ACADEMIA JOURNAL OF BIOLOGY 2018, 40(3): 113–121
DOI: 10.15625/2615-9023/v40n3.12601

EFFECT OF GROWTH REGULATORS ON THE *IN VITRO* PROPAGATION AND *EX. VITRO* ROOT FORMATION OF *Gnetum gnemon* var. *griffithii* Markgr.

Nguyen Thi Phuong Hoang*, Phan Xuan Huyen, Nguyen Thi Thanh Hang, Dinh Van Khiem

Tay Nguyen Institute for Scientific Research, VAST, Vietnam

ABSTRACT

This study is to investigate the *in vitro* propagation and *ex vitro* root formation of *Gnetum gnemon* var. *griffithii* Markgr (*G. gnemon)*, one of the nutrient-rich forest vegetables. The results showed that MS medium supplemented with 1.5 mg l^{-1} BAP was the most suitable combination for shoot formation after 90 days of culture. The combination between 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA gave the best *in vitro* rooting rate (40%) after 60 days culture. After 60 days tranfered to the greenhouse, plantlets which planted on the substrate of coconut fiber powder had a better growth (plant height of 6.14 cm, root length of 6.90 cm) than those on a combination substrate of coconut fiber powder and sand (1:1). In additional, plantlets treated with 3.0 mg l^{-1} IBA for 1 minute had the highest root formation rate (80%), the greatest root number per plantlet (2.80 roots), and the longest root (1.76 cm) when compared with those in different of treatment.

Keywords: Nodal segments, *PGRs*, root formation, shoot formation.

Citation: Nguyen Thi Phuong Hoang, Phan Xuan Huyen, Nguyen Thi Thanh Hang, Dinh Van Khiem, 2018. Effect of growth regulators on the *in vitro* propagation and *ex. vitro* root formation of *Gnetum gnemon* var. *griffithii* Markgr. *Academia Journal of Biology*, 40(3): 113–121. https://doi.org/10.15625/2615-9023/v40n3.12601.

**Corresponding author email*: phuonghoang2406@gmail.com

Received 30 May 2018, accepted 18 August 2018

INTRODUCTION

Harnessing and exploring the potentials of local resources to reduce the dependency on commercial food and medicines is an urgent and very practical call for many impoverished communities in the country and around the world. Domestication of forest-based floral resources like *Gnetum gnemon*, which young leaves and immature flowers are popularly eaten (in particular, 100 g of seeds contain 30 g of water, 11 g of protein, 1.7 g of lipid, 50 g of carbohydrate, and 1.7 g of ash. And 100g of young leaves include 75.1 g of water, 6.6 g of protein, 1.2 g of lipid, 9.1 g of carbohydrate, 6.8 g of fiber, 1.3g of ash, 224 mg of phosphorus, 151 mg calcium, 2.5 mg iron and vitamin A) would not only enhance household economy and nutrition but also promote ex-situ conservation of the species. However the species remain largely forest-based and unresponsive to natural cutting propagation. *Gnetum gnemon* is an important agroforest species in Southeast Asia and Melanesia (Thaman et al., 2000). Vietnam, *Gnetum gnemon* is distributed in Tay Nguyen, Quang Nam, Khanh Hoa provinces, etc. (Bullecer & Bullecer, 2011; Celis & Avalos, 2013; Pham, 1999; Vo, 2003; Won & Renner, 2006).

Moreover, it is of great value to medicine. *G. gnemon* is an excellent source of Vitamins A and C. *G. gnemon* is a versatile medicinal plant with a wide range of ethnobotanical utilizations, which are primarily based on the diverse patterns of secondary metabolites found in the plant. Stilbenoids were found to be responsible for the pharmacological effects of *G. gnemon* like inhibitory effect on tyrosinase activity, melanin biosynthesis and on multiple angiogenesis. Additionally, clinical studies suggested that stilbenoids were beneficial in the management of diabetes and cardiovascular

diseases. Resveratrol, a polyphenol biphenyl, and multiple hydroxyl groups and its derivatives such as resveratrol oligomers polyphenol have been emerging to be promising new sources of natural antioxidant. Resveratrol is also known to protect the plant while being invaded by vermin, or while climate is severe. *G. gnemon* resveratrol (in dimer form) seemed to be effective in preventing arteriosclerosis, cancer, Alzheimer's diseases and other life style diseases. In addition to *G. gnemon* resveratrol's anti-oxidative and antiviral activities, its probable role in reduction of visceral fat and increase stamina in human beings have attracted the global markets. Hence, from the available scientific data pertaining to the nutritive potency and antitoxicity effect of this plant, it can be inferred that *G. gnemon* is a safe food and can be utilized effectively as a basic raw material to develop a novel nutritious functional food. Exploration of its newer pharmacological properties in addition to the reported activities can fill up the gap for development of a novel compound with medicinal as well as nutritive properties (Barua et al., 2015).

