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Research Article
Effect of 6-Benzyladenine Purine, α-Naphthalene Acetic Acid, Coconut Water and Potato Extract on Micropropagation of *Ensete glaucum* from Vietnam

[1]Xuan Duong Vu, [1]Thi Tam Tien Ha, [1]Thanh Loan Pham, [2,3]Luong Thi Hien Dao and [2]Phi Bang Cao

[1]Institute of Applied Research and Development, Hung Vuong University, Nong Trang Wards, Viet Tri City, Phu Tho Province 35000, Vietnam
[2]Faculty of Natural Sciences, Hung Vuong University, Nong Trang Wards, Viet Tri City, Phu Tho Province 35000, Vietnam
[3]Ngo Gia Tu High School, Lap Thach, Vinh Phuc Province 15400, Vietnam

Abstract

Background and Objective: *Ensete glaucum* (Roxb.) Cheesman is widely dispersed and has ornamental value as well as potential medicinal benefits. This research aimed to discover the effect of BAP, NAA and two organic additives, coconut water and potato extract on shooting and rooting during *in vitro* propagation of *Ensete glaucum* collected from Xuan Son Nation Park, Phu Tho Province, Vietnam. **Materials and Methods:** For shoot production, sterilized shoot tip explants of *E. glaucum* (1 cm in height) were placed in cultured media containing BAP at different concentrations (0, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}) combined with 0.2 mg L^{-1} NAA for the first experiment. Coconut water (CW) (0, 50, 100, 150 and 200 mL L^{-1}) and potato extract (PE) (0, 10, 20, 30 and 40 g L^{-1}) combined with BAP at the optimum concentration obtained from the previous experiment was used for the second experiment. For rooting, explants of *E. glaucum* (3 cm in height) were transferred in cultured media containing different concentrations of NAA (0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}) combined with 0.2 mg L^{-1} BAP for the first experiment. In the second experiment, explants of *E. glaucum* were cultured in the medium added CW (0, 50, 100, 150 and 200 mL L^{-1}) and PE (0, 10, 20, 30 and 40 g L^{-1}) combined with NAA at the optimum concentration obtained from the previous experiment to investigate the effect of these organic additives. Obtained data were statistically analyzed by using Duncan's Multiple Range Test ($p = 0.05$). **Results:** The BAP at the concentration of 3 mg L^{-1} combined with 0.2 mg L^{-1} NAA gave the highest mean number of shoots produced per explant and shoot length while, 2 mg L^{-1} BAP combined with 0.2 mg L^{-1} NAA acquired the highest mean number of leaves. The NAA at 0.5 mg L^{-1} (combined with BAP at 0.2 mg L^{-1}) was the most favorable for the rooting of *E. glaucum*. Medium supplemented with CW (100 mL L^{-1}) or PE (30 g L^{-1} for shooting and 20 g L^{-1} for rooting, respectively) resulted in an increase in shoot multiplication and root generation during micropropagation of *E. glaucum*. **Conclusion:** The present study first represents an investigation of the effect of BAP, NAA, coconut water and potato extract on micropropagation of *E. glaucum* collected in Vietnam.

Key words: *Ensete glaucum*, micropropagation, 6-benzyladenine purine (BAP), α-naphthalene acetic acid (NAA), coconut water, potato extract

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Corresponding Author: Phi Bang Cao, Faculty of Natural Sciences, Hung Vuong University, Nong Trang Wards, Viet Tri City, Phu Tho Province 35000, Vietnam

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Ensete* Bruce ex Horaninow (*Ensete* or false banana), along with two genera *Musa* and Musella, belong to Musaceae[1,2]. There are currently 10 species of giant, herbaceous plants of this genus, all of which are indigenous to tropical Africa and Asia[2]. Among them, *Ensete glaucum* (Roxb.) Cheesman is diploid with 2n = 2x = 18 chromosomes and monocarpic with a dilated and distinctively glaucous basal pseudostem. It has banana-like fruits including a small number of large seeds. *Ensete glaucum* is widely dispersed throughout Asia and has been observed in China, India, Indonesia, Vietnam and many more countries[3]. *Ensete glaucum* has ornamental value due to its persistent green bracts and cone-shaped stem[4]. The pseudostem sap of *E. glaucum* has traditionally been used to treat especially diarrhea and dysentery. Recently, many phytochemical compounds have been determined in the pseudostem sap of *E. glaucum*, including flavonoids, reducing sugars, terpenoids, saponins, cardiac glycosides and alkaloids[5,6]. Recently, novel microcrystalline cellulose derived from *E. glaucum* (Roxb.) Cheesman biomass was evaluated as a sustainable biomaterial for drug delivery[7]. Moreover, *E. glaucum* has been considered one of the most cold-resistant and drought-tolerant species in Musaceae. Therefore, this plant has the potential to be a gene and germplasm source for banana breeding abiotic stress resistance, which will probably be necessary for the adaptation to a future climate that is more unpredictable and harsher[8].

