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Full Length Research Paper

Propagation of five high yielding clones of *Labisia pumila* var. *alata* and evaluation of their growth performances at nursery stage

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Five high yielding clones of *Labisia pumila* var. *alata* (DA5, TA7, TSA22, AA28 and TA8) were propagated through leaf cuttings. The objectives were to evaluate the rooting ability of the clones and to analyse the growth performances of the ramets produced from the cuttings. Cuttings were treated with rooting hormone and grown in an enclosed mist propagation chamber for 12 weeks. Clone TA7 showed the highest number of rooted cuttings with percentage of 100%, followed by clone TA8 with 95% successful rooted cuttings. In contrast, clone TSA22 showed the least number of rooted cuttings with the percentage of 85%. After being treated with hormone, the rooted cuttings were transplanted into a growing medium in polybag and hardened in an open mist propagation chamber before being transferred to the nursery. The growth performances of the ramets produced from the cuttings were observed at week 28 until week 36. Clone AA28 showed the best growth performances in collar diameter (2.62 mm), stem height (3.51 cm), leaf length (7.23 cm) and leaf width (3.62 cm). And in leaf production, clone TA8 produced the highest number of leaves per plant at 5.46 ± 0.26, followed by clone DA5 at 4.12 ± 0.26. It is inferred from this study that the stock plant condition plays a pivotal role in ensuring the quality of root development in the early stage while environmental conditions influence growth performance of the plantlets generally.

Key words: Cutting, growth performance, *Labisia pumila*, vegetative propagation.

INTRODUCTION

Labisia pumila (*L. pumila*) is an undershrub herb from the Myrsinaceae family. It is commonly known as kacip Fatimah in Malay. At least four varieties of *L. pumila* can be found in Malaysia but only three are regularly reported, namely var. *alata*, var. *pumila* and var. *lanceolata* (Sunarno, 2005). *L. pumila* is synonymous with the title 'queen of herbs' due to its medicinal value for women's health. Traditionally, it is used by the local people, particularly Malay women during post-natal care. The demand for *L. pumila* is estimated to expand annually due to its pharmacological activity (Azidah et al., 2012; Fathilah et al., 2013; Nik and Azidah, 2013), estrogenic activity and antioxidant content (Karimi et al., 2011; Lee et al., 2012), which are useful especially for women's health. Furthermore, the safety of *L. pumila* products for human consumption has been evaluated through toxicity testing (Wan et al., 2007; Singh et al., 2009).

In natural habitat, *L. pumila* usually lives in clumps and is reported to have slow growth rate (Mohd et al., 2006). The raw material of this species is continuously been exploited for various human purposes. In long term, this herbal plant may face a great loss in the natural habitat if less effort were put to cultivate it. *L. pumila* can be propagated through seed. But, it takes almost 16 weeks for the seedlings to be able to transferred into growing

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medium (Ahmad, 2013), which is not applicable for commercial production due to its slow growth. Mohd et al. (2000) and Mohd et al. (2002) also reported the slow growth in breeding of this species through seed.

Facing this challenge, Forest Research Institute Malaysia (FRIM) has established a germplasm of *L. pumila* with a total of 54 clones of *L. pumila* var. *alata* that has been screened for Total Phenolic Content (TPC) in attempt to produce high quality planting materials for sustainable supply (Farah et al., 2012a). The clones with high TPC were selected for further propagation process and used as planting materials in this study. The scope of this study is to evaluate the rooting ability of five selected clones of *L. pumila* var. *alata* using leaf cuttings and then to analyse the early growth performances of the plantlets for production of high quality planting stocks. It is hoped that the output of this research will be used as reference to assist the planters in their sustainable production of planting materials for the future.

MATERIALS AND METHODS

Planting materials

The five high yielding clones of *L. pumila* var. *alata* were labelled as DA5, TA7, TSA22, AA28 and TA8. The mother trees of these five clones were originally collected from four different forest reserves (F.R.) in Malaysia, namely Batu Papan F.R., Kelantan (clone DA5); Tembat F.R., Terengggganu (clone TA7 and clone TA8); Sungai Nipah F.R., Terengganu (clone TSA22); and Bukit Larut F.R., Perak (clone AA28). These mother trees were then planted as germplasm in FRIM. Only healthy mother trees with more than five leaves per plant were selected as planting stock. Leaf cuttings were made to propagate the clones in large numbers. The experiment was carried in the propagation house of FRIM on 11 January 2013.

Propagation of *L. pumila* var. alata via leaf cuttings

The leaf cutting technique was followed after Aminah et al. (2008). Leaves were cut into size of 30 cm^2 and rooting hormone (Seradix 1) was applied at the base of the cuttings. Cuttings were grown in an enclosed mist propagation chamber for 12 weeks. Sterile river sand was used as the propagation media. A total of 200 cuttings were produced from the five high yielding clones in a randomize complete block design (RCBD) with four replications.

