

Acclimatization of KFeFRIM01:

A Superior Clone of *Labisia pumila* var. *alata*

Syafiqah Nabilah, S. B.¹, Farah Fazwa, M. A.², Siti Suhaila, A. R.³, Norhayati, S.⁴,
Mohd Zaki, A.⁵, Masitah, M. T.⁶

^{1,2,4-6}Plant Improvement Programme, Forestry Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia

³Centre for Biotechnology Bioentrepreneur, Forestry Biotechnology Division, Forest Research Institute Malaysia, 52109 Kepong, Selangor, Malaysia

Abstract— *Labisia pumila* or commonly known as *kacip fatimah* is one of the popular medicinal plant in Malaysia. The constituents of this plant have been reported to possess anti cancer, antioxidant, anti-obesity and anti-inflammatory properties. The growth and production of *L. pumila* is greatly influenced by the environmental condition such as shade, humidity and growing media. In this study, the survivability of *L. pumila* var. *alata* (KFeFRIM01) plantlets derived from tissue culture technique using temporary immersion system were analyzed during pre and post acclimatization process. The plantlets were pre acclimatize in different potting media namely 100% sand and 100% jiffy and grown in plastic growth chamber with different percentage of shade (0%, 50% and 70%). After a month, the plants were transferred to the nursery for post acclimatization. Three experiments were conducted to evaluate the survivability of plants placed under different percentage of shade (70% and 50%), suitable growing media and watering requirement per day. The growth of the plants such as plant height, number of leaves, leaf length and leaf width were recorded during the acclimatization process. The analysis of variance (ANOVA) was conducted to evaluate the survivability and growth of this plant during the acclimatization process. KFeFRIM01 was found to grow better in 0% shade compared to 50% shade and 70% shade. However, there were no significant difference recorded in the usage of two potting medium (100% sand and 100% jiffy) on the growth of KFeFRIM01 during pre acclimatization process. While in post acclimatization, KFeFRIM01 placed under 50% shade produce better growth in term of leaves number ($8.44a \pm 0.20$), leaf length ($6.13a \pm 0.14$) and leaf width ($3.17a \pm 0.06$) compared to 70% shade. KFeFRIM01 plants planted in treatment 1-top soil: leaf compost: sand (2:3:1) gave the highest growth performance in all parameter measured. Whereas, KFeFRIM01 plants that watered twice per day (9AM & 4PM) have greater plant height ($8.83a \pm 0.34$) and leaves number ($7.53a \pm 0.39$) compared to plants watered once per day. The findings from this study are essential for mass production of *L. pumila* using tissue culture technology in future as a successful protocol for acclimatization of this plants obtained.

Keywords— adaptation, environmental condition, plant growth, survivability, clonal propagation.

I. INTRODUCTION

Labisia pumila is an herbal plant from Primulaceae family. The plant usually found in the tropical forest of Asian countries. This plant favors shady area and humus rich soil (Sunarno, 2005). *Labisia pumila* is very synonym in treating women health and due to that numerous of *L. pumila* based products are sold in the market. The most common method of *L. pumila* propagation is through cuttings (Aminah et al., 2008). However, for large scale production of planting materials, tissue culture technique using temporary immersion system (RITA™) is preferable (Syafiqah et al., 2016).

The process of tissue culture technique involved surface sterilization, culture initiation, shoots multiplication, *in vitro* or *ex vitro* rooting and acclimatization. The acclimatization process is a prerequisite in many species grown *in vitro* to ensure high plant survivability and vigorous growth when transferred to soil. This is because, in lab, tissue culture plantlets were developed within culture vessels under low level of light, high humidity, sufficient sugar and nutrients for heterotrophic growth (Preece & Sutter, 1991). Therefore, a step by step of acclimatization process is required for the plantlets to prevent transfer shock.

KFeFRIM01 is the superior clone of *L. pumila* var. *alata* discovered by Farah Fazwa et al. (2012) through clonal trial study. This clone has vigorous growth in terms of plant size and leaf number as well as high in total phenolic content (TPC) which act as antioxidant properties that essential to prevent free radical damage to our body. The clone was mass produced through *in vitro* propagation using RITA™. Thus, to ensure high survivability of the plants at *ex vitro* condition, a good acclimatization practice must be considered.

