RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 56 Issue 1: 70-75 (February 2022)

The Effects of 2,4-Dichlorophenoxyacetic Acid and α-Naphthaleneacetic Acid on Biomass Increment, Rhizogenesis and Somatic Embryogenesis of Suspension-cultured Dinh Lang Cells [*Polyscias fruticosa* (L.) Harms]

T.T.B. Phuong, V.P. Trung, N.H. An, N.D. Tuan, P.T.T. Nguyen

10.18805/IJARe.A-605

ABSTRACT

Background: Dinh Lang [*Polyscias fruticosa* (L.) Harms] is a medicinal plant widely grown in Vietnam, with proven note-worthy health benefits. However, Dinh Lang's amounts of triterpenoid saponins could not meet the need of the pharmaceutical industry. Thus, this study's purpose is to figure out the optimal condition for raising Dinh Lang's cell biomass, rhizogenesis and somatic embryogenesis to provide materials for bioactive compound productions.

Methods: Different 2,4-dichlorophenoxyacetic acid and α-naphthaleneacetic acid concentrations (0.5, 1.0, 1.5 and 2.0 mg/L) were examined to determine the best amount of each plant growth regulator for raising cells' biomass, rhizogenesis and somatic embryogenesis. In each treatment, two grams of eight-week-old calli were cultured in 50 mL of liquid MS medium.

Result: It is demonstrated by the results that liquid MS medium containing 1.5 mg/L α-naphthaleneacetic acid has the capacity of producing the highest numbers of somatic embryos (489 embryos per flask) and rooted cells (259.5 cells per flask), while the fresh weight of cells cultured in the medium given 1.5 mg/L 2,4-dichlorophenoxyacetic acid reached its peak of 5.7 g.

Key words: Callus, Embryogenesis, *Polyscias fruticosa* (L.), Rhizogenesis, Suspension culture.

INTRODUCTION

Dinh Lang [*Polyscias fruticosa* (L.) Harms, Araliaceae] is a dicot evergreen shrub and distributes in Southeast Asian countries, including Vietnam. The secondary metabolites in the leaves and roots of this plant (mainly polyacetylenes and saponins) have note-worthy health benefits such as reducing inflammation, neutralizing toxins, anti-bacterial effects, promoting diuresis, reducing fever and anti-dysentery effects. Thus, Dinh Lang has long been utilized as a tonic in Asian countries (Huan *et al*., 1998).

However, the amounts of triterpenoid saponins in Dinh Lang are not sufficient for meeting the demand of the pharmaceutical industry. Therefore, the cell culture of [*P. fruticosa* (L.) Harms] could be used as an effective method of increasing biomass to produce large amounts of secondary metabolites without depending on the traditionally cultivated plants (Ahn *et al*., 1996).

Despite studies on cell suspension culture and somatic embryogenesis of several species in the Araliaceae family, including *Panax ginseng* (Ahn *et al*., 1996; Arya *et al*., 1993; Chang and Hsing, 1980) and *Acanthopanax koreanum* Nakai (Choi *et al*., 1997), there has been no published paper about producing somatic embryos, rooted cells and large biomass of Dinh Lang by using cell suspension culture.

Considering the lack of studies on Dinh Lang cell suspension culture, in this article, two auxins (2,4-D and NAA) were tested to initially determine the most suitable auxin and its optimal concentration for biomass raising, *in vitro* rhizogenesis and somatic embryogenesis of suspension-cultured Dinh Lang cells. By this means, a large source of Dinh Lang somatic embryos, roots and cell biomass could be created for bioactive compounds extraction and micropropagation.

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MATERIALS AND METHODS

This study (8/2019-4/2020) was conducted in the Department of Biology, Hue University of Sciences, 77 Nguyen Hue St., Vietnam.

The plant materials of the study were primary calli induced from the lower parts of Dinh Lang [*P. fruticosa* (L.) Harms] leaves. To obtain the calli, the leaves had been

Department of Biology, Hue University of Sciences, 77 Nguyen Hue St., Thua Thien Hue, Vietnam.

