In vitro propagation of *Homalomane occulta*, A medicinal herb from Vietnam

Nhung Dang Thi, Thao Nguyen Huynh Yen, Minh Nguyen Hoang Nhat, Thanh Pham and Trang Nguyen Thi Quynh*
University of Education, Hue University, Hue 53000, VIETNAM
*ntqtrang@hueuni.edu.vn; nguyenthiquynhtrang@dhsphue.edu.vn

Abstract

Homalomane occulta (H. occulta) is an herbal plant with many great traditional medicinal values. In general, H. occulta is mainly propagated naturally from seeds or rhizomes, with a relatively low multiplication rate. They also have slow growth rates and depend highly on seasonal and weather conditions. Propagation using tissue culture was reported to have many advantages compared with traditional propagation methods. This research aims to study the in vitro propagation of H. occulta, a medicinal herb from Vietnam. Our study showed that the rhizome section carrying the bud is the most effective culture organ, sterilized with 0.1% $HgCl_2$ for 8 minutes, which has an excellent disinfection effect and a high survival rate (82.87%). The suitable medium for in vitro shoot regeneration of H. occulta is MS medium with sucrose (30 g/L) and agar (8 g/L) supplemented with 4.0 mg/L BAP (2.10 shoots/explant).

The appropriate medium for rapid multiplication and formation of shoot clusters and roots is essential MS medium supplemented with 1.0 mg/L TDZ (with 5.20 shoots/explant, 9.40 roots/shoot clumps). When transferred from an in vitro environment to an open garden, seedlings have a 100% survival rate.

Keywords: *In vitro* propagation, Multiple shoot clumps, *Homalomena occulta*, Shoot regeneration, root formation.

Introduction

Homalomena, a genus in the Araceae family, is a group of plants with high medicinal value[11]. They are primarily found in Southeast Asia's humid, mountainous regions[2]. *Homalomena* has been traditionally used as herbal medicine in many countries, especially in Vietnam, where it holds significant value for indigenous communities in the Truong Son region[1]. Recent scientific research has revealed that extracts and isolated compounds from various species of the *Homalomena* genus possess noteworthy pharmacological properties including anti-inflammatory[0,10], anti-osteoporotic[8], antimicrobial[14], acetylcholinesterase inhibition[15], anti-cancer effects[10], beta-secretase enzyme inhibition[17] and the promotion and differentiation of bone marrow stem cells[4]. This demonstrates that the *Homalomena* genus is a valuable source of medicinal materials with the potential to improve human health.

**** Author for Correspondence***

However, in Vietnam, some *Homalomena* species are listed in the Red Book with some rare species such as *H. Vietnam*, *H. pierreana, H. conchinchinensis* and *H. occulta*[7]. *H. occulta* has a long history of use in traditional medicine as a digestive stimulant, tonic and rheumatism treatment[3]. Previous studies by Yang et al[16] and Ye et al[17] also demonstrated its potential anti-osteoporotic and anti-inflammatory activities.

In recent times, the increased awareness of the medicinal properties of *Homalomena* has led to a surge in the production and use of herbal products derived from these plants. This has resulted in unsustainable and uncontrolled harvesting, putting certain *Homalomena* species at risk of extinction and jeopardizing the long-term supply of medicinal materials for humans. Furthermore, *Homalomena* plants are mainly propagated naturally from seeds or rhizomes, with a relatively low multiplication rate (one mother plant yielding only 3-5 offspring). They also have slow growth rates and depend highly on seasonal and weather conditions. Presently, some *Homalomena* species worldwide are being researched for *in vitro* propagation due to the advantages of rapid multiplication in a short period[9,11]. It is essential to research developing an *in vitro* propagation process for *H. occulta* to support conservation efforts and this medicinal resource's sustainable development.

Material and Methods

Plant Materials: Plants of *H. occulta* were collected in the A Luoi region, Thua Thien Hue province.

The effect of sterilization time and culture organ on the creation of initial material: The experiment includes 12 formulas corresponding to 6 disinfection time points and four different organs including the root tip and rhizome (The rhizome was cut into small sections, each section containing one bud), leaf and petiole. Healthy, undamaged explants (2 cm) were excised from the mother plants and were cleaned with tap water followed by a 5-minute wash with diluted soap. They were then rinsed under tap water and washed with sterile distilled water. The preliminary sterilization was done with 70% ethanol for 30 seconds followed by 0.1% $HgCl_2$ solution for 5-20 minutes to determine the most suitable sterilization duration. Afterwards, the samples were rinsed with sterile distilled water three times before being transferred to the nutrient medium.

Shoot regeneration: Natural rhizomes with shoot explants (inheriting experiment 1), after sterilization, were cultured on an essential MS medium with 30 g/L sucrose and 8 g/L agar, supplemented with different concentrations of TDZ

(0.0-8.0 mg/L), BAP (0.0-8.0 mg/L), or KIN (0.0-8 mg/L) for shoot regeneration. The results were collected after 12 weeks of culture.

Multiple shoot clumps induction and root formation: Shoots regenerated from the above process were separated and further cultured on an essential MS medium with 30 g/L sucrose, 8 g/L agar, supplemented with growth-regulating substances such as TDZ (0.0-2.0 mg/L), BAP (0.0-2.0 mg/L), KIN (0.0-2.0 mg/L), or combination with NAA (0.0-2.0 mg/L), IBA (0.0-2.0 mg/L) and coconut water (5-20%) to investigate shoot multiplication, shoot growth and root production *in vitro*. The results were collected after 12 weeks of culture.