To date, there is lack of study reported on the acclimatization of *L. pumila* to *ex vitro* condition. Therefore, this study was conducted with the objective to determine the suitable requirements needed by the plants during pre and post acclimatization

process in order to increase the survivability and the growth of the plants. High survivability of the plants could ensure sustainable supply of raw materials to the industry. In future, Malaysia can be one of the main exporters of high quality *L. pumila* and reduce the dependency on imported raw materials as well as from the wild.

II. MATERIAL AND METHOD

Experiment 1- Acclimatization of plant in growth chamber (Pre-acclimatization)

In vitro rooted of KFeFRIM01 plantlets were used in this study. The plantlets with the height of 2-5 cm were taken out from RITA™ and washed under running tap water to remove the traces of medium from the plantlets surface. After that, the plantlets were dipped into Thiram solution (fungicide) for few seconds to disinfect the plants. About 120 plantlets of KFeFRIM01 were planted in two different potting medium; 100% jiffy and 100% sand within three replicates. The plantlets were kept in a transparent plastic chamber (1.5 m x 1.0 m size) with different percentage of shade (0%, 50% and 70%). The plantlets were watered once per day for 30 seconds to maintain the humidity. The survival rates and growth such as stem height, number of leaves, leaf length and leaf width of the plants were recorded before and after acclimatization process. The environmental data such as temperature and humidity in each acclimatization bed were also monitored. The pre acclimatization process for FaFaF01 was conducted in a transparent plastic chamber for a month period.

Experiment 2- Acclimatization of plant in green house (Post-acclimatization)

The plants of KFeFRIM01 were subjected for three different experiments during post acclimatization. The survival rates and growth such as stem height, number of leaves, and leaf length and leaf width of the plants were recorded once per month. The post acclimatization process took about three month period.

i) Shade percentage

The plants were placed under different percentage of shade; 70% and 50%.

ii) Growing media

Three treatments of growing media containing combination of soil, compost and sand were tested for FaFaF01.

Treatment 1- Top soil: leaf compost: sand (2:3:1)

Treatment 2 - Top soil: leaf compost: cocopeat: sand (2:2:1:1)

Treatment 3 - Top soil: cocopeat: sand (2:3:1)

iii) Watering schedule

The plants were watered daily based on three types of schedule:

Schedule 1: Twice per day at 9.00 am and 4.00 pm

Schedule 2: Once per day at 9.00 am

Schedule 3: Once per day at 4.00 pm

III. RESULTS AND DISCUSSION

3.1 Effects of different potting media on the growth of KFeFRIM01 during pre acclimatization

After one month of acclimatization in transparent plastic chamber, it was observed that plantlets in 100% jiffy had 94% survival rate while 100% sand recorded 88.3% (Figure 1). In other study, Muhammad Fuad et al. (2015) reported that the usage of 100% jiffy during acclimatization of *Eurycoma longifolia* (tongkat ali) gave 100% of survival rate while 100% sand gave 67% of survival rate. The ability of 100% jiffy in retaining moisture compared to 100% sand could be the factor of high survivability. This is because during acclimatization, minimal watering is provided in order to harden the plants. In terms of growth, analysis of variance (ANOVA) shows there was significant difference between two potting media (100% sand and 100% jiffy) except for leaf length variable. Based on Figure 2, plantlets in 100% sand had higher stem height (3.76 ± 0.07 cm) and number of leaves (5.81 ± 0.11) compared to 100% jiffy. In contrast, the leaf width of KFeFRIM01 was found greater in 100% Jiffy (1.40 ± 0.03) than 100% sand. While the leaf length of KFeFRIM01 in 100% jiffy and 100% sand was 2.21 ± 0.05 cm and 2.20 ± 0.05 cm respectively. The findings from this study, suggest that 100% jiffy was the suitable potting media for *L. pumila* var. *alata* (KFeFRIM01) during pre acclimatization.

