova and brought to Bucharest, Romania (Figure 1). All experiments in this study were

carried out in the Plant Micropropagation Laboratory, part of the Research Center for Studies of Food Quality and Agricultural Products, of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania.



Figure 1. *Euonymus fortunei* 'Emerald 'n' Gold' (left) and 'Emerald Gaiety' (right) shoots

For culture initiation, two variants of MS (Murashige and Skoog, 1962) media supplemented with growth regulators were used (as detailed in Table 1).

Table 1. Composition of media variants used for *in vitro* inoculation of *Euonymus fortunei* cultivars

No.	Media variant	Media composition
1.	I1	MS + 1 mg/L BAP + 0.5 mg/L IAA + 0.5 mg/L GA_3
2.	I2	MS + 1 mg/L BAP + 0.5 mg/L IAA + 0.5 mg/L GA_3 + 0.2% (v/v) PPM

The difference consisted in the addition of 0.2% (v/v) of commercial stock solution of Plant Preservative Mixture™ (PPM) in variant I2, corresponding to 2 mL PPM per litre of culture medium. Commonly used as an ingredient in culture media, especially for woody species, Plant Preservative Mixture™ (PPM) is a biocide with broad-spectrum that contains 0.1305% 5-Chloro-2-methyl-3(2H)-isothiazolone and 0.0459% 2-methyl-3(2H)-isothiazolone as active ingredients (Plant Cell Technologies, 2026). To minimize tissue browning, the culture media was enriched with 200 mg/L polyvinylpyrrolidone (PVP) and 1 g/L activated charcoal. To stimulate shoot regeneration, 1 mg/L 6-benzylaminopurine (BAP), 0.5 mg/L indole acetic acid (IAA) and 0.5 mg/L gibberellic acid (GA_3) were added to the media, prior to sterilization in the autoclave. Surface sterilization was performed sequentially using 1% citric acid, 0.5% sodium dichloroisocyanurate (NaDCC) for 5 min, 70% ethanol for 60 s, and 0.3% mercury(II) chloride ($HgCl_2$) for 5-7 min. Due to the high toxicity of mercury (II) chloride ($HgCl_2$), all handling was performed using appropriate laboratory safety precautions, and mercury-containing waste was collected separately and disposed of in accordance with institutional hazardous-waste procedures. After the explants were rinsed with sterile distilled water, they were inoculated on the two variants of media used for culture initiation: I1 and I2 (Table 1). In order to reduce phenolic oxidation, the explants were incubated for 10 days at 6°C, in complete darkness. Following this, they were transferred to the growing room, where they were grown at 22-25°C, under white, red and blue LED lights and a 16 hours photoperiod (Figure 2). All contaminated explants (including those contaminated with endophytic bacteria were removed from the experiment).

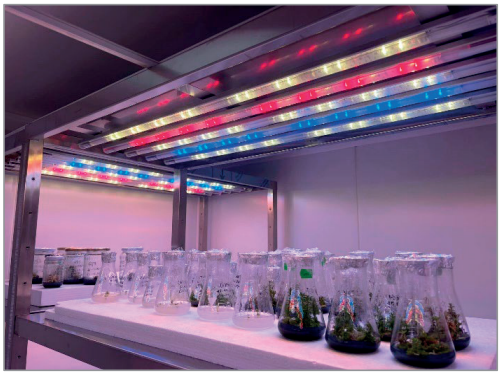


Figure 2. Culture conditions used for *in vitro* cultivation of *Euonymus fortunei* cultivars

