RAPID MASS PRODUCTION OF ELITE CLONE OF LABISIA PUMILA VAR. ALATA (KFeFRIM01) FOR SUSTAINABLE SUPPLY OF HIGH QUALITY PLANTING MATERIALS

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ABSTRACT

Labisia pumila is listed as one of the highly-valued herbal plant that has bright future in the herbal industry. Recent study has found that the plant contains bioactive antioxidant compounds such as recorcinols, flavonoid and phenolic. Labisia pumila has been discovered in various fields of applications, particularly in pharmaceutical and cosmeceutical purposes. As the application of L. pumila continuously being explored, many healthcare products with the incorporation of L. pumila will be developed and consequently increase the demand for raw material supply. High quality raw materials will be the added value in these newly developed products. Therefore, it is important to screen plants with outstanding characteristics among the plant variety. A study on identification of elite planting materials of L. pumila has been done by FRIM. Six superior clones of L. pumila has been evaluated through clonal trial and one of the elite clone namely KFeFRIM01 has been identified having good growth performances, and survivability and containing high total phenolic content (TPC) and primary metabolites. As the elite clone identified, cultivation of its in large number through tissue culture should be applied to cater the industry demand. This study consists of two main objectives, i) to develop large scale of KFeFRIM01 using tissue culture technique, Temporary Immersion System (TIS) and ii) to evaluate the growth performances and determine the chemicals compounds of KFeFRIM01 planting materials at fields trial. The outputs from this study are important to ensure sustainable supply of elite planting materials of KFeFRIM01 for high quality health products. It is anticipated that FRIM will be able to facilitate herbal industries for high quality planting materials for its commercial plantation.

Key words: temporary immersion system, planting material supply, commercialization

INTRODUCTION

Labisia pumila (L. pumila) is an undershrub herb from the Primulaceae 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). Labisia pumila is synonymous with the title ‘queen of herbs’ due to its medicinal value for women’s health. It has been listed as one of the high-value herbal product that has bright future in the herbal industry. Recent study has found that the plant contains bioactive antioxidant compounds such as recorcinols, flavonoid and phenolic.

L. pumila has been discovered in various fields of applications, particularly in pharmaceutical and cosmeceutical purposes. As the application of L. pumila continuously being explored, many newly developed products will be invented and consequently
increase the demand of raw material supply. A quality raw material will be the added value in the product developed. Therefore, it is important to screen the plants variety which has better characteristics than the common one. A study on identification of elite planting materials of L. pumila has been done by FRIM. One elite clone of L. pumila has been identified in having good growth performance and survivability as well as containing high total phenolic content (TPC) and primary metabolites (Norhayati et al. 2016). This clone has been registered under FRIM’s Invention Disclosure with the name of KFeFRIM01.

Currently, the industries are facing the issue of insufficient supply high quality raw materials. This is because the industries have to depend on the supply from the wild where the quality of the raw materials is uncertain and the supply is not sustainable. Study by FRIM, Rohana et al. (2017) found that 83% of L. pumila raw materials were harvested from natural forests and only 17% were cultivated. Theoretically, L. pumila can be propagated through tissue culture (Hartinie and Jualang, 2007; Nor ‘Aishah et al., 2013; Syafiqah Nabilah et al., 2015) and cuttings (Aminah et al., 2008; Rozihawati, 2008 & Farah Fazwa et al., 2013). However, tissue culture technique is more preferable since it able to mass produce planting materials in a relatively shorter period of time compared to conventional method (cutting). A study on tissue culture using TIS showed not only the technique promote mass production but also improves the survival rates during and after acclimatization of tissue culture-derived plantlets, hence quality of planting materials determined (Siti Suhaila et al. 2013). It is also reported that tissue culture derived plantlets of L. pumila only required 6 months before transplanted to field, whereas cutting need more than 9 months (Farah Fazwa et al. 2017).

The incorporation of L. pumila in many health products for women consumptions has becominga trend nowadays. However, one of the constraints to develop this species (L. pumila) is the lack of producible tissue culture protocol which can mass produce the raw material for commercialization purposes. In future, the raw materials of L. pumila will face great extinction if it is continuously harvested from the wild and less effort were put on its cultivation. Therefore, this study is conducted with the objectives to develop large scale of KFeFRIM01 planting materials using tissue culture technique, Temporary Immersion System (TIS) and to evaluate the growth performances and determine the chemicals compounds of KFeFRIM01 planting materials.