Similar to other *Ensete* species, *E. glaucum* plants do not naturally produce side shoots or suckers due to their high apical dominance. Therefore, they normally reproduce by seeds[2]. However, macro-propagation techniques are used to clonally regenerate cultivated *Ensete*. Cutting the top of an *Ensete* plant at the vegetative phase results in hundreds of new shoots emerging from the bulb[9]. The genetic divergence of wild and native *Ensete* is currently at important risk of loss due to habitat loss, intensification of agriculture, change of climate and introduction of high-yield genotypes[3]. Especially, *E. glaucum* has been classified as critically endangered under the criteria of IUCN[4]. Thus, it was especially required a method of conservation and propagation of *E. glaucum*. The *E. glaucum* seeds and zygotic embryos have been well conserved by *in vitro* culture and cryopreservation procedures in India[10]. To date, *in vitro* propagation of only a few economically significant *Ensete* species was communicated in the previous reports[9,11,12]. Therefore, this work was carried out to develop micropropagation techniques for *E. glaucum*.

MATERIALS AND METHODS

Study area: The research was performed from March, 2021 to October, 2021. The experiments were carried out at Hung Vuong University (Phu Tho Province, Vietnam).

Materials: Fifteen *E. glaucum* plants and seeds were collected at Xuan Son National Park (Phu Tho Province, Vietnam). These fifteen plants and the germinated plants were kept at greenhouse-grown plants at Hung Vuong University.

Research procedure: Shoot tip explants of *E. glaucum* were collected and detached from plants 60/80 cm tall and kept in the greenhouse. The cut shoot tips were sterilized as a routine procedure in the laboratory: Sterilized with 10% H_2O_2 for 5 min, further sterilized with 2.5% NaClO for 10 min after being cleaned with sterile water and then washed with sterile water 3 times. The sterilized shoot tip explants were vertically cultured into Murashige and Skoog (MS) medium containing sucrose (30 g L^{-1}), agar (6.5 g L^{-1}) and activated charcoal (0.5 g L^{-1}). After 30 days, the sterilized shoot explants were used for the further shoot multiplication experiment.

Shoot multiplication: Shoot explants of similar height were collected and then vertically inoculated into MS medium adding sucrose (30 g L^{-1}), agar (6.5 g L^{-1}), activated charcoal (0.5 g L^{-1}), α-naphthalene acetic acid (NAA) 0.2 (mg L^{-1}) and various concentrations of 6-benzyladenine purine (BAP) (0, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}). Each treatment represented 60 explants and three repeated experiments were conducted. The proliferation coefficient of shoots, number of leaves per explant and the length of the shoot were calculated after 40 and 60 days of culture.

Shoot explants with similar growth status were inoculated into MS medium supplementing with sucrose (30 g L^{-1}), agar (6.5 g L^{-1}), activated charcoal (0.5 g L^{-1}), BAP at the optimum concentration (mg L^{-1}) obtained from the previous experiment and different concentrations of coconut water (CW) (0, 50, 100, 150 and 200 mL L^{-1}) or potato extract (PE) (0, 10, 20, 30 and 40 g L^{-1}). Each treatment represented 60 explants and three repeated experiments were conducted. The proliferation coefficient of shoots, the number of leaves per explant and the shoot length were calculated after 40 and 60 days of culture.

Production of root: Regenerated shoots longer than 3 cm containing 2-3 leaves were collected and transferred into the 1/2 MS rooting medium supplementing sucrose (30 g L^{-1}),

agar (6.5 g L^{-1}), activated charcoal (0.5 g L^{-1}), BAP 0.2 (mg L^{-1}) and various NAA concentrations (0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}). Each treatment represented 30 explants and three repeated experiments were conducted. The rooting rate, number of roots per explant and the length of root were calculated after 15, 30 and 45 days of culture.

To investigate the effect of CW and PE on the rooting of *E. glaucum*, the regenerated shoots longer than 3 cm containing 2-3 leaves were collected and transferred into the 1/2 MS rooting medium supplementing sucrose (30 g L^{-1}), agar (6.5 g L^{-1}), activated charcoal (0.5 g L^{-1}), NAA 0.5 (mg L^{-1}) and various CW concentrations (0, 50, 100, 150 and 200 mL L^{-1}) or PE (0, 10, 20, 30 and 40 g L^{-1}). Each treatment represented 30 explants and three repeated experiments were conducted. The rooting rate, number of roots per explant and root length were calculated after 15, 30 and 45 days of culture.

Parameters: The highest shoot or the longest root of explant were used to calculate the shoot length or root length. Shoot length after 40 and 60 days of culture and root length after 15, 30 and 45 days of culture were measured by using electronic caliper (Mitutoyo, Japan).