Acclimatization: *In vitro* plantlets, after 12 weeks of culture with a shoot length of about 3-4 cm and 3-4 roots and at least 2-3 leaves, were removed from the flasks and washed thoroughly in running tap water. They were then transferred to the plastic pots containing media including sand and organic soil (1:1). They were placed in a greenhouse. The survival rate of *in vitro* plants was observed after four weeks.

Monitoring Indicators: Survival sample rate (%), infected sample rate rate (%), death sample rate (%), number of shoots per explant (shoots/explant), number of leaves per shoot (leaves/shoot), shoot height (cm), number of roots (roots/shoot); root length (cm) were monitored.

Culture conditions: The culture medium was a basic MS medium with 30 g/L sucrose and 8 g/L agar, supplemented with growth-regulating substances from the auxin and cytokinin groups at varying concentrations, depending on the research objectives. The culture medium was sterilized at 120°C, 1 atm for 15 minutes. The samples were cultured under aseptic conditions at 22–25°C, with a light intensity of 2000–3000 lux and a 16-hour photoperiod.

Statistical analysis: The experiment was repeated three times with 30 samples for each experiment. Data were processed using SPSS 20.0 software for One-factor Analysis of Variance (ANOVA).

Results and Discussion

The effect of sterilization time and culture organ on the creation of initial material: Sterilize samples with $HgCl_2$ at intervals of 5-10 minutes on different organs include root tip segments, rhizomes carrying bud, leaves and petioles to determine the organ to be cultured.

Table 1
The effect of sterilization time with 0.1% $HgCl_2$ and culture organ on the creation of initial material

Time (min)	Section	Infected sample rate (%)	Death sample rate (%)	Survival sample rate (%)
5	Root tip	$85.03^{a} \pm 1.36$	$0.00^{p} \pm 0.00$	$14.97^{p} \pm 1.36$
	Rhizome	$54.87^{g} \pm 1.35$	$0.00^{p} \pm 0.00$	$45.13^{f} \pm 1.35$
	Leaves	$77.25^{c} \pm 1.13$	$0.00^{p} \pm 0.00$	$22.75^{m} \pm 1.13$
	Petiole	$66.58^{e} \pm 0.89$	$0.00^{p} \pm 0.00$	$33.42^{ik} \pm 0.89$
6	Root tip	$81.21^{b} \pm 0.99$	$0.00^{p} \pm 0.00$	$18.79^{o} \pm 0.99$
	Rhizome	$40.82^{ik} \pm 0.84$	$0.00^{p} \pm 0.00$	$59.18^{e} \pm 0.84$
	Leaf	$60.83^{f} \pm 1.36$	$0.00^{p} \pm 0.00$	$39.17^{g} \pm 1.36$
	Petiole	$69.76^{d} \pm 1.04$	$0.00^{p} \pm 0.00$	$30.24^{kl} \pm 1.04$
7	Root tip	$63.65^{f} \pm 1.02$	$7.48^{m} \pm 0.40$	$28.86^{l} \pm 1.01$
	Rhizome	$25.25^{n} \pm 1.04$	$3.56^{o} \pm 0.32$	$71.17^{c} \pm 1.05$
	Leaf	$55.51^{g} \pm 1.38$	$15.19^{k} \pm 0.60$	$29.28^{l} \pm 1.50$
	Petiole	$51.31^{h} \pm 0.94$	$16.23^{i} \pm 0.38$	$32.44^{ik} \pm 1.00$
8	Root tip	$49.39^{h} \pm 0.53$	$11.35^{l} \pm 0.34$	$32.25^{g} \pm 0.64$
	Rhizome	**$11.17^{o} \pm 0.96$**	**$5.95^{n} \pm 0.51$**	**$82.87^{a} \pm 0.86$**
	Leaf	$43.48^{i} \pm 1.16$	$34.33^{e} \pm 0.46$	$22.18^{m} \pm 1.25$
	Petiole	$40.18^{k} \pm 0.81$	$21.05^{h} \pm 0.29$	$38.76^{g} \pm 0.92$
9	Root tip	$31.34^{m} \pm 0.94$	$25.22^{g} \pm 0.38$	$43.42^{f} \pm 1.00$
	Rhizome	$10.40^{o} \pm 1.02$	$10.46^{l} \pm 0.57$	$79.13^{b} \pm 0.87$
	Leaf	$40.96^{ik} \pm 0.67$	$45.34^{c} \pm 0.46$	$13.69^{p} \pm 0.83$
	Petiole	$35.01^{l} \pm 0.62$	$30.06^{f} \pm 0.38$	$34.91^{h} \pm 0.48$
10	Root tip	$28.88^{m} \pm 1.02$	$37.86^{d} \pm 0.38$	$33.25^{ik} \pm 1.03$
	Rhizome	$9.37^{o} \pm 1.04$	$25.02^{g} \pm 0.35$	$65.59^{d} \pm 1.20$
	Leaf	$35.94^{l} \pm 1.37$	$51.00^{a} \pm 0.44$	$13.04^{p} \pm 1.24$
	Petiole	$29.65^{m} \pm 0.97$	$46.90^{b} \pm 0.48$	$23.43^{m} \pm 1.06$

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($P \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

The results were assessed after four weeks of culture. The results are presented in table 1. Table 1 data shows that the organs used for culture have very different responses to different levels of sterilization time. Root tip, leave and petiole samples had higher death and infection rates than rhizome-carrying buds at all experimental periods. The death rate of these samples is proportional to the increase in sterilization time. Some living samples are also fragile and difficult to regenerate. Thus, using the vegetative organs of the root tip, leaf and petiole to create starting materials is almost fruitless for the *H. occulta*.

