Malaysian Journal of Fundamental and Applied Sciences Vol.10, No.1 (2014) 49-52

MJFAS Malaysian Journal of Fundamental and Applied Sciences
ISSN:2289-5981 e-ISSN:2289-599X
UTM UNIVERSITI TEKNOLOGI MALAYSIA

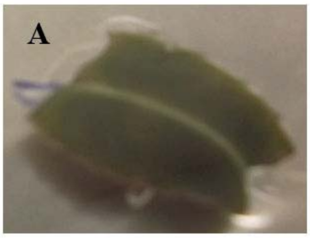
Plant growth regulator effect on adventitious roots induction of *Labisia pumila*

Nor'Aishah Hasan[1]*, Sobri Hussein[2] and Rusli Ibrahim[2]

[1]*Department of Biology, Faculty of Applied Science, UiTM, 72000, Negeri Sembilan, Malaysia*
[2]*Bioprocess and Agrotechnology Division, ANM, 43000, Selangor, Malaysia*
**Corresponding Author: aishahnh@ns.uitm.edu.my (N.A. Hasan)*

Article history :
Received 25 March 2013
Revised 15 June 2013
Accepted 16 July 2013
Available online 2 August 2013

GRAPHICAL ABSTRACT



ABSTRACT

Labisia pumila or locally known as 'Kacip Fatimah' is an herbaceous plant commonly used for dysentery, flatulence, dysmenorrhoea and gonorrhoea. A study was undertaken to investigate the effect of different plant growth regulators at various concentrations on adventitious root induction from the leaf explants of *Labisia pumila*. Murashige and Skoog (MS) medium supplemented with auxins; indole-acetic acid (IAA), indole-butyric acid (IBA) and naphtaleneacetic acid (NAA) each at the concentrations of 0, 1, 3, 5, and 7 mg/L were evaluated for their effects on adventitious root induction. No adventitious roots formed in the control medium devoid of any PGRs. Among the plant growth regulators (PGRs) used, IBA showed the best adventitious roots formation for all explants, followed by NAA and IAA. Leaf explants cultured on MS medium supplemented with 5 mg/L of IBA showed the best rooting ability. In this treatment, 72.4 ± 9.3 % of rooting, 17.8 ± 9.4 roots per explants and 0.123 ± 0.096g of dry weight were recorded. NAA and IAA showed ability to induce adventitious roots from leaf however the rooting ability was relatively lower that IBA treatment. The highest rooting ability for NAA and IAA was achieved in 7 mg/L and 3 mg/L with (71.2 ±10.9 % and 10.6 ± 3.6%) of rooting, (14.9 ± 0.669 and 1.60 ± 0.179) roots per explants and (0.044 ± 0.002g and 0.014 ± 0.001g) of dry weight, respectively.

Keywords: Adventitious roots, Auxin, Labisia pumila, Plant Growth Regulators, Secondary metabolites

http://dx.doi.org/10.11113/mjfas.v10n1.70

1. INTRODUCTION

Older generation were using plants as medicine and the knowledge was passed down orally. Nowadays, plants are used as alternative sources of secondary metabolites [1]. The discovery of medicinal plant for an active compound has been an interest for researcher to find novel treatment against variety of illness.

Labisia pumila is one of the valuable medicinal plants and has been used by locals as protective medicine before and after childbirth [2]. Interest in this plant was further enhanced due to its potential as a remedy for dysentery, flatulence, dysmennorhoea and gonorrhoea [8]. It was also found to have a high flavonoids and phenolic contents that are responsible for pharmacological activities. The key issues in commercialization of herbal-based-products are standardized and consistency of materials. Moreover, most plants produced a small quantity of secondary metabolites. Besides, other factors such as over-collecting, unsustainable agriculture practice, urbanization, pollution and climate change contribute to the plant extinction. Therefore, tissue culture techniques can be an alternative approach to maintain the sustainability supply of plant materials for producing bioactive compounds continuously under artificially controlled conditions [10]. Adventitious root cultures have also been proven to be suitable for continuous production of biochemical and molecular investigation of plant secondary metabolites.

