PRODUCTION OF SUPERIOR CLONE *Labisia pumila* var *Alata* FOR FUTURE PLANTING STOCK

Syafiqah Nabilah Samsul Bahari[1, 2], Farah Fazwa Md Ariff[1], Nor Hasnida Hassan[1], Siti Suhaila A. Rahman[1], Nor Hayati Saffie[1], Mohd Zaki Abdullah[1] and Mohamad Osman[2]

[1] Forest Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor,
[2]Faculty of Plantation and Agroforestry, Universiti Teknologi MARA, Shah Alam, Selangor, MALAYSIA

Tel: +603-62797156; Fax: +603-62731314; E-mail: syafiqah@frim.gov.my

ABSTRACT

Labisia pumila is well known as the queen of herb in Malaysia due to its phytoestrogenic activity that is beneficial to women health. Since the raw material supply is limited, this study aimed to identify feasible propagation methods for future planting stock production of this herb. Two vegetative propagation techniques were investigated in this study, namely tissue culture and leaf cuttings. In tissue culture method, a combination of MS media with 0.5 mg L^{-1} NAA gave fast response in shoot development of *L. pumila* var. *alata* compared to other treatment media. The *in vitro* leaves explant were able to produce 4.33 ± 0.39 shoots per explants after being cultured for 16 weeks. The established plantlets from tissue culture were acclimatised in four different potting media, namely sand (100%), cocopeat (100%), jiffy (100%) and sand:cocopeat 50:50). Jiffy (100%) was found to be the best potting media during the acclimatisation of the plantlets compared to other treatment. While for leaf cuttings, the shoot development on rooted cuttings were observed only after 19 to 30 weeks of cutting period. The leaf cuttings can produce about 1.50 ± 0.65 shoots per cutting in 30 weeks interval. The growth performance of the plants from tissue culture and leaf cuttings were further evaluated at nursery stage. The results showed that tissue culture plants have more leaves (7.80 ± 0.22) compared to leaf cuttings (4.98 ± 0.18). Meanwhile, plants from leaf cuttings have better plant size over tissue culture plants in terms of leaf length, leaf width and collar region. However, the different leaf number and plant size did not reflect the total biomass of the plants. The ANOVA results showed no significant difference in fresh and dry weight of the plants from both sources of propagation where tissue culture plant recorded 2.55g ± 0.15 fresh weight and 0.41g ± 0.03 dry weight while leaf cuttings plant recorded 2.87g ± 0.14 fresh weight and 0.48g ± 0.05 dry weight. The total phenolic content (TPC) was also evaluated in this study. Both tissue culture and leaf cutting plant did not show significant value of TPC with 326.21±13.37 (Gallic acid equivalent) and 320.78±17.19 mg GAE $100g^{-1}$ respectively. The analysis showed that tissue culture method was more preferable since it was able to produce fast and higher number of shoots compared to leaf cuttings.

Keywords: *Labisia pumila*, leaf cuttings, micropropagation, shoots

INTRODUCTION

Labisia pumila or locally known as kacip fatimah is listed as one of the high-value herbal products that has bright future in the herbal industry. It is well recognised in containing phytoestrogen that is essential for women health care. Traditionally, it has been used in the form of water decoction for confinement mother. The advancement of biotechnology in Malaysia has discovered the benefit of *L. pumila* in various fields of applications, particularly in pharmaceutical and cosmeceutical purpose. Its positive effects include regulating body weight (Fazliana et al. 2009), preventing photoaging (Hyun-kyung et al. 2010) and possessing anti-bacterial and anti-fungal activities (Karimi et al. 2011; Ali and Khan 2011).

As the application of *L. pumila* is continuously being explored, many new products will be invented; consequently this increases the demand of raw material supply. It has been reported that 50% of the raw materials used by the local herbal industry came from forests (Zurinawati 2004). Over time, our herbal industry will face insufficient supply of local raw materials to feed the growing industry. Hence, mass propagation of *L. pumila* in commercial scale is relevant to be conducted to avoid such

problem. This herbal plant is naturally propagated through seeds which have slow growth rate in its own habitat. The quality and quantity of the plant available in the natural habitat is also uncertain (Mohd Setafarazi et al. 2001). As the demand on this herbal plant keep increasing by the herbal industry, a feasible propagation method of *L. pumila* should be explored. In this study, two vegetative propagation methods namely micropropagation and leaf cutting were evaluated on *L. pumila* var. *alata* based on their growth performances, total biomass and total phenolic contents.