Statistical analysis: Means were compared using Duncan's Multiple Range Test with a significance level of 5% (p = 0.05).

RESULTS AND DISCUSSION

Effect of BAP, NAA, coconut water and potato extract on *in vitro* shoot multiplication of *E. glaucum*: To investigate the effect of BAP combined with 0.2 mg L^{-1} NAA on the shoot multiplication, shoot explants with similar growth status (1 cm of length) were cultured on MS medium adding sucrose (30 g L^{-1}), agar (6.5 g L^{-1}) and activated charcoal (0.5 g L^{-1}), NAA 0.2 (mg L^{-1}) and different BAP concentrations (0, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}). Obtained results were shown in Table 1.

Shoot proliferation on medium containing BAP at all studied concentrations and NAA at 0.2 mg L^{-1} was greater than on free-hormone medium. At 40 DC, the significantly highest mean number of shoots per explant (4.12 shoots/explant) was regenerated from medium added with BAP at 3.0 mg L^{-1} combined with NAA at 0.2 mg L^{-1}, followed by 4.0 mg L^{-1} BAP treated medium (3.79 shoots/explant), 2.0 mg L^{-1} BAP treated medium (3.37 shoots/explant) and 1 mg L^{-1} BAP treated medium (2.66 shoots/explant), while the lowest average shoot number (1.74 shoots/explant) was recorded on medium without plant growth regulators (Table 1). Similarity at 60 DC, medium supplemented with BAP at 3.0 mg L^{-1} combined with NAA at 0.2 mg L^{-1} gave the highest average shoot number of shoots (4.2 shoots/explant) while the lowest mean number of shoots (2.25 shoots/explant) noted on free plant growth regulator medium (Table 1).

At 40 DC, the significantly ($p<0.05$) highest mean number of shoot length was recorded from medium supplemented 3.0 or 2.0 mg L^{-1} BAP in combination with 0.2 mg L^{-1} NAA (3.04 and 2.99 cm, respectively). However, the lowest average shoot length (2.57 cm) was noted on medium added 4.0 mg L^{-1} BAP in combination with 0.2 mg L^{-1} NAA (Table 1). Thus, at 60 DC, medium containing BAP at 3.0 mg L^{-1} in combination with NAA at 0.2 mg L^{-1} gave a higher mean number of shoot length (4.37 cm) than on medium-added with other concentrations of BAP and on free plant growth regulator medium (Table 1). On other hand, the use of the combination between 2.0 mg L^{-1} BAP and 0.2 mg L^{-1} NAA gave the highest value in terms of leaf number per shoot (3.28 leaves/shoot at 40 DC and 3.87 leaves/shoot at 60 DC, respectively) (Table 1). In brief, BAP at 3.0 mg L^{-1} was found to be optimum among four studied concentrations (ranging from 1.0 to 4.0 mg L^{-1}) for shooting *in vitro E. glaucum*.

In order to discover the effect of CW and PE on the shoot multiplication of *E. glaucum*, shoot explants with similar growth status (1 cm of length) were cultured on MS medium adding sucrose (30 g L^{-1}), agar (6.5 g L^{-1}), activated charcoal (0.5 g L^{-1}), 3 mg L^{-1} BAP (the concentration gave the better result in terms of shoot multiplication than other investigated concentrations) and different concentrations of CW (0, 50,

Table 1: Effect of BAP and NAA on *in vitro* multiplication of *E. glaucum*

Treatment		Number of shoots per explant		Shoot length (cm)		Leaf number per shoot	
BAP (mg L^{-1})	NAA (mg L^{-1})	40 DC	60 DC	40 DC	60 DC	40 DC	60 DC
0.0	0.2	1.74±0.71^{e}	2.25±0.72^{e}	2.91±0.45^{b}	3.91±0.39^{b}	2.76±0.82^{c}	2.92±0.85^{c}
1.0	0.2	2.66±0.78^{d}	3.00±0.69^{d}	2.87±0.42^{b}	3.93±0.40^{b}	2.67±0.68^{c}	3.55±0.96^{b}
2.0	0.2	3.37±0.86^{c}	3.74±0.70^{c}	2.99±0.34^{a}	3.99±0.34^{b}	3.28±0.79^{a}	3.87±0.74^{a}
3.0	0.2	4.12±0.78^{a}	4.20±0.66^{a}	3.04±0.34^{a}	4.37±0.41^{a}	2.99±0.80^{b}	3.58±0.75^{b}
4.0	0.2	3.79±0.92^{b}	3.96±0.70^{b}	2.57±0.36^{c}	3.93±0.33^{b}	2.95±0.71^{b}	2.95±0.95^{c}

Within a column, means followed by the different letter (a, b, c, d and e) are significantly different following to Duncan's Multiple Range Test (p = 0.05) and DC: Day of culture

Table 2: Effect of the combination between coconut water and BAP on *in vitro* multiplication of *E. glaucum*

