

# IN VITRO ROOTING OF CLINACANTHUS NUTANS AS AFFECTED BY COMBINATION OF DIFFERENT MURASHIGE & SKOOG SALT AND IBA CONCENTRATIONS

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# IN VITRO ROOTING OF CLINACANTHUS NUTANS AS AFFECTED BY COMBINATION OF DIFFERENT MURASHIGE & SKOOG SALT AND IBA CONCENTRATIONS

By

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**Degree of Bachelor of Horticultural Science** 

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## **APPROVAL SHEET**

I certify that this research project report entitled "*In vitro* rooting of *Clinacanthus nutans* as affected by combination of different Murashige & Skoog salt and IBA concentrations" has been examined and approved as a partial fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of the degree of Bachelor of Horticultural Science in the Faculty of Agriculture, Universiti Putra Malaysia, Serdang Selangor Campus.

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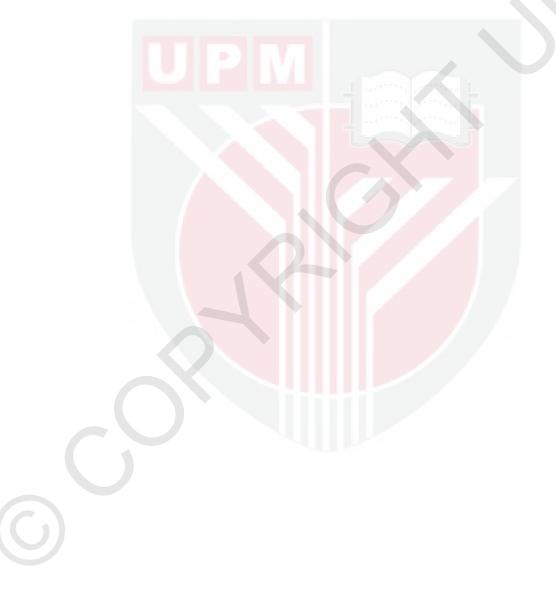
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# LIST OF ABBREVIATIONS

Indole acetic acid	IAA
Indole butyric acid	IBA
2,4-Dichlorophenoxyacetic acid	2,4-D
6-Benzylaminopurine	ВАР
Naphthalene acetic acid	NAA
Murashige and Skoog medium	MS
Gamborg B5 medium	В5
Linsmaier and Skoog medium	LS
Nitsch and Nitsch medium	NN
Sodium hydroxide	NaOH
Hydrochloric acid	HCl
Quarter strength Murashige and Skoog medium	<sup>1</sup> ⁄4 MS
Half strength Murashige and Skoog medium	½ MS
Milligram per Litre	mg/L
Pound per square inch	psi
Plus-minus sign	±
Metre squared per second	$m^{-2} s^{-1}$
Micromole	μmol
Statistical Analysis Software	SAS

 $\bigcirc$ 

## ABSTRACT

*Clinacanthus nutans* is a medicinal plant widely grown in tropical Asia and locally known "Belalai Gajah" or Sabah Snake Grass. Low reproductive capacity and nonuniform plant materials of *Clinacanthus nutans* propagated by stem cutting had brought to a study on the best combination of MS salt and IBA concentrations for rooting of *Clinacanthus nutans* explant under *in vitro* condition. This experiment was carried out at *In Vitro* Laboratory, Department of Agriculture, Universiti Putra Malaysia and conducted using Completely Randomized Design (CRD). Medium with full strength and half strength of Murashige and Skoog (1962) medium formulation were supplemented with indole butyric acid (IBA) at 0, 0.1, 1.0, 2.5 and 5.0 mg/L respectively. Shoot of axenic cultures of *Clinacanthus nutans* with a size of 2.5 cm was cultured in these treatments with ten replicates each. It has been observed that medium with half strength MS salt supplemented with 0.1 mg/L IBA gave good rooting of *Clinacanthus nutans* as it produced 90% of explant rooted with two roots formed per explant and a reasonable root length of 1.38 cm. This treatment gives an economical benefit by reducing the cost of planting material production.