Adventitious roots are roots develop on excised aerial plants parts (shoots or stems), or from an unusual point of origin on the plant (old roots that have undergone secondary metabolite). Adventitious roots are natural, grown vigorously in phytohormone supplemented medium and have shown tremendous potentialities of accumulation of valuable secondary metabolites [6].

Extensive studies in plant cell cultures optimization are therefore necessary to increase cell biomass and production of bioactive compounds and this will directly reduce the production cost for future commercial production. Plant growth regulators are the important factor affecting secondary metabolite production. Auxin is essential to induce cell differentiation and to maintain cell proliferation in vitro. The amount of auxin in medium is critical in controlling the growth and morphogenesis of plant tissues. Generally a high concentration or a low concentration of auxin supplemented in medium could promote or inhibit cell growth. Many synthetic auxin are extensively used because of their stability such as 2, 4-

dichlorophenopxyacetic acid (2, 4-D), 1-napthaleneacetic acid (NAA) and indole-3-butyric acid (IBA). In view of the importance of adventitious roots as well as the limited research on *L. pumila*, thus this study was carried out to investigate the effect of plant growth regulators at various concentrations on adventitious roots induction.

2. EXPERIMENTAL

2.1 Plant materials

In vitro plantlets of *Labisia pumila* that had been cultured on basal Murashige and Skoog (MS) medium were used as plant materials in this study. Only young and fresh leaf explants were selected throughout the experiment.

2.2 Culture medium

Full strength of MS medium was used in this study. The medium was prepared from each stock solution consisting of all the macronutrients, micronutrients, vitamins and Fe-NaEDTA. The auxins, indole-3-acetic acid (IAA) (Duchefa Biochemie, Netherlands), indole-3-butyric acid (IBA) (Duchefa Biochemie, Netherlands) and α-naphtaleneacetic acid (NAA) (Duchefa Biochemie, Netherlands) as plant growth regulator (PGR) were added singly to the medium at appropriate concentration. A total of 5% of sucrose (w/v) (Fischer Scientific, USA) was added into the media as a carbon source. The pH of the medium was adjusted to 5.8 ± 0.1 with 0.1 M NaOH or 0.1 M HCl prior to the addition of 0.8% (w/v) agar (Fisher Scientific, USA) and autoclaved at 121^{o}C for 15 min. approximately 25mL of the sterile medium were the poured into the 90 mm x 15 mm disposable sterile Petri dish (AxyDish, China) under laminar flow.

2.3 Effect of different plant growth regulators at various concentrations on leaf explants

Leaf explants were used to induce adventitious roots from *L. pumila*. The leaf (0.5 cm x 0.5 cm) explants were aseptically excised from intact in vitro plant. The study of leaf explants was initiated by placing the abaxial (lower) surface of the explants facing to the medium. In this study, three different auxins (IAA, IBA and NAA) at the concentration of 1, 3, 5 and 7 mg/L were used. The MS medium without any PGR was used as the control medium in each treatment. A total of 5 explants were cultured on each Petri dish and 3 replicate were prepared. All the treatments also were repeated twice. All the cultures were incubated in a culture room and allowed to grow at 25^{o}C ± 1^{o}C.

2.4 Data collection

The initiation of root explants was recorded on the basis of visual observations. Results were expressed as mean ± standard deviation. Observation on explants, the rooting percentage (%), dry weight (g), and number of roots formed per explants and any morphology changes were recorded continuously for 4 weeks. As for dry weight, the adventitious root induced in each treatment were excised and collected after 4 weeks of culture. Prior to blotting of the roots, the weight of the filter paper was determined and recorded. The roots were then collected on filter paper and allowed to air dry for 15 minutes. In order to measure the dry weight, the roots collected were oven-dried at mild temperature until a constant weight was obtained.