Treatment		Number of shoots per explant		Shoot length (cm)		Leaf number per shoot	
CW (mL L^{-1})	BAP (mg L^{-1})	40 DC	60 DC	40 DC	60 DC	40 DC	60 DC
0.0	3.0	4.08 ± 0.65^{c}	4.11 ± 0.71^{d}	3.08 ± 0.36^{a}	4.41 ± 0.40^{a}	2.96 ± 0.67^{c}	3.56 ± 0.75^{c}
50	3.0	4.41 ± 0.80^{b}	4.65 ± 0.79^{c}	2.87 ± 0.36^{c}	3.99 ± 0.46^{cd}	2.80 ± 0.67^{d}	3.85 ± 0.76^{ab}
100	3.0	4.96 ± 0.89^{a}	5.35 ± 0.70^{a}	2.99 ± 0.38^{b}	4.17 ± 0.45^{b}	3.71 ± 0.67^{a}	3.93 ± 0.63^{a}
150	3.0	4.86 ± 0.97^{a}	5.04 ± 0.66^{b}	3.04 ± 0.35^{ab}	4.07 ± 0.38^{c}	3.24 ± 0.61^{b}	3.72 ± 0.63^{b}
200	3.0	4.04 ± 0.64^{c}	4.64 ± 0.86^{c}	2.57 ± 0.39^{d}	3.91 ± 0.45^{d}	3.06 ± 0.60^{c}	3.22 ± 0.82^{d}

Within a column, means followed by the different letters (a, b, c and d) are significantly different following to Duncan's Multiple Range Test (p = 0.05), CW: Coconut water and DC: Day of culture

Table 3: Effect of the combination between potato extract and BAP on *in vitro* multiplication of *E. glaucum*

Treatment		Number of shoots per explant		Shoot length (cm)		Leaf number per shoot	
PE (g L^{-1})	BAP (mg L^{-1})	40 DC	60 DC	40 DC	60 DC	40 DC	60 DC
0.0	3.0	4.08 ± 0.65^{e}	4.11 ± 0.71^{e}	3.08 ± 0.36^{c}	4.18 ± 0.38^{c}	2.96 ± 0.67^{d}	3.56 ± 0.75^{c}
10	3.0	4.35 ± 0.88^{d}	4.82 ± 0.65^{d}	3.18 ± 0.34^{b}	4.28 ± 0.44^{b}	3.22 ± 0.65^{c}	4.18 ± 0.69^{b}
20	3.0	4.85 ± 0.70^{c}	5.13 ± 0.68^{c}	3.39 ± 0.40^{a}	4.33 ± 0.44^{ab}	3.88 ± 0.60^{a}	4.44 ± 0.76^{a}
30	3.0	5.34 ± 0.91^{a}	5.71 ± 0.85^{a}	3.48 ± 0.46^{a}	4.41 ± 0.40^{a}	3.97 ± 0.67^{a}	4.41 ± 0.86^{a}
40	3.0	5.03 ± 0.66^{b}	5.41 ± 0.95^{b}	3.42 ± 0.52^{a}	4.13 ± 0.45^{a}	3.69 ± 0.83^{b}	4.12 ± 0.65^{b}

Within a column, means followed by the different letters (a, b, c, d and e) are significantly different following to Duncan's Multiple Range Test (p = 0.05), PE: Potato extract and DC: Day of culture

100, 150 and 200 mL L^{-1}) or PE (0, 10, 20, 30 and 40 g L^{-1}). Obtained results were represented in Table 2 and 3.

Additionally, the medium supplemented with CW at different concentration (50, 100, 150 and 200 mL L^{-1}) in combination with 3.0 mg L^{-1} BAP gave the higher mean number of shoot than the medium without CW, except medium adding 200 mL L^{-1} CW at 40 DC. Similarly, the higher values of leaf number of *in vitro E. glaucum* were observed on most of the medium supplementing CW at different concentrations, except 200 mL L^{-1}, compared to medium without CW. In contrast, the shoot length values on medium free CW were higher than on most of medium containing CW at both 40 and 60 DC (Table 2).

Supplementing PE at all concentrations (10, 20, 30 and 40 g L^{-1}, respectively) led the better results in terms of shoot proliferation and elongation as well as leaf number than free PE treatment (Table 3). The highest values of a number of shoots were recorded on medium supplemented with 30 g L^{-1} PE (5.34 shoots/explant at 40 DC and 5.71 shoots/explant at 60 DC, respectively). Shoot length values from medium adding 20, 30 and 40 mg L^{-1} PE was higher than from medium supplementing 10 g L^{-1} PE or non-adding PE. The significantly highest mean number of leaves per explant was regenerated from mediums added with 20 or 30 g L^{-1} PE, followed by 10 or 40 mg L^{-1} PE supplemented medium, while the lowest average leaf number was observed on medium without PE (Table 3). The obtained result suggested that supplementation of CW or PE at a favorable concentration in the medium had a significant role in shoot proliferation and further development.