Acclimatization of rooted cuttings

After 12 weeks of treatment with the hormone, the rooted cuttings were transplanted into a growing medium in 8' × 6' polybag. The growing media used was a combination of soil, compost and sand in the ratio of 3: 2: 1. The rooted cuttings were grown in an open mist propagation chamber for another 12 weeks for hardening. Then they were transferred to the nursery for growth assessment. Shoot development was observed in the meantime. At the nursery, the environment data such as temperature and humidity were recorded from 8:30 h to 16:00 h (Table 3). The nursery was shaded with black plastic netting (70% light intensity).

Data collection

Evaluation of rooted cuttings

The numbers of leaves rooted were recorded at week 3 until week 12 and the data were then subjected to analysis of variance (ANOVA) to determine the effect of genotypes on the number of leaves rooted in 12 weeks using Statistical Analysis System (SAS) package, Version 9.1.

Evaluation of plant growth performance

The growth of the five high yielding clones of *L. pumila* var. *alata* from leaf cuttings were observed after 28 weeks of transplanting. The growth was evaluated once per month until week 30. Among the parameters observed were stem height (cm), collar diameter (mm), leaf number, leaf length (cm) and leaf width (cm). The data were analysed by using SPSS software, Version 22.0 to evaluate and compare the growth performances between the clones.

RESULTS AND DISCUSSION

Results at 12 weeks after planting in the closed chamber showed that all the five clones tested gave rooting of more than 85%. The analysis of variance in percentage of rooted cuttings showed a significant difference between the five high yielding clones as the value of P was less than the probability level of 0.05 ($P < 0.05$). Tukey's multiple comparison test was run to see the mean difference between the five clones. The results are displayed in Table 1.

Based on Tukey's test in Table 1 there was a significant difference observed in clones TA7 and TSA22 at confidence level of 95% for percentage of rooted cuttings. Clone TA7 had the significantly highest number of leaf rooted with the total of 100.0 (Table 1), while in Figure 1 the rooting percentages of *L. pumila* cuttings were plotted by weeks. Cuttings started to root at week 3 for most of the clones especially for clones TSA22 and DA5. These results are in line with a study done by Rozihawati et al. (2005) which also reported that all parts of *L. pumila* stem cuttings started rooting at week 3. It was observed that rooting of the cuttings did not show a substantial increment after 9 weeks in most of the clones. Rooting of AA28 clone was slightly slower compared to the other clones within weeks 3 to 5. However, it started

Table 1. Rooting ability of five high yielding clones of *L. pumila* var. *alata* at 12 weeks after hormone treatment.

Clone	Rooted cuttings (%)	Unrooted cuttings (%)	Dead cuttings (%)
DA5	92.5ab	2.5a	5.0a
TA7	100.0a	0.0a	0.0a
TSA22	85.0b	10.0a	5.0a
AA28	92.5ab	2.5a	5.0a
TA8	95.0ab	2.5a	5.0a

Means followed by the same letters are not significantly different at P<0.05.

Table 2. Vegetative growth characteristics of 9 month old plantlets from the five high yielding clones of *L. pumila* var. *alata*.

Clone	Parameter				
	Collar diameter (mm)	Stem height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)
DA5	1.88bc ± 0.07	3.43ab ± 0.16	4.12 b ± 0.26	7.10a ± 0.23	2.55c ± 0.09
TA7	1.81cd ± 0.07	3.04bc± 0.16	3.72bc± 0.26	6.43b ± 0.23	2.70c ± 0.09
TSA22	2.04b ± 0.07	2.34d ± 0.17	1.61d ± 0.30	6.86ab± 0.27	3.31b ±0.10
AA28	2.62a ± 0.06	3.51a ± 0.16	3.30c ± 0.26	7.23a ± 0.23	3.62a ± 0.09
TA8	1.68d ± 0.07	2.83c ± 0.16	5.46a ± 0.26	6.82ab± 0.23	2.60c ± 0.09

Means with the same letter are not significantly different.

Table 3. Environment data under shade house from 8:30 h to 16:00 h.

Temperature (°C)	Relative humidity (%)
26 - 29	56 - 66

to show better rooting in week 6. The slow and poor rooting of cuttings for some clones was probably due to the limited supply of nutrients available (Aminah et al., 2008) while Rozihawatti et al. (2005) stated that the mortality of the cuttings might be caused by fungi attack or low carbohydrate content in the cutting part.

Evaluation of plant growth performance

For growth performance evaluation, the results show that clone AA28 which is the parent was collected from Bukit Larut F. R. which had the highest values of collar diameter (2.62 mm), stem height (3.51 cm), leaf length (7.23 cm) and leaf width (3.62 cm). This finding is in line with the study done by Farah et al. (2012b) where the *in situ* clone of *L. pumila* var. *alata* at Bukit Larut F. R. gave the highest reading of collar diameter (7.30 mm) and leaf width (9.00 cm). The richness of soil macronutrients at the study site indicates the excellent growth of *L. pumila* var. *alata* at Bukit Larut F. R. This indirectly influences the phenotypic characteristics of clone AA28. Meanwhile, clone TSA22, which originate from Sungai Nipah F. R., gave higher readings of collar diameter (2.04 mm) and leaf width (3.31 cm) as compared to the other three clones. It is reported that Sungai Nipah F. R. has average soil macronutrient levels which are conducive to the survival of *L. pumila* var. *alata* accessions (Farah et al., 2012b).