MATERIALS AND METHODS

Development of large scale planting materials of KFeFRIM01 using temporary immersion system (TIS)

Nine media treatments consist of MS medium with different strength (¼ MS, ½ MS, MS) and various concentrations of 6-Benzylaminopurine (BAP) in the range of 0.1, 0.15 and 0.3 mg/L were tested to accelerate the growth of KFeFRIM01 in RITAM™ system as shown in Figure 1. The in vitro nodal segments were used as explants in this study.

Multiplication of KFeFRIM01 through leaf cuttings

Leaf cuttings of KFeFRIM01 were also conducted as control for field trial. Multiplication by leaf cuttings was carried out on 11th January 2016 (Figure 2). About 700 cuttings were produced from three different leaf parts (top, middle, and bottom). Cuttings were placed in a mist propagation chamber for 12 weeks before hardening process.
Acclimatization of plantlets

The plantlets of KFeFRIM01 produced from both sources of propagation were transferred to FRIM’s greenhouse and hardened in a weaning chamber for a month period. Then, the plantlets were transferred to planting bed in the greenhouse for acclimatization processes. After 3 months of acclimatization, the plantlets were transferred and grown at nursery.

Establishment of field trials at 3 locations

In this study the field trial were established at FRIM, Kepong and two FRIM’s substation located at SPF Mata Ayer, Perlis and SPF Maran, Pahang. The locations were selected based on their climatic and environmental conditions. At each selected site, the plants were planted in a Randomized Complete Block Design (RCBD) with three experimental blocks. In total, there were 540 plants were tested for field trial at three locations.

Assessment of growth performances and total phenolic compounds

The plant growth assessment were measured based on stem height, number of leaf, leaf length, leaf width and collar region. The data were collected monthly in a period of nine months. The quantification of TPC was analysed by using Folin Ciocelteau (FC) reagent following the method by Singleton & Rossi (1965) with slight modifications (Vimala et al., 2003). TPC analysis was conducted at 3, 6 and 9 months after planting at three locations.

RESULTS AND DISCUSSION

Plant growth performances of KFeFRIM01 in temporary immersion system

The plant growth performances of KFeFRIM01 grown in RITA™ system were evaluated. Stem height development, number of leaves, leaf length and leaf width were measured in 4 weeks old cultures (Figure 3). The analysis of variance (ANOVA) showed there were significant differences at \( p < 0.05 \) between different media treatment with plant height and number of leaves. The mean ± standard error of plant growth in each media treatment was presented in Table 1. From the analysis, \( 1/2 \) MS + 0.15 mg/L BAP gave the highest growth of KFeFRIM01 in terms of plant height (5.42 ± 0.29 cm) and number of leaves (5.83 ± 0.49). This media combination may be able to accelerate the production of KFeFRIM01 in large scale.
Means followed with similar alphabets were not significant at p < 0.05.

KFeFRIM01 at three research plots may be due to some external factors such as soil series, rainfall distribution and temperature (Table 6).

Whereas, in terms of leaf number, tissue culture and leaf cutting plants at FRIM, Kepong, SPF Mata Ayer, Perlis and SPF Maran, Pahang had greater stem height compared to leaf cutting plants.

Analysis of the growth performances of KFeFRIM01 after nine months planted at SPF Mata Ayer, Perlis showed SPF Maran, Pahang recorded the highest values for all measured parameters (Table 6).

Growth performances of leaf cuttings

Several parameters of rooted cutting plants were collected such as stem height, leaf number, leaf length and leaf width (Table 2). ANOVA showed that middle part gave the highest value compared to top and bottom for stem height (0.87 cm ± 0.10), leaf number (0.67 ± 0.08) and leaf width (1.46 cm ± 0.16). For leaf length, the bottom part recorded the lowest value (0.49 ± 0.15) compared to others. It was observed that cuttings from middle part gave better roots and shoots performances might due to sufficient sugars and less transpiration occurred compared to other parts. However in terms of planting materials production, it can be concluded that any part of the leaves can be used for multiplication by leaf cuttings.

