Malays. Appl. Biol. (2015) 44(1): 115–118

PRELIMINARY STUDY OF *IN VITRO* PROPAGATION OF A SELECTED HIGH YIELDING CLONE OF KACIP FATIMAH (*Labisia pumila* VAR. *alata*)

SYAFIQAH NABILAH, S.B.[1,3*], FARAH FAZWA, M.A.[1], NOR HASNIDA, H.[2], SITI SUHAILA, A.R.[2], NORHAYATI, S.[1], NAZIRAH, A.[2], MUHAMMAD FUAD, Y.[2], HALIZA, I.[2] and MOHAMAD, O.[3]

[1]Plant Improvement Programme, Forest Biotechnology Division, Forest Research Institute Malaysia, 52109, Kepong, Selangor, Malaysia.
[2]Tissue Culture Laboratory, Forest Biotechnology Division, Forest Research Institute Malaysia, 52109, Kepong, Selangor, Malaysia.
[3]Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
[*]E-mail: syafiqah@frim.gov.my

ABSTRACT

Kacip Fatimah *(Labisia pumila),* an undershrub herb from the Myrsinaceae family, is traditionally used as medicine, particularly by Malay women during post-natal care. The development of *L. pumila* as a medicinal herb has led to its commercialization and increasing demand. To ensure its sustainable supply, the propagation of kacip Fatimah on a large scale such as through tissue culture needs to be explored. In this study, the leaves of a selected high yielding clone of kacip Fatimah, clone AA28, were used as explants. After surface sterilization, the explants were cultured on MS basal medium containing 0.5 mg/l NAA with different concentrations of BAP ranging from 0.05 to 0.15 mg/l for shoot induction. The explants cultured in MS medium containing 0.5 mg/l NAA in combination with 0.05 mg/l BAP produced the highest mean number of shoots per explants (10 ± 0.1). The shoot multiplication study is in progress and will be reported in a future paper.

Key words: *Labisia pumila*, shoot induction

INTRODUCTION

Labisia pumila (*L. pumila*) is one of the herbal plants with bright future in the global market. In Malaysia, *L. pumila* has been listed as one of the five important herbal plants that have potential to contribute to the gross national income (GNI) *via* the National Key Economic Area (NKEA) initiative. The initial phase of the entry project point (EPP) of the NKEA is to ensure sufficient supply of raw materials for research and development (R&D) and clinical trials before production takes place (PEMANDU, 2013). *Labisia pumila* is in high demand due to its phytoestrogenic activity deemed essential to protect the body against hormonal dependent cancers such as breast, uterine and prostate cancers (Lee *et al.*, 2012).

In its natural habitat, *L. pumila* was reported to have slow growth rate (Mohd Noh *et al.*, 2002). Propagation through seed *via* the conventional method takes a long time for germination to occur. Plus, the seedlings produced from seed are not homogenous and not suitable for commercial plantation. Nevertheless, the raw material of this species is continuously been exploited for various human purposes. In the long run, this herbal plant may face depletion in its natural habitat if little effort is put to cultivate it. This problem could be overcome by producing a large number of plantlets through tissue culture.

Previously, Hartinie and Azlan (2007) had successfully developed the *in vitro* germination and plantlet establishment of *L. pumila* using seeds as the explants. Ling *et al.* (2013) discovered the comparative effects of plant growth regulators (PGRs) on *in vitro* leaf and stem explants of *L. pumila* var. *alata*. Recently, Nor' Aishah and Sobri (2013) studied the response of stem and leaf explants of *L. pumila* to different PGRs in the formation of adventitious roots.

* To whom correspondence should be addressed.

In this paper, we focus on the *in vitro* propagation of a high yielding clone of *L. pumila* var. *alata* using leaf explants. The high yielding clone was obtained from a previous study by Farah Fazwa *et al*. (2012) on the selection of *L. pumila* varieties that yielded high phenolic contents for establishing planting stock. To ensure only good and quality raw material supply to the industry, the Forest Research Institute Malaysia (FRIM) has successfully identified five clones of *L. pumila* var. *alata* that contain high total phenolic contents (TPC). Thus, a further step is taken by producing these high yielding clones on a large scale *via* tissue culture. This study highlights the preliminary study done on one of the high yielding clones, namely clone AA28.

