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Ensuring Sustainability of Kacip Fatimah (*Labisia Pumila*) Through Ex-Situ Conservation

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Abstract

Labisia pumila or locally known as Kacip Fatimah of the family Myrsinaceae is one of the popular herbal species in Malaysia. The leaves or the whole plant are being traditionally used to treat women internal problem and health. Other medicinal uses of the plant are as a treatment for dysentery, flatulence, dysmenorrhoea and gonorrhoea. The increasing demand of the plants for those usages has lead to the over-exploitation in the wild and might endanger the species if no conservation activities are being carried out. Beside the danger of extinction, the species also experience severe genetic loss and shortage of raw materials. Realizing to this, Plant Improvement Programme of Forest Research Institute Malaysia (FRIM) has taken an initiative to ex-situ conserve the species through the establishment of germplasm and development of breeding strategy. The purpose is to avoid extinction of the species and to produce high quality planting materials for commercial production. This paper discusses the collection, selection, propagation and establishment of clonal bank/germplasm of the species. It is anticipated that with the initiative, the sustainability of the species can be ensured to support the industries demand in the future.

1. Introduction

Kacip Fatimah or scientifically known as *Labisia pumila* (Myrsinaceae) is a well-known herb for multipurpose uses in general women health. Other medicinal uses of the plant are as a treatment for dysentery, flatulence, dysmenorrhoea and gonorrhoea (Burkill 1966). With the growing interest in L. pumila as a source of new pharmaceutical products and the increasing demand for herbal products in Malaysia, the demand for its raw materials is also increasing (Mohd. Azmi & Awang Noor 2001). In Malaysia, most of the raw materials are sourced from the wild (Kumari et al. 1998 & Mohd Setafarzi 2001) which is uncertain of quantity and quality to meet the market demands. In order to reduce the dependence of getting raw material of the species from natural forests, Plant Improvement Programme of Forest Research Institute Malaysia has come up with ex-situ conservation through establishment of germplasm and development of breeding strategy in order to produce high quality planting materials of the species for commercial production.

Plant germplasm is the most important components of conservation, and is the lifeblood of plant breeding without which breeding is impossible to conduct (Mohamad et al. 2012). Germplasm provides

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the basic materials used to initiate a breeding program. Through plant breeding, germplasm can be improved for better performance of the crops.

The objective of this paper is to highlight the collection, selection, propagation and establishment of clonal bank/germplasm of the species. This is the first stage of a breeding program of these species in order to generate improved and high quality plant materials to meet increasing demand by the herbal industry.

2. Materials and Methods

2.1. Collection of mother plants

Three varieties were investigated namely, *L. pumila* var. *pumila* (Figure 1) and *L. pumila* var. *alata* (Figure 2) and *L. pumila* var. *lanceolata* (Figure 3) A total of 150 mother plants were collected from five provenances for each variety and were given different code numbers (Table 1).

Figure 1: *Labisia pumila* var. *pumila*

Figure 2: *Labisia pumila* var. *alata*

Figure 3: *Labisia pumila* var.

Table 1: Details for the collected locations of Labisia. pumila var. pumila and Labisia pumila var.alata and Labisia pumila pumila var.lanceolata in Peninsular Malaysia

Variety	Location
L. pumila var. pumila	Gunung Inas FR, Kulim, Kedah Pondok Tanjung FR, Taiping, Perak Tembat FR, Kuala Berang FR, Terengganu Sungai Nipah FR, Kemaman, Terengganu Batu Papan FR, Gua Musang, Kelantan
L. pumila var. alata	Gunung Inas FR, Kulim, Kedah Bukit Larut FR, Taiping, Perak Pasoh FR, Jelebu, N. Sembilan Panti FR, Kota Tinggi, Johor Tekai FR, Jerantut, Pahang
L. pumila var. lanceolata	Betong FR, Maran, Pahang Pulau Selimbar, Tasik Kenyir, Terengganu Gunung Berembun FR Negeri Sembilan Korbu FR, Kuala Kangsar, Perak Gunung Belumut, Kluang, Johor

At each population, some environmental data were recorded such as temperature, relative humidity and light intensity, while data on annual rainfall distribution were gathered from Meteorology Department. All collected mother plants were tagged, wrapped and placed in plastic bags before they were transported and replanted in the nursery of FRIM.

2.2. Selection of high yielding planting materials

These mother plants were then raised in nursery for few months before transplanted to plots. Selections of individual plants were carried out within

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this germplasm for further improvement activities. Early selections of the plants were done based on the traits of total phenolic content and growth performance. Total phenolic content were determined using Folin-Ciocalteu reagent according to the method of Singleton & Rossi (1965) with slight modifications. The results were calculated according to the standard curve and expressed as gallic acid equivalents (GAE). Several studies on *L. pumila* to examine the relationship between total phenolic content and antioxidant activities (Khairul et al., 2005; Mohamad Norhaiza et al., 2009; Choi, 2010; Ehsan et al., 2011) have found that there was strong positive correlation between total phenolic content and antioxidant activities.

2.3. Propagation of high quality planting materials

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. Only propagation of L. pumila var. alata was carried out through leaf cuttings technique (Rozihawati 2005; Aminah et al. 2008; Farah Fazwa et al. 2013). 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).