There have been worldwide published studies on the *in vitro* shoot formation of *G. gnemon.* In the study of Noochum & Te-chato (2003), the culture medium supplemented with 1.53 mg l^{-1} BAP in combination with 0.25 mg l^{-1} IBA show the best bud regeneration from the young leaf of *G. gnemon*. Handayani (2012) reported that 2–3 mg l^{-1} KIN combined with 0.1 mg l^{-1} IAA or 1.5–2 mg l^{-1} KIN combined with 0.3–5 mg l^{-1} IAA led to the highest shoot growth rate. However, there has not been any published study on the *in vitro* root formation of *G. gnemon.* Goenadi & Sudharama (1995) in combinations with BA, the addition of humic acids at 400 mg l^{-1} yielded the fastest growth of *G. gnemon*.

Bullecer & Bullecer (2011) studied the cuttings of *G. gnemon*, which were soaked in a mixture of 500 ppm indole-3-butyric acid (IBA) for 1 hour, producing the result that IBA was the best response for root formation. In Vietnam, there have not been any published studies on the *in vitro* propagation of *G. gnemon.*

Therefore, we conduct this research to investigate the effects of growth regulators on the *in vitro* shoot propagation and root formation, as well as the *ex vitro* root formation. The results aim to contribute to building the process of *in vitro* propagation and *ex vitro* culture of *G. gnemon*. It is essential to preserve and develop a nutrient-rich forest vegetable and a natural herbal resource as well.

MATERIALS AND METHODS

Materials

One-year-old nodal segments without leaf (1.0–1.5 cm) *Gnetum gnemon* var. *griffithii* Markgr obtained from Duc Trong district Lam Dong province were used for this study. They were thoroughly rinsed under running tap water for 30 minutes, then washed with soap. Next, the explants were dipped in alcohol 70% for 1 minute. For surface sterilization, the explants were dipped in 0.1% $HgCl_2$ (w/v) for 10 minutes, then washed in sterile distilled water 3 to 4 times, till the sterilant was removed completely. After sterilization, the nodal segments were then cut at both ends prior to inoculation on culture medium. The explants were cultured on MS (Murashige & Skoog, 1962) medium supplemented with 1.5 mg l^{-1} 6-benzylaminopurine (BAP), 30 g l^{-1} sucrose (Bien Hoa Sugar Factory, Dong Nai, Vietnam) and 8 g l^{-1} agar (Ha Long Food Co., Hai Phong, Vietnam). The pH of the medium was adjusted to 6.0 before sterilization (Noochum & Te-chato, 2003). Nodal segments containing single axillary buds were used as the source materials for the experiment.

Experimental design and culture conditions

The MS medium was used as the culture medium. Depending on the objective of each experiment, it was added with 6-benzylaminopurine (BAP) (0.5–3.0 mg l^{-1}), Kinetin (KIN) (0.5–3.0 mg l^{-1}), Indole-3-butyric acid (IBA) (0.1–2.0 mg l^{-1}), α-naphthalenacetic acid (NAA) (0.1–2.0 mg l^{-1}), indole acetic acid (IAA) (0.1–2.0 mg l^{-1}) or BAP (0.1–2.0 mg l^{-1}) combined with NAA (0.5 mg l^{-1}), sucrose 30 g l^{-1} and agar 8 g l^{-1}. For *in vitro* culture, the cultures were incubated at 25 ± 2°C during photoperiod of 10 hours per day.

In the greenhouse, the substrates are coconut fiber powder and a combination of coconut fiber powder and sand. The greenhouse was equipped with the rain cover and shading net

blocking out 70% of light. It had the temperature of 20–25°C and the humidity of 60–70%.