Multiplication on media with BAP or mT

Following culture establishment and stabilization, explants were transferred to MS media containing either 6-benzylaminopurine (BAP) or meta-Topolin (mT) and indole acetic acid (IAA), as detailed in Table 2. For each medium variant, 10 explants were used per cultivar (n = 10 explants per treatment). The multiplication experiment was conducted following two subculture cycles. Data were recorded after a total culture period of 70 days. Media was prepared using stock solutions prepared individually for each element. Sucrose was supplemented in concentration of 30 g/L and the media was solidified with 7 g/L agar. To prevent oxidation, 100 mg/L

polyvinylpyrrolidone (PVP) and 1 g/L activated charcoal were added.
The pH value was adjusted at 5.70 and the media was sterilized in the autoclave for 20 minutes, at 121°C. All plant growth regulators (BAP, mT, and IAA) were filter sterilized with PES syringe filters (0.22 µm) and added after sterilization in the autoclave. Media was poured in sterile 250 ml Erlenmeyer flasks and covered with aluminum foil. Culture conditions were the same as before (Figure 1).

Table 2. Composition of media variants used for *in vitro* inoculation of *Euonymus fortunei* cultivars

No.	Media variant	Media composition
1.	M1	MS + **2 mg/L BAP** + 0.2% Plant Preservative Mixture™ (PPM) + 1g/L activated charcoal
2.	M2	MS + **2 mg/L BAP** + **1 mg/L IAA** + 0.2% Plant Preservative Mixture™ (PPM) + 1g/L activated charcoal
3.	M3	MS + **2 mg/L mT** + 0.2% Plant Preservative Mixture™ (PPM) + 1g/L activated charcoal
4.	M4	MS + **2 mg/L mT** + **1 mg/L IAA** + 0.2% Plant Preservative Mixture™ (PPM) + 1g/L activated charcoal

At the end of the 70-day multiplication period, biometric measurements were performed. The following parameters were evaluated: (i) mean number of shoots per explant, determined by counting the shoots regenerated from each explant; (ii) mean shoot length (mm), measured using a digital caliper; and (iii) mean fresh callus mass (g), determined by carefully excising the callus tissue from the explants and weighing it immediately using an analytical balance. The measurements were performed at the end of the multiplication experiment.

Statistical analyses
All statistical analyses were conducted using Microsoft Excel 2021 in combination with the Real Statistics Resource Pack (www.real-statistics.com). For each evaluated parameter, descriptive statistics were generated to summarize the data, with results expressed as the mean ± standard error (SE). The homogeneity of variances was assessed using Levene's test before selecting the appropriate statistical approach. Data satisfying the assumption of equal variances were analyzed by one-way analysis of variance (ANOVA). When significant treatment effects were identified, pairwise comparisons were carried out using Tukey's honestly significant difference (HSD). Statistical significance was established at a threshold of $p < 0.05$.

RESULTS AND DISCUSSIONS

Use of Plant Preservative Mixture™ (PPM) was proven to be effective in reducing endophytic bacteria contamination. After 16 days, surface sterilization of explants resulted in 55.56% and 48.38% uncontaminated explants, whereas the addition of PPM increased the percentage to 88.89% and 95.71% in 'Emerald 'n' Gold' and 'Emerald Gaiety', respectively (Figure 3).

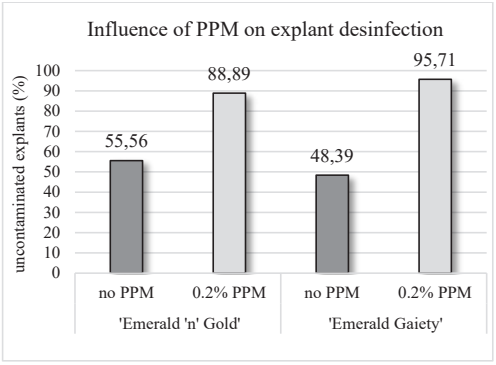


Figure 3. Influence of PPM on the percentage of explant disinfection

The beneficial effect of PPM observed in the present study is consistent with previous reports of its application in the establishment of woody plant material. Niedz and Bausher (2002) reported that PPM reduced contamination in greenhouse- and field-derived Citrus sinensis buds, although the effectiveness depended on the sterilization treatment used. PPM has also been successfully incorporated into culture systems for several woody species, including *Malus* spp. (Romadanova et al., 2022), *Corylus avellana* (Kushnarenko et al., 2025), *Rubus idaeus* (Trzewik et al., 2025), *Vitis vinifera* (Bettoni et al., 2019), and *Cryptocarya massoy* (Fajrisani et al., 2026).

