# SELECTION OF HIGH YIELDING GENOTYPES OF *SENNA ALATA* FOR FUTURE BREEDING PROGRAMME

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## ABSTRACT

*Senna alata (L.) Roxb. or locally known in Malaysia as Gelenggang is an important medicinal and ornamental flowering tree in the family of Fabaceae. This plant can grow up to 12 m high and can be found mostly in tropical countries with warm and humid environments such as Africa and Southeast Asia. Senna alata leaf extract has been reported to have various pharmacological activities including antibacterial, cytotoxicity, anti-inflammatory, antidiabetic, antihepatotoxic, hepatoprotective effects, antiseptic, antiviral and exhibited strong DPPH radical scavenging activities. The highest content of major flavonoid glycoside compound, kaempferol-3-O-gentiobioside (K3G), which has anti-inflammatory effect was detected in leaf extracts of of C. alata. Due to the pharmaceutical potential of the species, this research has been conducted to screen the K3G compound on 12 genotypes of S. alata collected from four wild populations in Peninsular Malaysia. The main objective is to select superior genotypes of the species which contains high chemical constituents for future production of high-quality planting materials. This paper also highlights the process of collection, propagation via seeds, establishment of germplasm as part of the process in screening of high-quality genotypes of the species.*

Key words: herbal medicine, high quality planting materials, germplasm, anti-inflammatory

## INTRODUCTION

The World Health organization (WHO) appraised that 80% of the population in developing countries relied on herbal medicine (Ekor, 2013). Recently, it was discovered that one-third of commonly used drugs were obtained from natural sources (Oluwole et

al., 2020). Hence, medicinal plants with diverse pharmacophore are being scientifically investigated. A tropical plant that has shown potential in this regard is *Senna alata*.

*Senna alata* also known as *Cassia alata*, is a widely distributed herb of the Fabaceae family. In Malaysia, it is known as gelenggang or daun kurap. Elsewhere it is commonly known as candle bush, Christmas candle, craw-craw plant, acapulo, ringworm bush or ringworm plant. This shrub originated from Argentina, and currently can be found in Asia and Africa (Bradley *et al*., 2019). The plant is large and can be reached heights of 1 to 2 m, and has greenish branches. This plant is easily identified from its morphological characteristics. It has pinnate leaves, oblong-elliptical with rounded corners leaves with large inflorescence and orange-yellow flowers. The fruit is tetragonal, winged pods, black, glabrous and up to 50 quadrangular seeded (Globinmed, 2020).

In Ayurvedic, Sinhala, Chinese, and African traditional medicine, various parts of *S. alata* is used in therapeutic activities. In northern Nigeria, a decoction of stem, leaf, and root is used to treat wound, skin respiratory tract infection, burns, diarrhoea and constipation (Uwazie *et al*., 2020). In Malaysia, fresh leaves of the plant are used to treat skin rashes, mycosis, and dermatitis. The frequent use of *S. alata* leaves is more than that of roots, flowers and roots. Whereas in Nigeria, the plant has been processed into capsules, pellet and tea for preventing diseases and maintaining good health (Oluwole et al. 2020). *Senna alata* has been reported to have a variety of bioactive compounds. The major compounds found are kaempferol and glycosides (such as kaempferol-3-O-gentiobioside and kaempferol-3-O-β-d-glucopyranoside) (Naowaboot and Wannasiri, 2016). This compound has contributed to various pharmacological activity such as anti-inflammatory, antimicrobial, anti-obesity, anti-malarial and hepatoprotective activity (Ranjanie et al. 2019). Other chemical constituents found are phenolics (rhein, chrysaphanol, kaempferol, aloeemodin, and glycosides), anthraquinones (alatinone and alatonal), fatty acids (oleic, palmitic, and linoleic acids), steroids, and terpenoids (sitosterol, stigmasterol, and campesterol) (Liu et al. 2009).

Looking at the pharmaceutical potential of the species, an initiative has been taken by Forest Research Institute Malaysia (FRIM) to screen the kaempferol-3-O-gentiobioside compound on 12 genotypes of *S. alata* collected from four wild populations in Peninsular Malaysia. The main objective of this study is to select high yielding genotypes of the species which contain high chemical constituents for future production of high-quality planting materials. This paper also highlights the process of collection, establishment of germplasm and seed germination rate as part of the process in screening of high-quality genotypes of the species.