Effects of cytokinin on shoot formation

The *in vitro* nodal explants were cultured on MS medium supplemented with BAP (0; 0.5; 1; 1.5; 2; 3 mg l^{-1}) and KIN (0; 0.5; 1; 1.5; 2; 3 mg l^{-1}), 30 g l^{-1} sucrose, 8 g l^{-1} agar. The pH of the medium was adjusted to 6.0 before sterilization. Thirty explants were cultured for each treatment. The data was collected after 90 days of culture.

Effects of growth regulators on the in vitro root formation

The *in vitro* explants were cultured on MS medium supplemented with IBA (0; 0.1; 0.5; 1; 2 mg l^{-1}), NAA (0; 0.1; 0.5; 1; 2 mg l^{-1}), IAA (0; 0.1; 0.5; 1; 2 mg l^{-1}) and BAP (0; 0.1; 0.5; 1; 2 mg l^{-1}) in combination with NAA (0.5 mg l^{-1}), 30 g l^{-1} sucrose, 8 g l^{-1} agar. The pH of the medium was adjusted to 6.0 before sterilization. Thirty explants were cultured for each treatment. The data was collected after 60 days of culture.

Effects of different substrates on the adaptability of plantlets in the greenhouse

The plantlets of *G. gnemon*, with stems, leaves and roots after 3–month *in vitro* culture, were transplanted onto the two kinds of substrate including coconut fiber powder and combination coconut fiber powder and sand (1:1). Thirty shoots were planted for each treatment. The data was collected after 60 days.

Effects of IBA (0; 1; 2; 3; 4; 5 mg l^{-1}) on the ex vitro root formation

The *in vitro* 3–month shoots in dark green were used as the source materials for the experiment. The shoots were soaked in IBA (0; 1; 2; 3; 4; 5 mg l^{-1}) for 1 minute, then transplanted on the substrate of coconut fiber powder. Thirty shoots were planted for each treatment. The data was collected after 60 days.

The growth measurements

All *in vitro* plants treated in each experiment were taken for destructive measurement of the number shoots number of roots, shoot and root lengths and root formation rate.

Statistical analyses

All treatments were in triplicates and each one was replicated with 10 culture vessels.

All the data were analyzed for statistical analysis using Analysis of variance (ANOVA). The significant differnce between the means were compared with Duncan multiple range test (DMRT), LSD - test at $p \leq 0.01$; $p \leq 0.05$ or T-test for two treatment level $p \leq 0.05$, performed with the program MSTATC version 1.2 (Michigan State University, USA) (Duncan, 1955).

RESULTS AND DISCUSSION

Effects of cytokinin on shoot formation

BAP and KIN belong to the cytokinin group. BAP is one of the most commonly used substances in plant tissue culture. KIN plays an important role in cell division and stimulating the shoot formation.

Nodal explants of *G. gnemon* were cultured on MS medium supplemented with different concentrations of BAP and KIN for multiple shoot formation after 90 days of culture (Table 1). The results showed that BAP and KIN had positive effects on the shoot formation and growth. However, different concentrations resulted in different shoot formation. The medium without cytokinins produced only 1.20 shoots per explant.

By increasing the concentration to 0.5–1 mg l^{-1} BAP, the shoots were produced in the higher number (from 2.50 to 3.20 shoots per explant) with a rise in length (4.37–5.24 cm) (Fig. 1a and Table 1). MS medium supplemented with 1.5 mg l^{-1} BAP was the best combination for the shoot formation and growth with the high number of shoots (4.00 shoots per explant) reaching 6.48 cm in length. When the concentrations of BAP increased to 2 and 3 mg l^{-1}, the number of shoots was higher (4.10 and 4.80 shoots per explant respectively), but the shoot length was lower (3.79 cm and 3.61 cm respectively) and some variations were observed. Therefore, BAP with the low concentration stimulated the shoot growth but it would inhibit the development when raising its concentration. This result is similar to Noochum & Techato (2003), supplemented with 1.53 mg l^{-1} BA combining 0.25 mg l^{-1} IBA in MS medium show the best bud regeneration from young leaves of *G. gnemon.*