Corresponding Author: T.T.B. Phuong, Department of Biology, Hue University of Sciences, 77 Nguyen Hue St., Thua Thien Hue, Vietnam. Email: ttbphuong@hueuni.edu.vn

How to cite this article: Phuong, T.T.B., Trung, V.P., An, N.H., Tuan, N.D. and Nguyen, P.T.T. (2022). The Effects of 2,4-Dichlorophenoxyacetic Acid and α-Naphthaleneacetic Acid on Biomass Increment, Rhizogenesis and Somatic Embryogenesis of Suspension-cultured Dinh Lang Cells [*Polyscias fruticosa* (L.) Harms]. Indian Journal of Agricultural Research. 56(1): 70-75. DOI: 10.18805/IJARe.A-605.

Submitted: 19-10-2020 **Accepted:** 01-07-2021 **Online:** 25-09-2021

cultured on MS media (Murashige and Skoog, 1962) given 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) or 1.0 mg/L α-naphthaleneacetic acid (NAA) for 15 days before the beginning of this study.

To find the most suitable amount of time for callus culture, firm, light-yellow primary calli were aseptically separated into 2-to-3-millimeter piles, sub-cultured on solid MS media supplemented with 1.0 mg/L NAA or 1.0 mg/L 2,4-D in different culture durations (2, 4, 6, 8 and 10 weeks). The optimal culture duration would then be used to create the source of cells for suspension culture.

To identify the optimal auxin concentrations for Dinh Lang *in vitro* biomass increment, rhizogenesis and somatic embryogenesis, two grams of 8-week-old calli were cultured in a 250 mL Erlenmeyer flask containing 50 mL of liquid MS medium given 30 g/L sucrose and different concentrations of NAA or 2,4-D (0.5, 1.0, 1.5 and 2.0 mg/L). All the flasks were shaken at 120 rpm. The best auxin and its optimal concentration for suspension culture of Dinh Lang cells were determined by the following factors:

- The release and clusterization of cultured cells and the numbers of somatic embryos and rooted cells.
- The fresh biomass showing the growth level of the cells.

The release and clusterization of cells in the flasks were observed after 4, 8 and 12 weeks of culture. After 12 weeks, all suspension cultures were poured out and vacuum filtered. The cultured cells were then washed on the vacuum filter by using double distilled water. Finally, the fresh weight of the cells and numbers of somatic embryos and rooted cells per flask were determined.

All cultures were incubated at the temperature of 25 ± 2°C and the photon flux density of 68 µmol/m^{2}s. Liquid media (pH 5.8) were autoclaved at 121°C, 1 atm in 30 minutes and poured into 250 mL Erlenmeyer flasks capped by two nylon sheets, with 50 mL each. Solid media (pH 5.8) were sterilized as mentioned above and distributed into 100 mL glass bottles covered by two nylon sheets. Each glass bottle has 20 mL of solid medium.

The means in each experiment were analyzed by ANOVA and compared by Duncan's test (Duncan, 1955) with an alpha of 0.05. All experiments were done in triplicates so that the sample size of each treatment was 30.

RESULTS AND DISCUSSION

Optimizing callus culture duration

After being cultured for two weeks on MS medium supplemented with 1.0 mg/L 2,4-D, the calli did not show any sign of strong growth. Calli's size commenced to increase fast in the fourth week of culture and continued to the eighth week. In this week, Dinh Lang calli (firm and light yellow) reached their maximum sizes. The brown color and death of calli were observed in the tenth week of culture (Table 1, Fig 1).

In contrast to calli cultured on MS medium containing 1.0 mg/L 2,4-D, the ones stimulated by 1.0 mg/L NAA began to grow fast from the sixth week. In the eighth week, calli with the largest sizes were observed. However, the growth level of calli cultured on the medium with 1.0 mg/L NAA was lower than on the medium with 1.0 mg/L 2,4-D. The brown areas indicating dead cells also appeared in the tenth week of culture (Table 2, Fig 2).