MATERIAL AND METHODS

Micropropagation of *L. pumila* var. *alata*

In vitro leaves of *L. pumila* var. *alata* obtained from culture initiation stage were used as the explants in this study. The leaves explants were cultured in eight treatments of Murashige and Skoog (MS) medium containing 0.5 mg L^{-1} NAA, 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, 0.5 mg L^{-1} BAP, 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} NAA and 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA. MS medium without any plant growth regulator was set as control. Four leaf exlants were cultured in each treatment and replicate thrice. Cultures were incubated at 23 ± 2 °C with 16-h photoperiod.

Acclimatisation of tissue culture plantlets

The established plantlets produced were transferred into greenhouse for acclimatisation process. Four different potting media consisting of sand (100%), cocopeat (100%), jiffy (100%), and sand:cocopeat (50:50) were used during the acclimatisation . All of the plantlets were acclimatised in the glass chamber for two months (8 weeks). The growth performances of plantlets such as shoot height, number of leaf/explant, leaf length and leaf width were evaluated before and after the acclimatisation process.

Post acclimatisation process

After the acclimatisation process, the *L. pumila* var. *alata* were shifted to nursery. The growing medium was replaced with top soil: compost: sand in the ratio of 2:3:1.

Leaf cuttings of *L. pumila* var. *alata*

Matured leaf of *L. pumila* var. *alata* was used in this study. The leaves were cut into three different parts i.e. upper, middle and lower (Figure 1). The cuttings were treated immediately with Seradix 1 (0.1% IBA) before being inserted into the rooting medium. The cuttings were propagated in a mist propagation chamber for 12 weeks. A total of 180 cuttings were produced in a Completely Randomized Design (CRD) within three replicates. The observation on rooted cuttings was made during 3 to 12 weeks of cuttings.

Acclimatisation of rooted cuttings

After 12 weeks, the rooted cuttings (Figure 2) were transferred into growing medium consisting of top soil: leaf compost: sand (2:3:1). The rooted cuttings were acclimatized in an open mist propagation chamber for three weeks before being transferred to the nursery. The production of shoot was recorded during week 13 to week 30 after cuttings.

Figure 1. Determination of upper, middle and lower part of leaf cuttings

Figure 2. Rooted cuttings from different leaf part

Growth monitoring and data collection

A total of 60 ramets propagated through tissue culture and leaf cuttings were raised and maintained at FRIM's nursery. A known composition of fertilizer (Floramas) (NPKMgSCa, 20:15:10:1:3:2 + 1% trace elements) was applied to the plants in two weeks interval. The plant growth parameters were measured based on stem height, number of leaf, leaf length, leaf width and collar region. The data were collected once per month starting from acclimatisation stage until harvesting stage (9 months old). For total biomass (fresh and dry weights), the assessment was conducted during harvesting stage. The plants were randomly harvested and cleaned from remaining soils before weight. After fresh weight was recorded, the sample was dried in oven dryer (MEMMERT) for 48 hours. The dry weight was recorded when the balance gave constant reading.

The percentage of dry weight was calculated based on:-

$$\text{Dry weight (\%)} = \frac{\text{Dry weight (g)}}{\text{Fresh weight (g)}} \times 100$$

The percentage of moisture content was calculated by:-

$$\text{Moisture (\%)} = \frac{[\text{Fresh weight (g)} - \text{Dry weight (g)}]}{\text{Dry weight (g)}} \times 100$$

Assessment of total phenolic content

After 9 months of planting, the leaves samples were randomly harvested for total phenolic content analysis. Leaves were selected for the analysis because it contains high total phenolic content as compared to the other part of the plant (Karimi et al. 2011). The quantification of TPC were analysed by using Folin Ciocelteau (FC) reagent following the method by Singleton & Rossi (1965) with slight modifications (Vimala et al. 2003) The absorbance was measured at 725 nm by using UV Spectrophotometer with the unit of Gallic acid equivalent mg per 100g dry weight (GAE-TPC mg $100g^{-1}$).

Statistical analysis

Analysis of variance was used to analyse the variance between the treatments in both propagation techniques by using SPSS software version 22.

RESULTS AND DISCUSSION

Effects of plant growth regulator on shoot initiation

Among the eight treatment media, leaf explants of *L. pumila* var. *alata* gave response to MS + 0.5 mg L^{-1} NAA, MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA during 16 weeks of culture. The analysis of variance showed the eight treatment media gave significant difference at $p < 0.05$ on the mean number of shoots produced. The highest shoot formation was observed in the MS + 0.5 mg L^{-1} NAA with 4.33 ± 0.39 shoots per explants. The shoot formation was also observed in MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA with 4.17 ± 0.63 shoots per explant and MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA with 2.42 ± 0.26 shoots per explant. Meanwhile, leaf explant of *L. pumila* var. *alata* cultured in MS (control), MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, MS + 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, MS + 0.5 mg L^{-1} BAP and MS + 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} NAA did not show any response during the 16 weeks culture period.