Similar to BAP, KIN with the concentration of 1.5 mg l^{-1} was found as the best response for shoot formation and growth with 3.40 shoots per explant and reaching 6.95 cm in length (Fig. 1b and Table 1). By the treatment of 0.5 and 1 mg l^{-1} KIN, it promoted the shoot formation (1.70 and 2.80 shoots per explant respectively) and the shoot length as well (4.35 and 6.50 cm respectively). Increasing the concentrations of KIN to 2 and 3 mg l^{-1} produced the higher number of shoots (3.40 and 4.00 shoots respectively), yet they showed several variations and seemed short–lived. The shoot length was also lower (5.46 and 4.90 cm respectively). Same with Handayani (2012) used a combination of KIN and IAA in the study on shoot growth of *Gnetum gnemon* Linn, reporting that combinations of IAA and KIN with the rates of 0.1 mg l^{1} and 2.0–3.0 mg l^{-1} or 0.3–5.0 mg l^{-1} and 1.5–2.0 mg l^{-1}, respectively, have the highest growth of shoot. As a result, increasing the concentration of KIN helped enhance the shoot formation and growth but it would inhibit the growth in length at the high concentrations.

Table 1. Effects of BAP and KIN on shoot formation of *G. gnemon* after 90 days of culture

Treatment code	Plant growth regulators	No. of Shoots/explants	Shoot length (cm)
	BA		
C0	0.0	1.20^{d*}	2.25^{e}
B1	0.5	2.50^{c}	4.37^{c}
B2	1.0	3.20^{bc}	5.24^{b}
B3	1.5	4.00^{ab}	6.48^{a}
B4	2.0	4.10^{a}	3.79^{d}
B5	3.0	4.80^{a}	3.61^{d}
	ANOVA	**	**
	CV (%)	13.97	2.98
	KIN		
C0	0.0	1.20^{c*}	2.25^{f}
K1	0.5	1.70^{c}	4.35^{e}
K2	1.0	2.80^{b}	6.50^{b}
K3	1.5	3.40^{ab}	6.95^{a}
K4	2.0	3.40^{ab}	5.46^{c}
K5	3.0	4.00^{a}	4.90^{d}
	ANOVA	**	**
	CV (%)	19.36	2.69

**significant at $p \leq 0.01$. Means in the same column followed by the same letters are not significantly different according to Duncan Multiple Range test

Thus, the MS medium supplemented with 1.5 mg l^{-1} BAP or 1.5 mg l^{-1} KIN performed best in the formation and growth of *G. gnemon* shoots. Between the two cytokinins, BAP showed better response than KIN.

Effects of IBA, NAA, IAA and BAP in combination with NAA on *in vitro* root formation

The rooting of the *in vitro* grown shoots is an integral part before transferring them to the greenhouse. For some plant species, the rooting happens spontaneously during the *in vitro* shoot formation. However, they were found to be inadequate for transferring. Therefore, it's essential to use the growth regulators to enhance the healthy root formation of the shoots, then transfer them from the culture medium to greenhouse.

In *in vitro* propagation, there have been numerous published researches using IBA, NAA and IAA as well as the regulators of cytokinin group combing with auxin to study the root formation and plant growth.

In this study, rooting formation was observed onto the MS media supplemented with different concentrations of IBA, IAA and NAA (0.1–2.0 mg l^{-1}) and BA (0.1–2.0 mg l^{-1}) combined with NAA (0.5 mg l^{-1}) within 60 days (Table 2 and Figs 1c, 1d, 1e, 1f).