### ABSTRAK

Clinacanthus nutans adalah tumbuhan ubatan yang tumbuh secara meluas di Asia tropika dan nama tempatannya dikenali sebagai Belalai Gajah atau 'Sabah Snake Grass'. Kajian mengenai kombinasi terbaik MS media dan kepekatan IBA untuk pengakaran dalam keadaan *in vitro* dilakukan kerana *Clinacanthus nutans* mempunyai kapasiti pembiakan yang rendah dan anak pokok tidak seragam apabila dibiak menggunakan kaedah keratan batang. Eksperimen ini dijalankan di Makmal In Vitro, Jabatan Pertanian, Universiti Putra Malaysia dan dilakukan dengan menggunakan Rekabentuk Rawak penuh (CRD). Formulasi kekuatan penuh dan kekuatan separuh media Murashige dan Skoog (1962) ditambah dengan asid indole butyric (IBA) pada 0, 0.1, 1.0, 2.5 dan 5.0 mg/L. Pucuk 'axenic' hasil sub-kultur daripada Clinacanthus *nutans* dengan saiz 2.5 cm dibiakkan dalam rawatan ini dengan menggunakan sepuluh replika. Pemerhatian menunjukkan bahawa medium dengan separuh kekuatan garam MS ditambah dengan 0.1 mg/L IBA memberikan pengakaran yang baik pada Clinacanthus nutans kerana ia menghasilkan 90% akar yang terbentuk dengan dua akar terbentuk bagi setiap eksplan dan panjang akar yang munasabah iaitu 1.38 cm. rawatan ini memberikan manfaat ekonomi dengan mengurangkan kos pengeluaran bahan tanaman.

## **1.0 INTRODUCTION**

Currently, the studies conducted on herbs in Malaysia are still under developing level and more research should be established for improving its production. One of the medicinal plants which can give benefits in the medicine line through proven research is *Clinacanthus nutans*. This medicinal plant has become well identified and research on it has been widened by several discoveries. This plant is a perennial herb widely grown in tropical Asia, especially in Malaysia, Vietnam, Indonesia, and Thailand. It is locally known as 'Belalai Gajah' or Sabah Snake Grass and it belongs to the Acanthaceae family.

Statistically, there are about 80% of the populations of some Asian and African countries still vastly use herbal medicine for some aspects of basic health care (Edgar et al., 2002). Malaysia is regarded as a country with a very rich biological diversity in the world. The tropical rainforests are rich for the growth of various types of herbs (Yusof, 2002).

*Clinacanthus nutans* is the most important species from the Acanthaceae family and it has been used as vital herbal medicine in tropical Asia and considered as one of the largest sources of medicinal plants used in traditional herbal medicine (Fazil et al., 2016). *Clinacanthus nutans* has diverse and potential medicinal uses in treating skin rashes, insects and snake bites, skin lesion caused by virus and diabetes (Lau et al., 2014). There is a research claim that, *Clinacanthus nutans* can treat several diseases including cancer. Arullappan et al. (2014) observed that the leaf extracts of *Clinacanthus nutans* are effective as antioxidant with antiproliferative effect on human cancer cell lines. Their study has been proven as an efficient alternative side treatment for cancer.

*Clinacanthus nutans* is usually propagated by stem cuttings and seeds. However, a limited number of plant materials can be produced using cuttings while non-uniform plant materials can be produced using seeds. In addition, it will cause low reproductive capacity due to slow growth and germination.

In order to fulfil high demand towards this plant, tissue culture technique is applied in order to regenerate the explant for conservation and rapid multiplication of a plant species (Bouhouche & Ksiksi, 2007). Therefore, *in vitro* propagation is another alternative for mass production of uniform planting materials. It does not require much labour and is also less time consuming.