In our study, Ding Lang calli began to grow in the second week of culture. This result is in line with the work of Bhat *et al*. (2020), claiming that *Solanum melongena* calli were observed after 15 days. However, the time needed for callus to start growing could be shorter or longer than two weeks depending on the cultured species. Specifically, while *Curcuma caesia* calli were observed after five weeks of culture, it only took 7-8 days for *Cajanus cajan*'s calli to be induced from epicotyl explants (Abubakar and Pudake, 2019; Padmavathi *et al*., 2017).

The results in Table 1 and Table 2 prove that 2,4-D is

Table 1: Effect of culture duration on characteristic and growth of calli cultured on MS medium supplemented with 1.0 mg/L 2,4-D.

Culture duration (week)	Characteristics	Color	Growth level
2	Soft, watery	White	+
4	Soft, watery	White	++
6	Friable, less watery	Light greenish yellow	+++
8	Strongly grown, firm	Greenish yellow	++++
10	Having tiny white spots and brown areas	Brownish yellow	-

Note (applied for Table 1 and 2): The growth levels of calli were determined by their diameters: ++++ Extremely strong (>2 cm); +++ strong (1-2 cm); ++ middling (0.5-1 cm); + weak (~0.5 cm); - no growth.

Fig 1: Induced calli cultured in MS medium supplemented with 1.0 mg/L 2,4-D in the 6th week (A), 8th week (B) and 10th week (C).

more suitable than NAA for Dinh Lang callus cell multiplication. 2,4-D was also considered the most efficient plant growth regulator for callus culture (Fonseka and Aluthgamage, 2020). This might explain why 2,4-D is often used for callus induction of species in the Araliaceae family. Specifically, Chang and Hsing (1980) used 1.0 mg/L 2,4-D to induce *Panax ginseng* C.A. Meyer calli. Moreover, Kim *et al.* (2019) reported that 2.0 mg/L is the optimal 2,4-D concentration for callus induction of interspecific ginseng hybrid between *P. ginseng* and *P. quinquefolius*, with a 100% induction rate.

There are also agreements among the results in Table 1, Table 2 and the ones in the study of other authors. Particularly, Thach *et al.* (2016) demonstrated that NAA could not stimulate the highest growth level of Dinh Lang calli (++++, profuse callus formation). Moreover, 2,4-D was also proven to be more efficient than NAA at enhancing callus induction and growth of other species, including *Centella asiatica* L. Urban, with the maximum induction rate of 83.33% (Tan *et al.*, 2010).

Effect of 2,4-D on callus suspension culture

As is shown in Table 3, 1.5 mg/L is the optimal 2,4-D concentration for callus suspension culture. In particular, the released callus cells strongly grew in the 8 week before forming large and dense clusters containing a high number of somatic embryos and several rooted cells in the 12 week.

Table 4 and Fig 3 present more clearly the positive effect of 1.5 mg/L 2,4-D. Specifically, calli cultured in basal MS medium could not survive and then became a thin layer of dead cells in the 12 week of culture. There were insignificant increases of callus biomass cultured in MS media given 0.5 mg/L and 1.0 mg/L 2,4-D. Additionally, no somatic embryos and rooted cells were observed in those media. However, calli in MS medium supplemented with 1.5 mg/L 2,4-D formed 13 somatic embryos per flask, several rooted cells (1.2 roots/flask) and the heaviest biomass of 5.7 g in the 12 week of culture.

Effect of NAA on callus suspension culture

Of all the tested media, MS medium with 1.5 mg/L NAA could produce the highest quantities of somatic embryos and adventitious roots. Particularly, the number of somatic embryos began to surge in the 8 week. Moreover, long and thick adventitious roots, which formed large root clusters, were obtained in the 12 week of culture (Table 5).

The stimulatory impacts of 1.5 mg/L NAA on rhizogenesis and somatic embryogenesis are specifically clarified in Table 6 and Fig 4. In particular, there was no

Table 2: Effect of culture duration on characteristic and growth of calli cultured on MS medium supplemented with 1.0 mg/L NAA.