## MATERIAL AND METHODS

### Collection of *Senna alata* genotypes from wild populations

A total of 120 genotypes of *S. alata* were identified from four populations in Peninsular Malaysia such as i) Kuala Selangor, Selangor ii) Raub, Pahang and iii) Ketereh, Kelantan and iv) Kuala Pilah, Negeri Sembilan. A few phenotypically superior genotypes showing good growth, full of branches, superior height, and diameter were selected for the study. The stumps of selected plants were dug out whereas the matured fruit pods were brought back to FRIM, Kepong (Figure 1). These materials were transplanted into polybags and the seeds were used for germination. All collected stumps were labelled differently such as BG1-BG30 (Kuala Selangor, Selangor), CG1-CG30 (Raub, Pahang), DG1-DG30 (Ketereh, Kelantan) and NSG1-NSG30 (Kuala Pilah, Negeri Sembilan).

**Figure 1. Collection and preparation of Senna alata stumps from four populations**

The topographic information such as coordinates, altitudes, dates of assessment and morphological data were also recorded. The data are shown in Table 1 and Table 2.

**Table 1: Topographic information of *Senna alata* genotypes from four populations**

| Populations | Genotype Code | GPS Points | Altitude |
|---|---|---|---|
| Kuala Selangor, Selangor | BG | N3 21'51.6 E101 19'21.5 | 9 m |
| Raub, Pahang | CG | N3 56'28.2 E101 50'45.0 | 135 m |
| Ketereh, Kelantan | DG | N5 34'68.9 E102 13'89.3 | 48 m |
| Kuala Pilah, N. Sembilan | NSG | N2 44'33.9 E102 08'88.7 | 115 m |

**Table 2: Morphological data of *Senna alata* genotypes from four populations**

| Populations | No. of clumps | Height (m) | Diameter (cm) | Leaf length (cm) | Leaf width (cm) |
|---|---|---|---|---|---|
| BG | 1-3 | 1.0-4.2 | 1.2-6.8 | 10.0-16.3 | 4.0-7.8 |
| CG | 1-5 | 0.5-5.3 | 0.7-7.5 | 10.5-17.2 | 3.5-6.5 |
| DG | 1-8 | 1.02-2.56 | 1.0-5.4 | 10.2-18.5 | 4.4-7.8 |
| NSG | 2-9 | 1.03-2.97 | 1.0-4.0 | 9.3-17.4 | 3.8-7.5 |

**Maintenance of *Senna alata* stumps at nursery**

It is vital to maintain the stumps of *S. alata* at nursery condition in order to make sure that the plants get appropriate treatment and have higher percentage of survival. It is also the process to understand their requirement such as fertilizer regime and water supply. All stumps were planted in polybags with the size of 10" x 10" and maintained for three months before being planted at germplasm. All plants were irrigated with water sprinkler system twice a day.

**Establishment of germplasm**

After three months, all the grown stumps were planted as a germplasm collection located at FRIM, Kepong. The planting distance was 1.5 m x 1.5 m. The growth data at the germplasm were collected monthly. The parameters measured were height (cm), diameter (mm) and the size of the silara (x and y). After 12 months of planting, only genotypes with good growth performance and reached the minimum height of 1.0 meter were choose for further chemical analysis.

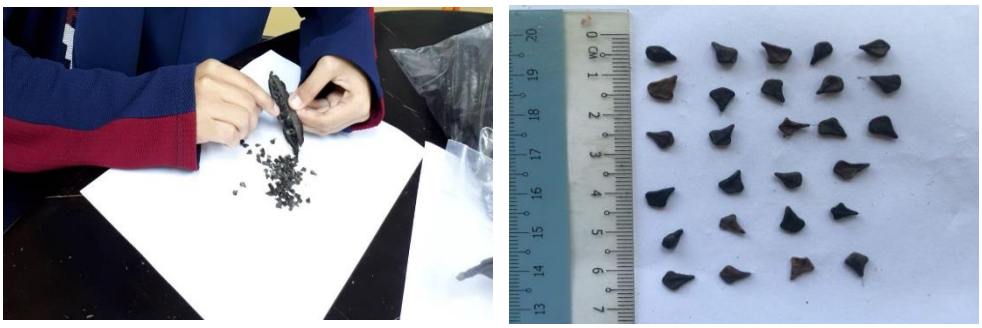
**Quantitative analysis of kaempferol 3-*O*-gentiobioside using High Performance Liquid Chromatography (HPLC)**