Together with the present results, these studies support the usefulness of PPM as an additional measure for controlling persistent microbial contamination during the establishment of woody explants.

The effectiveness and phytotoxicity of surface disinfectants are also strongly dependent on their concentration and exposure time. In Euonymus koopmannii, for example, prolonged treatment with 5% sodium hypochlorite caused tissue injury and death of axillary buds (Kali et al., 2024). For field-derived nodal segments of Euonymus bungeanus, Cui et al. (2025) reported successful sterilization using 75% ethanol for 30 s followed by 0.1% $HgCl_2$ for 7 min. In the present study, NaDCC was incorporated into the sterilization procedure, followed by $HgCl_2$ treatment and the addition of PPM to the culture medium. The high proportion of uncontaminated explants obtained with PPM suggests that controlling microorganisms that persist after surface sterilization was particularly important for the establishment of the mature E. fortunei material used in this study.

The literature also indicates that no single disinfectant is universally optimal. NaDCC has been reported to provide effective surface sterilization with relatively limited tissue damage compared with some other disinfectants (Parkinson et al., 1996; Caton, 2008; Simran & Narasimhan, 2021). Reddy et al. (2025), for example, found 0.5% NaDCC to be more effective than 1% sodium hypochlorite or 1% calcium hypochlorite for sterilizing rhizome explants of Siphonochilus aethiopicus. Conversely, Mihaljević et al. (2013) reported that 1–2% NaDCC resulted in a higher contamination rate and lower survival of sour cherry axillary buds than some alternative disinfectants. These contrasting findings emphasize that sterilization efficiency depends on explant type, physiological condition, plant species, disinfectant concentration, and exposure duration. In the present study, the addition of PPM after surface sterilization provided an additional means of reducing contamination and consequently increased the proportion of explants suitable for culture establishment.

Information regarding the efficient sterilization of shoot tips and nodal segments of *Euonymus* remains relatively limited, as several previous studies on *in vitro* propagation or conservation have used seeds as starting material (Smith & Jernstedt, 1989; Biahoua & Bonneau, 1999; Chen et al., 2006; Shang et al., 2008; Thammina et al., 2011, 2012; Woo et al., 2021). For young shoot tips of *Euonymus verrucosus*, Kirillov et al. (2022) recommended 70% ethanol for 1 min followed by treatment with 0.025% thimerosal and a 7% aqueous sodium hypochlorite-based commercial bleach solution. The present results therefore provide additional information on the establishment of mature shoot-derived explants of *E. fortunei*, particularly regarding the potential contribution of PPM to contamination control.

Multiplication

Average number of shoots obtained from each explant

Both varieties, 'Emerald 'n' Gold' and 'Emerald Gaiety' reacted similarly when cultivated on the four media variants (Figure 4). Highest number of shoots was obtained on media variant M4 (2 mg/L mT and 1 mg/L IAA): 4.71 ± 2.05 shoots in 'Emerald 'n' Gold' and 4.4 ± 2.65 shoots on 'Emerald Gaiety'. Media variant M3 (2 mg/L mT) produced fewer shoots, however the recorded values were higher than the ones obtained on BAP-supplemented media: 3.3 ± 1.0 shoots ('Emerald 'n' Gold') and 2.6 ± 1.02 shoots ('Emerald Gaiety').