3. Results and Discussion

3.1. Determination of total phenolic contents

The results (in the unit of gallic acid equivalent mg 50.0 g-1) showed that the range of total phenolic contents for var. *alata* and var. *pumila* extracts was from 1720.77 to 2927.79 mg 50 g-1 and 633.65 to 2792.57 mg 50 g-1 respectively (Table 2). The results also indicated that *L. pumila* contains comparable high phenolic compound as compared with other herbal species (Hafzan et al., 2005), such as for kesum (*Polygonum minus*) (20.8 ± 0.6 mg 100 g-1) and jarum tujuh bilah (*Pereskia gradifolia*) (17.2 ± 2.2 mg 100 g-1). Both L. *pumila* varieties contain high phenolic content of more than 1000 mg 50 g-1 and can be considered as plants with high phenolic content as classified by Vimala (2010) (pers. comm). However, for L. *pumila* var. *lanceolata*, analysis of total phenolic content is still on going.

Table 2. Range and mean of total phenolic content for both *Labisia pumila* varieties

L. pumila	**TPC [mg 50 g^{-1}] Min-Max Value**	**Mean TPC [mg 50 g^{-1}]**	**Min-Max Value [mg 50 g^{-1}]**
Var. *alata*	1720.77 - 2927.79	2523.54 a	2497.74 - 2549.34
Var. *pumila*	633.65 - 2792.57	1759.76 b	1692.96 - 1826.56

3.2. Selection of high yielding plants

Based on the total phenolic content screening results, 119 genotypes were selected. A total of 63 out of 150 var. *alata* genotypes with total phenolic content higher than the average (2523.54 mg 50 g^{-1}) were selected. While for var. *pumila*, only 56 out of 150 with total phenolic content higher than the average (1759.76 mg 50 g^{-1}) were selected. These superior genotypes were further mass propagated using cutting technique.

3.3. Propagation of high quality planting materials

Rooting and sprouting on cuttings occur with 3-4 weeks after planting. Results at 12 weeks after planting showed that all tested clones gave rooting of more than 90% (Farah Fazwa et al. 2013; Syafiqah Nabilah et al. 2013) (Figure 4). The results of this experiment were used for the production of planting stocks of this species.

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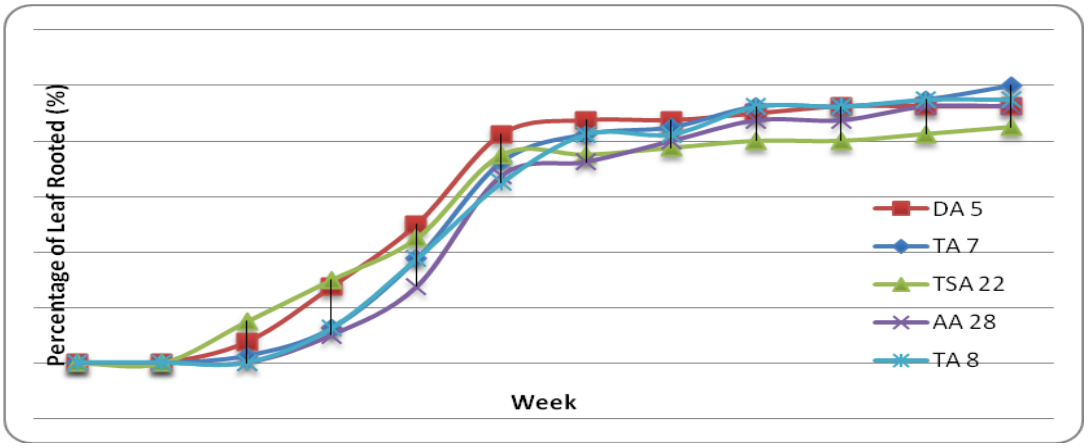

Figure 4: Percentage of leaf rooted at 12 weeks after planting

3.4. Evaluation of plant growth performance

The growth data of the five high yielding clones of *L. pumila* var. *alata* plantlets were recorded at the age of 7 months to 9 months, which is the optimum age for harvesting the plants (Aminah & Farah Fazwa, 2012). About 96% of the rooted cuttings had successfully developed into plantlets. Significance differences were found between the clones with respect to all parameters studied at 5% level of significance (Table 3).

Table 3: Vegetative growth characteristics of 9 month old plantlets from the five high yielding clones of *Labisia 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	3.43cd ± 0.16	4.12 c ± 0.26	7.10b ± 0.23	2.55a ± 0.09
TA7	1.81ab ± 0.07	3.04bc± 0.16	3.72bc± 0.26	6.43a ± 0.23	2.70a ± 0.09
TSA22	2.04c ± 0.07	2.34a ± 0.17	1.61a ± 0.30	6.86ab± 0.27	3.31b ±0.10
AA28	2.62d ± 0.06	3.51d ± 0.16	3.30b ± 0.26	7.23b ± 0.23	3.62 c ± 0.09
TA8	1.68a ± 0.07	2.83b ± 0.16	5.46d ± 0.26	6.82ab± 0.23	2.60 a ± 0.09

3.5. Establishment of Germplasm

Germplasm for *L. pumila* was established in 2010 at FRIM, Field 13 covering an area of 0.8 hectare (Figure 5). This area was planted with a total of 2250 mother plants with planting spacing of 30 cm x 30 cm between rows and between plants (Figure 6).

Figure 5: Germplasm of *Labisia pumila* established in FRIM

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Figure 6: *Labisia pumila* plants with planting spacing of 30 cm x 30 cm

4. Conclusion

Sustainability through ex-situ need to be continued as it will be the main genetic sources for further research in genetic or plant breeding program. Thus the principal to sustain any plants included herbs species such as *L. pumila* has to be understood by many peoples to avoid the loss of diversity. All the planting materials must be properly managed to encourage and facilitate their use by plant breeders and other researchers.

In terms of commercialization, by having this germplasm, the selected high yielding materials are ready for mass production in the future to fulfill the demand from herbal industries. We anticipated that in future the industry players would sooner or later, seek FRIM help in providing high quality planting materials for the species of *E. longifolia* and *L. pumila*.

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