In earlier investigations, a similar multiplication behavior was described in *Ensete* species. The BAP at a concentration of 10 µM or 20 µM allowed generation of multiple shoots from both corm- and embryo-explants of *Ensete ventricosum* (Welw). Cheesman from southwestern Ethiopia[9]. The CW and PE were used to multiplicate *Ensete* shoot for the first time in this work. The increase of shoot production from medium supplemented with favorable concentrations of CW or PE in this study confirmed the effect of these organic additives in the regeneration of plants, including banana[13] or other plants[14,15].

Effect of BAP, NAA, coconut water and potato extract on *in vitro* root production of *E. glaucum*: To explore the effect of NAA combined with 0.2 mg L^{-1} BAP on the rooting of *E. glaucum*, shoot explants with similar growth status (longer than 3 cm containing 2-3 leaves) were transferred on 1/2 MS medium supplementing sucrose (30 g L^{-1}), agar (6.5 g L^{-1}) and activated charcoal (0.5 g L^{-1}), BAP (0.2 mg L^{-1}) and various NAA concentrations (0, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}). The results were displayed in Fig. 1 and Table 4.

The rooting rate of *in vitro E. glaucum* on medium containing NAA at all studied concentrations and BAP at 0.2 mg L^{-1} was higher than on medium without NAA at 45 DC (Fig. 1). The significantly ($p<0.05$) highest mean rooting rate (86.67%) was recorded from medium added with NAA at 0.5 mg L^{-1} in combination with BAP at 0.2 mg L^{-1}, followed by 1.0 mg L^{-1} NAA treated medium (81.11%), 1.5 mg L^{-1} NAA treated medium (73.33%) and 0.2 mg L^{-1} NAA treated medium (66.67%), while the lowest average rooting rate value (5.56%) was recorded on medium without NAA (Fig. 1).

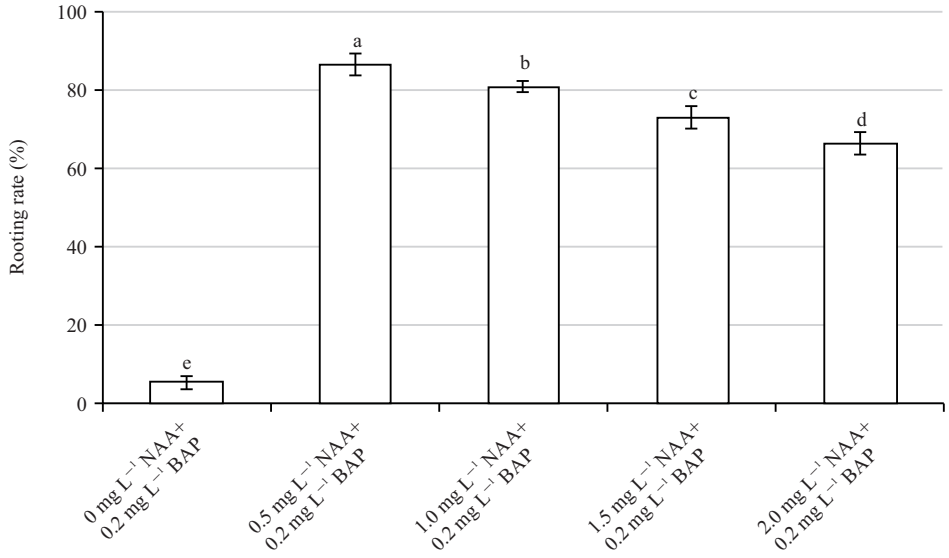

Fig. 1: Effect of NAA combined with BAP on rooting rate of *in vitro E. glaucum* at 45 days of culture

Means followed by the different letter (a, b, c, d and e) are significantly different following to Duncan's Multiple Range Test (p = 0.05) and DC: Day of culture

Table 4: Effect of growth regulators on rooting of *in vitro E. glaucum*

Treatment		Number of roots per explant			Root length (cm)		
NAA (mg L^{-1})	BAP (mg L^{-1})	15 DC	30 DC	45 DC	15 DC	30 DC	45 DC
0.0	0.2	2.42 ± 0.78^{d}	7.59 ± 0.81^{d}	9.39 ± 1.07^{e}	2.48 ± 0.40^{c}	4.20 ± 0.39^{c}	4.70 ± 0.49^{b}
0.5	0.2	4.27 ± 0.65^{a}	9.07 ± 0.79^{a}	12.10 ± 1.19^{a}	3.15 ± 0.41^{a}	4.60 ± 0.48^{a}	5.13 ± 0.52^{a}
1.0	0.2	4.12 ± 0.70^{a}	8.67 ± 0.95^{b}	11.76 ± 1.11^{b}	3.10 ± 0.37^{a}	4.45 ± 0.53^{b}	5.05 ± 0.51^{a}
1.5	0.2	3.84 ± 0.72^{b}	8.38 ± 0.79^{c}	10.99 ± 1.24^{c}	3.03 ± 0.36^{ab}	4.38 ± 0.54^{b}	4.80 ± 0.53^{b}
2.0	0.2	3.54 ± 0.77^{c}	8.31 ± 0.79^{c}	10.37 ± 0.98^{d}	2.95 ± 0.34^{b}	4.19 ± 0.36^{c}	4.51 ± 0.44^{c}