Assessment of growth performances of KFeFRIM01

The growth performances of KFeFRIM01 after nine months planted at three research plots were as shown in Table 3-5. The Analysis of Variance (ANOVA) showed there is significant difference in the growth performances of KFeFRIM01 produced through tissue culture and leaf cuttings at p<0.05. The overall assessment showed KFeFRIM01 sourced from tissue culture planted at FRIM, Kepong, SPF Mata Ayer, Perlis and SPF Maran, Pahang had greater stem height compared to leaf cutting plant. Whereas, in terms of leaf number, tissue culture and leaf cutting plants did not show any significant different. In contrast, leaf cutting plant showed greater leaf length and collar diameter at all research plot while leaf width development is greater at FRIM, Kepong and SPF Mata Ayer, Perlis. Overall assessment showed SPF Maran, Pahang recorded the highest values for all measured parameters (Table 6). This finding is in line with the study done by Norhayati et al. (2016) where the clone of L. pumila var. alata planted at SPF Maran, Pahang gave the best growth performances. The differences in the growth performances of KFeFRIM01 at three research plots may be due to some external factors such as soil series, rainfall distribution and temperature.

<table>
<thead>
<tr>
<th>Treatment media</th>
<th>Plant height (cm)</th>
<th>No. of leaves</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>¼ MS + 0.10 mg/L BAP</td>
<td>4.88 ± 0.39bc</td>
<td>4.17 ± 0.27c</td>
<td>3.00 ± 0.17a</td>
<td>1.46 ± 0.10b</td>
</tr>
<tr>
<td>½ MS + 0.10 mg/L BAP</td>
<td>3.96 ± 0.36bc</td>
<td>3.75 ± 0.18c</td>
<td>3.08 ± 0.21a</td>
<td>1.50 ± 0.09a</td>
</tr>
<tr>
<td>MS + 0.10 mg/L BAP</td>
<td>4.27 ± 0.31bc</td>
<td>4.38 ± 0.47bc</td>
<td>3.08 ± 0.25a</td>
<td>1.42 ± 0.10ab</td>
</tr>
<tr>
<td>¼ MS + 0.15 mg/L BAP</td>
<td>5.42 ± 0.29a</td>
<td>5.83 ± 0.49a</td>
<td>2.79 ± 0.13a</td>
<td>1.29 ± 0.07ab</td>
</tr>
<tr>
<td>½ MS + 0.15 mg/L BAP</td>
<td>4.21 ± 0.21bc</td>
<td>3.79 ± 0.19a</td>
<td>3.25 ± 0.08a</td>
<td>1.67 ± 0.08ab</td>
</tr>
<tr>
<td>MS + 0.15 mg/L BAP</td>
<td>3.55 ± 0.27c</td>
<td>4.73 ± 0.30bc</td>
<td>3.23 ± 0.25a</td>
<td>1.45 ± 0.11ab</td>
</tr>
<tr>
<td>¼ MS + 0.30 mg/L BAP</td>
<td>4.67 ± 0.32ab</td>
<td>5.44 ± 0.50bc</td>
<td>3.33 ± 0.20a</td>
<td>1.39 ± 0.11ab</td>
</tr>
<tr>
<td>½ MS + 0.30 mg/L BAP</td>
<td>4.27 ± 0.32bc</td>
<td>4.36 ± 0.43bc</td>
<td>2.72 ± 0.15a</td>
<td>1.41 ± 0.09ab</td>
</tr>
<tr>
<td>MS + 0.30 mg/L BAP</td>
<td>3.56 ± 0.27c</td>
<td>4.56 ± 0.26bc</td>
<td>3.34 ± 0.20a</td>
<td>1.50 ± 0.09ab</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Leave part</th>
<th>N</th>
<th>Stem height (cm)</th>
<th>Leaf number</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top</td>
<td>90</td>
<td>0.56 ± 0.09a</td>
<td>0.43 ± 0.07a</td>
<td>1.75 ± 0.27a</td>
<td>1.00 ± 0.15a</td>
</tr>
<tr>
<td>Middle</td>
<td>90</td>
<td>0.87 ± 0.10a</td>
<td>0.67 ± 0.08a</td>
<td>2.52 ± 0.28a</td>
<td>1.46 ± 0.16a</td>
</tr>
<tr>
<td>Bottom</td>
<td>90</td>
<td>0.19 ± 0.06b</td>
<td>0.14 ± 0.04c</td>
<td>0.49 ± 0.15b</td>
<td>0.24 ± 0.07d</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Propagation method</th>
<th>Stem height (cm)</th>
<th>No. of leaves</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Collar diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture</td>
<td>7.04 ± 0.25a</td>
<td>6.02 ± 0.25a</td>
<td>12.63 ± 0.51b</td>
<td>5.78 ± 0.22a</td>
<td>4.67 ± 0.10b</td>
</tr>
<tr>
<td>Leaf cuttings</td>
<td>6.77 ± 0.17a</td>
<td>5.97 ± 0.17a</td>
<td>15.45 ± 0.40a</td>
<td>6.95 ± 0.16a</td>
<td>5.64 ± 0.09a</td>
</tr>
</tbody>
</table>