In terms of productivity, clone TA8 recorded the highest number of leaves (5.46) per plants, followed by clones DA5 and TA7 at 4.12 and 3.72 leaves per plant respectively. Based on the previous study of Farah et al. (2012b), the clones of *L. pumila* var. *alata* collected from these forest reserves gave highest mean values for number of leaves per plant due to the high relative humidity in these study areas. In its natural habitat, a matured plant of *L. pumila* var. *alata* was claimed to have 8 to 10 total number of leaves per plant (Tan et al., 2010). Siti et al. (2012) have noted the leaf production of *L. pumila* var. *alata* grown in different amounts of biochar to be in the range of 6.50 to 7.75 leaves per plant. The overall leaf production of the clones studied was observed to increase month by month. However, some of the clone, for example, TSA22, with mean value of 1.61, resulted in less production of leaves due to the pest attack. By tradition, people harvest the roots or whole plants of *L. pumila* for decoction. This technique might be harmful to conservation of the stock plants and give limited yields. Harvesting of the leaves is recommended in *L. pumila* plantation due to their high availability plus the mother plants which can be conserved as stock plants (Ab et al., 2012). The leaves of *L. pumila* have

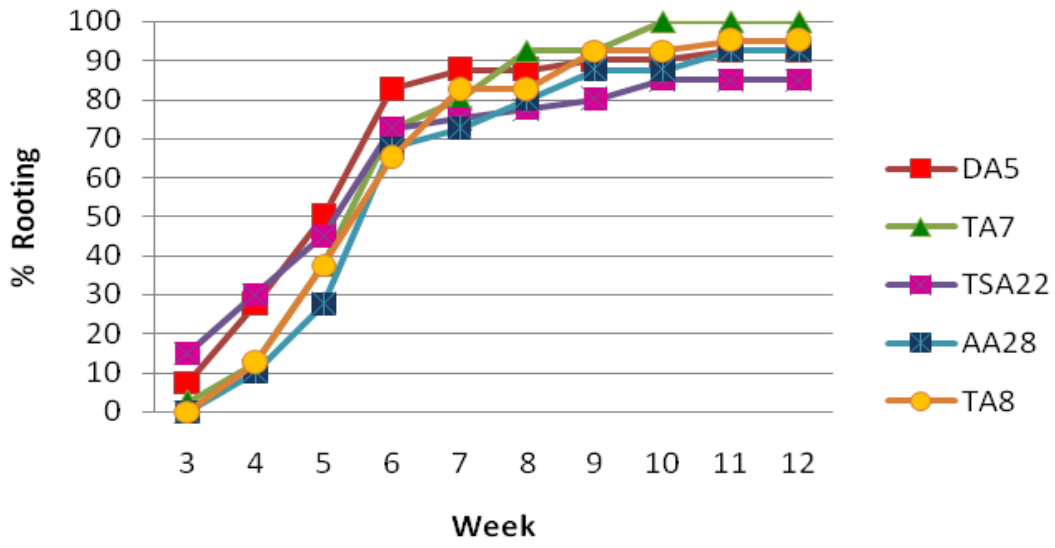

Figure 1. Rates of rooting of the five high yielding clones of *L. pumila* var. alata.

also been reported to contain high contents of antioxidants such as total phenolic acid and total flavonoid which are beneficial for the pharmacological industry (Norhaiza et al., 2009; Karimi et al., 2011). It can be seen that leaf production is the major concern in *L. pumila* plantation. At this early stage, it may be too soon to clarify the clone which gave the best growth. Further data collection is needed to justify the performance of each clone.

The environment condition at the nursery was recorded from 8:30 to 16:00 h (Table 3). *L. pumila* might be sensitive to direct sunlight as the leaves will become wilt and dry. Mohd et al. (2006) reported that *L. pumila* growth under shade house (60-70% shade) produced 22.1 ton ha^{-1} fresh weight compared to only 9.8 ton ha^{-1} under thinned jungle (50-70% shade). Thus, cultivation under heavy shade is recommended for better performance of *L. pumila*. It is also important to provide humid condition at *L. pumila* cultivation site to enhance their productivity.

In general, there are various factors that affect the regeneration of plants from cuttings. The physiological condition of the stock plant reflecting its nutrition status exerts a strong influence on the development of roots and shoots from cuttings taken from the plant. Low nitrogen - high carbohydrate balance in stock plants seems to favour rooting in many cases (Hudson and Dale, 1968). In addition, heavy shade, soil humidity and no water logging are the most favorable conditions for the growth of *L. pumila*. Thus, in future study it is recommended to manipulate the growing condition for better production of *L. pumila*.

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