The multiplication of explant culture began with the formation of adventitious root (Figure 3(a)) followed by shoot induction (Figure 3 (b)). The first adventitious root formation was observed after 22.00 ± 0.93 days of culture in MS + 0.5 mg L^{-1} NAA. The adventitious root formation in MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA were observed only after 29.25 ± 0.86 and 45.17 ± 1.05 days respectively. Shoot induction was observed after 65.7 ± 0.62 days of culture in MS + 0.5 mg L^{-1} NAA and later 79.00 ± 0.92 days in MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and 84.75 ± 0.86 days in MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA.

Based on the media optimisation, *L. pumila* var. *alata* favored MS media with low concentration of BAP and high concentration of NAA for regeneration. This finding was similar with Victor (2005) where the low concentration of BAP (0.1 mg L^{-1}) with high concentration of NAA (0.9 mg L^{-1}) had stimulates extensive explants enlargement in *Nicotiana tobacum* (tobacco). Meanwhile, no regeneration was observed in MS media containing high concentration of BAP with low concentration of NAA. It was also observed that no regeneration occurred in equal concentration of BAP and NAA. According to Trigiano and Gray (2004), high concentration of cytokinin usually inhibits the root development as occurred in MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA, MS + 0.5 mg L^{-1} BAP and MS + 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} NAA.

After 16 weeks of growing in culture medium, the growth data of established shoot in MS + 0.5 mg L^{-1} NAA, MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA were recorded as shown in Table 2. Four variables were evaluated such as shoot height, number of leaf produced per explants, leaf length and leaf width in each shoot. Of all the variables tested, the highest mean of shoot growth was obtained in MS + 0.5 mg L^{-1} NAA followed by MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA. Fast shoot induction occurred in MS + 0.5 mg L^{-1} NAA which was one of the factors that led to the highest growth performance of the shoots compared to other treatments. In this experiment, it can be concluded that treatment media with MS +

0.5 mg L^{-1} NAA, MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA were found to be ideal for the shoot multiplication of *L. pumila* var. *alata* whilst MS + 0.5 mg L^{-1} NAA gave the fastest and highest shoot production.

Table 1. Response of *L. pumila* var *alata* to different concentration of BAP and NAA

Treatment	Mean no. of shoots per explants	Time to first adventitious root formation (day)	Time to first shoots induction (day)
MS (control)	0	0	0
MS + 0.5 mg L^{-1} NAA	4.33a±0.40	22.00a±0.93	65.75a±0.62
MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	4.17b±0.63	29.25b±0.86	79.00b±0.93
MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	0	0	0
MS + 0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	0	0	0
MS + 0.5 mg L^{-1} BAP	0	0	0
MS + 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} NAA	0	0	0
MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA	2.42b±0.26	45.17c±1.05	84.75c±0.86

Means with the same letters are not significantly different at 0.05 level of confidence

Table 2. Growth performance of *L. pumila* var. *alata* after 16 weeks of culture in three treatment media

Treatment	Shoot height (cm)	No. of leaf per explant	Leaf length (cm)	Leaf width (cm)
MS + 0.5 mg L^{-1} NAA	3.10a±0.14	3.10a±0.10	1.40a±0.07	1.09a±0.04
MS + 0.1 mg L^{-1} BAP + 0.5 mg L^{-1} NAA	2.63b±0.07	2.69b±0.11	1.33a±0.04	0.88b±0.03
MS + 0.5 mg L^{-1} BAP + 1.0 mg L^{-1} NAA	1.97c±0.06	2.47c±0.19	1.08b±0.04	0.74c±0.12

Means with the same letters are not significantly different at 0.05 level of confidence