Culture duration (week)	Characteristics	Color	Growth level
2	Soft, watery	White	+
4	Soft, watery	White	+
6	Friable	White	++
8	Firm, rooty	Light greenish yellow	+++
10	Having brown areas	Light brownish yellow	-

Fig 2: Induced calli cultured in MS medium supplemented with 1.0 mg/L NAA in the 6th week (A), 8th week (B) and 10th week (C).

Table 3: Characteristics of callus cells cultured in liquid MS medium supplemented with 2,4-D.

2,4-D concentration (mg/L)	Cultured callus cells' characteristic		
	After 4 weeks	After 8 weeks	After 12 weeks
0.0	Cells were released	Cell growth deteriorated fast	Flasks contained dead cells and opaque medium.
0.5	Cells were released	Released cells multiplied and increased in number and size	A low number of small senescent cell clusters was formed.
1.0	Cells were released	Released cells multiplied and increased in number and size	A high number of small cell clusters appeared. No somatic embryos and adventitious roots were observed.
1.5	Cells were released	Released cells multiplied and increased in number and size	Large and dense cell clusters were formed. somatic embryos and thin roots were formed.
2.0	Cells were released	Released cells multiplied and increased in number and size	Senescent cells were gradually deteriorated.

embryo and adventitious root in the medium given 0.5 mg/L NAA. Additionally, low numbers of 5.5 somatic embryos and 1.0 rooted cells were obtained from MS medium added with 1.0 mg/L NAA. However, these two values were rocketed by 1.5 mg/L NAA, with 489 embryos/flask and 259.5 roots/flask.

2,4-D is widely used for somatic embryogenesis and proven in studies on several species to possess a higher capacity of stimulating somatic embryo formation than NAA (Arya *et al.*, 1993; Choi *et al.*, 1997; Luo *et al.*, 1999). However, in the case of Dinh Lang, the results in our study demonstrate that NAA is better than 2,4-D at stimulating somatic

Table 4: Effect of 2,4-D on rhizogenesis and embryogenesis of cultured Dinh Lang callus cells.

2,4-D	Cell		Somatic embryo			Adventitious root		
concentration (mg/L)	Fresh weight (g)	Percentage (%)	Embryos /flask	Fresh weight (g)	Percentage (%)	Roots/ flask	Fresh weight (g)	Percentage (%)
0.0	0.00^{c*}	0.00	0.00^{b}	0.00^{b}	0.00	0.00^{a}	0.00^{a}	0.00
0.5	2.89^{b}	100.00	0.00^{b}	0.00^{b}	0.00	0.00^{a}	0.00^{a}	0.00
1.0	3.11^{b}	100.00	0.00^{b}	0.00^{b}	0.00	0.00^{a}	0.00^{a}	0.00
1.5	5.70^{a}	85.84	13.00^{a}	0.93^{a}	14.01	1.20^{a}	0.01^{a}	0.15
2.0	2.89^{b}	100.00	0.00^{b}	0.00^{b}	0.00	0.00^{a}	0.00^{a}	0.00

*Note (applied for Table 4 and 6): Within a column, means having a letter in common are not significantly different at the 5% level by Duncan's test.

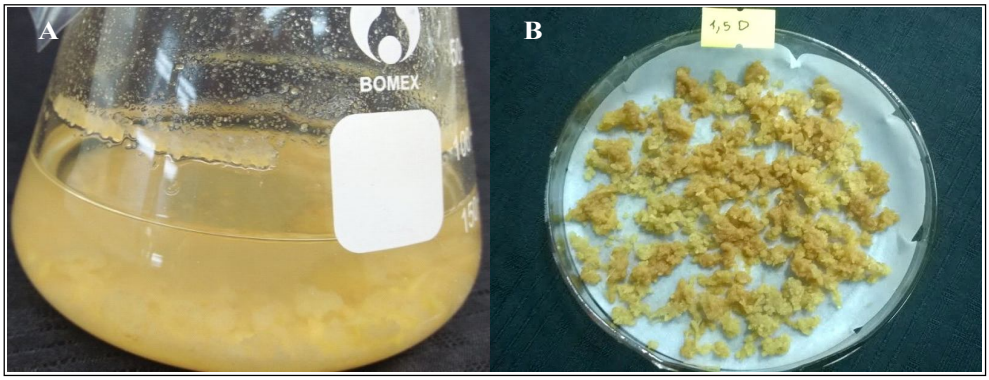

Fig 3: Suspension culture of Dinh Lang cells in liquid MS medium supplemented with 1.5 mg/L 2, 4-D (A) and the biomass obtained from this medium (B).