At the five and 6-minute sterilization time points, the rate of infected samples was high (40.82-85.03%), the rate of survival samples was low (14.97-59.18%) and especially there were no dead samples in all organs using culture. Continuing to increase the sterilization time to 7 minutes and 8 minutes, the survival rate increased from 22.18% to 82.87%, reaching the highest value in the part of rhizome carrying bud is 82.87%. The infected samples decreased gradually from 63.65% to 11.17%; however, dead samples began to appear at a rate of 3.56% (Rhizome sample) to 34.33% (Leaf sample). Sterilization time increased to 9 minutes and 10 minutes, the survival rate and infected samples rate gradually decreased and the death rate gradually increased in all starting material sources, up to 51% (with leaf sample).

In general, compared to other exploration times and other starting material sources, disinfecting the rhizome-carrying bud with $HgCl_2$ for 8 minutes gives the best results. The infected rate decreased sharply by 11.17% and the rate of dead samples was low at only 5.95%, the rate of survival samples increased to 82.87%. Therefore, to create a source of starting material for *in vitro* propagation of *H. occulta*, the vegetative organ is part of the rhizome that carries the bud more efficiently than root tip, petiole and leaf. Sterilization time is 8 minutes for a high sample survival rate reaching 82.87%.

Effect of various cytokinins on shoot regeneration of *H. occulta:* Rhizome samples carrying the bud after sterilization were cultured on MS basic medium with 30 g/L sucrose, 8 g/L agar and individually supplemented with various cytokinins including TDZ, BAP and KIN with concentrations ranging from 0.5 to 8.0 mg/L for shoot regeneration. The research results were monitored for 12 weeks of culture (Table 2 and figure 1).

The data in table 2 shows that depending on the type and concentration of cytokinin, the effect on shoot regeneration differs. After 12 weeks of culture, adding three cytokinins significantly increased shoot formation compared to the media without plant growth regulators. Some environments are also capable of creating shoot clumps. The number of shoots per explant ranges from 0.90 to 1.40 shoots (for TDZ), from 1.00 to 2.10 shoots (for BAP) and from 0.70 to 1.20 shoots (for KIN). Among them, MS medium supplemented with a BAP concentration of 4 mg/L gave the highest result with 2.10 shoots. Monitoring the shoot height, the cultured samples all reached 1.05 - 2.96 cm (for TDZ), 1.04 - 3.38 cm (for BAP) and 0.50 - 1.48 cm (for KIN). The number of leaves/shoot also ranged from 0.67 to 3.11 leaves (for TDZ), from 1.96 to 4.10 leaves (for BAP) and 0.84 to 2.17 leaves (for KIN).

Table 2
Effect of various cytokinins on shoot regeneration of *H. occulta*

Plant growth hormone (mg/L)		No. of shoot	Length of shoot	No. of leave/shoot	Percent response of shoot [%]
0.0		$0.65^{d} \pm 0.29$	$0.55^{h} \pm 0.07$	$0.96^{h} \pm 0.12$	$72.06^{e} \pm 2.16$
TDZ	0.5	$1.00^{bc} \pm 0.21$	$1.32^{f} \pm 0.06$	$2.62^{c} \pm 0.07$	$85.96^{c} \pm 1.80$
	1	$1.40^{abc} \pm 0.16$	$2.46^{c} \pm 0.05$	$3.11^{b} \pm 0.07$	$95.47^{a} \pm 1.26$
	2	$1.20^{bc} \pm 0.20$	$2.96^{b} \pm 0.04$	$2.01^{e} \pm 0.07$	$88.13^{c} \pm 1.52$
	4	$1.00^{bc} \pm 0.25$	$1.75^{e} \pm 0.03$	$1.27^{g} \pm 0.03$	$87.58^{c} \pm 1.58$
	8	$0.90^{bc} \pm 0.23$	$1.05^{g} \pm 0.05$	$0.67^{i} \pm 0.09$	$79.51^{d} \pm 1.32$
BAP	0.5	$1.50^{abc} \pm 0.22$	$1.04^{g} \pm 0.05$	$2.27^{d} \pm 0.07$	$89.85^{bc} \pm 1.12$
	1	$1.60^{abc} \pm 0.54$	$1.72^{e} \pm 0.06$	$2.02^{de} \pm 0.08$	$96.77^{a} \pm 1.47$
	2	$1.80^{ab} \pm 0.38$	$2.47^{c} \pm 0.07$	$3.28^{b} \pm 0.05$	$98.85^{a} \pm 2.15$
	4	**$2.10^{a} \pm 0.27$**	**$3.38^{a} \pm 0.05$**	**$4.10^{a} \pm 0.09$**	**$95.03^{a} \pm 1.40$**
	8	$1.00^{bc} \pm 0.21$	$2.06^{d} \pm 0.05$	$1.96^{e} \pm 0.08$	$94.56^{ab} \pm 1.80$
KIN	0.5	$0.90^{bc} \pm 0.23$	$1.48^{f} \pm 0.04$	$1.44^{fg} \pm 0.07$	$89.09^{c} \pm 1.62$
	1	$1.20^{bc} \pm 0.20$	$1.11^{g} \pm 0.05$	$2.17^{de} \pm 0.07$	$90.19^{bc} \pm 1.38$
	2	$1.00^{bc} \pm 0.25$	$1.38^{f} \pm 0.04$	$1.39^{fg} \pm 0.09$	$87.42^{c} \pm 1.21$
	4	$0.80^{c} \pm 0.24$	$1.34^{f} \pm 0.06$	$1.57^{f} \pm 0.09$	$90.27^{bc} \pm 1.38$
	8	$0.70^{c} \pm 0.21$	$0.50^{h} \pm 0.04$	$0.84^{hi} \pm 0.07$	$80.65^{d} \pm 1.39$