Means followed with similar alphabets were not significant at p <0.05.

<table>
<thead>
<tr>
<th>Propagation</th>
<th>Stem height (cm)</th>
<th>No. of leaves</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
</tr>
</thead>
</table>
Table 5: Growth performances of KFeFRIM01 after 9 months planted at SPF Maran, Pahang

<table>
<thead>
<tr>
<th>Propagation method</th>
<th>Stem height (cm)</th>
<th>No. of leaves</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
<th>Collar diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue culture</td>
<td>7.72 ± 0.25a</td>
<td>5.83 ± 0.25a</td>
<td>14.57 ± 0.42b</td>
<td>6.52 ± 0.16a</td>
<td>4.75 ± 0.14b</td>
</tr>
<tr>
<td>Leaf cuttings</td>
<td>6.94 ± 0.22b</td>
<td>5.76 ± 0.28a</td>
<td>15.77 ± 0.45a</td>
<td>6.78 ± 0.19a</td>
<td>5.72 ± 0.10a</td>
</tr>
</tbody>
</table>

Means followed with similar alphabets were not significant at p < 0.05

Total phenolic contents of KFeFRIM01 at 3, 6 and 9 months after field planting

The values of TPC for every three months at each location were tabulated in Figure 5 for tissue culture plants and Figure 6 for leaf cuttings plants. At 3 months after planting, TPC values from tissue culture plants were high at SPF Mata Ayer, Perlis (467.3 mg/100g GAE) and SPF Maran, Pahang (450.3 mg/100g GAE) compared to FRIM Kepong (233.3 mg/100g GAE) (Figure 5). However, after 6 months of planting, TPC value at FRIM, Kepong (336.8 mg/100g GAE) started to increase while the others reduce; SPF Mata Ayer, Perlis (341.7 mg/100g GAE) and SPF Maran, Pahang (340.4 mg/100g GAE). After 9 months of planting, the TPC reduced at all locations but no significant difference at p < 0.05 for TPC values at SPF Maran, Pahang at 6 and 9 months after planting.

For cuttings plant, there was no significant difference at p<0.05 between TPC value at all locations after 3 months of planting. However, at 6 and 9 months after planting TPC value produced for all three locations were significant at p<0.05. SPF Maran, Pahang recorded the highest TPC (336.7 mg/100g GAE) followed by SPF Mata Ayer, Perlis (316.6 mg/100g GAE) and FRIM Kepong (283.3 mg/100g GAE) at 6 months after planting. At 9 months after planting, FRIM Kepong and SPF Maran showed increment in TPC value at 330.2 mg/100g GAE and 358.7 mg/100g GAE respectively.

From both TPC analysis, FRIM Kepong showed inconsistent trend of TPC production while SPF Mata Ayer, Perlis showed reduction in TPC value at 3, 6, and 9 months after planting. Whereas, SPF Maran, Pahang showed consistent production of TPC value in each months compared to others locations. Environmental factors such as annual average precipitation, sunshine duration, soil pH and soil organic matter could be the reason of variation in TPC production at all locations. This finding is similar with Liu et al. (2015) where ecological factors greatly influenced the secondary metabolites production in Sinopodophyllum hexandrum.

Figure 5: Total phenolic content of KFeFRIM01 from tissue culture plants after planting at different locations
CONCLUSION

The results from this study concluded that clone KFeFRIM01 can be mass produced using Temporary Immersion System (TIS) with the combinations of suitable media and growth hormone. The plants produced through the system showed good growth performances at field planting and the TPC production were present at each location in accepted quantity and quality.

REFERENCES


Figure 6: Total phenolic content of KFeFRIM01 from cuttings plants after planting at different locations