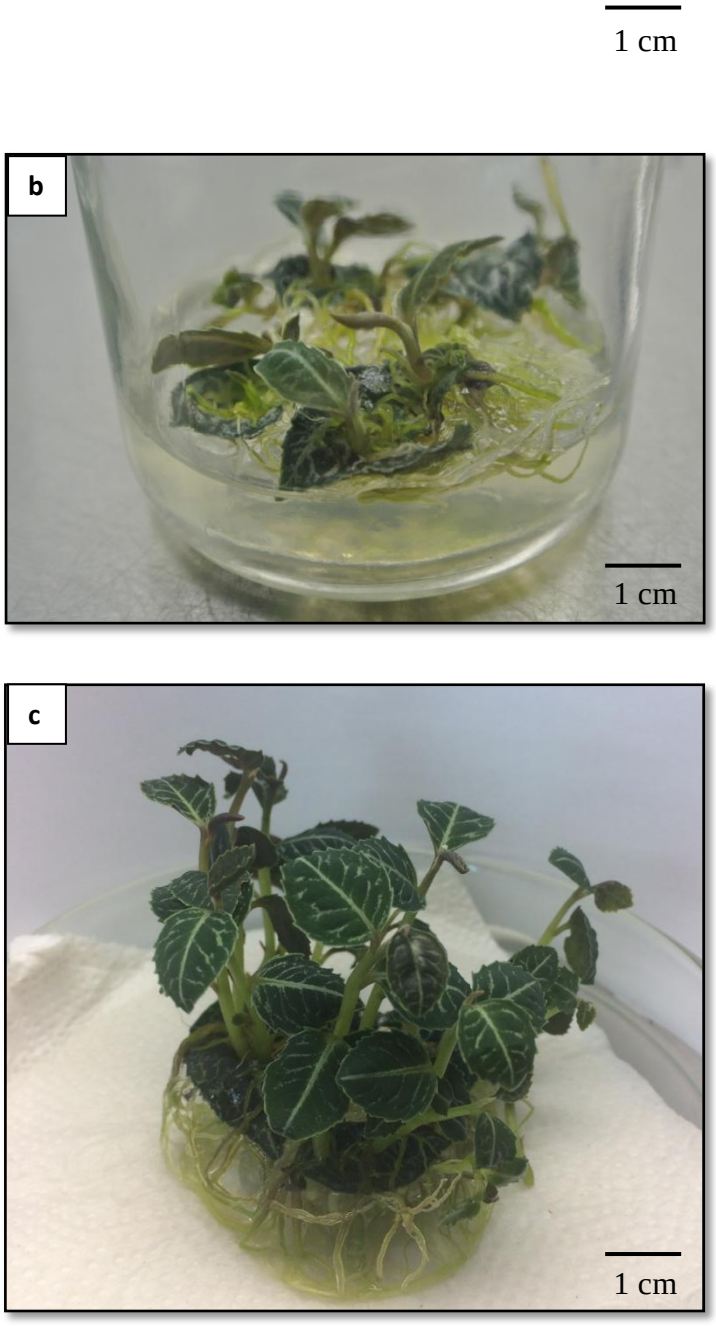

Figure 3. (a) Adventitious root formation in MS + 0.5 mg L^{-1} NAA; (b) Shoot induction in MS + 0.5 mg L^{-1} NAA at 10 weeks of culture; (c) Vigorous shoot of *L. pumila* var. *alata* taken out from MS + 0.5 mg L^{-1} NAA after 16 weeks of culture.

Table 3. Effects of different potting media on the growth of *L. pumila* var. *alata*

Potting media	Stem height (cm)	No. of leaf	Leaf length (cm)	Leaf width (cm)	No. of root	Root length (cm)
Cocopeat (100%)	$3.22^{a} \pm 0.22$	$3.30^{a} \pm 0.36$	$1.86^{a} \pm 0.19$	$1.15^{a} \pm 0.09$	$0.90^{a} \pm 0.28$	$0.91^{a} \pm 0.30$
Sand (100%)	$3.73^{a} \pm 0.33$	$5.72^{b} \pm 0.50$	$2.74^{b} \pm 0.23$	$1.62^{b} \pm 0.08$	$1.63^{a} \pm 0.30$	$1.72^{a} \pm 0.58$
Jiffy (100%)	$3.95^{a} \pm 0.29$	$6.75^{b} \pm 0.49$	$3.72^{c} \pm 0.26$	$2.00^{c} \pm 0.16$	$3.33^{b} \pm 0.58$	$1.86^{b} \pm 0.38$
Sand:cocopeat (50:50)	$4.07^{a} \pm 0.25$	$4.35^{a} \pm 0.44$	$2.26^{ab} \pm 0.19$	$1.28^{a} \pm 0.08$	$1.71^{a} \pm 0.46$	$4.62^{a} \pm 0.50$

The root performance of superior clone *L. pumila* var. *alata* plantlets were also evaluated after two months of acclimatisation. Plantlets grown in jiffy (100%) produced the highest number of primary root with the mean of 3.33 ± 0.58. While the root performance of plantlets in sand:cocopeat (50:50) and sand (100%) were not much different with 1.71 ± 0.46 and 1.64 ± 0.31 respectively. Plantlets grown in cocopeat (100%) potting medium showed poor rooting performance compared to others with only 0.90 ± 0.28 primary roots per plantlets. For the root length variables, the performance was shown by the total number of primary roots produced. The faster and greater number of roots produced will reflect the root length of the plantlets as shown in Table 3. The rooting performance of superior clone *L. pumila* var. *alata* plantlets in four different media were in ascending order of cocopeat (100%) < sand (100%) < sand:cocopeat (50:50) < jiffy (100%).