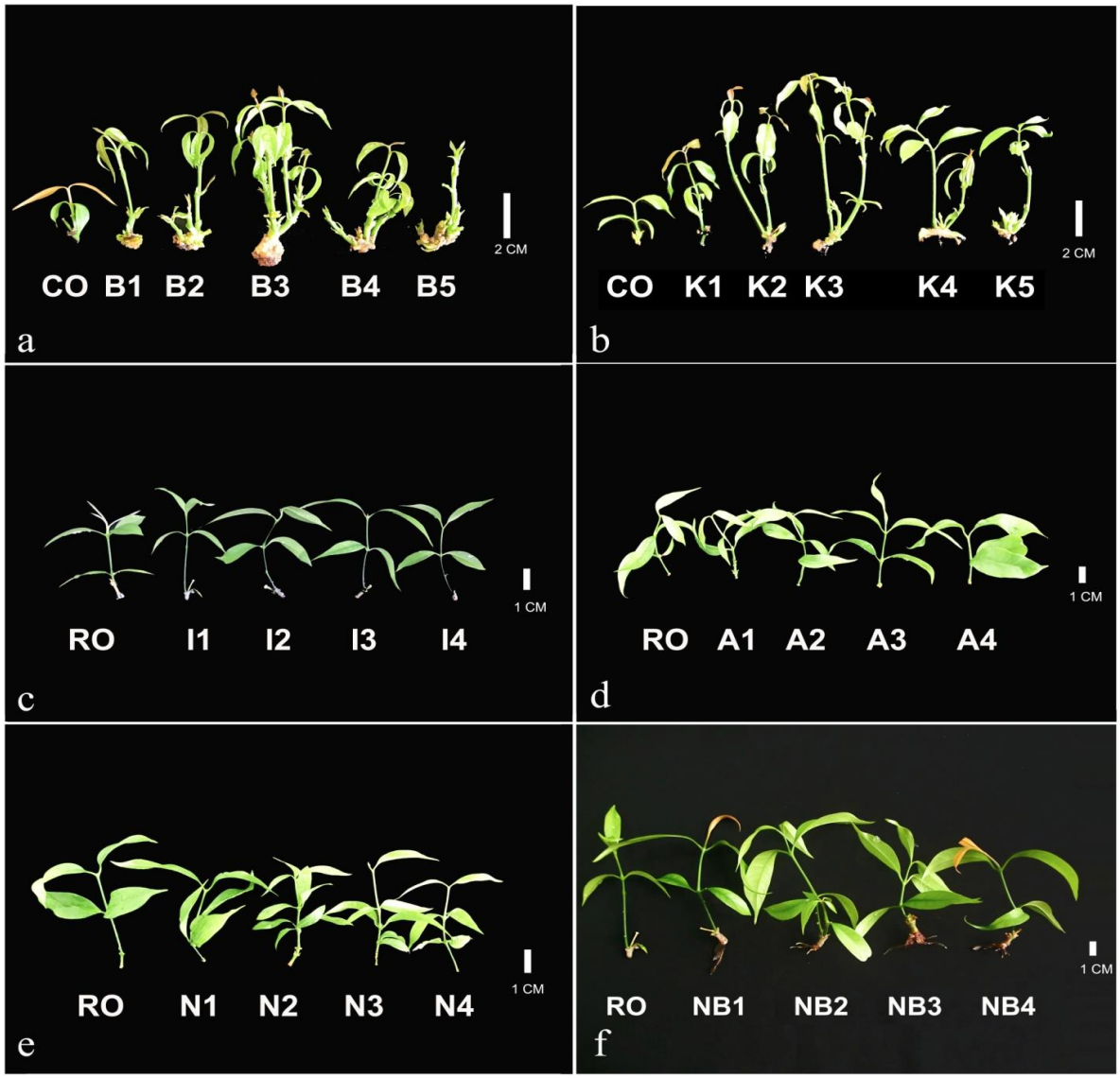

Figure 1. Study on *in vitro* propagation of *Gnetum gnemon* var. *griffithii* Markgr.
Notes: The treatment codes were the same as those shown in table 1, table 2

The results showed that all the shoots cultured on medium without any plant growth regulators and medium supplemented with 1–2 mg l^{-1} IBA or NAA or IAA did not regenerate any roots. They grew taller instead and became dark green. This suggested that IBA, NAA and IAA (0–2 mg l^{-1}) were inadequate for the *in vitro* root formation of *G. gnemon*, Noochum & Te-chato (2003) studied the *in vitro* propagation of *Gnetum gnemon* Linn., reporting that *in vitro* rooting was impossible. Currently, there is not any published research on the success of *G. gnemon*'s *in vitro* rooting formation in Vietnam and all over the world as well.

Furthermore, the maximum rooting response (40%), the highest number of roots (4.20 roots/shoot) and the root length of 1.35 cm were recorded on MS medium added with 1.0 mg l^{-1} BAP in combination with 0.5 mg l^{-1} NAA. In other media, the rooting rate ranged from 10 to 20%. This result also indicated that increasing the concentrations of BAP led to a rise in the number of roots but a fall in the length of root.