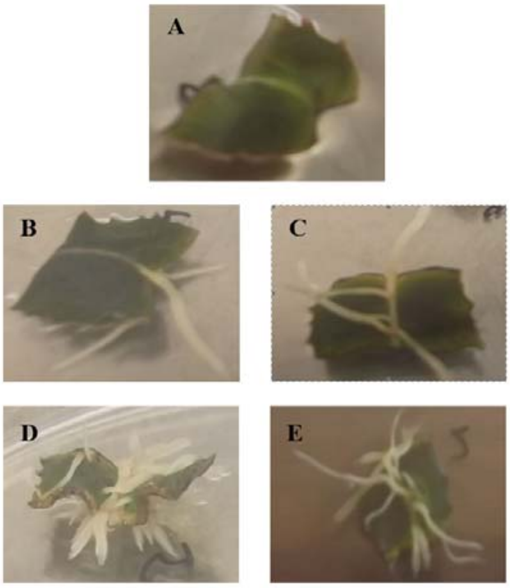
2.5 Statistical analysis

All data collected from each treatment were analyzed statistically using One Way Analysis of Variance (One-way ANOVA) followed by the Tukey's HSD test with the mean comparison was made by at least significant differences at the 5% probability level (p <0.05). All the statistical analysis was conducted using the statistical software of SPSS (version 12.0).

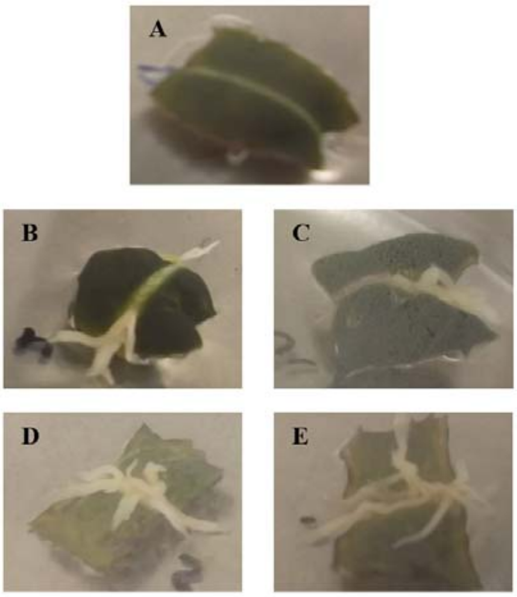
3. RESULTS & DISCUSSION

In the study of the effect of auxin concentrations on adventitious roots induction from leaf explants, it was found that no root was induced from control medium that did not contain any PGRs. However, medium supplemented with 1, 3, 5, and 7 mg/L of auxin showed different degree of adventitious roots formation.