Based on the results obtained, jiffy (100%) was the most suitable potting media for *L. pumila* var. *alata* plantlets. Muhammad Fuad et al. (2015) also obtained similar results during the acclimatisation of tongkat ali. Jiffy (100%) consists of sphagnum peat and coir fiber offer quicker rooting due to the air pruning that stimulates fibrous root development within the plug. The crop cycle can be shortened up to 25% to produce stronger and more compacts plants.

Effects of different leaf cuttings part on the percentage of rooted cuttings and shoot production

The number of rooted cuttings was measured during week 3 to week 12 of cutting period (Figure 4). Root development of superior clone *L. pumila* var. *alata* in this study can only be observed at week 3 of cutting period. This finding was also supported by Rozihawati et al. (2005); Aminah et al. (2008); Farah Fazwa et al. (2013) and Syafiqah Nabilah et al. (2014). Based on Figure 3, the lower part of leaf cuttings rooted early compared to upper and middle part. However, the use of different leaf cutting part has no significant effect on rooting percentage of the cuttings based on analysis of variance (ANOVA) (Table 4). The lower part produced the highest rooting percentage (90%) followed by middle part (84%) and upper part (81%). The total number of primary root was measured at week 12 of cutting period. The three different parts of leaf cuttings produced around 2.76 to 2.86 primary roots.

After 12 weeks of cutting period, the rooted leaf cuttings were transferred to growing medium to enhance shoot production. The first shoot emergence was observed at cuttings of lower part in week 19 (Figure 5). The middle part of leaf cuttings started to produce shoot at week 20 while upper part at week 24. Most of the rooted cuttings in three different parts successfully produced shoot at week 30. The high accumulation of endogenous auxin in the lower part of the leaf cuttings could be the factor of early rooting production in this study as the natural auxin flow in stem tissue is in a biseptal direction (apex to base) (Hartmann and Kester 1968).

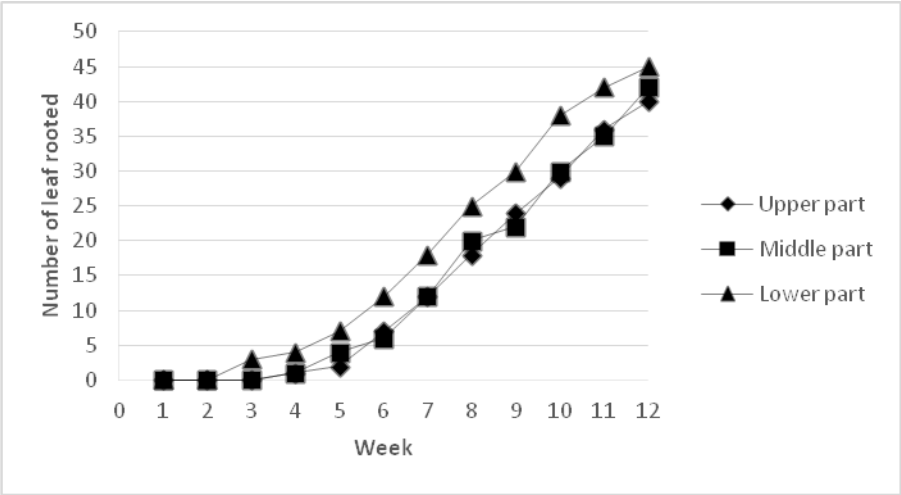

Figure 4. Number of rooted cuttings in different leaf cuttings part during the 12 weeks of observation

Table 4. Percentage of rooted cuttings and total number of primary root production in different leaf cuttings part

Source of variation	Levels	Rooting (%)	Total number of primary root
Leaf part	Upper	90.12[a]	2.76[a]
	Middle	83.84[a]	2.80[b]
	Lower	80.92[a]	2.86[a]

Means with the same letters are not significantly different at 0.05 level of confidence