Table 2. Effects of IBA, NAA, IAA and BA in combination with NAA on *in vitro* root formation

Treatment code	IBA (mg l^{-1})	IAA (mg l^{-1})	NAA (mg l^{-1})	BA (mg l^{-1})	No. of roots/explants	Root length (cm)	Root formation rate (%)
R0	0	0	0	0	0	0	0
I1	0.1				0	0	0
I2	0.5				0	0	0
I3	1.0				0	0	0
I4	2.0				0	0	0
A1		0.1			0	0	0
A2		0.5			0	0	0
A3		1.0			0	0	0
A4		2.0			0	0	0
N1			0.1		0	0	0
N2			0.5		0	0	0
N3			1.0		0	0	0
N4			2.0		0	0	0
NB1			0.5	0.1	1.80^{c*}	1.66^{a}	15^{bc}
NB2			0.5	0.5	3.00^{bc}	1.43^{ab}	20^{b}
NB3			0.5	1.0	4.20^{ab}	1.35^{b}	40^{a}
NB4			0.5	2.0	5.40^{a}	0.92^{c}	10^{c}
	ANOVA				**	**	**
	CV (%)				19.39	9.66	11.28

**significant at $p \leq 0.01$. Means in the same column followed by the same letters are not significantly different according to LSD-test

Thus, IBA, NAA and IAA are not suitable for the *in vitro* root formation. However a combination of 1 mg l^{-1} BAP and 0.5 mg l^{-1} NAA, stimulated the *in vitro* rooting of *G. gnemon*.

Effects of different substrates on the adaptability of plantlets in the greenhouse

The *in vitro* cultured plantlets of *G. gnemon* with stems, leaves and roots were transplanted onto the two kinds of substrate including coconut fiber powder and combination coconut fiber powder and sand (1:1). The adaptability and growth of *G. gnemon* plantlets after 90 days from transplantation were shown in the table 3 and Fig. 2a.

Table 3. Effects of different substrates on the adaptability of plantlets in greenhouse

Treatment code	Substrate	Plant height (cm)	Root length (cm)	Survival rate (%)
S1	Coconut fiber powder	6.14	6.90	90
S2	½ Coconut fiber powder + ½ Sand	5.12*	5.65^{*}	90^{ns}

ns, *: non-significant or significant at $p \leq 0.05$, respectively, according to T-test

In tissue culture, the transfer of grown plantlets from *in vitro* condition to greenhouse is a crucial stage. The in vitro plantlets are often cultured on the agar medium with high and stable humidity. Therefore, when being transplanted on the new substrate in greenhouse, they are easily dead due to the low air humidity.

The obtained results revealed that the survival rate of plantlets on the two substrates, coconut fiber powder and a mixture of coconut fiber powder and sand (1:1), both reached 90%. It suggested that the two substrates provided the airy space and appropriate humidity level for the acclimatization of *G. gnemon* in the greenhouse. However, while the plantlets on coconut fiber powder substrate were well grown with 6.14 cm height and 6.90 cm in root length, the plantlets planted on combination coconut fiber

powder and sand (1:1) just reach 5.12 cm height and 5.65 cm in root length. Currently, there are no published studies on transferring the *in vitro* plantlets to greenhouse.

As a result, the coconut fiber powder served as a better bed for the *ex vitro* grown plantlets of *G. gnemon* than combination coconut fiber powder and sand (1:1).

Effects of different IBA concentrations on *ex vitro* root formation

The *in vitro* shoots of *G. gnemon* in dark green were soaked in IBA solution at different concentrations of 0; 1; 2; 3; 4; 5 mg l^{-1} for 1 minute and then transplanted onto the substrate of coconut fiber powder. As being transferred to the natural environment, the *in vitro* shoots tend to easily get faded and dead. Therefore, after being immersed in IBA at different concentrations and transplanted on the cocopeat substrate, they needed covering with white nylon plastic to maintained the stable humidity for the shoots, then unwrapping and spraying water once every 3 or 4 days. After 60 days of taking care and observing, the ability to regenerate *ex vitro* roots of the shoots was shown in table 4 and Figs 2b, 2c, 2d. The results indicated that the shoots treated with IBA all regenerated the roots with different rates at different concentrations of IBA, as the following findings. The control treatment did not produce any root formation. When the concentration of IBA increased to 1 mg l^{-1} and 2 mg l^{-1}, the rate of root formation went up (35% and 60% respectively) with an increase in the number of roots (1.20 and 2.90 per explant respectively) and the root length as well (1.66 cm to 1.96 cm respectively). Next, by the treatment of 3 mg l^{-1} IBA, it led to the highest root formation rate at 80%. However, raising the IBA concentrations to 4 and 5 mg l^{-1} caused a decrease in the rooting rate, the number of roots and the root length (Table 4 and Fig. 2b). These results revealed that the low concentration of IBA speeded up the rate of root formation, the number of roots and the root length. The IBA at high concentration, by contrast, inhibited the root formation.