MATERIALS AND METHODS

Plant Materials

Five mother trees of *L. pumila* var. *alata* clone AA28 were grown at FRIM's nursery and the leaves were used as the explants for the tissue culture study. The explants were collected and washed under running tap water for 30 min followed by wiping with Teepol to remove the dirt and stains on the explant surfaces. The explants were rinsed with distilled water three times before soaking in 0.1% fungicide solution (THIRAM) with a drop of Tween 20 for one hour. After soaking, the explants were rinsed with distilled water three times and surface sterilized using 30% Clorox® with a drop of Tween 20 for 20 min. Then, the explants were rinsed with sterile distilled water three times and soaked in 70% ethanol with a drop of Tween 20 for 2 min. Again, the explants were rinsed with sterile distilled water three times before soaking in 20% Clorox® with a drop of Tween 20 for 10 min. The surface sterilization was completed after rinsing the explants with sterilized distilled water five times. The sterilized explants were then cut into pieces of 4-6 cm^2 size.

Initiation Media for Shoot Induction

The explants were cultured on MS basal medium (Murashige and Skoog, 1962) with the combination of 0.5 mg/L of 1-naphthaleneacetic acid (NAA) and each of three different concentrations of 6-benzylaminopurine (BAP) of 0.05, 0.1 and 0.15 mg/L. MS basal medium without any plant growth regulator (PGR) was used as control. Each treatment medium was assigned with one explant per bottle in three replicates. After 20 weeks in culture, observations of callus, root and shoot formations were recorded. All cultures were incubated in a growth room at 23 ± 2ºC for 16-h photoperiods.

Statistical Analysis

Experiments were repeated three times using a complete randomized block design. Analysis of variance (ANOVA) was carried out and differences between the means of the treatments were determined by Tukey's Multiple Comparison test at $p < 0.05$. The analysis of variance and means was carried out with Minitab 16 software.

RESULTS AND DISCUSSION

Clone AA28 is one of the high yielding clones of Kacip Fatimah that contain high total phenolic content. The cultivation of this species in large number could support our herbal industry by supplying high quality raw materials. After two weeks of culture, callus formation was observed in each treatment (Fig. 1a) except in MS basal medium (control). Adventitious roots started to initiate slowly from the callus after eight weeks of culture (Fig. 1b). Based on Tukey's test in Table 1, there was a significant difference observed in T1 at confidence level of 95%. Explants cultured in T1 showed the highest formation of adventitious roots (12.0 ± 2.3) in low concentration of NAA (0.5 mg/L) (Table 1). In comparison, 12.9 ± 1.6 numbers of roots were obtained per explant of *L. pumila* var. *alata* cultured in a high concentration of NAA (5 mg/L) (Nor' Aishah and Sobri, 2013). This finding shows that low concentration of NAA is sufficient to trigger formation of adventitious roots. After 16 weeks of culture, shoots started to initiate (Fig. 1c) and explants cultured in T1 produced the highest mean number of shoots (10.0 ± 0.1) compared with the other initiation media (Table 1). The combination of exogenous hormones (cytokinin BAP and auxin NAA) had facilitated in inducing and multiplying the *L. pumila* var. *alata* (clone AA28) plantlets but explants cultured in MS basal medium without any PGR (control) did not show any growth and turned necrotic after four weeks in culture. Using the combination of cytokinin and auxin in T1 medium is a promising approach to shoot initiation of *L. pumila* var. *alata* clone AA28. T1 medium has successfully initiated the highest mean number of shoots per explant. This medium will be used as a reference for the shoot multiplication study.