Table 5: Characteristics of callus cells cultured in liquid MS medium supplemented with NAA.

NAA concentration (mg/L)	Cultured callus cells' characteristic		
	After 4 weeks	After 8 weeks	After 12 weeks
0.0	Cells were released	Cell growth deteriorated fast	Flasks contained dead cells and opaque medium.
0.5	Cells were released	Released cells multiplied and increased in number and size	Cell clusters formed.
1.0	Cells were released	Released cells multiplied and increased in number and size	Higher numbers of cell clusters and somatic embryos were observed. Adventitious roots began to form. Cells in the medium are denser.
1.5	Cells were released	The number of somatic embryos formed by released cells significantly surged	The maximum number of long thick adventitious roots was reached. Induced roots formed large clusters.
2.0	Cells were released	Weak somatic embryogenesis was observed	A high number of short, thick and watery adventitious roots was observed. The number of root clusters was small.

Table 6: Effect of NAA on rhizogenesis and embryogenesis of cultured Dinh Lang callus cells.

NAA concentration (mg/L)	Cell			Somatic embryo			Adventitious root	
	Fresh weight (g)	Percentage (%)	Embryos /flask	Fresh weight (g)	Percentage (%)	Roots /flask	Fresh weight (g)	Percentage (%)
0.0	0.00^{c}	0.00	0.00^{c}	0.00^{c}	0.00	0.00^{c}	0.00^{b}	0.00
0.5	2.55^{a}	100.00	0.00^{c}	0.00^{c}	0.00	0.00^{c}	0.00^{b}	0.00
1.0	1.89^{ab}	96.92	5.50^{c}	0.05^{c}	2.56	1.00^{c}	0.01^{b}	0.51
1.5	0.1^{bc}	0.85	489.00^{a}	6.24^{a}	53.20	259.50^{a}	5.39^{a}	45.95
2.0	0.07^{c}	0.70	222.00^{b}	4.71^{b}	47.05	158.50^{b}	5.23^{a}	52.25

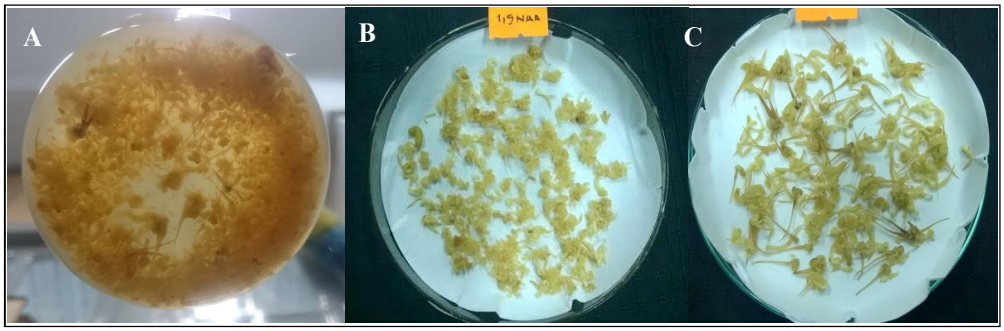

Fig 4: Suspension culture of Dinh Lang cells in liquid MS medium supplemented with 1.5 mg/L NAA (A); somatic embryos (B) and adventitious roots (C) obtained from this medium.

embryogenesis, with the number of NAA-induced embryos being approximately 38 times higher than that of 2,4-D-induced ones. This finding is similar to that observed by several authors such as Ozcan *et al.* (1993), Ozias *et al.* (1989) and Yang *et al.* (2018). Specifically, Ozcan *et al.* (1993) claimed that the number of *Pisum sativum* L. somatic embryos stimulated by NAA was higher than that in the medium added with 2,4-D. Ozias *et al.* (1989) concluded that the percentage of *Arachis hypogaea* L. nodular outgrowths in the medium given 20 mg/L NAA (72%) was larger than in the medium supplemented with 4 mg/L 2,4-D (24%). Yang *et al.* (2018) demonstrated that NAA at the concentration of 1.0 mg/L could stimulate a higher number of *Koelreuteria paniculata* Laxm. somatic embryos (54.75% induction rate) than all tested 2,4-D concentrations.