Within a column, means followed by the different letters (a, b, c, d and e) are significantly different following to Duncan's Multiple Range Test (p = 0.05) and DC: Day of culture

All treatments added NAA led to an increase in the number of roots compared to treatment without NAA at all three points, 15, 30 and 45 DC, respectively. The lowest root numbers were recorded from a medium supplemented with only 0.2 mg L^{-1} BAP (without NAA). These values reached 2.42, 7.59 and 9.39 roots/explants at 15, 30 and 45 DC, respectively. At 15 DC, the use of 0.5 or 1.0 mg L^{-1} NAA combined with 0.2 mg L^{-1} BAP generated the highest number of roots (4.27 and 4.12 roots/explant, respectively). However, supplementation of 0.5 mg L^{-1} NAA combined with 0.2 mg L^{-1} BAP gave the best results in terms of root number at 30 (9.07 roots/explant) and 45 DC (12.10 roots/explant) (Table 4).

In terms of root length of *in vitro E. glaucum*, higher values were observed from medium containing NAA at all studied concentrations compared to free NAA medium at 15 DC but a decrease of root length was recorded when NAA concentration reached 2.0 mg L^{-1}. At 30 and 45 DC, treatments supplementing NAA ranged from 0.5 to 1.5 mg L^{-1} NAA generated higher mean root length compared to treatment without NAA at 30 and 45 DC (Table 4). Among four studied concentrations, NAA at 0.5 mg L^{-1} was optimum for rooting *in vitro E. glaucum*.

To investigate the effect of a combination between CW and 0.5 mL L^{-1} NAA on the rooting of *E. glaucum*, shoot explants were cultured on 1/2 MS medium supplementing sucrose (30 g L^{-1}), agar (6.5 g L^{-1}) and activated charcoal (0.5 g L^{-1}), NAA (0.5 mg L^{-1}) and CW at different concentrations (0, 50, 100, 150 and 200 mL L^{-1}) or PE (0, 10, 20, 30 and 40 g L^{-1}). The results were represented in Fig. 2 and 3, Table 5 and 6, respectively.

Medium added with CW at different concentrations (50, 100 and 150 mL L^{-1}) combined with 0.5 mg L^{-1} NAA produced a higher mean rooting rate than the medium without CW, while the rooting rate of *E. glaucum* from medium supplemented with 200 mL L^{-1} CW was not significantly different from free CW medium (Fig. 2). The highest rooting rate of *E. glaucum* was recorded in

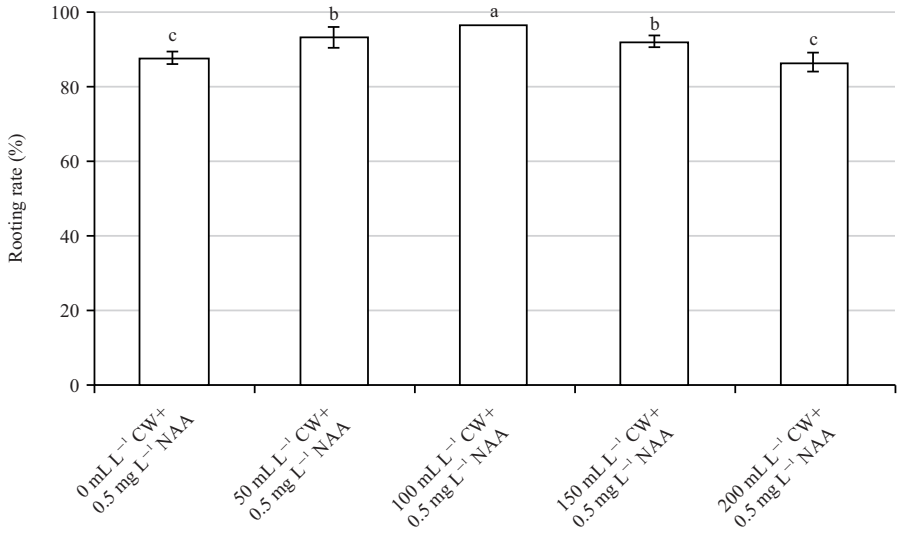

Fig. 2: Effect of combination between coconut water and 0.5 mL L^{-1} NAA on rooting rate of *in vitro E. glaucum* at 45 days of culture