A total of 12 genotypes with the height of 1.0 m and above were selected from germplasm for chemical screening. HPLC analysis was conducted on a WATERS HPLC system equipped with a photodiode array (PDA). Samples were prepared in methanol by ultrasonication for 15 minutes. Sample solutions were then filtered using 0.45 µm PTFE membrane filter before eluted through a Phenomenex Luna C18 column (5 µm, 2 mm x 250 mm). A gradient elution was carried out with a mobile phase consists of 0.1% formic acid in water (A) and acetonitrile (B). The gradient profile used was 10% - 40% B in 20 min, 40% - 50% B in 20 min, 50% - 90% B in 10 min and equilibrate for 5 min. The flow rate was 1 mL/min and injection volume was 10 µL. Kaempferol 3-*O*-gentiobioside was detected by measuring the absorbance at 280 nm. For quantification analysis, a series of concentrations of kaempferol 3-*O*-gentiobioside in the range of 5 µg/mL to 1000 µg/mL were prepared and a calibration curves was plotted.

**Germination of *Senna alata* Seeds from Selected Genotypes**

Besides chemical screening, performance of the 12 genotypes were also evaluated in terms of their germination rate. Seeds were extracted manually and air dried at a normal temperature (Figure 2). Only complete dried seeds were chosen for germination studies. A total of 100 *S. alata* seeds from each of 12 selected genotypes were sown in a germination tray with 100% of sand medium. The tray was placed under 50% shade and equipped with complete irrigation system. The mist sprinkler was set up for 1 minute for three times per day. Data on germinated seeds were collected every two days.

**Figure 2. Seeds of Senna alata were extracted manually**



## RESULTS AND DISCUSSIONS

### Growth performance of 12 genotypes *Senna alata*

After 12 months of growth at the germplasm plot, only 12 genotypes of *S. alata* were selected for further chemical screening. It was observed that most of the selected genotypes had height of more than 1.0 m with diameter range of between 1.2-5.8 m. In terms of leaf characteristics, all genotypes recorded more than 10.0 cm of leaf length, whereas more than 5.0 m for leaf width. The growth performance for the 12 genotypes are shown in Table 3.

**Table 3: Growth performance of 12 genotypes *Senna alata* 12 months after planting at germplasm**

| Genotype | No of Clumps | Height (m) | Diameter (cm) | Leaf Length (cm) | Leaf Width (cm) |
|---|---|---|---|---|---|
| BG20 | 6 | 3.0 | 5.80 | 11.7 | 5.5 |
| BG24 | 2 | 2.0 | 3.10 | 11.0 | 5.4 |
| BG25 | 2 | 1.9 | 2.20 | 11.3 | 5.4 |
| BG29 | 3 | 1.2 | 2.40 | 10.6 | 5.3 |
| BG30 | 2 | 3.0 | 3.20 | 11.9 | 6.2 |
| NSG12 | 2 | 1.4 | 1.20 | 14.5 | 5.7 |
| CG30 | 5 | 2.1 | 2.80 | 15.2 | 5.3 |
| CG32 | 3 | 2.5 | 1.60 | 15.2 | 5.7 |
| DG23 | 2 | 1.9 | 1.50 | 17.8 | 7.5 |
| DG21 | 4 | 1.4 | 1.20 | 12.0 | 6.4 |
| BG6 | 4 | 1.8 | 3.20 | 12.9 | 5.5 |
| BG17 | 1 | 1.2 | 2.20 | 10.9 | 5.0 |