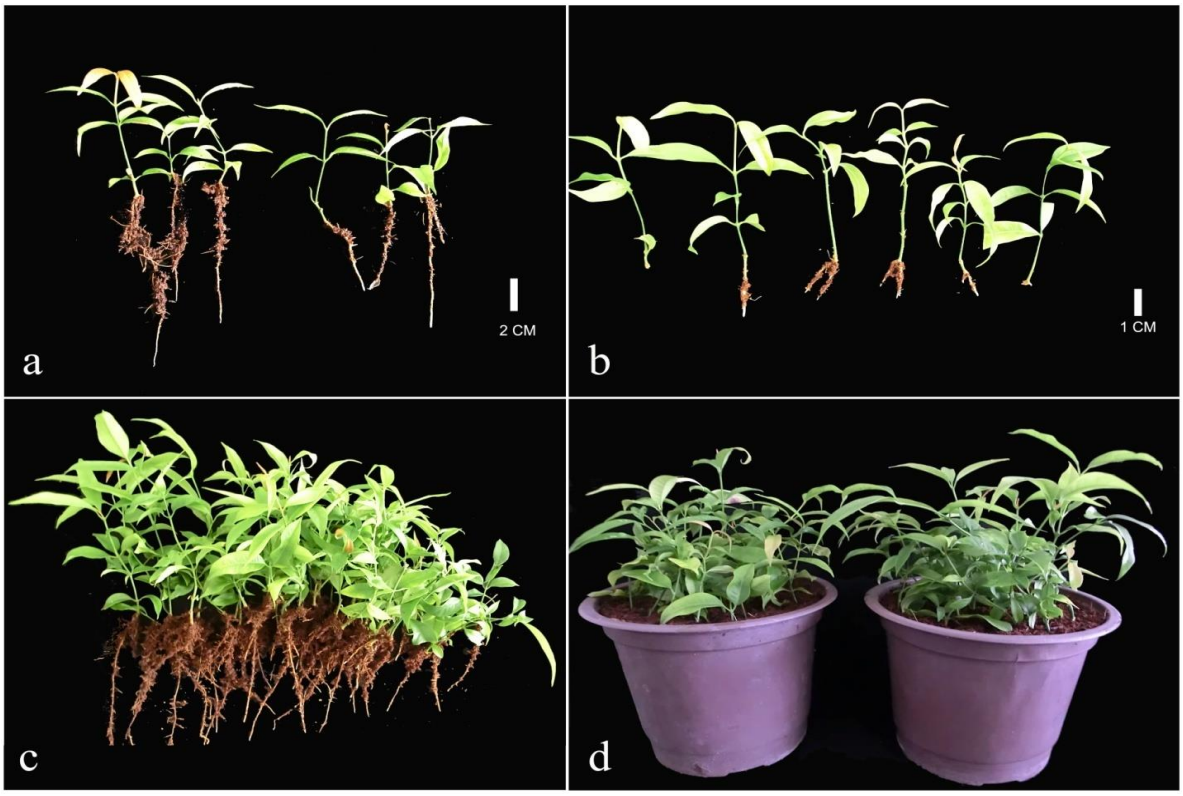

Figure 2. Study on *ex vitro* of *Gnetum gnemon* var. *griffithii* Markgr
Notes: The treatment codes were the same as those shown in table 3, table 4