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($P \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

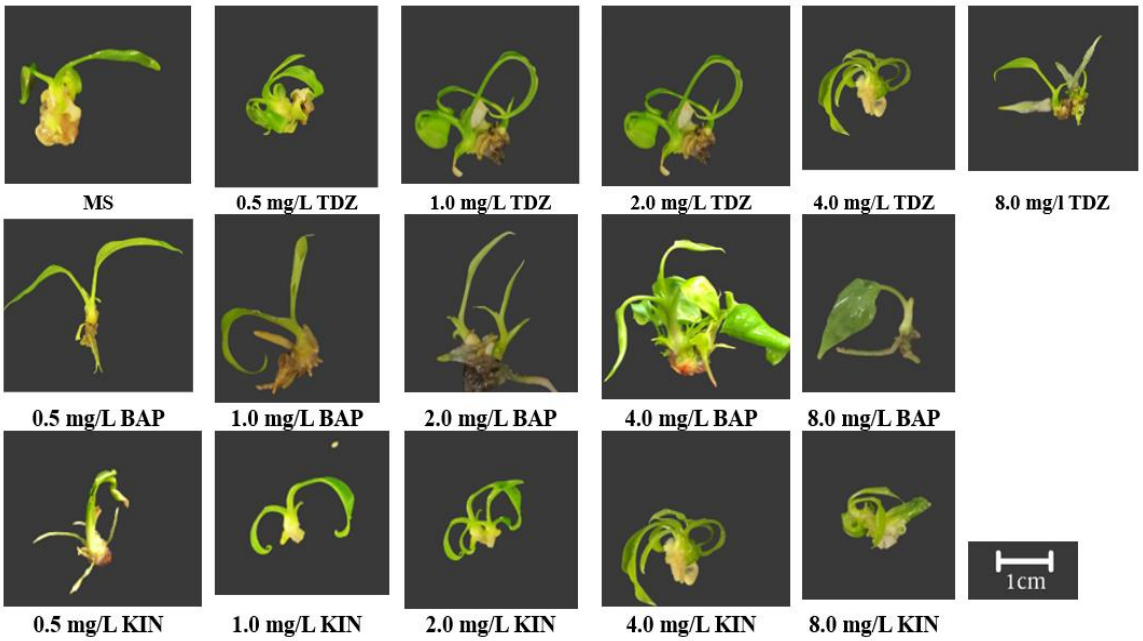

Figure 1: Effect of various cytokinins (TDZ, BAP and KIN) on shoot regeneration of *H. occulta*

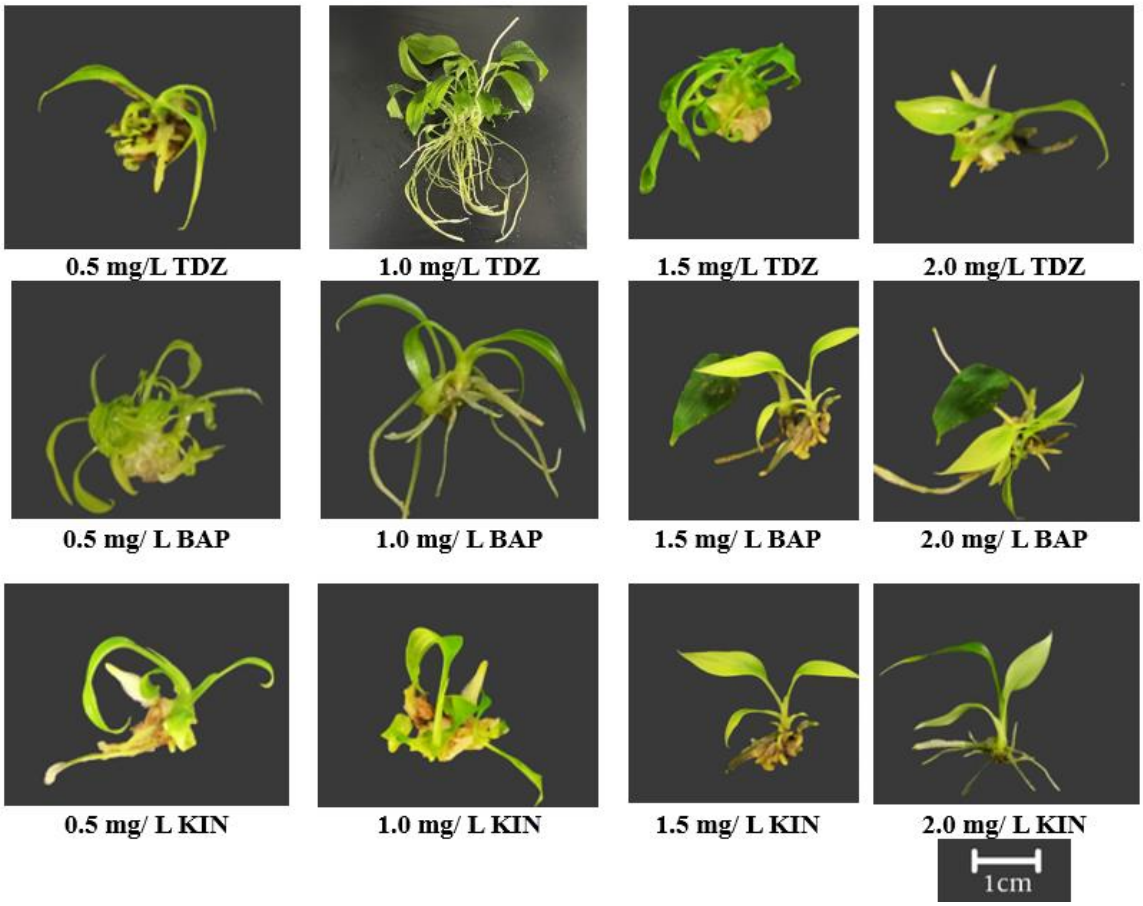

Figure 2: Effect of various cytokinins on shoot multiplication, shoot growth and root production of *H. occulta*

Thereby, it can be seen that the number of shoots in the KIN medium was significantly reduced compared to the TDZ and BAP medium. This result is also consistent with the study of Raomai et al[9] in *in vitro* propagation of *H. aromatica.* Of the three cytokinins tested, BAP is the best result in shoot formation of *H. occulta* from rhizome carrying bud. The number of shoots, shoot length and leaves/shoots decreased with the continued increase in BAP concentration (Table 2).