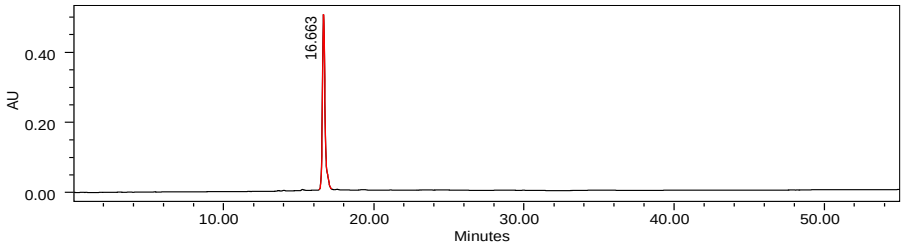
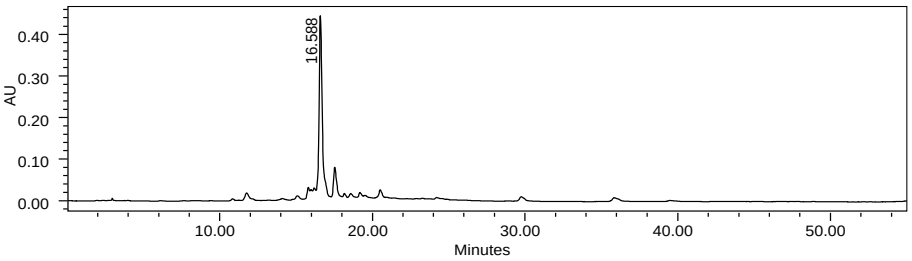
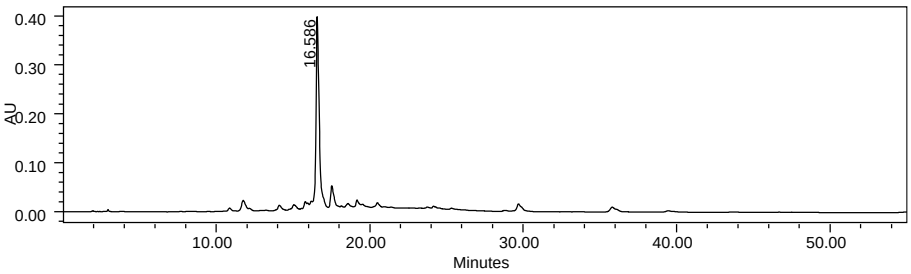
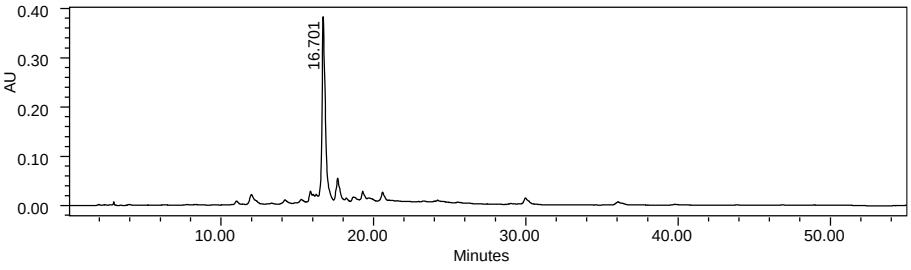
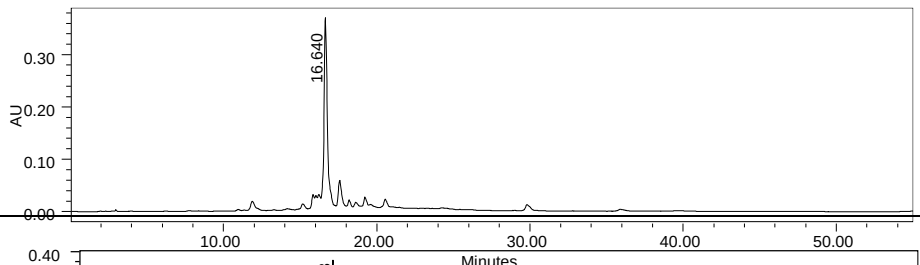
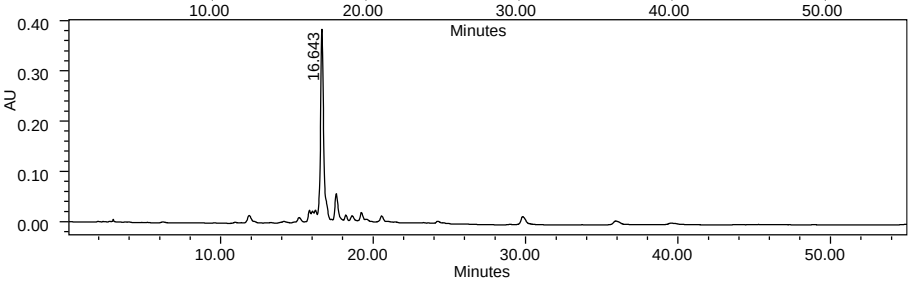
### Selection of superior genotypes of *Senna alata*