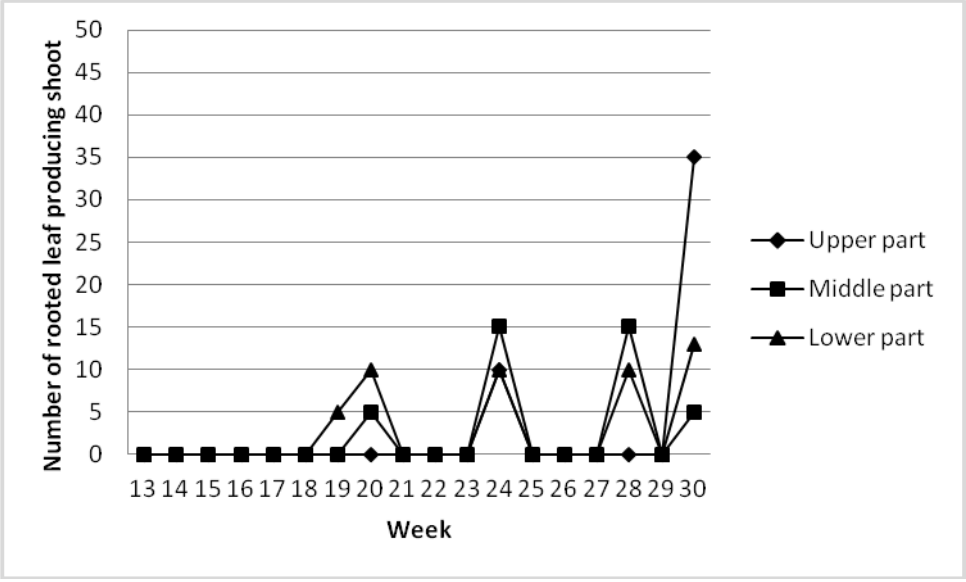

Figure 5. Observation on shoot production of three different cutting parts

Plant growth performance

The ANOVA has been conducted for the growth performances of superior clone *L. pumila* var. *pumila* at nine months of age. The analysis shows there is significant difference in the growth performances of plants produced from tissue culture and leaf cuttings except for stem height variable. The mean and standard errors (SE) of growth performance data were presented in Table 5.

Growth performances of the plants after nine months indicated that plant produced through tissue culture have greater leaf number compared to leaf cuttings plants. However, the latter propagation method has larger size of plant in terms of leaf length, leaf width and collar region. While, the shoot length of plants from both propagation method does not show any significant value with 5.13 ± 0.21 cm for tissue culture plants and 5.15 ± 0.21 cm for leaf cuttings plants.

Each shoot of tissue culture plants able to produce 7.80 ± 0.22 leaves number while leaf cutting plants only produced 4.98 ± 0.18 leaves number. On the other hand, the size of tissue culture plants (leaf length: 7.31 ± 0.39 cm; leaf width: 3.45 ± 0.11 cm) was observed smaller compared to leaf cuttings plants (leaf length: 8.56 ± 0.37 cm; leaf width: 4.10 ± 0.16 cm). Similar to leaf size, the collar region of *in vitro* plants was also smaller than leaf cuttings plants with 3.08 ± 0.13 mm and 3.31 ± 0.11 mm respectively.

The addition of plant growth hormones during *in vitro* propagation possibly influenced the vigorous leaf production and size of tissue culture plants. This finding is similar with Nehra et al. (1992), where the influence of plant growth hormones on callus regenerants of strawberry caused shorter petiole length and smaller leaf size but more leaves and runners under greenhouse condition. Furthermore, the tissue culture plants need gradual process of acclimatisation after *ex vitro* transplantation to adapt with the new environment and grow normally (Pospisilova et al., 1999).

Table 5. Growth performances of *L. pumila* var. *alata* from different source of propagation method at nine months of age

Source of propagation	Stem height (cm)	No. of leaf	Leaf length (cm)	Leaf width (cm)	Collar region (mm)
Tissue culture	5.13±0.21[a]	7.80±0.22[a]	7.31±0.39[b]	3.45±0.11[b]	3.08±0.13[b]
Leaf cuttings	5.15±0.21[a]	4.98±0.18[b]	8.56±0.37[a]	4.10±0.16[a]	3.31±0.11[a]

Means with the same letter are not significantly different at 0.05 level of confidence

Total biomass

The ANOVA test showed no significant difference between the fresh weight, dry weight, percentage of dry weight and percentage of moisture contents of *L. pumila* var. *alata* produced from tissue culture and leaf cuttings. Means of all the variables tested were recorded in Table 6.

In previous discussion, the growth performance of leaf cutting plants was observed greater than tissue culture plants in terms of leaf size and collar region. However, the difference in size of the plants did not influence the biomass and moisture content of the plant produced either through tissue culture or leaf cuttings. From the analysis, the percentage of dry weight in *L. pumila* var. *alata* was in the range of 15.97 ±0 .89 % to 16.44 ±0 .90 % while the moisture content was 83.55 ± 0.90 % to 84.02 ± 0.89 %. In other study, Anee et al. (2014) has reported the moisture content in leaf of selected clones from *L. pumila* var. *alata* was in the range of 73.78 ± 5.13 to 79.91 ± 0.67 whereas *L. pumila var. pumila* in the range of 72.03 ± 0.76 to77.74 ± 0.85. In large scale plantation of *L. pumila*, it is estimated that 4,000 to 5,000 kg of fresh weight can be obtained from 45,000 plants planted in one hectre field whereas about 800 to 1,000 kg of dry weight gain after drying process.