Means followed by the different letters (a, b and c) are significantly different following Duncan's Multiple Range Test (p = 0.05) and DC: Day of culture

Table 5: Effect of the combination between coconut water and NAA on rooting of *in vitro E. glaucum*

Treatment		Number of roots per explant			Root length (cm)		
CW (mg L^{-1})	NAA (mg L^{-1})	15 DC	30 DC	45 DC	15 DC	30 DC	45 DC
0.0	0.5	4.18±0.68^{d}	9.12±0.88^{d}	11.16±1.03^{c}	3.19±0.37^{b}	4.49±0.53^{d}	5.10±0.50^{b}
50	0.5	4.49±0.77^{c}	9.73±0.99^{c}	11.02±1.04^{c}	3.31±0.45^{ab}	4.83±0.54^{c}	5.25±0.52^{b}
100	0.5	5.09±0.71^{a}	10.20±0.74^{a}	12.49±0.99^{a}	3.44±0.52^{a}	5.34±0.55^{a}	5.47±0.51^{a}
150	0.5	4.89±0.80^{ab}	10.03±0.93^{ab}	11.62±1.36^{b}	3.29±0.46^{b}	5.03±0.48^{b}	5.19±0.51^{b}
200	0.5	4.70±0.79^{bc}	9.80±0.66^{bc}	10.59±0.91^{d}	3.19±0.41^{b}	4.80±0.58^{c}	4.82±0.51^{c}

Within a column, means followed by the different letters (a, b, c, d and e) are significantly different following Duncan's Multiple Range Test (p = 0.05), CW: Coconut water, DC: Day of culture

Table 6: Effect of the combination between potato extract and NAA on rooting of *in vitro E. glaucum*

Treatment		Number of roots per explant			Root length (cm)		
PE (g L^{-1})	NAA (mg L^{-1})	15 DC	30 DC	45 DC	15 DC	30 DC	45 DC
0.0	0.5	4.18±0.68^{d}	9.01±0.79^{b}	11.16±1.03^{c}	3.19±0.37^{b}	4.46±0.55^{e}	5.10±0.50^{b}
10	0.5	4.59±0.83^{bc}	8.97±0.83^{b}	11.90±1.12^{b}	2.99±0.47^{c}	4.96±0.55^{c}	5.15±0.48^{b}
20	0.5	5.18±0.93^{a}	9.63±0.97^{a}	12.34±0.91^{a}	3.47±0.46^{a}	5.60±0.57^{a}	5.65±0.43^{a}
30	0.5	4.82±0.79^{b}	9.46±0.64^{a}	11.68±1.29^{b}	3.46±0.49^{a}	5.18±0.48^{b}	5.22±0.48^{b}
40	0.5	4.57±0.74^{c}	9.59±0.96^{a}	10.76±1.11^{d}	3.24±0.47^{b}	4.76±0.47^{d}	4.81±0.47^{c}

Within a column, means followed by the different letters (a, b, c and d) are significantly different following Duncan's Multiple Range Test (p = 0.05), PE: Potato extract and DC: Day of culture

medium added with combination between 100 mL L^{-1} CW and 0.5 mg L^{-1} NAA (Fig. 2).

Additionally, the medium supplemented with CW at different concentrations (50, 100, 150 and 200 mL L^{-1}) combined with 0.5 mg L^{-1} NAA led a higher mean number of roots than the medium without CW at 15 and 30 DC. However, at 45 DC, compared to free CW, medium adding 100 or 150 mL L^{-1} CW produced a higher mean number of the root while, medium supplementing 200 mL CW generated a lower average of root number (Table 5). Supplementation of CW caused different effects on root elongation of *E. glaucum* depending on treatments. Root length varied between 3.19 cm and 3.44 cm at 15 DC, 4.49 cm and 5.34 cm at 30 DC and 4.82 cm and 5.47 cm at 45 DC, respectively (Table 5). The longest roots were observed with 100 mL L^{-1} CW combined with 0.5 mg L^{-1} NAA treatment. However, the use of CW above 150 mL L^{-1} decreased root elongation at 45 DC (Table 5).

In brief, the best rooting rate, rooting coefficient and root elongation was observed with 100 mL L^{-1} CW.