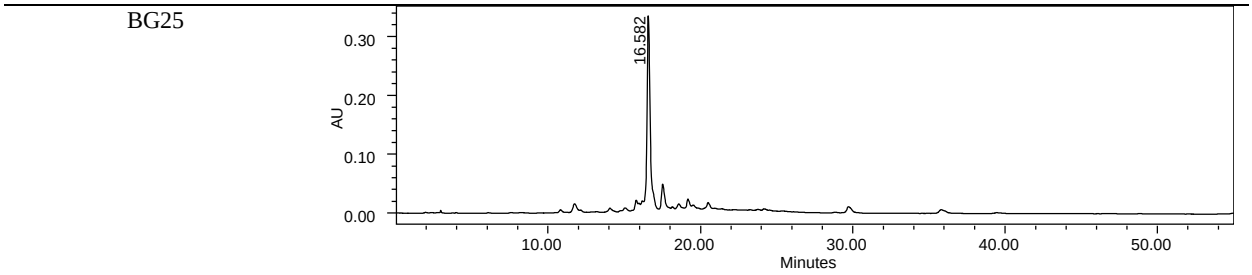
All samples from 12 genotypes of *S. alata* exhibited the kaempferol-3-O-gentiobioside constituent. Results showed that the concentration of kaempferol-3-O-gentiobioside from 12 genotypes have the minimum value of 0.43 ± 4.68% (w/w) and maximum value of 0.84 ± 7.47% (w/w) which were presented during the injection time of 17 minutes (Table 4). However, for further selection as superior genotypes, only six genotypes were selected due to higher concentration of kaempferol-3-O-gentiobioside which are more than the average of 0.56% (w/w). The six genotypes that categorized as superior were BG29 (0.84 ± 7.47% w/w), DG23 (0.80 ± 4.51% w/w), DG21 (0.76 ± 4.86% w/w), BG30 (0.71 ± 1.94% w/w), BG20 (0.66 ± 4.48% w/w) and BG25 (0.59 ± 5.11% w/w). The HPLC profiles for the six superior genotypes of *S. alata* were shown in Figure 3. Previously, it was reported that other herbal plant that also contains of kaempferol-3-O-gentiobioside constituent were *Arabidopsis thaliana* leaves (Markus & Guido, 1999) and *Cornus Canadensis* (Bain & Denford, 1979). Superior genotypes usually referred to good growth characteristics and/or contained high quality active ingredients (Zobel and Talbert, 1984). High quality materials will be the added value to the end products. Therefore, it is important to screen the genotypes which has better characteristics than the common one. A few investigations on selection of superior genotypes was previously conducted by FRIM on selected species such on *Citrus hystrix* (Farah Fazwa et al., 2005); *Citrus microcarpa* (Farah Fazwa et al., 2007); *Labisia pumila* (Farah Fazwa et al., 2012) and *Chromolaena odorata* (Farah Fazwa et al., 2019).

**Table 4. Percentage of kaempferol-3-O-gentiobioside ± RSD w/w for 12 selected *Senna alata* genotypes**

| Genotype no. | Percentage of KG3 ± RSD w/w |
|---|---|
| BG 29 | 0.84 ± 7.47 |
| DG 23 | 0.80 ± 4.51 |
| DG 21 | 0.76 ± 4.86 |
| BG 30 | 0.71 ± 1.94 |
| BG 20 | 0.66 ± 4.48 |
| BG 25 | 0.59 ± 5.11 |
| BG 6 | 0.52 ± 1.30 |
| BG 24 | 0.50 ± 3.44 |
| CG 30 | 0.50 ± 4.41 |
| BG17 | 0.46 ± 2.91 |
| NSG 12 | 0.43 ± 4.68 |