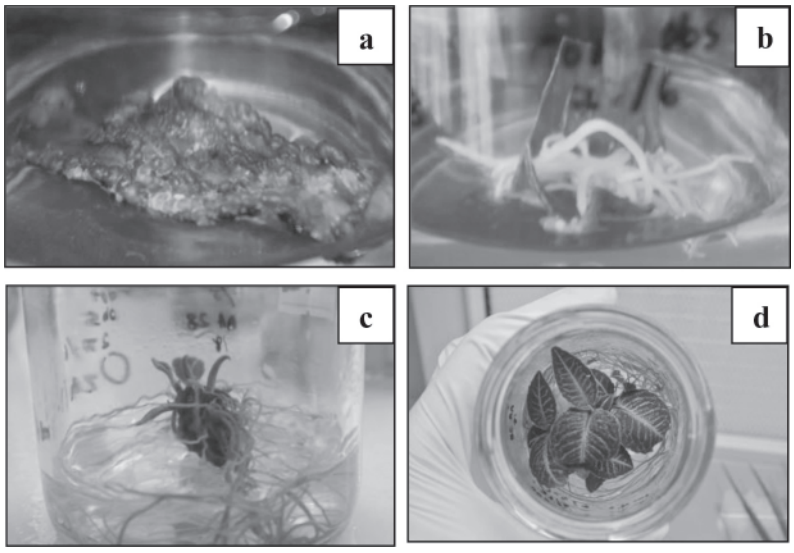

Fig. 1. Plant regeneration from leaf explants of *L. pumila* var. *alata* clone AA28. a) Callus formation after 2 weeks in T1 culture medium; b) Development of adventitious roots in T1 medium; c) Shoot formation at 16 weeks of culture in T1 medium; d) Shoots of *L. pumila* var. *alata* clone AA28 after 20 weeks in culture in T1 medium.

Table 1. Effects of plant growth regulators on root and shoots initiations from leaf explants of *L. pumila* var. *alata* clone AA28

Media	NAA concentration (mg/L)	BAP concentration (mg/L)	Mean no. of adventitious roots per explant	Mean no. of shoots per explant	Mean of plant height (cm)	Mean of leaf length (cm)	Mean of leaf width (cm)
T0	0	0	NA	NA	NA	NA	NA
T1	0.5	0.05	12.0a ± 2.3	10.0a ± 0.1	1.8ab ± 0.3	2.3a ± 0.3	1.3a ± 0.3
T2	0.5	0.1	5.0bc ± 3.8	6.0ab ± 0.3	1.8ab ± 0.3	2.1ab ± 0.1	1.2ab ± 0.3
T3	0.5	0.15	6.5ab ± 1.8	5.0bc ± 0.2	2.7a ± 0.2	2.0bc ± 0.2	1.2ab ± 0.3

NA = not applicable.
Means followed by different letters (a, b, c) within each row are significantly different at $p < 0.05$ by Tukey's multiple comparison tests. Mean ± SD.

ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Agriculture (MOA) for the National Research Grant Scheme (NRGS) (NH0612A013), and the Tissue Culture Laboratory and Plant Improvement Programme, FRIM for making this research a success.

REFERENCES

Farah Fazwa, M.A., Maiden, H., Mohamad, O. & Ab. Rasip, A.G. 2012. Selection of high yielding accessions of *Labisia pumila* (kacip Fatimah) for the production of superior planting stock. *Journal of Tropical Medicinal Plants.* **13(1)**: 17-27.

Hartinie, M. & Azlan, J.G. 2007. *In vitro* germination and plantlet establishment of *Labisia pumila*. *Scientia Horticulturae.* **115**: 91-97.

Lee, S.C., Norliza, A.L., Sze, Y.L., Chew, T.L., Mohamad Roji, S. & Ramlan, A.A. 2012. Flavonoids and phenolic acids from *Labisia pumila* (kacip Fatimah). *Food Chemistry*. **127**: 1186-1192.

Ling, A.P.K., Tan, K.P. & Hussein, S. 2013. Comparative effects of plant growth regulators on leaf and stem explants of *L. pumila* var. *alata. Journal of Zhejian University Science B*. **14(1)**: 621-631.

Mohd Noh, H.J., Mohd Jelani, B. & Mohd Akhir, A.H. 2002. Prestasi pertumbuhan empat variati kacip Fatimah di ladang semaian Sungkai, Perak. In: *Proceedings of Seminar on Medicinal and Aromatic Plants.* Chang Y.S., Mastura M. & Nurhanan M.Y. (Eds.). Forest Research Institute Malaysia (FRIM), Kepong, 20-21 August 2002. pp. 25.

Murashige, T. & Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiological Plant*. **15**: 473-497.

Nor' Aishah, H. & Sobri, H. 2013. Adventitious root induction of *Labisia pumila* in response to plant growth regulators and different type of explants. *Science Letters.* **7(1)**: 9-18.

Performance Management & Delivery Unit (PEMANDU). 2013. Economic Transformation Programme. Retrieved at etp.pemandu.gov.my.