FACILITATION OF THE RHIZOGENESIS AND CAULOGENESIS OF *EPIPREMNUM AUREUM* BY *IN VITRO* MICROPROPAGATION AND DIFFERENT MURASHIGE-SKOOG CULTURE MEDIA FORMULATION

FACILITAREA RIZOGENEZEI ŞI A CAULOGENEZEI LA SPECIA *EPIPREMNUM AUREUM* PRIN MICROPROPAGARE *IN VITRO* ŞI FOLOSIREA DIFERITELOR FORMULĂRI ALE MEDIULUI DE CULTURĂ MURASHIGE-SKOOG

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Abstract. *Epipremnum aureum is a houseplants cultivated in Romania for its decorative appearance given by the presence of large heart-shaped leaves. In vitro micropropagation is one of the most used laboratory techniques for the multiplication of plants under controlled conditions (humidity, temperature, photoperiod). As E. aureum is of commercial interest, in vitro micropropagation by direct organogenesis from petiole and leaf explants grown on Murashige-Skoog (MS) basal culture medium supplemented with both a carbon source (sucrose) and a gelling agent (agar-agar) is proposed. Depending on the aim, the medium was supplemented with different growth hormones. Thus, α-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA) were added for rhizogenesis, and in the case of shoots regeneration the MS medium was supplemented with kinetin (Kin) and 6-benzyladenine (BA). Both root and adventitious shoot regeneration efficiency was higher from petiole explants compared to the use of leaf fragments.*

Key words: rhizogenesis, *Epipremnum aureum*, *in vitro* micropropagation

Rezumat. *Epipremnum aureum este o plantă de apartament cultivată în România pentru aspectul său decorativ dat de prezența unor frunze mari sub formă de inimă. Micropropagarea in vitro reprezintă una dintre cele mai utilizate tehnici de laborator pentru înmulțirea plantelor în condiții controlate (umiditate, temperatură, fotoperioadă). Datorită faptului că E. aureum prezintă interes comercial, se propune micropropagarea in vitro prin organogeneză directă din explante de pețiol și frunze cultivate pe mediu de cultură bazal Murashige-Skoog (MS) la care s-a adăugat atât o sursă de carbon (zaharoză), cât și un agent de gelifiere (agar-agar). În funcție de scopul urmărit, mediul a fost suplimentat cu diferiți*

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hormoni de creștere. Astfel, pentru rizogeneză a fost adăugat acid α-naftaleneacetic (ANA) și 6-benziladenină (BA), iar pentru obținerea de lăstari mediul MS a fost suplimentat cu kinetină (Kin) și 6-benziladenină (BA). Eficiența regenerării rădăcinilor și a lăstarilor adventivi a fost mai mare de la explantele de pețiol, comparativ cu utilizarea fragmentelor de frunze.

Cuvinte cheie: rizogeneză, *Epipremnum aureum*, micropropagare *in vitro*

INTRODUCTION

Epipremnum aureum, known also as „Money plant", belongs to the kingdom *Plantae*, subkingdom *Viridiplantae*, infrakingdom *Streptophyta*, superphylum *Embryophyta*, phylum *Tracheophyta*, subphylum Spermatophytina, class *Liliopsida*, superoder *Lilianae*, order *Alismatales*, family *Araceae*, genus *Epipremnum* Schott, species: *Epipremnum aureum* (Linden & André) G.S.Bunting (Panchal *et al*., 2022, https://www.gbif.org/species/2868323/metrics, https://www.gbif.org/species/102191837).

Many green plants have the ability to purify the air by removing some volatile compounds such as benzene, formaldehyde, and xylene either through stomatal uptake (Newman and Reynolds, 2004) or by adsorbing on their leaf surface (Orwell *et al*., 2004), in this category also being included the *E. aurem* (sau asa: *E. aurem* being included in this category), which led to its current use as an indoor plant. Additionally, the Money plant's antibacterial, antioxidant, and anticancer properties make it a successful tool in homeopathic treatment (Panchal *et al*., 2022). Moreover, *E. aureum* has gained the Royal Horticultural Society's Award of Garden Merit (https://www.gbif.org/species/113559470, https://www.rhs.org.uk/plants/pdfs/agm-lists/agm-ornamentals.pdf).

Based on International Institute for Sustainable Development data (https://sdg.iisd.org/news/world-population-to-reach-9-9-billion-by-2050), population growth—which is expected to reach 9.9 billion by 2050—is one of the major global issues. This implies that, in order to achieve fast plant development, we must discover substitute alternatives that are practical, affordable, and simple to use. Over time, one of the most popular laboratory methods for the regulated multiplication of plants (temperature, humidity, photoperiod) has been plant-tissue culture, also known as in vitro micropropagation (Abdalla *et al*., 2022).

The main aim of this study was to facilitate the process of rhizogenesis for *E. aureum* by using the Murashige-Skoog culture medium enriched with nutrients (6-benzyladenine, α-naphthaleneacetic acid, kinetin), carbon source (sucrose) and gelling agent (agar-agar).