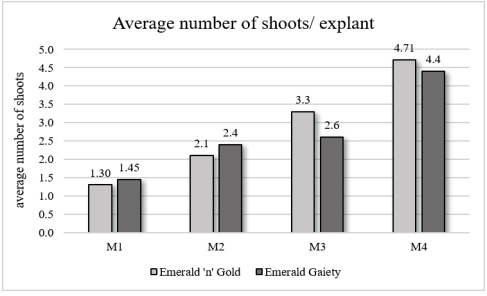


Figure 4. Average number of shoots obtained from each explant

The synergic interaction of meta-Topolin with auxins such as NAA, IBA and 2,4-D is well documented, by increasing the number of shoots, shoot length and stimulating organogenesis in species such as *Eucomis autumnalis* (Masondo et al., 2015), *Coleonema*

album (Fajinmi et al., 2014), *Manihot esculenta* (Chauhan & Taylor, 2018) *Hypericum frondosum* (Meyer et al., 2009) or *Huernia hystrix* (Amoo et al., 2009). mT-containing media also produced shoots without visible signs of oxidative damage (Figure 5). Previous studies have suggested that mT may reduce some of the undesirable effects associated with BAP, including oxidative stress and tissue damage, which can negatively affect plant growth and subsequent acclimatization (Keshari et al., 2020). The absence of visible oxidation in the mT treatments in the present study (Figure 5), together with their greater shoot proliferation, further supports the suitability of mT for multiplication of the investigated *E. fortunei* cultivars.

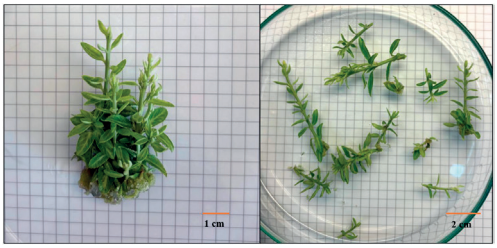


Figure 5. *E. fortunei* 'Emerald Gaiety' shoots obtained on media with 2 mg/L meta-Topolin and 1 mg/L indole acetic acid

The lowest shoot regeneration was observed on media M1 (2 mg/L BAP), with an average of 1.30 ± 0.46 ('Emerald 'n' Gold') and 1.45 ± 0.66 shoots ('Emerald Gaiety') and media M2 (2 mg/L BAP and 1 mg/L IAA), with an average of 2.1 ± 1.0 'Emerald 'n' Gold') and 2.4 ± 0.8 shoots ('Emerald Gaiety'). However, the difference between M1 and M2 was not statistically significant according to Tukey's HSD test ($p > 0.05$). Thus, although the numerical increase following IAA supplementation was apparent, it should not be interpreted as a statistically confirmed improvement. However, for shoot regeneration of *Euonymus* species, most studies focus on using BAP in combination with NAA (Shang et al., 2006). In *E. alatus*, adventitious shoot formation from hypocotyl segments can be achieved on media with 0.3 mg/L BAP and 0.1 mg/L NAA (Smith & Jernstedt, 1989). For direct shoot regeneration, Kali et al. (2024) recommend using media with BAP (1 mg/L), NAA (0.5 mg/L), and KIN (0.2 mg/L), both for nodal segments and internode explants, reaching a regeneration potential up to 90%. In *E. fortunei* var. *radicans,* Shang et al. (2009) obtained an average of 5.1 shoots from hypocotyl explants cultivated on media with 1.0 mg/L BAP and 0.01 mg/L NAA, with a regeneration frequency of 94%. For further proliferation of the regenerated shots, the concentration of NAA can be increased to 0.1 mg/L. *Euonymus bungeanus* plantlets can be successfully regenerated from leaf explants on induction media with 0.5 mg/L BAP and 0.5 mg/L NAA under red radiation and then transferred to media with 1 mg/L BAP and 0.5 mg/L NAA (Ning et al., 2021).

All multiplication media contained the same concentration of PPM (0.2%, v/v); therefore, PPM was not an experimental factor in the comparison among M1–M4. Consequently, differences in shoot proliferation observed among these treatments are interpreted primarily in relation to the cytokinin type and the presence or absence of IAA. Nevertheless, because PPM may have biological effects in addition to its antimicrobial activity, its potential contribution to plant growth cannot be completely excluded. The constant PPM concentration across all multiplication treatments, however, allows the relative effects of BAP and mT to be compared under the same PPM conditions.