Table 6. Biomass and moisture content of plants from different source of propagation method at nine month age

Source of propagation	Fresh weight (g)	Dry weight (g)	Dry weight (%)	Moisture content (%)
Tissue culture	2.55±0.15[a]	0.41±0.03[a]	15.97±0.89[a]	84.02±0.89[a]
Leaf cuttings	2.87±0.14[a]	0.48±0.05[a]	16.44±0.90[a]	83.55±0.90[a]

Means with the same letter are not significantly different at 0.05 level of confidence

Total phenolic content

The total phenolic contents of plants sourced from tissue culture and leaf cuttings were also estimated at the age of nine months. The ANOVA test showed no significant difference between the total phenolic contents of the plants in both propagation methods as shown in Figure 6.

At nine months of age, the total phenolic content in tissue culture plants was about 326.21 ± 13.37 mg GAE $100g^{-1}$ and leaf cuttings plants yield about 320.78 ± 17.19 mg GAE $100g^{-1}$. In comparison with Farah Fazwa et al. (2012), the screening of total phenolic contents in *L. pumila* var. *alata* collected from five populations was in the range of 971.42 ± 59.53 to 2680.80 ± 25.17 mg GAE $50g^{-1}$. Since it is collected from wild, the exact age of the plants is unknown. Age can be one of the factors that influence the difference yield of total phenolic contents in these two studies. Achakzai et al (2009) also reported that aged plant parts usually contained greater level of secondary metabolites.

Different standard used by different investigators also may give different reading of the total phenolic contents. For example, Norhaiza et al. (2009) recorded the values of total phenolic contents in *L. pumila* were between 2.53 to 2.22 mg per g of fresh weight. Whereas Mohd Hafiz et al. (2011) recorded the amount of total phenolic contents in *L. pumila* ranging from 0.23 to 1.01 mg GAE per g dry weight.

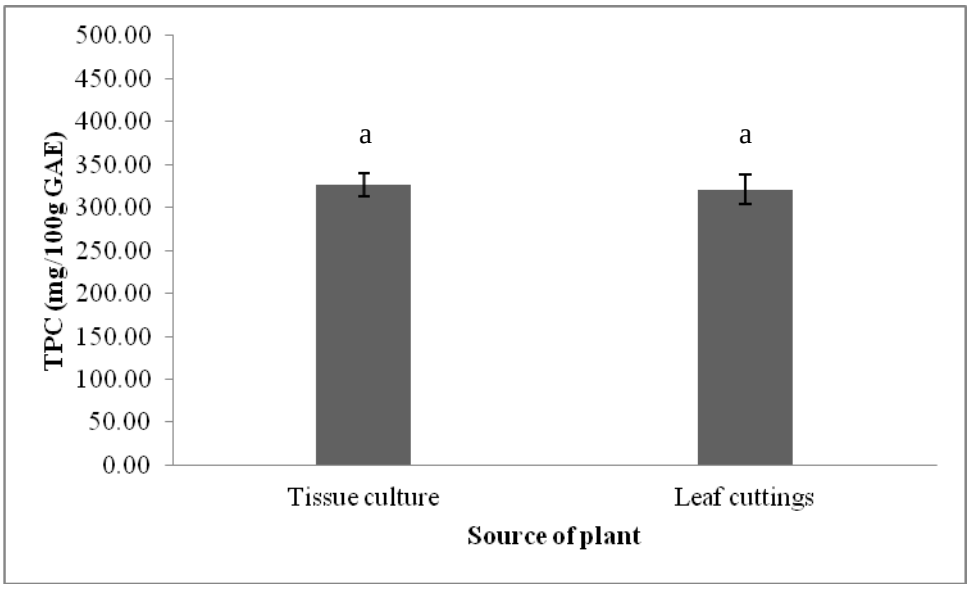

Figure 6. Total phenolic contents of Clone KF08 source from tissue culture and leaf cuttings at nine months of age.

CONCLUSIONS

The current results suggested that tissue culture is the feasible propagation method for multiplication of *L. pumila* var. *alata* in large scale. The process of plantlets establishment through tissue culture (24 weeks) was more rapid than leaf cuttings (30 weeks) and the number of shoots produced in tissue culture (4.33) technique was higher compared to leaf cuttings (1.50). The results from this study can be the baseline and reference for the mass production of *L. pumila* species in future.

REFERENCES

Achakzai AKK, Achakzai P, Masood A, Kayani SA and Tareen RB, 2009. Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. *Pak J Bot* **41**(5), 2129-2135.

Anee Suryani S, Farah Fazwa MA, Zunoliza A, Norhayati S, Syafiqah Nabilah SB and Mohd Zaki A, 2014. Total phenolic content of Labisia pumila var alata from selected forest reserve in Peninsular Malaysia**.** Poster presented at the 13th Malaysia Society of Applied Biology Symposium: The New era, The Legend Resort, Cherating Kuantan, Pahang, 8-10 Jun 2014.