Table 4. Effects of different IBA concentrations on *ex vitro* root formation

Treatment code	IBA (mg l^{-1})	No. of roots/explants	Root length (cm)	Root formation rate (%)
IB0	0	0.00	0.00	0
IB1	1.0	1.20^{c*}	1.66^{c}	35^{c}
IB2	2.0	2.90^{a}	1.96^{a}	60^{b}
IB3	3.0	2.80^{ab}	1.76^{b}	80^{a}
IB4	4.0	2.00^{abc}	1.45^{d}	40^{c}
IB5	5.0	1.18^{bc}	0.26^{e}	30^{c}
	ANOVA	**	**	**
	CV (%)	20.7	3.45	12.35

**significant at $p \leq 0.01$. Means in the same column followed by the same letters are not significantly different according to LSD-test

As a consequence, IBA at the concentration of 3.0 mg l^{-1} concentration is the best for the *ex vitro* root formation of *G. gnemon*. This result is different from Bullecer & Bullecer's research (2011), reporting that IBA at the concentration of 500 ppm produced the highest average number of *G. gnemon*'s roots.

CONCLUSION

In this research, we successfully investigated the propagation of *G. gnemon* from its nodal segment on MS medium supplemented with 1.5 mg l^{-1} BAP or 1.5 mg l^{-1} KIN. In addition, BAP with the concentration of 1 mg l^{-1} combined with 0.5 mg l^{-1} NAA was the best combination for the *in vitro* rooting. Being transferred to the greenhouse, the plantlets on the substrate of coconut fiber powder grew better than on combination coconut fiber powder and sand (1:1). Examining the *ex vitro* root formation, IBA with the concentration of 3 mg l^{-1} showed the highest results in the *ex vitro* root formation of *G. gnemon*. The results of this research help to build the process of *in vitro* propagation and *ex vitro* culture of *G. gnemon*.

***Acknowledgements**:* The authors would like to thank Tay Nguyen Institute for Scientific Research for funding the completion of this research.

REFERENCES

Barua C. C., Haloi P., Barua I. C., 2015. "*Gnetum gnemon* Linn.: A Comprehensive Review on its Biological Pharmacological and Pharmacognostical Potentials", Inter. J. Pharmaco n. *Phytochem. Res*, 7(3): 531–539.

Bullecer R. C., Bullecer G. H. C., 2011. Growth Response of Bago (*Gnetum gnemon*) Cuttings to Various Rooting Agents. *Asian. J. Biodivers*: 172–182. https://doi.org/10.7828/ajob.v2i1.97.

Celis G., Avalos G., 2013. Acclimation of seedlings of *Gnetum leyboldii* Tul. (Gnetaceae) to light changes in a tropical rain forest. *Rev. Biol. Trop*. 61(4): 1859–1868. https://doi.org/10.15517/rbt.v61i4.12857.

Vo Van Chi, 2003. Volume 1–Dictionary of common plants. Science and Technics Publishing, Ha Noi, Vietnam.

Duncan D. B., 1955. Multiple range and F tests. Biometrics, 11: 1–42. https://doi.org/10.2307/3001478.

Goenadi D. H., Sudharama I. M., 1995. Shoot initiation by humic acids of selected tropical crops grown in tissue culture. *Plant. Cel. Rep*., 15: 59–62. https://doi.org/10.1007/BF01690254.

Handayani T. T., 2012. Effect of IAA and Kinetin on growth of shoot tip explant of melinjo (*Gnetum gnemon* Linn.) Universitas Lampung, Bandar Lampung, Indonesia. (in Indonesian with English abstract).

Pham Hoang Ho, 1999. Volume 1 - An illustrated flora of Vietnam. Tre Publishing, Hanoi, Vietnam, pp.215.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue. *Physiol. Plant*, 15: 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Noochum P., Te-chato S., 2003. Factors affecting proliferation and elongation of shoots of Phak Liang (*Gnetum gnemon* Linn.) through tissue culture technique. Songklanakarin *J. Sci. Technol*., 25(5): 565–575. (in Thai language with English abstract)

Thaman R. R., Elevitch C. R., Wilkinson K. M., 2000. Multipurpose Trees for Agroforestry in the Pacific Islands, Permanent Agriculture Resources, Holualoa, Hawaii, 3–48.

Won H., Renner S. S., 2006. Dating dispersal and radiation in the Gymnosperm *Gnetum* (Gnetales)-clock calibration when outgroup relationships are uncertain, *Syst. Biol*., 55(4): 610–622. https://doi.org/10.1080/10635150600812619.