MATERIAL AND METHOD

Stem fragments with leaves of 5-10 cm were washed under running tap water for 20 min. Stems were divided into nodal segments, each containing a lateral shoot, and young leaves were separately collected. Explants were disinfected using 70%

ethanol for 1 min, followed by sterilization in separate containers (stems separated from leaves) containing 20% Clorox solution (1.2% NaOCl) and a few drops of Tween 20 for 20 min, under shaking condition. Both nodal segments and leaves were rinsed three times with sterile distilled water. The ends of the knotted fragments - slightly blackened after sterilization - were removed and placed on either rhizogenesis medium (1) or caulogenesis medium (2). The petiole was cut into 1 cm segments and the leaves into 1 cm^2 pieces. Petioles and leaf fragments were grown together or separately on the two types of media, as follows:

1. rhizogenesis medium: MS basal medium supplemented with 0.1 mg/L 1-naphthaleneacetic acid (NAA), 1 mg/L 6-benzyladenine (BA), 3% (w/v) sucrose and 0.7% (w/v) agar-agar

2. caulogenesis medium: MS basal medium supplemented with 0.5 mg/L kinetin (Kin), 1 mg/L 6-benzyladenine (BA), 3% (w/v) sucrose and 0.7% (w/v) agar-agar.

The last step was transferring of the newly regenerated plantlets into planting pots containing a mixture of peat (66.37%), vermiculite (13.40%), perlite (13.36%) and compost (6.85%).

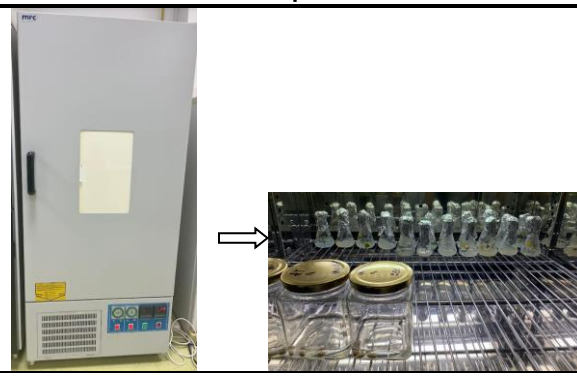
After being removed from sterile containers and cleaned with distilled water, plantlets with roots and leaves were placed into 15 cm plastic pots that contained a particular nutrient substrate. To avoid desiccation losses and photoinhibition phenomena, the *ex vitro* experimental condition were gradually changed ensuring a relative humidity of 70%, pH=7, and 21°C during the entire acclimatization process (Qu *et al.,* 2002; Wang *et al*., 2007).

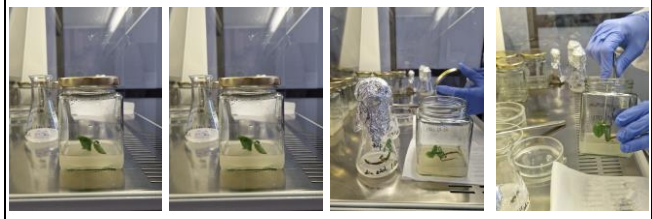
RESULTS AND DISCUSSIONS

The significance of *E. aureum in vitro* micropropagation has been emphasized by numerous investigations over time *(*Qu *et al*., 2002; Villafuerte *et al*., 2022; Wang *et al.,* 2007; Yadav *et al*., 2021; Zhao *et al*., 2012). Consequently, the laboratory work details in this study were carried out using the data from the previously described research, as table 1 illustrates.

Table 1

SPECIFICATIONS FOR *EPIPREMNUM AUREUM'S* LABORATORY WORK *IN VITRO* MICROPROPAGATION AND *EX VITRO* OBSERVATION

Work stage details	Work photos
The plant growth chamber, which provides accurate conditions of light, temperature, and humidity, is where the experiment begins. Placing of the explants (leaf, petiole and single nodal shoot fragments) in MS medium to initiate organogenesis.	

Root regeneration from single nodal shoot fragment placed on MS medium variant (1) – 21 days after inoculation).	
Two lateral shoots formation from single nodal shoot fragment placed on MS medium variant (2) – 21 days after inoculation. The leaf fragment has run out (on the right of the explant); aerial root appeared (on the left of the explant).	
Three leaves formation after 100 days post inoculation.	
For shoot and roots growth, explants were maintained by repeated subculture (every two-three week) on MS basal medium supplemented with proper growth hormones (1 and 2 media variants) for several month (next pictures).	
Three leaves formation after 100 days post-inoculation. The leaf fragment has run out (on the right of the explant); aerial root appeared (on the left of the explant).	
Soil potting mixture for acclimatization: perlite, vermiculite, peat and compost.	

E. aureum acclimatization conditions.	

Regeneration of *E. aureum* plants was successfully achieved due to effective rhizogenesis and caulogenesis media, using a plant growth chamber with appropriate internal temperature and humidity parameters, as well as by ensuring proper *ex vitro* conditions.

CONCLUSIONS

1. The main aim of this study was to facilitate the process of rhizogenesis for *Epipremnum aureum* by using the Murashige-Skoog culture medium enriched with nutrients, carbon source and gelling agent.

2. *In vitro* micropropagation by direct organogenesis from petiole and leaf explants grown on MS medium supplemented with both a carbon source (sucrose) and a gelling agent (agar-agar) were applied.

3. α-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA) were added for rhizogenesis, and in the case of shoots regeneration the MS medium was supplemented with kinetin (Kin) and 6-benzyladenine (BA).

4. Of the three types of explants (leaf, petiole and single nodal shoot fragments), only the uninodal stem fragments regenerated roots and leaves, as well as aerial roots with role of hanging on the support.

5. The soil mixtures used and the high humidity level (minimum 70%) maintained during the acclimatization period contributed to the effectiveness of *ex vitro* adaptation of the *E. aureum* plant.

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