Recently, there is a study on *Clinacanthus nutans* which was carried out by Nur Syazwani (2017) for her Final Year Project. Her study was focused on shoot multiplication using node cultures which were supplemented with various kinetin concentration. However, there is no study about *in vitro* rooting of *Clinacanthus nutans* treated with different MS salt formulation and IBA concentration have been done.

Besides that, the objective in defining two different MS medium formulation of full and half MS would help in maximizing the root initiation under *in vitro* condition and lead to an idea on minimizing the cost of medium preparation.

Therefore, this study was aimed to determine the best combination of MS salt and IBA concentrations for rooting of *Clinacanthus nutans* explant under *in vitro* condition.



## REFERENCES

- Arullappan, S., Rajamanickam, P., Thevar, N., & Kodimani, C. C. (2014). In vitro screening of cytotoxic, antimicrobial and antioxidant activities of *Clinacanthus nutans* (Acanthaceae) leaf extracts. Tropical Journal of Pharmaceutical Research, 13 (9), 1455–1461.
- **Bidarigh S. and Azarpour E. (2013).** Evaluation of the effect of MS medium levels on rooting in micro cuttings of tea (*Camellia sinensis* L.) under *In-vitro* culture condition. ARPN Journal of Agriculture and Biological Sciences. 8 (1), 24-28.
- Bouhouche, N. & Ksiksi, T. (2007). An efficient *In vitro* plant regeneration system for the medicinal plant *Teucrium stocksianum* Boiss. Plant Biotechology Report; 1 (4), pp 179-184.
- Briggs B. A. and S. M. McCulloch. (1984). Progress in micro propagation of woody plants in the United States and western Canada. Combined Proceedings. International Plant Propagators' Society. 33: 239-248.
- Dahab, A. M. Abou, Habib, Afaf M. A., Hosni, Y. A. and Gabr, A. M. M. (2004). Effect of MS-salt strength, sucrose and IBA concentration and acclimatization media on *Ruscus hypoglossum* L. micropropagation. Arab Journal Biotechnology, 8 (1): 141-154.
- Dewir, Y. H., El-Mahrouk, M. E., Murthy, H. N. and Paek, K. Y. (2015). Micropropagation of *Cattleya*: Improved *In vitro* rooting and acclimatization. Horticultural Environmental Biotechnology, 56 (1): 89-93.

- Edgar J, Elias B, Adnan B (2002). Biotechnology and the developing world. Electronic Journal of Biotechnology; 5 (1): 112-117.
- Fadel, Dani & Kintzios, Spiridon & Economou, A.S. & Moschopoulou, Georgia
  & Constantinidou, Helen-Isis. (2010). Effect of different strength of medium on organogenesis, phenolic accumulation and antioxidant activity of spearmint (*Mentha spicata* 1.). The Open Horticulture Journal, 3 (1): 31-35.
- Faisal M., Ahmad N., Anis M., Alatar A. A. and Qahtan A. A. (2018). Auxincytokinin synergism in vitro for producing genetically stable plants of *Ruta* graveolens using shoot tip meristems. Saudi Journal of Biological Sciences 25 (2): 273 – 277.
- Fazil, F. N. M., Azzimi, N. S. M., Yahaya, B. D., Kamalaldin, N. A., Zubairi, S. I. (2016). Kinetics extraction modelling and antiproliferative activity of *Clinacanthus nutans* water extract. Hindawi Publishing Corporation, The Scientific World Journal.
- Fong, S., (2015). Genetic, phytochemical and bioactivity studies of *Clinacanthus nutans* (Burm. f.) Lindau (Acanthaceae), Doctor of Philosophy (PhD), Applied Science, RMIT University.
- **George E. F., Debergh P. C., (2008).** Micropropagation: uses and methods. In: plant propagation by tissue culture (Edn 3) (George EF et al. eds), pp. 29 64.
- Gunasekaran U. (2014). Callus induction and plant regeneration studies of *Clinacanthus nutans* (Sabah snake grass). Bachelor thesis, Selangor: Universiti Tunku Abdul Rahman.