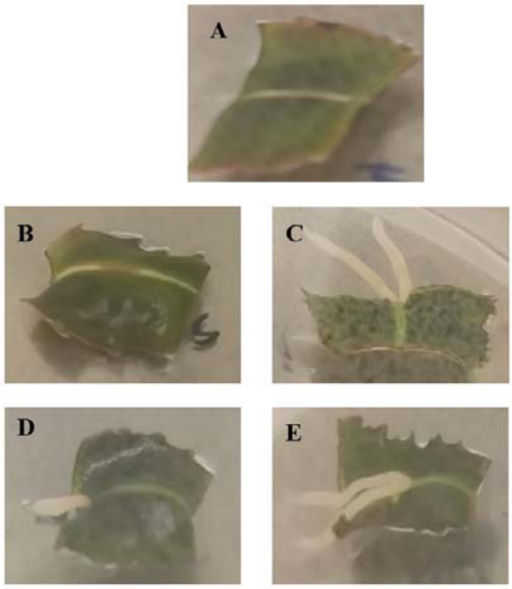
Generally, IBA was the best PGR in the induction of adventitious roots from leaf explants of *L. pumila* compared to NAA and IAA. All the IBA treatments successfully induced adventitious roots. As the concentration of IBA was increased from 1 to 5 mg/L, the percentage of explants forming roots, the number of roots per explants and the dry weight of roots also increased (Figure 1). Medium supplemented with 5 mg/L of IBA exhibited the highest rooting efficiency among all the concentration of IBA tested with 72.4 ± 9.3 % of rooting, 17.80 ± 9.4 roots per explants and 0.123 ± 0.096g of dry weight. When the concentration of IBA were increased from 5 to 7 mg/L, the percentage of rooting was decreased from (72.4 ± 9.3 % to 61.0 ± 5.2%), (17.80 ± 9.4 to 10.86 ± 1.81%), roots per explants and (0.123 ± 0.096g to 0.118 ± 0.01g) of dry weight. In current study, we consider that treatment with 5 mg/L of IBA was optimal in the adventitious roots formation from leaf explants. Similar studies had been reported by Ling et al., (2009) using *Centella asiatica*. Comparing with NAA and IAA, IBA has a unique characteristic of the rooting process because of its high ability to promote root initiation [7]. In other hand, according to Zolman et al., (2000), superior activity in rooting response also might be due to the differences in transport, uptake, degradation or metabolism of the plant used. IBA also induced rooting over a longer period of time compare to IAA because it is active and has a slower rate of conjugation. This had been reported in many plant species such as *Malus pumila*, *Pinus sylvestris*, *Populus tremula*, *Pyrus communis*, and *Vitis vinifera* and *Olea europaea* [4].



Scheme 1 Effect of IBA at various concentrations on the adventitious roots induction of *Labisia pumila*; (a) 0 mg/L, (b) 1 mg/L, (c) 3 mg/L, (d) 5 mg/L, (e) 7 mg/L



Scheme 2 Effect of NAA at various concentrations on the adventitious roots induction of *Labisia pumila*; (a) 0 mg/L, (b) 1 mg/L, (c) 3 mg/L, (d) 5 mg/L, (e) 7 mg/L



Scheme 3 Effect of IAA at various concentrations on the adventitious roots induction of *Labisia pumila*; (a) 0 mg/L, (b) 1 mg/L, (c) 3 mg/L, (d) 5 mg/L, (e) 7 mg/L

NAA was the second best PGR in adventitious root induction. Treatment with 7 mg/L recorded (71.2 ± 10.9%) of rooting, (14.93 ± 0.669) roots per explants and (0.044 ± 0.002g) of dry weight of roots marked as the highest value among all the concentration tested (Figure 2). Increasing NAA concentration from 1 to 7 mg/L showed an increasing value of rooting efficiency which expressed as percentage of rooting, number of explants formed and dry weight of roots. NAA was most effective auxin in stimulating rooting and correlated the faster uptake than IAA in the present study. This was proved by studied made by Ercisli et al., (2002) using *Baliospermum mintanum* and Mung bean [3]. The lower efficiency of NAA adventitious root induction could also explained by the fact of the relationships between levels of endogenous IAA and adventitious formation. It was suggested that the endogenous applied synthetic NAA have not been efficiently oxidized IAA for plant cell utilization. Thus, in the condition of insufficient supply of IAA, the explants will slow lower ability in rooting initiation. Besides, more energy are used up to converting synthetic auxins, NAA to natural form IAA before have been used by explants lead to insufficient energy for cell growth and development during root formation [5].

The least effective PGR in rooting response of *L. pumila* was IAA. MS supplemented with 3 mg/L of IAA was observed to have the highest rooting efficiency with (10.6 ± 3.6%) of rooting, (1.6 ± 0.179) roots per explants formed and (0.014 ± 0.001g) of dry weight of roots (Figure 3). As the hormone concentration increased from 3 to 7 mg/L, the percentage of rooting, number of roots per explants and dry weight of roots showed a decreasing value. However, studied made by Ling et al., (2009) discovered a better rooting in the IAA treatment for *Orthosiphon stamineus*. According to [9], IAA was naturally synthesized in apical part of plants likes leaves and shoot apices, then it will be transported downward to lower part of plant until it reaches the rooting zone. The reason of lower rooting in IAA might be due to factors like species dependent, in which different species were reported to response better in IAA.