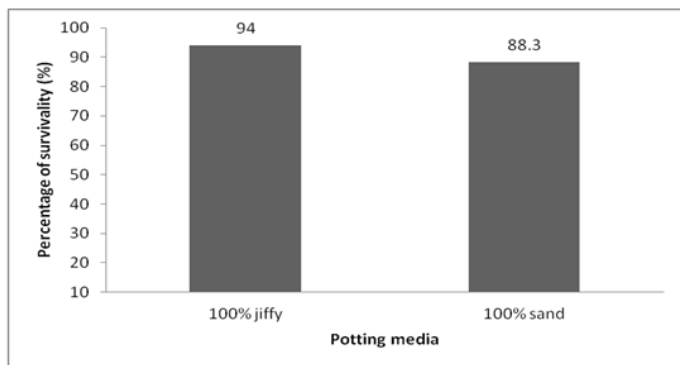


FIGURE 1: PERCENTAGE OF KFeFRIM01 SURVIVALITY IN 100% JIFFY AND 100% SAND

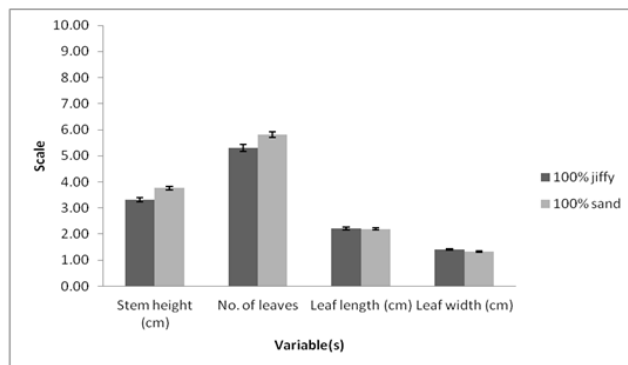


FIGURE 2: GROWTH OF KFeFRIM01 IN 100% JIFFY AND 100% SAND

3.2 Effects of different shade percentage on the growth of KFeFRIM01 during pre acclimatization

The plantlets of KFeFRIM01 were acclimatized in different percentage of shade for one month. The survivality of the plantlets was recorded 100% in 0% (no shade) followed by 50% shade (94%) and 70% shade (80%) (Figure 3). The ANOVA test was conducted to determine the effects of different shade percentage on the growth of KFeFRIM01. The result shows that there was significant difference between different percentage of shade with the growth of KFeFRIM01 (Figure 4). Plantlets of KFeFRIM01 grown in 0% (no shade) recorded the highest growth for all variables measured followed by 50% shade and 70% shade. The findings revealed that *L. pumila* var. *alata* (KFeFRIM01) can be acclimatized in transparent plastic chamber without shade (0%) and increasing the percentage of shade from 50% to 70% may contribute to mortality effect and slow growth. This finding is in line with Ginting et al. (2015) where the higher the percentage of shades, the lower the number of tiller in upland rice plants.

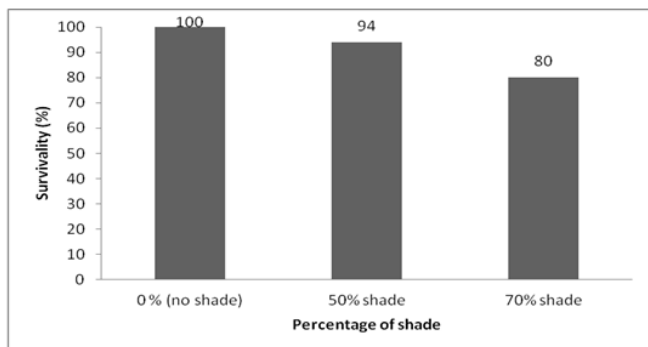


FIGURE 3: PERCENTAGE OF KFeFRIM01 SURVIVALITY IN DIFFERENT PERCENTAGE OF SHADE

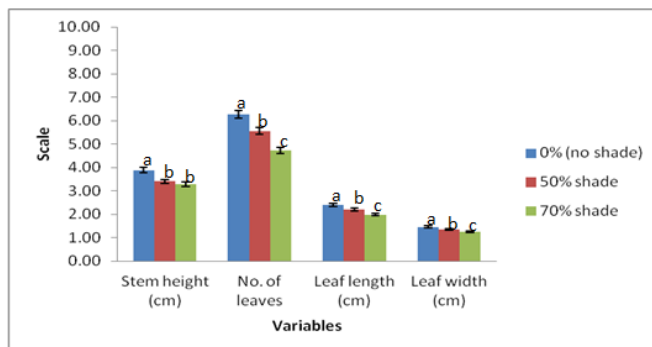


FIGURE 4: GROWTH OF KFeFRIM01 IN DIFFERENT PERCENTAGE OF SHADE

3.3 Effects of different shade percentage on the growth of KFeFRIM01 during post acclimatization stage.

During post acclimatization, the plants of KFeFRIM01 were placed at two different shade houses with 50% and 70% shade for three months. The growth performances of the plantlets were monitored monthly and presented in Table 1.