Average shoot length

Shoot length also varied among the multiplication treatments (Figure 6). The highest numerical mean shoot length was 34.17 ± 4.94 mm on M2 (2 mg/L BAP and 1 mg/L IAA) for 'Emerald Gaiety' and 34.10 ± 9.99 mm on M3 (2 mg/L mT) for 'Emerald 'n' Gold', followed by 34.05 ± 9.64 mm on M4 (2 mg/L mT and 1 mg/L IAA) for 'Emerald Gaiety'. Lowest results were obtained on media M1 (2 mg/L BAP), 23.93 ± 5.76 mm for 'Emerald Gaiety' and 24.76 ± 5.11 mm (2 mg/L BAP and 1 mg/L IAA) for 'Emerald 'n' Gold'. Tukey's HSD test indicated that M2, M3, and M4 did not differ significantly from one another, whereas significant differences were detected between M1 and M2 ($p < 0.05$), M1 and M3 ($p < 0.05$), and M1 and M4 ($p < 0.05$). Thus, although the numerical ranking of M2, M3, and

M4 differed between cultivars, these differences should not be interpreted as statistically significant.

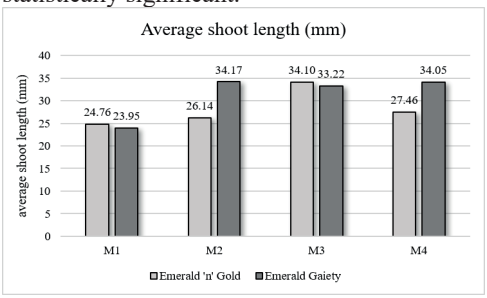


Figure 6. Average shoot length (mm)

Even though results regarding shoot length are similar between the media variants, shoots obtained on media with BAP (M1 and M2) exhibited shoot tip necrosis. Necrosis is a negative effect attributed to BAP during the multiplication phase, as excessive accumulation of cytokinin 9-glucosides has been associated with the occurrence of shoot tip necrosis in plants cultured *in vitro* (Bairu et al., 2011). In *Persea americana*, the use of mT has successfully alleviated dieback of the shoot apex, premature leaf abscission, and tissue necrosis (Hiti Bandaralage et al., 2015). Moreover, culture on media with BAP can also cause rooting inhibition and other abnormal growth patterns that have been attributed to the accumulation of biologically inactive storage forms, which are gradually released over time (Werbrouck et al., 1996). Such inactive reserves originate from the glucosylation of cytokinins such as BAP on the purine ring, either directly as glucosides or through the action of β-glucosidase enzymes (McGaw et al., 1985; Krishna Vrundha et al., 2021)

Average basal callus mass

Regarding the growth of basal callus, results show significant differences between the culture media variants ($p < 0.05$). In Figure 7 it can be observed that the highest mass (g) was obtained on the two media variants containing BAP: 1.90 ± 0.32 g for 'Emerald 'n' Gold' and 1.87 ± 0.32 g in 'Emerald Gaiety' on M2 and 1.81 ± 0.30 g in 'Emerald Gaiety' and 1.80 g in 'Emerald 'n' Gold' on M1; however, results from Tukey's HSD test revealed no significant differences between these two values. Lowest basal callus formation was observed on the explants cultivated on the two media variants containing mT instead of BAP: 0.77 ± 0.17 g in 'Emerald 'n' Gold' and 1.11 ± 0.47 g in 'Emerald Gaiety' on variant M3 and 1.54 ± 0.45 g in 'Emerald Gaiety' and 1.65 ± 0.44 g in 'Emerald 'n' Gold' on variant M4. Results from Tukey's HSD Test have shown that M3 and M4 to be statistically different ($p < 0.05$); however, no statistically significant differences were recorded between M4 and media variants M1 and M2 ($p > 0.05$ in both cases).