In particular, the BAP supplement formula of 4.0 mg/L is the most suitable environment for shoot regeneration in this variety.

Effect of various cytokinins on shoot multiplication, shoot growth and root production of *H. occulta:* The individual effects of TDZ, BAP and KIN with lower concentrations (0.5-2.0 mg/L) on the ability to create shoot multiplication, shoot growth and root production of *H. occulta* were studied. After 12 weeks of culture, the results show that the medium supplemented with TDZ, BAP and KIN all give a higher shoot growth and root production than the control (MS), from 77.01 to 96.85% (Percent response of shoot) and 83.33 to 95.77% (Percent response of root), compared to is 75.99% and 74.79% in MS medium without plant growth regulators (Table 3, Figure 2).

In the case of supplementing BAP, the culture medium adds 0.5 mg/L BAP gave the number of shoots with 4.30 shoots/explant. The shoots' growth ability was 2.27 cm in height/shoot, 3.09 leaves/shoot, 6.73 roots/shoot and 1.25 cm in length/root. The shoot condition is also good. Continuing to increase BAP concentration to 1.0-2.0 mg/L, the number of shoots decreased sharply from 3.00 to 1.70 shoots/explant, the height/shoot and number of leaves/shoot also decreased only reaching 1.64 -1.13 cm/shoot and 2.70-1.74 leaves/shoot. The length/root and number of roots/shoot also decreased, only reaching 1.57 -0.56 cm/root and 5.34-3.53 leaves/shoot (Table 3).

In the case of supplementing TDZ, the number of shoots increased with the increase in concentration from 0.5 - 1.0 mg/L; the highest number of shoots and roots reached 5.20 shoots/sample and 9.40 roots/shoot in MS medium supplemented with 1.0 mg /L TDZ. This formula has the best shoot growth - shoot height reaching 2.64 cm and number of leaves/shoot reaching 3.91 leaves, root length was 4.13 cm and shoot condition is the best of all experiences. Continuing to increase TDZ concentration to 1.5-2.0 mg/L, the number of shoots decreased sharply from 1.30 to 1.00 shoots/explant, the height/shoot and number of leaves/shoot also decreased, only reaching 1.95-1.18 cm/shoot and 2.65-2.27 leaves/shoot.

Table 3
Effect of various cytokinins on shoot multiplication, shoot growth and root production of *H. occulta*

Plant growth hormone (mg/L)		No. of Shoot	Length of shoot	No. leave/ shoot	Percent response of shoot [%]	No. of root	Length of root	Percent response of root [%]
0.0		0.6^{e} ± 0.47	0.65^{k} ± 0.40	1.04^{h} ± 0.19	75.99^{g} ± 1.30	1.64^{f} ± 0.35	0.58^{fg} ± 0.26	74.79^{e} ± 3.54
TDZ	0.5	1.50^{de} ± 0.26	0.65^{k} ± 0.16	1.35^{fgh} ± 0.11	82.82^{ef} ± 1.78	3.23^{e} ± 0.36	0.73^{f} ± 0.26	83.56^{d} ± 1.76
	1	**5.20^{a} ± 0.33**	**2.64^{a} ± 0.13**	**3.91^{a} ± 0.16**	**93.36^{abc} ± 1.28**	**9.40^{a} ± 0.65**	**4.13^{a} ± 0.18**	**95.77^{a} ± 2.02**
	1.5	1.30^{de} ± 0.36	1.95^{c} ± 0.15	2.65^{c} ± 0.10	93.40^{abc} ±1.72	3.75^{de} ± 0.52	0.97^{e} ± 0.15	94.36^{ab} ± 2.25
	2	1.00^{de} ± 0.21	1.18^{f} ± 0.12	2.27^{d} ± 0.13	94.80^{ab} ± 2.05	5.67^{bc} ± 0.65	1.47^{c} ± 0.10	85.72^{cd} ± 2.77
BAP	0.5	4.30^{b} ± 0.30	2.27^{b} ± 0.26	3.09^{b} ± 0.15	96.85^{a} ± 2.63	6.73^{b} ± 0.63	1.25^{d} ± 0.25	90.93^{abcd} ± 2.32
	1	3.00^{c} ± 0.33	1.40^{e} ± 0.15	2.11^{de} ± 0.21	89.14^{cd} ± 1.37	5.34^{bcd} ± 0.55	1.57^{c} ± 0.21	84.33^{cd} ± 2.81
	1.5	2.10^{cd} ± 0.75	1.13^{fg} ± 0.21	2.70^{c} ± 0.14	89.11^{cd} ± 1.42	4.87^{cde} ± 0.38	1.04^{e} ± 0.17	85.24^{cd} ± 2.85
	2	1.70^{cde} ± 0.42	1.64^{d} ± 0.19	1.74^{ef} ± 0.12	90.75^{bc} ± 1.03	3.53^{e} ± 0.31	0.56^{fg} ± 0.14	85.16^{bcd} ± 2.28
KIN	0.5	1.00^{de} ± 0.21	0.96^{gh} ± 0.12	1.90^{de} ± 0.11	79.87^{fg} ± 1.16	7.00^{b} ± 0.70	1.23^{d} ± 0.11	92.74^{abc} ± 2.23
	1	1.20^{de} ± 0.24	1.18^{f} ± 0.17	2.16^{d} ± 0.14	89.07^{cd} ± 1.19	5.65^{bc} ± 0.83	2.19^{b} ± 0.23	90.35^{abcd} ± 3.03
	1.5	0.90^{de} ± 0.23	0.81^{hk} ± 0.15	1.48^{fg} ± 0.12	85.10^{de} ± 1.77	4.34^{cde} ± 0.49	0.68^{f} ± 0.18	86.61^{bcd} ± 3.19
	2	0.60^{e} ± 0.16	1.22^{f} ± 0.17	1.17gh ± 0.10	77.01^{fg} ± 1.50	3.48^{e} ± 0.58	0.45^{g} ± 0.25	83.33^{d} ± 2.83