**TABLE 1
EFFECTS OF DIFFERENT SHADE PERCENTAGE ON THE GROWTH OF KFeFRIM01 DURING POST ACCLIMATIZATION STAGE.**

Treatment	Parameter				
	Plant height (cm)	No of leaves	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)
50 % shade	5.04b ± 0.13	8.44a ± 0.20	6.13a ± 0.14	3.17a ± 0.06	2.63b ± 0.07
70 % shade	5.51a ± 0.20	6.87b ± 0.33	5.15b ± 0.24	2.64 b ± 0.12	2.88a ± 0.07

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

Based on ANOVA, the growth of KFeFRIM01 under different percentage of shade is significant at $p < 0.05$. The plants placed under 50% shade produce better growth in term of leaves number ($8.44a \pm 0.20$), leaf length ($6.13a \pm 0.14$) and leaf width ($3.17a \pm 0.06$) compared to 70% shade. However, plants placed under 70% shade had greater plant height ($5.51a \pm 0.20$) and collar diameter ($2.88a \pm 0.07$) than 50% shade. The difference in results may relate to the photosynthetically active radiation (PAR) that vary between the two shade used. Similar results were reported for red and blue colour shading net, which cause reduction in the amount of incident radiation and affecting the induction of multi-shoot plants of torch ginger (Rodrigues et al. (2015)). *L. pumila*, the leaves production is more important than other parameter since leaves contribute to the high biomass production. Therefore, it is suggested that the plants of *L. pumila* were placed under 50% shade for high production of leaves and biomass.

3.4 Effects of different growing media on the growth of KFeFRIM01.

The plants of KFeFRIM01 were transferred from jiffy to three different growing media during the post acclimatization period. Based on ANOVA, there is significant different in the growth of KFeFRIM01 planted at different growing media at $p < 0.05$. Plants planted in treatment 1-top soil: leaf compost: sand (2:3:1) gave higher growth performance in all parameter measured followed by treatment 2-top soil: leaf compost: cocopeat: sand (2:2:1:1) and treatment 3-top soil: cocopeat: sand (2:3:1) (Table 2). The combination of leaf compost into substrate in treatment 1 may influence the growth performances of *L. pumila*. According to Roy (1914), leaf compost is not normally considered as fertilizer as it is too low in nutrient content. However, it serves primarily as an organic amendment and a soil conditioner that improves the physical, chemical, and biological properties of soils.

TABLE 2
EFFECTS OF DIFFERENT GROWING MEDIA ON THE GROWTH OF KFeFRIM01.

Treatment	Parameter				
	Plant height (cm)	No of leaves	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)
T1- Top soil: leaf compost: sand (2:3:1)	$6.58a \pm 0.28$	$8.20a \pm 0.39$	$10.0a \pm 0.27$	$4.80a \pm 0.13$	$3.47a \pm 0.13$
T2- Top soil: leaf compost: cocopeat: sand (2:2:1:1)	$6.60a \pm 0.29$	$7.13ab \pm 0.33$	$9.84a \pm 0.42$	$4.58a \pm 0.19$	$3.17a \pm 0.09$
T3- Top soil: cocopeat: sand (2:3:1)	$5.29b \pm 0.16$	$6.60b \pm 0.42$	$7.06b \pm 0.22$	$3.50b \pm 0.10$	$2.79b \pm 0.10$

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

3.5 Effects of different watering schedule on the growth of KFeFRIM01

Based on ANOVA, the effects of different watering schedule were significant at $p < 0.05$ for parameters; leaves number and leaf length only. Plants that watered twice per day (9AM & 4PM) have greater plant height ($8.83a \pm 0.34$) and leaves number ($7.53a \pm 0.39$) compared to plantlets watered once per day. Whereas, plants watered at 9AM daily had greater growth of leaf length ($12.06a \pm 0.38$), leaf width ($5.76a \pm 0.16$) and collar diameter ($4.39a \pm 0.85$) compared to plants watered at 4PM and 9AM & 4PM. The plants that watered once per day may result in drought stress and disturb the growth of the plants as reported by Jaleel et al. (2008).