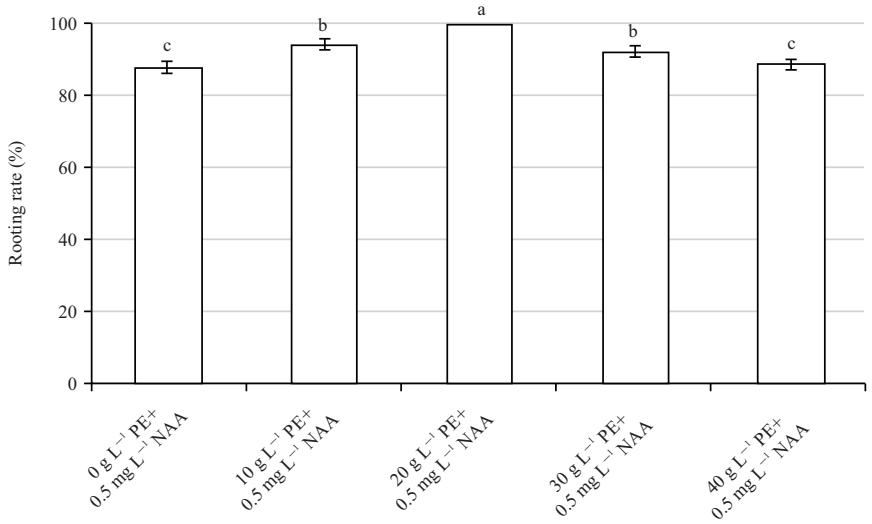

Fig. 3: Effect of combination between potato extract and 0.5 mL L^{-1} NAA on rooting rate of *in vitro E. glaucum* at 45 days of culture
Means followed by the different letters (a, b and c) are significantly different following to Duncan's Multiple Range Test (p = 0.05) and DC: Day of culture

On other hand, medium supplemented with PE at three different concentrations (10, 20 and 30 g L^{-1}, respectively) combined with 0.5 mg L^{-1} NAA generated a higher rooting rate than the medium without PE, while, the rooting rate of *E. glaucum* from medium supplemented 40 g L^{-1} PE was not significantly different from free PE medium (Fig. 3). Addition of 20 g L^{-1} PE gave the highest rooting rate of *E. glaucum* in compared to other treatments (Fig. 3).

Similarly, medium-adding PE resulted in varied effects on root production and elongation of roots depending on treatments. The highest values of root number and root length were recorded in a medium supplementing 20 g L^{-1} PE. At 15 and 30 DC, the use of PE at different concentrations (10, 20, 30 and 40 g L^{-1}) combined with 0.5 mg L^{-1} NAA led a higher mean number of roots than the medium without CW, except 10 g L^{-1} PE at 30 DC. At 45 DC, compared to free CW, medium supplementing 10 to 30 g L^{-1} PE produced a higher mean number of the root while, medium adding 40 mg L^{-1} PE generated a lower average of root number (Table 6). Root length of *in vitro E. glaucum* varied from 2.99 cm to 3.47 cm at 15 DC, from 4.46 cm to 5.60 cm at 30 DC and 4.81 cm and 5.65 cm at 45 DC, respectively (Table 5). Our result indicated that the addition of CW or PE at a convenient concentration in the medium increased root production and elongation.

For *in vitro* rooting of *Ensete*, different kinds of auxin were used such as IBA and IAA[9]. However, the effect of medium supplementing NAA at different concentrations on *in vitro Ensete* rooting was first described in this work. Additionally, a positive effect of CW and PE at the favorable concentration on rooting of *Ensete* was reported for the first time in this study, too. While NAA and these organic additives were efficiently used for rooting *Musa*[16,17], the related species of *Ensete*, or other plants[14,15]. To date, the micropropagation of *E. glaucum* has been poorly reported in the literature. Therefore, our obtained results may allow describing a micropropagation protocol for this rare *Ensete* species using two organic additives, coconut water and potato extract, respectively. At the same time, the obtained *in vitro* explants will be material for the cryopreservation of *E. glaucum*. In addition, an investigation on *ex vitro* acclimatization of micropropagated *E. glaucum* plants is necessary for the near future to be able to transfer *in vitro E. glaucum* plants into the greenhouse as well as the natural environment.

CONCLUSION

This study provided the investigation effect of BAP, NAA and two organic additives (CW and PE) on the shooting and rooting of *E. glaucum* collected from Xuan Son Nation Park, Vietnam, during the micropropagation process. Medium supplementing of 3.0 mg L^{-1} combined with 0.2 mg L^{-1} NAA was optimum and favorable for shooting *in vitro E. glaucum*. The use of medium-added CW (100 mL L^{-1}) increased the efficiency of both stages, shooting and rooting of *in vitro E. glaucum*. Medium supplemented with PE at a concentration of 30 g L^{-1} resulted in good shoot production while 20 g L^{-1} led to a significant increase in root production. Therefore, further research is needed in this regard to improve micropropagation techniques for this cultivar.

SIGNIFICANCE STATEMENT

To date, *Ensete glaucum* is not widely known while, this species has ornamental value and potential medicinal benefit. As a result, only limited *in vitro* investigations have been conducted in the recent past. Studies investigating the effect of BAP on *in vitro* shoot production and NAA on rooting of *E. glaucum*. Particularly, the effect of CW and PE on both stages, shooting and rooting, of *E. glaucum* micropropagation for the first time. Obtained results could help to establish a micropropagation protocol for the rapid propagation and conservation of this species.

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