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($P \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

Table 4
Effect of various combination of TDZ and NAA, IAA on shoot multiplication, shoot growth and root production of *H. occulta*

Plant growth hormone (combinative with TDZ 1.0 mg/L)		No. of shoot	Length of shoot	No. of leave/ shoot	Percent response of shoot [%]	No. of root/ shoot	Length of root	Percent response of root [%]
TDZ 1.0 mg/L		5.20^{a} ± 0.33	2.64^{a} ± 0.13	3.91^{a} ± 0.16	93.36^{abc} ± 1.28	9.40^{a} ± 0.65	4.13^{b} ± 0.18	95.77^{a} ± 2.02
NAA (mg/L)	0.5	1.70^{de} ± 1.52	0.36^{gh} ± 0.25	1.15^{e} ± 0.36	84.72^{def} ± 3.69	1.00^{de} ± 0.21	1.60^{c} ± 0.22	87.28^{b} ± 2.94
	1	2.50^{bcd} ± 0.34	0.58^{ef} ± 0.15	1.80^{c} ± 0.22	91.36^{bc} ± 2.29	1.70^{cde} ± 0.55	2.80^{c} ± 0.51	90.17^{b} ± 7.74
	1.5	2.40^{cde} ± 2.26	0.34^{gh} ± 0.36	2.27^{b} ± 0.40	88.86^{cde} ± 2.29	1.10^{de} ± 0.37	2.60^{c} ± 0.73	81.96^{b} ± 2.36
	2	2.20^{cde} ± 0.24	0.23^{h} ± 0.17	1.72^{c} ± 0.32	83.62^{efg} ± s1.21	1.00^{de} ± 0.21	1.90^{c} ± 0.23	87.20^{b} ± 3.90
IBA (mg/L)	0.5	2.00^{de} ± 0.29	0.35^{gh} ± 0.22	1.39^{d} ± 0.29	90.38^{bcd} ± 1.96	1.70^{cde} ± 0.26	4.70^{ab} ± 1.72	88.43^{b} ± 2.28
	1	2.30^{cde} ± 0.30	0.69^{e} ± 0.31	2.33^{b} ± 0.34	96.42^{ab} ± 1.53	2.40^{cd} ± 0.65	2.20^{c} ± 0.51	83.93^{b} ± 2.81
	1.5	2.71^{bc} ± 0.29	1.44^{b} ± 0.25	2.62^{b} ± 0.27	94.64^{abc} ± 2.17	3.00^{c} ± 0.61	2.70^{c} ± 0.36	89.81^{b} ± 2.46
	2	3.00^{b} ± 0.51	1.02^{d} ± 0.16	2.60^{b} ± 0.30	99.45^{a} ± 0.41	5.70^{b} ± 0.85	5.60^{a} ± 3.81	90.51^{b} ± 1.80
Coconut water (%)	5	1.50^{e} ± 1.66	0.40^{gh} ± 0.31	1.15^{e} ± 0.25	68.74^{i} ± 2.36	0.60^{e} ± 0.22	1.20^{c} ± 0.13	90.48^{b} ± 2.31
	10	2.00^{de} ± 0.21	1.23^{c} ± 0.24	1.82^{c} ± 0.17	81.79^{fgh} ± 2.95	1.10^{de} ± 0.23	1.40^{c} ± 0.16	81.79^{b} ± 3.92
	15	1.80^{de} ± 0.24	0.67^{e} ± 0.22	2.07^{c} ± 0.22	77.32^{gh} ± 1.99	1.20^{de} ± 0.29	1.60^{c} ± 0.26	72.61^{c} ± 2.63
	20	1.60^{de} ± 0.22	0.48^{fg} ± 0.26	1.21^{e} ± 0.16	75.93^{h} ± 1.75	1.00^{de} ± 0.25	1.50^{c} ± 0.22	83.91^{b} ± 3.74

Note: According to Duncan's multiple range tests, means that are followed by the same letter are not substantially different for each column and treatment ($P \leq 0.05$). Values reflect the means and standard errors of cultured explants, with n = 30 for each treatment.

Results in the medium supplementing of KIN (0.5-2.0 mg/L) were higher than the control (MS medium), but it was still generally lower than BAP and TDZ supplement formulas. The highest number of shoots is obtained in MS medium with 1.0 mg/L TDZ with 5.20 shoots/sample and the best shoot quality is with 2.64 cm in height/shoot, 3.91 leaves/shoot, 9.40 roots/sample and 4.13 cm in length/root. The shoots are dark green and healthy. From there, it can be concluded that the MS medium culture supplement 1.0 mg/L TDZ is the best result formation shoot for *H. occulta* propagation.