In addition to the great positive effect on somatic embryogenesis, Table 4 and Table 6 also clarified that NAA is more suitable than 2,4-D for inducing adventitious roots from suspension-cultured cells. Several studies also mentioned this observation. In particular, Ozcan *et al.* (1993) did not found *Pisum sativum* adventitious roots in the medium containing 2,4-D. Moreover, Sofiari *et al.* (1997) reported that there was a shift (created by NAA) from *Manihot esculenta*'s secondary somatic embryos to adventitious roots in several cultivars ("Gading", "MCol1505", "Line 11", "TMS90853" and "Adira 1"), while 2,4-D was not able to induce rooted cells. Additionally, Geneve and Kester (1990) proved that 100 μM NAA could stimulate an optimal number of 30.5 *Cercis canadensis*'s rooted cells, which could not be obtained using 2,4-D. Finally, Lazzeri *et al.* (1987) claimed that *Glycine max* adventitious roots were only formed in media containing NAA.

CONCLUSION

The most strongly growing calli were formed in solid MS medium given 1 mg/L 2,4-D after eight weeks of culture. Therefore, these are the best culture duration and growth stimulant for callus multiplication of Dinh Lang.

While the heaviest biomass (5.7 g) was obtained in liquid MS medium added with 1.5 mg/L 2,4-D, 1.5 mg/L NAA is more suitable than 2,4-D for cell's rhizogenesis and somatic embryogenesis, with 489 embryos/flask and 259.5 cells/flask, respectively.

REFERENCES

Abubakar, A.S. and Pudake, R.N. (2019). Sterilization procedure and callus regeneration in black turmeric (*Curcuma caesia*). Agricultural Science Digest. 39(2): 96-101. DOI: 10.18805/ag.D-4714.

Ahn, C.H., Yoon, W.M., Ahn, I.O. (1996). Effects of auxin and sucrose on cell growth and production of phenolic compounds in cell suspension culture of *Panax ginseng* C.A. Meyer. Journal of the Korean Society for Horticultural Science. 37: 340-344.

Arya, S., Arya, I.D., Eriksson, T. (1993). Rapid multiplication of adventitious somatic embryos of *Panax ginseng*. Plant Cell, Tissue and Organ Culture. 34: 157-162. DOI: https://doi.org/10.1007/BF00036096.

Bhat, S.G., Arulananthu, G., Ramesh, N. (2020). *In-vitro* Callus Induction and Regeneration of Brinjal (*Solanum melongena* L.) through Cotyledon. Indian Journal of Agricultural Research. A-5451. DOI: 10.18805/IJARe.A-5451.

Chang, W.C. and Hsing, Y.I. (1980). Plant regeneration through somatic embryogenesis in root-derived callus of ginseng (*Panax ginseng* C.A. Meyer). Theoretical and Applied Genetics. 57: 133-135. DOI: https://doi.org/10.1007/BF00253888.

Choi, Y.E., Kim, J.W., Soh, W.Y. (1997). Somatic embryogenesis and plant regeneration from suspension cultures of *Acanthopanax koreanum* Nakai. Plant Cell Reports. 17: 84-88. DOI: 10.1007/s002990050357.

Duncan, D.B. (1955). Multiple range and multiple *F* tests. Biometrics. 11: 1-42. DOI: https://doi.org/10.2307/3001478.