**Figure 3: HPLC profiles of Kaempferol 3-O-gentiobioside from six superior genotypes of Senna alata**

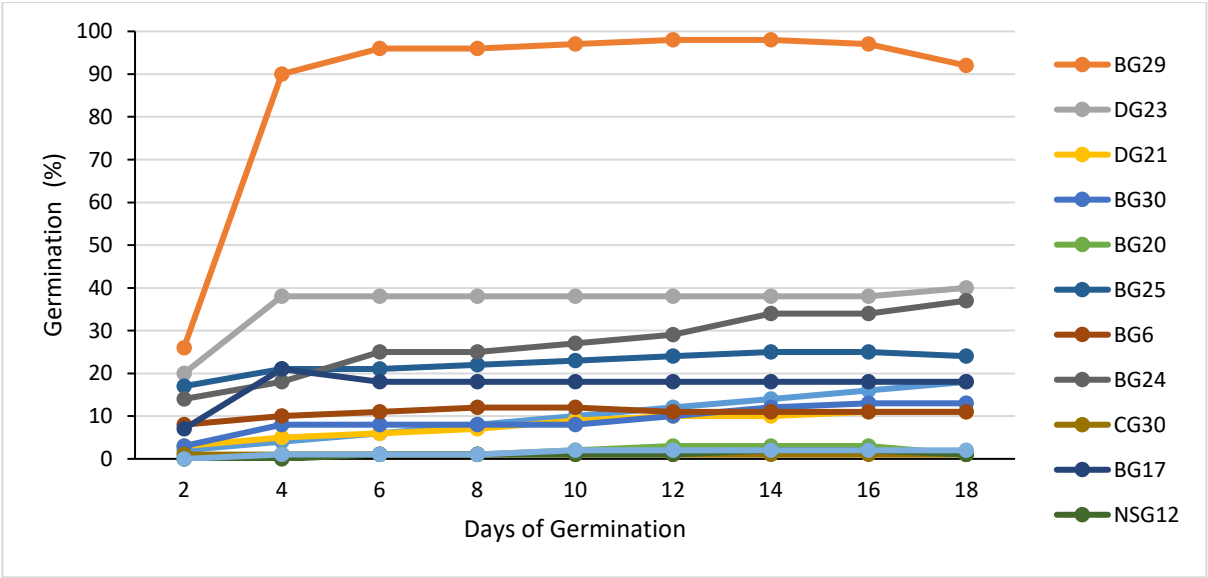
| Genotypes | HPLC Chromatogram |
|---|---|
| Standard<br>*Kaempferol 3-O-gentiobioside* |  |
| BG29 |  |
| DG23 |  |
| DG21 |  |
| BG30 |  |
| BG 20 |  |




**Seed Germination Rate of Selected *Senna alata* Genotypes**

Results in Figure 4 showed that seeds from genotype BG29 gave higher number (>90%) of germination compared to others after two days of germination. Previous study reported that the seeds of *S. alata* after water soaked for 24hr resulted in better germination percentage of around 76.80% (Thirupathi, 2012). The number of seeds germinated increased drastically from 22 to 90 after four days of germination. Besides BG29, the highest germination rate was also recorded by genotypes DG23 and BG24 with the percentage of almost 40%. Whereas, other seeds showed germination rate below than 30%. Genotype CG30 recorded the lowest germination rate (<5%) compared to others, might be due to the less viability of the seeds. The results indicated that only seeds of *S. alata* from genotype BG29 has the highest seed viability. This finding showed that this plant has high potential to be selected as good parent material for future breeding programme. In addition, according to Kumar et al. (2008), seed characteristics such as seed length of different provenance is important criteria in order to produce maximum germination percentage. Besides that, seeds sources from different agroclimatic zone also gave an effect to the seed viability. According to Zobel & Talbert (1984), the ensuring seed production are coming from good genotype individuals. The mother trees which are pollinated by good genotype individuals, minimal selfing or breeding which then produced a vigour and good generation. Thus, all mother trees have different viability due to different genotype and even phenotype which have different seeds size.

**Figure 4. Germination rate of Senna alata seeds from twelve genotypes**




**CONCLUSION**

The six superior genotypes of *Senna alata* which contained high concentrations of kaempferol 3-O-gentiobioside will be further investigated by conducting genotype x environment interaction study. Prior to that, the best mass production method for the species has to be developed. The selected superior genotypes will provide the opportunity to use these basic planting materials to initiate a breeding programme for the species. Through selection, plants from different origins can be improved to develop new clone or varieties. The established germplasm is also a method of genetic conservation and to sustain the production of quality planting materials in the future. In addition, seed germination screening provides a great opportunity to the tree breeder to screen and capture natural variation, besides providing information on the raw material for breeding. As conclusion, outputs from the study are not only beneficial to the plant breeder in the aspect of producing new variety but also to herbal industries in producing high quality herbal products.

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