Effect of various combinations of TDZ and NAA, IAA on shoot multiplication, shoot growth and root production of *H. occulta*: The shoot explants were cultured on MS medium additionally with 1.0 mg/L TDZ in combination with different concentrations of NAA (0.5-2.0 mg/L), IBA (0.5-2.0 mg/L) and coconut water (from 5 - 20%) on shoot multiplication, shoot growth and root production. Research results after 12 weeks of culture are shown in table 4 and figure 3. The results in table 4 show that all MS media supplemented with TDZ combined with NAA, IBA and coconut water generally reduce the number of shoots compared to MS media supplemented with 1.0 mg/L TDZ. The number of shoots only reached 1.50-3.00 shoots/sample and the number of roots reached 0.60 - 3.00 roots/sample.

The growth of shoots and roots was lower than the MS medium, only adding 1.0 mg/L TDZ. It is probably due to NAA, IBA and coconut water weakening the positive effect of TDZ in multiple shoot clumps induction in *H. occulta*. Stanly et al[11] on micropropagation of *Homalomena pineodora* from Malaysia reported the deleterious effect of IBA on growth and multiplication. Moreover, this finding was also indicated in Yu e al[17] report and Trang et al[13] report on the lotus. Therefore, MS medium supplemented with 1.0 mg/L TDZ is the most suitable medium for shoot multiplication, shoot growth and root production of *H. occulta.*

Survival rate when transferring the seedling from *an in vitro* environment to an open garden: The *in vitro* plantlets with approximately 3-4 cm in height and well-developed roots were planted in pots on sand and organic soil mediums

with a ratio of 1:1 and were placed in the greenhouse. After four weeks, plants grew well with much bigger leaves in the greenhouse. The survival rate of transplanting plants was 100% (Figure 4).

Conclusion

The suitable environment for *in vitro* shoot regeneration of *H. occulta* is MS medium with 30 g/L sucrose and 8 g/L agar supplemented with 4.0 mg/L BAP (2.10 shoots/explant). The appropriate environment for rapid multiplication and formation of shoot clusters and roots is essential MS medium supplemented with 1.0 mg/L TDZ (5.20 shoots/explant, 9.40 roots/shoot clumps). When transferred from an *in vitro* environment to an open garden, seedlings have a 100% survival rate.

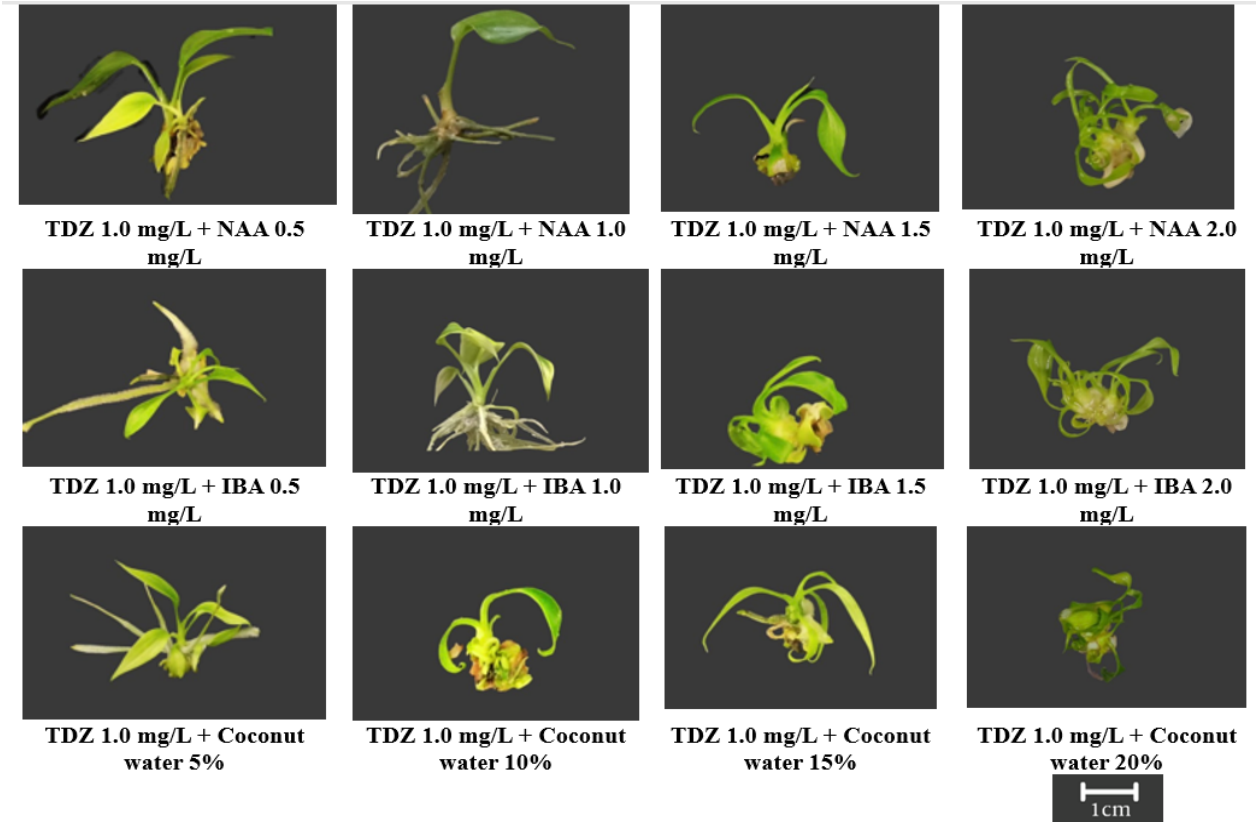

Figure 3: Effect of various combination of TDZ and NAA, IAA on shoot multiplication, shoot growth and root production of *H. occulta*

Figure 4: Regenerated plants of *H. occulta* growing in plastic pots after 4 weeks in the greenhouse

Acknowledgement
This work was supported by the University of Education, Hue University (Project No T.22-TN.SV-03).