Fonseka, D.L.C.K., Aluthgamage, H.N. (2020). Callus Production Protocol for [*Aegle marmelos* (L.) Corr.]: As a Tool for Extraction of Secondary Metabolites. Agricultural Science Digest. DOI: 10.18805/ag.D-300.

Geneve, R.L. and Kester, S.T. (1990). The initiation of somatic embryos and adventitious roots from developing zygotic embryo explants of [*Cercis canadensis* (L.) cultured] *in vitro*. Plant Cell, Tissue and Organ Culture. 22: 71-76. DOI: https://doi.org/10.1007/BF00043701.

Huan, V.D., Yamamura, S., Ohtani, K., Kasai, R., Yamasaki, K., Nham, N.T., Chau, H.M. (1998). Oleanane saponins from *Polyscias fruticosa*. Phytochemistry. 47: 451-457. DOI: 10.1016/s0031-9422(97)00618-3.

Kim, J.Y., Adhikari, P.B., Ahn, C.H., Kim, D.H., Kim, Y.C., Han, J.Y., Kondeti, S., Choi, Y.E. (2019). High frequency somatic embryogenesis and plant regeneration of interspecific ginseng hybrid between *Panax ginseng* and *P. Quinquefolius*. Journal of Ginseng Research. 43: 38-48. DOI: 10.1016/j.jgr.2017.08.002.

Lazzeri, P.A., Hildebrandt, D.F., Collins, G.B. (1987). Soybean somatic embryogenesis: Effect of hormones and culture manipulations. Plant Cell, Tissue and Organ Culture. 10: 197-208. DOI: https://doi.org/10.1007/BF00037304.

Luo, J.P., Jia, J.F., Gu, Y.H., Liu J. (1999). High frequency somatic embryogenesis and plant regeneration in callus cultures of *Astragalus adsurgens* Pall. Plant Science. 143: 93-99.

Murashige, T. and Skoog, F.A. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 15: 473-497. DOI: https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Ozcan, S., Barghchi, M., Firek, S., Draper, J. (1993). Efficient adventitious shoot regeneration and somatic embryogenesis in pea. Plant Cell, Tissue and Organ Culture. 34: 271-277. DOI: https://doi.org/10.1007/BF00029716.

Ozias-Akins, P. (1989). Plant regeneration from immature embryos of peanut. Plant Cell Reports. 8: 217-218. DOI: https://doi.org/10.1007/BF00778536.

Padmavathi, Thangella, A.V., Fakrudin, B. (2017). *In vitro* callus induction and regeneration of multiple shoots through callus derived from leaf and epicotyl explants in pigeon pea (*Cajanus cajan* L.). Legume Research. 40(5): 785-794. DOI: 10.18805/LR-3615.

Sofiari, E., Raemakers, C.J.J.M., Kanju, E., Danso, K., Lammeren, A.M., Jacobsen, E., Visser, R.G.F. (1997). Comparison of NAA and 2,4-D induced somatic embryogenesis in Cassava. Plant Cell, Tissue and Organ Culture. 50: 45-56. DOI: https://doi.org/10.1023/A:1005844414258.

Tan, S.H., Musa, R., Ariff, A., Maziah, M. (2010). Effect of Plant Growth Regulators on Callus, Cell Suspension and Cell Line Selection for Flavonoid Production from Pegaga [*Centella asiatica* (L.) Urban]. American Journal of Biochemistry and Biotechnology. 6: 284-299. DOI: https://doi.org/10.3844/ajbbsp.2010.284.299.

Thach, B.D., Linh, L.N.T., Cang, D.T., Ben, T.T., Giang, T.T.L., Suong, N.K. (2016). Protocol establishment for multiplication and regeneration of [*Polyscias fruticosa* (L.) Harms]. An important medicinal plant in Vietnam. European Journal of Biotechnology and Genetic Engineering. 3: 31-37.

Yang, X., Yang, X., Guo, T., Gao, K., Zhao, T., Cheng, Z., An, X. (2018). High-Efficiency Somatic Embryogenesis from seedlings of *Koelreuteria paniculata* Laxm. Forests. 9: 769. DOI: https://doi.org/10.3390/f9120769.