- Harahap F., Roedhy P., Suharsono, Cicik S., Suci R. (2014). *In vitro* growth and rooting of mangosteen (*Garcinia mangostana* L.) on medium with different concentrations of plant growth regulator. HAYATI Journal of Biosciences, 21 (4): p. 151-158.
- Hussey, G., (1983). *In vitro* propagation of horticultural and agricultural crops. In Plant Biotechnology. Mantell, S. H. and Smith, H. (Eds.). Cambridge University Press, Cambridge, pp: 111-138.
- Kamathi, S., Rajalakshmi, G., Savetha, S., Ayyappadas, M.P. (2011). In vitro regeneration of *Passiflora foetida* L., J. Res. Biol., 8, pp. 653-659.
- Koilpillai, Y. Justin, and S. Wilson (2010). In vitro propagation of Graptophyllum pictum L. (Acanthaceae) – a medicinal plant. Department of Biotechnology, Sathyabama University, Chennai-119, India. Journal of Pharmacy Research, 3 (9), 2201-2202.
- Kunsorn P, Ruangrungsi N, Lipipun V, Khanboon A, Rungsihirunrat K. (2013). The identities and anti-herpes simplex virus activity of *Clinacanthus nutans* and *Clinacanthus siamensis*. Asian Pacific Journal of Tropical Biomedicine, 3(4):284-290.
- Lakshmanan, P. and Gabriel, J. J. (2015). *In vitro* regeneration of *Ruellia Tuberosa* L. (Acanthaceae) through direct organogenesis. International Journal of Recent Scientific Research, 6 (5), p. 3988-3990.
- Lau, K., Lee, S., Chin, J., (2014). Effect of the methanol leaves extract of *Clinacanthus nutans* on the activity of acetylcholinesterase in male mice. Journal of Acute Disease; (1), 22–25.

- Li J. R. and Eaton G. W. (1984). Growth and rooting of grape shoot apices *in vitro*. Hort Science. 19: 64-65.
- Mat Ali, R., Abu Samah, Z., Musaadah Mustapha, N. & Hussein, N. (2010). ASEAN herbal and medicinal plants. ASEAN secretariat. Jakarta, Indonesia.
- Milliam, S. and Sharma, S. K. (2007). Soil free technique. Potato Biology and Biotechnology. Elsevier. Amsterdam, The Netherland, p. 705-716.
- Murashige T. and T. Skoog. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiology, 15: 473-497.
- Naik S. K., Pattnaik S., Chand P. K. (1999). *In vitro* propagation of pomegranate (*Punica granatum* L. cv. Ganesh) through axillary shoot proliferation from nodal segments of mature tree. Sciences Horticulture. 79: 175-183.
- Nur Syazwani Zakaria (2017). The effect of different kinetin concentration on shoot multiplication of *Clinacanthus nutans* node culture. A Final Year Project report submitted to the Faculty of Agriculture, Universiti Putra Malaysia.
- Odutayo, O. I., Amusa, N. A., Okutade O. O. and Ogunsanwo, Y. R. (2007). Determination of the sources of microbial contaminants of cultured plant tissues. Plant Pathology Journal, 6 (1), 77-81.
- Pacurar, D. L., Perrone, I. and Bellini, C. (2014). Auxin is a central player in the hormone cross-talks that control adventitious rooting. Physiologia Plantarum, 151: 83-96.