References
1. Bogner J. and Nguyen V.D., A new *Homalomena* species *(Araceae)* from Vietnam, *Willdenowia*, **38(2)**, 527-531 **(2008)**

2. Boyce P.C. and Yeng W.S., Studies on Homalomeneae (Araceae) of Borneo I. Four New Species and Preliminary Thoughts on Informal Species Groups in Sarawak, *Gard. Singap*, **60(1)**, 1- 29 **(2008)**

3. Chi V.V., The dictionary of medicinal plant in Vietnam, In Vol. 2, Hanoi, Medical Publishing House, 872-873 **(2012)**

4. Hu Y.M., Liu C., Cheng K.W., Sung H.H.Y., Williams L.D., Yang Z.L. and Ye W.C., Sesquiterpenoids from *Homalomena occulta* affect osteoblast proliferation, differentiation and mineralization *in vitro*, *Phytochemistry*, **69(12)**, 2367-2373 **(2008)**

5. Kumar N. and Reddy M.P., *In vitro* Plant Propagation: A Review, *Journal of Forest Science*, **27(2)**, 61-72 **(2011)**

6. Nguyen L.T.K., Hoang H.N.T., Do T.T., Tran T.V.A., Nguyen H.T. and Ho D.V., Sesquiterpenoids from the rhizomes of *Homalomena pendula* and their anti-inflammatory activities, *Natural Product Research*, **37(15)**, 2559-2567 **(2023)**

7. People's Committee of An Giang province, First draft of planning for conservation and development of medicinal plants applying high technology in An Giang province to 2020 and orientation to 2030, (https://media.angiang.gov.vn/QD_Quyhoach_KH/2015/Du%20thao%20QH%20cay%20duoc%20lieu.pdf **(2014)**

8. Pham T.V., Ngo H.P.T., Thi T.D.N., Khoa N.H., Thi N.H.H. and Pham T., Volatile Constituents and Anti-Osteoporotic Activity of the n-Hexane Extract from *Homalomena gigantea* Rhizome, *Natural Product Communications*, **17(9)**, 1934578X2 21125433 **(2022)**

9. Raomai S., Kumaria S. and Tandon P., *In vitro* propagation of *Homalomena aromatica* Schott., an endangered aromatic medicinal herb of Northeast India, *Physiology and Molecular Biology of Plants*, **19**, 297-300 **(2013)**

10. Sánchez L.A., Olmedo D., López-Pérez J.L., Williams T.D. and Gupta M.P., Two new alkylresorcinols from *Homalomena* wendlandii and their cytotoxic activity, *Nat. Prod. Commun.*, **7(8)**, 1043-1046 **(2012)**

11. Stanly C., Bhatt A., Sulaiman B. and Keng C.L., Micropropagation of *Homalomena pineodora* Sulaiman & Boyce (Araceae): a new species from Malaysia, *Horticultura Brasileira*, **30**, 39-43 **(2012)**

12. Tiwari S., Upadhyay N., Singh B.K., Singh V.K. and Dubey N.K., Chemically Characterized Nanoencapsulated *Homalomena* aromatica Schott, Essential oil as Green Preservative Against Fungal and A§atoxin B1Contamination of Stored Spices based on *in Vitro* and in Situ E¨cacy and Favourable Safety Profile on Mice, https://doi.org/10.21203/rs.3.rs-421736/v1, 1-57 **(2021)**

13. Trang N.T.Q., Hong H.T.K., Huong V.T.M. and Long D.T., *In vitro* Propagation of Red Lotus (*Nelumbo nucifera* Gaertn) - An Aquatic Edible Plant in Vietnam, *Agricultural Science Digest*, **41(Special Issue)**, 129-136 **(2021)**

14. Van H.T., Le_N.T., Huynh N.T.A., Vo H.S., Le T.T., Chu H.V. Truong H.A.V. and Nguyen Q.H., Chemical composition and antibacterial activities of essential oils from rhizomes and aerial parts of *Homalomena cochinchinensis* (Araceae), *Nat. Prod. Res.*, **36**, 1-4 **(2021)**

15. Wong K.C., Hamid A., Eldeen I.M.S., Asmawi M.Z., Baharuddin S., Abdillahi H.S. and Staden J.V., A new sesquiterpenoid from the rhizomes of *Homalomena sagittifolia*, *Nat. Prod. Res.*, **26(9)**, 850-858 **(2012)**

16. Yang J.L., Dao T.T., Tran H.T., Zhao Y.M. and Shi Y.P., Further sesquiterpenoids from the rhizomes of *Homalomena occulta* and their anti inflammatory activity, *Bioorg. Med. Chem. Lett*., **29(10)**, 1162-1167 **(2019)**

17. Ye J., Yin P. and Xiao M.T., New aromatic compounds from the rhizomes of *Homalomena occulta*, *Phytochem. Lett.*, **21**, 57-60 **(2017)**

18. Yu X., Sheng J., Zhao L., Diao Y., Zheng X., Xie K., Zhou M. and Hu Z., *In vitro* plant regeneration of lotus (*Nelumbo nucifera*), *Open Life Sciences*, **10**, 142-146 **(2015)**.

(Received 14th September 2024, accepted 19th October 2024)