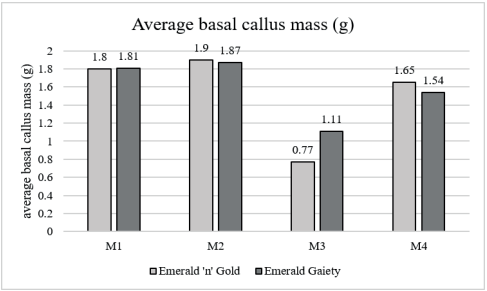


Figure 7. Average basal callus mass (g) per explant

Results obtained by Clapa et al. (2014) have also shown that media with mT reduces the formation of basal callus, compared to BAP during *in vitro* multiplication of *Paulownia* sp.. Moreover, besides a higher growth, the basal callus obtained on the media variants containing BAP progressively turned brown, accompanied by the tip necrosis of the shoots (Figure 8).

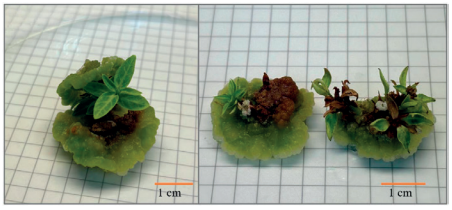


Figure 8. Effects of high concentrations of BAP – callus and shoot tip necrosis – on *E. fortunei* 'Emerald 'n' Gold' explants

Moyo et al. (2011) observed that explants cultivated on media with high concentrations of BAP resulted in the formation of non-organogenic basal callus, which was not able to differentiate into any organs. Moreover, the callus progressively turns brown during the

culture, which may be associated with the accumulation of biologically inactive cytokinin N7- and N9-glucoside conjugates, thereby reducing the availability of active cytokinins for further shoot proliferation, which may also explain the reduced number of shoots obtained from the explants cultivated on media supplemented with BAP (van Staden & Crouch, 1996; Auer, 1997; Moyo et al., 2011).

CONCLUSIONS

This study presents an efficient *in vitro* establishment and shoot multiplication protocol for the ornamental cultivars *Euonymus fortunei* 'Emerald 'n' Gold' and 'Emerald Gaiety'. The addition of 0.2% (v/v) Plant Preservative Mixture™ (PPM) during culture establishment substantially reduced visible contamination, increasing the proportion of contamination-free explants from 55.56% to 88.89% in 'Emerald 'n' Gold' and from 48.39% to 95.71% in 'Emerald Gaiety'. During the multiplication phase, meta-topolin (mT) was more effective than 6-benzylaminopurine (BAP) under the conditions tested. The highest shoot numbers were obtained on medium containing 2 mg/L mT and 1 mg/L IAA (M4), with 4.71 shoots per explant for 'Emerald 'n' Gold' and 4.40 shoots per explant for 'Emerald Gaiety', compared with 1.30 and 1.45 shoots per explant, respectively, on the BAP-only medium (M1). In addition, mT-containing media were associated with lower basal callus formation, while shoot tip necrosis and progressive basal callus browning were predominantly observed in BAP-containing treatments. These findings indicate that mT, particularly in combination with IAA, represents a promising alternative to BAP for the multiplication of *E. fortunei* cultivars.

The present study contributes to the limited information available on *in vitr*o propagation of these *E. fortunei* cultivars, particularly by providing comparative evidence on the responses of two ornamental cultivars to mT- and BAP-based multiplication media. However, the study was limited to two cultivars and explants collected during a single sampling period, and PPM was evaluated at only one concentration. Furthermore, rooting, *ex vitro* acclimatization, genetic fidelity, and long-term field performance were not assessed. Therefore, the protocol should be considered a promising strategy for the establishment and multiplication phases rather than a complete production system. Further studies addressing rooting and acclimatization, as well as performance under environmental conditions relevant to urban and degraded sites, are required to validate its practical application. Nevertheless, the efficient production of healthy shoots may provide a useful basis for the subsequent development of planting material for sustainable urban greening, green infrastructure, and the rehabilitation of degraded urban sites where the use of these ornamental *E. fortunei* is considered environmentally appropriate.

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