- Pospíšilová, J., Tichá, I., Kadleček, P., Haisel, D. and Plzáková, Š., (1999). Acclimatization of micropropagated plants to *ex vitro* conditions. Biol. Plant. 42:481–497.
- Raya, K. B., Ahmad, S. H., Farhana, S. F., Mohammad, M., Tajidin, N. E., and Parvez, A., (2015). Changes in phytochemical contents in different parts of *Clinacanthus nutans* (Burm. f.) lindau due to storage duration, *Bragantia*. 74 (4), pp. 445–452.
- Roger P. Hangarter, Michael D. Peterson and Norman E. Good. (1980). Biological activities of indoleacetylamino acids and their use as auxins in tissue culture. Plant physiology, 65, pp. 761 767.
- Rout G.R., A. Mohapatra and S. Mohan Jain. (2006). Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnology Advances. pp. 1-30.
- Sadeghi F, Yadollahi A, Kermani MJ, Eftekhari M. (2015). Optimizing culture media for in vitro proliferation and rooting of Tetra (*Prunus empyrean* 3) rootstock. Journal of Genetic Engineering and Biotechnology, 13(1):19-23.
- Sharma SK. (2011). Plant taxonomy. 2nd ed. New Delhi: Pacific Book International; p. 414.
- Sharma T., Modgil & Thakur M., (2007). Factors affecting induction and development of in vitro rooting in apple rootstock. Indian Journal of Experimental Biology, 45:824 – 829.

- Shekhawat, M. S., N. Kannan, M. Manokari, and C. Ravindran, (2015). In vitro regeneration of shoots and ex vitro rooting of an important medicinal plant Passiflora foetida L. through nodal segment cultures. Journal of Genetic Engineering and Biotechnology, 13 (2), pp. 209–214.
- Shekhawat, M. S., M. Manokari and C. P. Ravindran, (2016). Micropropagation, micromorphological studies and *in vitro* flowering in *Rungia pectinata* L. Research Article Hindawi Publishing Corporation Scientifica.
- Skoog, F. and Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symposium of the Society of Experimental Biology, 11, 118–130.
- Srikun N. (2017). In vitro propagation of the aromatic herb Strobilanthes tonkinensis Lindau. Agriculture and Natural Resources, 51 (1): 15-19.
- The Plant List (2013). The Plant List Website: Angiosperms-Acanthaceae-Clinacanthus-Clinacanthus nutans (Burm. F.) Lindau. Version 1.1. Available: <u>http://www.theplantlist.org/tp11.1/record/kew-2727901</u> (Retrieved on 11 February 2018).
- **Torres KC., editor. (1989).** Tissue culture techniques for horticultural crops. New York, London: Chapman and Hall.
- Vijendra, Poornima D. & Jayanna, S. G. & Kumar, Vadlapudi & Hari, Gajula & Rajashekar, J & Sannabommaji, Torankumar & Basappa, Giridhara & C. M.,
  A. (2017). Rapid *in vitro* propagation *of Lucas aspera* Spreng. A potential multipurpose Indian medicinal herb. Industrial Crops and Products, 107:281-287.

- Wiart, C. (2006). Medicinal plant of the Asia-Pacific: Drugs for The Future, Singapore. World Scientific Publishing Co. Pte Ltd, Singapore. P. 380.
- Wortley, A.H, (2007). Pollen wall development in flowering plants. Pubmed, 174(3), 483-98.
- Yadav, A. S., Yogesh Badkhane, Ajit K. Sharma, Sajad Hussain Bakhshi and D.
  K. Raghuwanshi. (2011). Effect of different plant growth regulators on *In-vitro* propagation of *Barleria Prionitis* L. a threatened medicinal plant. International Journal of Pharma and Bio Sciences. 2 (1) p. 438-444.
- Yian S., S. & Ismail, A. & B. Yin, K. (2013). Perspective and insight on *Clinacanthus nutans* Lindau in traditional medicine. International Journal of Integrative Biology, 14 (1):7.
- Yoke K. Y. (2013). *Clinacanthus nutans* extracts are antioxidant with antiproliferative effect on cultured human cancer cell lines. Evidence-Based Complementary and Alternative Medicine. 1 (1), p 1-8.
- Yusof, Z. A. M. (2002). Potential of developing cosmetics and cosmeceutical products from medicinal plant. The proceedings of the seminar on medicinal and aromatic plants, p. 1-17.