Table 1. Effect of different auxins at various concentrations on the adventitious roots of *L. pumila*

Plant Growth Regulators	Concentration (mg/L)	Percentage of rooting (%)	No. of roots per explants	Dry weight of roots (g)
IBA	0	0.0 ± 0.0 a	0.0 ± 0.0 b	0.0 ± 0.0 b
	1	32.2 ± 8.5 a	4.8 ± 0.4 a	0.029 ± 0.002 a
	3	36.4 ± 2.5 a	5.4 ± 1.5 b	0.059 ± 0.015 b
	5	72.4 ± 9.3 a	17.8 ± 9.4 a	0.123 ± 0.096 a
	7	61.0 ± 5.2 a	10.8 ± 1.8 a	0.118 ± 0.01 a
NAA	0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	21.3 ± 5.2 a	3.2 ± 0.6 a	0.002 ± 0.0004 a
	3	30.6 ± 3.6 a	4.6 ± 0.5 b	0.008 ± 0.0004 b
	5	63.0 ± 12.5 a	12.9 ± 1.6 a	0.032 ± 0.008 a
	7	71.2 ± 10.9 a	14.9 ± 0.6 b	0.044 ± 0.002 b
IAA	0	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a
	1	1.3 ± 0.4 a	0.2 ± 0.1 b	0.001 ± 0.001 b
	3	10.6 ± 3.6 a	0.6 ± 0.1 a	0.014 ± 0.001 a
	5	4.5 ± 1.3 a	0.6 ± 0.1 a	0.005 ± 0.001 a
	7	4.8 ± 0.8 a	0.7 ± 0.1 b	0.005 ± 0.001 b

Values represent means ± standard deviations for three replications.
Values followed by same letter are significantly different at the $p \leq 0.05$ level, according to the Tukey test.

4. CONCLUSION

Adventitious roots culture can be an alternative approach in production of secondary metabolites. Thus further studies can be carried out by focusing the optimum parameter such as different sucrose concentration and medium pH in order to establish a root culture system for large scale and commercial production.

ACKNOWLEDGEMENT

The authors are grateful to Universiti Teknologi MARA and Malaysian Nuclear Agency for the facilities and support during the research work.

REFERENCES

[1] S. Ercisli, O. Anpali, A. Esitken, A. and U. Sahin, Gartenbauwissenschaft, 67 (2002) 34.
[2] A. J. Jamia, and P. J. Houghton, Seminar on Natural Products, (2000) 118.
[3] K. Karin, H. Maria, and L. Desana, Plant Growth Regulation, 46 (2005) 1.
[4] S. Krisantini, M. Johnston, R.R. William, and C. Beveridge, Scientia Horticulturae, 107 (2006) 171.
[5] A. P. K. Ling, M. F. Chin, and S. Hussein, Plant Science and Biotechnology, 3 (2009) 36.
[6] H. N. Murthy, E. J. Hanh, and K. Y. Paek, Chinese Journal of Biotechnology, 24 (2008) 711.
[7] A. Qaddoury and M. Amssa, Botanical Bulletin of Academia Sinica, 45 (2004) 127.
[8] Z. Rozihawati, H. Aminah, and N. Lokman, Malaysian Science (2003) 112.
[9] K. Terasaka, J. J. Blakeslee, B. Titapiwatanakun, W. A. Peer, A. Bandyopadhyay, and S. N. Makam, Plant Cell, 17 (2005) 2922.
[10] T. Thorpe, C. Stasolla, E. C. Yeung, G. J. De Klerk, A. Roberts, and E. F. George, Plant Propagation by Tissue Culture, Springer, Dordrecht, 2008.
[11] B. K. Zolman, A. Yoder, and B. Bartel, Genetics, 156 (2000) 1323.