TABLE 3
EFFECTS OF DIFFERENT WATERING SCHEDULE ON THE GROWTH OF KFeFRIM01.

Watering schedule	Parameter				
	Plant height (cm)	No of leaves	Leaf length (cm)	Leaf width (cm)	Collar diameter (mm)
9AM, 4PM	$8.83a \pm 0.34$	$7.53a \pm 0.39$	$11.70a \pm 0.34$	$5.67a \pm 0.16$	$4.11ab \pm 0.16$
9AM	$8.65ab \pm 0.42$	$7.40a \pm 0.45$	$12.06a \pm 0.38$	$5.76a \pm 0.16$	$4.39a \pm 0.85$
4PM	$7.60b \pm 0.39$	$5.97b \pm 0.40$	$10.37b \pm 0.52$	$5.13a \pm 0.29$	$3.90b \pm 0.19$

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

IV. CONCLUSION

During the acclimatization process, the percentage of shade, type of substrate and water regime can significantly affect the growth, development and morphogenesis of *L. pumila*. Therefore, the finding from this study can be the reference for the key players to ensure high survivality of the plants during acclimatization stages.

ACKNOWLEDGEMENTS

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

REFERENCES

- [1] Aminah, H. Naimah, C.L. Mohd Zaki, A. & Lokmal, N. (2008). Rooted leaf cuttings of *Labisia pumila*. *Journal of Tropical Medicinal Plants*, 9(1), 101-104.
- [2] Farah Fazwa, M. A., Maideen, H., & Mohamad, O. (2012). Selection among two varieties of *Labisia pumila* that yield high phenolic contents for establishing plant stock and further cultivation. *Journal of Tropical Medicinal Plants*, 13(1).
- [3] Farah Fazwa, M.A., Norhayati, S., Syafiqah Nabilah, S.B., Mohd Zaki, A. & Mohd Asri, L. (2014). Establishment of Four Clonal Trial Plots of *Labisia pumila* In Frim. Poster presented at The 13th Symposium Malaysian Society of Applied Biology 2014. 8-10 June 2014.
- [4] Ginting, J., Damanik, B. S. J., Sitanggang, J. M., & Muluk, C. (2015). Effect Of Shade, Organic Materials And Varieties On Growth And Production Of Upland Rice. *International Journal of Technology Enhancements and Emerging Engineering Research*, 4(1), 68-74.
- [5] Jaleel, C. A., Gopi, R., Sankar, B., Gomathinayagam, M., & Panneerselvam, R. (2008). Differential responses in water use efficiency in two varieties of *Catharanthus roseus* under drought stress. *Comptes Rendus Biologies*, 331(1), 42-47.
- [6] Muhammad Fuad, Y., Hassan, N. H., Abdullah, N., Rahman, A., Suhaila, S., Ismail, H. & Koter, R. (2015). Acclimatization of *Eurycoma longifolia* (Tongkat Ali) Plantlets to Ex Vitro Conditions. *Journal of Tropical Resources and Sustainable Science*, 3(1), 129-131.
- [7] Preece, J. E., & Sutter, E. G. (1991). Acclimatization of micropropagated plants to the greenhouse and field. *Micropropagation: Technology and Application*, 71-93.
- [8] Rodrigues, M., Paiva, P. D. D. O., Freitas, R. T. D., Mansur, T. D. O. F., Paiva, R., & Barbosa, J. P. R. A. D. (2015). Growth and photosynthetic responses during ex vitro acclimatization of *Etlingera elatior* (Jack) rm smith (torch ginger). *Acta Scientiarum. Agronomy*, 37(4), 495-504.
- [9] Roy, L. F. (1914). Using leaf compost. The State University Of New Jersey. Rutgers Cooperative Extension. *Fact sheet*, 117.
- [10] Sunarno, B. (2005). Revision of the genus *Labisia* (Myrsinaceae). *Blumea-Biodiversity, Evolution and Biogeography of Plants*, 50(3), 579-597.
- [11] Syafiqah, N. S. B., Farah, F. M. A., Norhasnida, H. Siti Suhaila, A. R., Norhayati, S., & Mohamad, O. (2016). Production of Superior Clone *Labisia pumila* var. *alata* For Future Planting Stock. *J. Trop. Plant Physiol*, 8, 32-43.