Ali Z and Khan IA, 2011. Alkyl phenols and evaluation of an aqueous extract of *Labisia pumila*, saponins from the roots of *Labisia pumila* a Malaysian herb (Kacip Fatimah). *Phytochemistry* **72**, 2075-2080.

Aminah H, Naimah CL, Mohd Zaki A. and Lokmal N, 2008. Rooted leaf cuttings of *Labisia pumila*. *J Trop Med Plants* **9**(1), 593-599.

Karimi E, Hawa ZEJ and Sahida A, 2011. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of *Labisia pumila* Benth. *Molecules* **16**, 4438-4450.

Farah Fazwa MA, Maideen H and Mohamad O, 2012. Selection among two varieties of *Labisia pumila* that yield high phenolic contents for establishing plant stock and further cultivation. *J Trop Med Plants* **13**(1), 17-27.

Farah Fazwa MA, Norhayati S, Syafiqah Nabilah SB and Mohd Adi Faiz AF, 2013. Evaluation of rooting ability of five superior genotypes of *Labisia pumila* var. *alata* on sand media. Proceedings of Soil Science Conference of Malaysia. Pp 329-333.

Fazliana M, Wan Nazaimoon WM, Gu HF and Östenson CG, 2009. *Labisia pumila* extract regulates body weight and adipokines in ovariectomized rats. *Maturitas* **62**(1), 91–97. http://doi.org/10.1016/j.maturitas.2008.10.004.

Hartmann T and Kester E, 1983. Plant Propagation. Prentice-Hall, Inc. Englewood Cliffs, New Jersey. pp. 298.

Hyun-kyung C, Dong-hyun K, Jin Wook K, Sulaiman N, Mohamad Roji S and Chang SP, 2010. *Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation. *J Biosci Bioeng* **109**(3), 291–296.

Lars S, 1993. Vegetative propagation: Guidelines on air layering and cuttings. FAO Corporate Document Repository. Retrieved from http://www.fao.org/docrep/006/ad224e/AD224E00.htm

Mohd Setefarzi MN and Mansor P, 2001. Status penggunaan herba oleh pengilang tempatan dan potensi penanaman di Malaysia. Proceedings of the Seminar on Medicinal and Aromatic Plants pp. 139-146.

Muhammad Fuad Y, Hassan NH, Abdullah N, Rahman A, Suhaila S, Ismail H and Koter R, 2015. Acclimatisation of *Eurycoma longifolia* (Tongkat Ali) Plantlets to *Ex Vitro* Conditions. *J Trop Resources Sustainable Sci* **3**(1), 129-131.

Murashige T and Skoog F, 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol Planta* **15**, 473-497.

Nehra NS, Kartha KK, Stushnott C and Giles KL, 1992. The influence of plant growth regulator concentrations and callus age on somaclonal variation in callus culture regenerants of strawberry. *Plant Cell Tiss Org* **29**(3), 257-268.

Norhaiza M, Maziah M. and Hakiman M, 2009. Antioxidative properties of leaf extracts of a popular Malaysian herb, *Labisia pumila*. *J Med Plants Res* **3**, 217-223.

Pospóšilová J, Tichá I, Kadleček P, Haisel D & Plzáková Š, 1999. Acclimatisation of micropropagated plants to *ex vitro* conditions. *Biol Planta* **42**(4), 481-497.

Rozihawati **Z, Azmy M and Aminah H,** 2005. Pembiakan tampang melalui keratan bahagian batang *Labisia pumila* (Kacip Fatimah). Proceedings of The Seminar on Medicinal and Aromatic, Pp. 368-373.

Singleton VL and Rossi JA, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* **16**(3), 144-158.

Syafiqah Nabilah SB, Farah Fazwa MA, Norhayati S, Mohd Zaki A and Mohamad O, 2014. Propagation of five high yielding clones of *Labisia pumila* var. *alata* and evaluation of their growth performances at nursery stage. *Int J Agric Sci Res* **3**(7), 121-125.

Trigiano RN and Gray DJ, (Eds.). 2004. *Plant Development and Biotechnology*. CRC Press.

Victor PG, 2005. Plant Growth Regulators in Plant Tissue Culture and Development. In Trigiano R. and Gray, D. (Eds), *Plant Development and Biotechnology* (Pp. 87-100). Boca Raton, Florida: CRC.

Vimala S, Adenan MI, Ahmad AR, Shahdan R, Adenan MI, Ahmad AR and Shahdan R, 2003. Nature's choice to wellness: antioxidant vegetables/ulam. *Nature's choice to wellness: antioxidant vegetables/ulam.*

Zurinawati ZA, 2004. Medicinal Plants and Products Malaysian Industry Status Report. In *Proceedings of Medicinal Herbs & Plants: Scope for Diversified and Sustainable Extraction*, 22-26 July 2004, Bangalore, India.