# COLLECTION AND PROPAGATION OF *SENNA ALATA* FOR THE ESTABLISHMENT OF GERMPLASM FOR FUTURE BREEDING PROGRAM

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**ABSTRACT**

*Senna alata (L.) Roxb. or locally known as Gelenggang is an important medicinal and ornamental flowering tree from the family of Fabaceae. It is an annual and occasionally biannual herb with an average height of 1 to 4 m. This herb is found in tropical countries with warm and humid environments such as Africa and Southeast Asia. Senna alata has been reported to have various pharmacological activities including antibacterial, cytotoxicity, anti-inflammatory, antidiabetic, antimalaria, antifungal, antihepatotoxic, hepatoprotective effects, antiseptic, and antiviral. This herb also has several nutritional constituents that helps in formulation of herbal drugs and dietary supplements. As this herb getting attention for pharmacology activities, it is needed for this herb to be cultivated in new ecosystem and managed under the good agricultural practices. However, good planting materials should be provided before it can be commercially cultivated. Realising to this, plant breeders from Forest Research Institute Malaysia (FRIM) have taken an initiative to conduct extensive collections of S. alata mother plants from four natural populations throughout Peninsular Malaysia. The four populations are Kuala Selangor, Selangor; Raub, Pahang; Ketereh, Kelantan; and Kuala Pilah, Negeri Sembilan. A total of 30 stumps of from S. alata mother plants were collected from each population. Matured fruit pods from selected mother plants were also collected. All stumps were hardened at FRIM's nursery for about 6 months before they were transplanted to germplasm plot for future breeding program. In future, it was hoped that the selection of high-quality cultivars with good phenotypic characteristics and quality bioactive compounds could be produced.*

Key words: breeding, collection, gelenggang, germplasm, growth performance, propagation.

## INTRODUCTION

*Senna alata* or *Cassia alata* (candle bush) is a shrub and small tree in a family of Fabaceae. In Malaysia, it was locally known as "gelenggang" and "daun kurap". It is an annual and occasionally biannual herb with an average height of 1 to 4 m. This shrub has a beautiful look as their inflorescence looks like a yellow candle. The fruit is up to 25 cm long and is a straight pod with numerous seeds. Most of the species of *Senna* are self-incompatible and reproduced by seed. This herb can be found in tropical countries with warm and humid environments such as Africa and Southeast Asia. This species is easy to grow in an open area, disturbed areas that are not too dry and also being able to grow in flooding shrubland.

Cultivation of *S. alata* is not reported yet by any country either in Africa or Southeast Asia because it is considered as a weed. So, normally their distributions can be seen surrounding area of orchard, plantations and disturbed area (ILDIS, 2016). However, this herb has long been traditionally used for the treatment of constipation and dermatophyte infections as it has active constituents for the laxative properties and as antifungal active agents (Chatsiriwej, 2006). In traditional way, they just rubbed the pounded leaves and mix with lime and apply to human skin as a remedy for ringworm and other cutaneous disease. A decoction of the leaves may also be taken as a purgative (Burkill, 1966).

In biological and pharmaceutical activities studies, *S. alata* has been reported to have a variety of bioactive compounds. The major compounds found in Methanolic extract are kaempferol and glycosides (such as kaempferol-3-O- gentiobioside and kaempferol-3-O-β-d-glucopyranoside) (Naowaboot and Wannasiri, 2015). 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 that is found in the plant 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).

As this herb getting a great attention for pharmacology activities, it is extremely worrying if it adversely affects the wild habitat of this species due to over harvesting, improper collection practices and pollution (Dajic-Stevanovic et al. 2012) for extensive research and product. Thus, cultivation is the only way to stop or reduce harvesting pressure on this wild plants. However, this cultivation of raw materials of medicinal plants should be carried out in line with the terms of good agricultural practices (GAPs) either it is grown as single field crops, intercropping systems or plantation crops. In the same time, good stocks of planting materials should be ready and available before it can be cultivated for commercial purposes.

For these reasons, breeding program is important. Many breeders were emphasized on breeding for direct improvement of yield as well as agronomic characteristics such as standability and adaptability that may involved with breeder making choice as to the parents or starting material to be used to create segregating population. Then, it will involve either with selection or without selection until the purpose of producing homozygous lines completed before the best line were released as improved pure-line cultivars or improved germplasm. This is what happened in any breeding cycle. Next, the final product which is pure-line cultivars is the aim for grown by the farmer.

Therefore, plant breeders from Forest Research Institute Malaysia (FRIM) aim similar final product of breeding in potential medicinal plants. So, initiative has been taken to conduct extensive collection of good source *S. alata* mother plants from four natural populations throughout Peninsular Malaysia and planted them as *ex-situ* conservation and in a specific environment through germplasm establishment. After that, planting materials collected were then tested for their propagation response through seed germination and air layering technique after several months planted in germplasm based on the growth selection. Therefore, this paper will discuss on the collection technique and the best techniques for propagation of *S. alata* before it released as cultivar.

## MATERIALS AND METHODS

### Plant Materials

*Senna alata* plants with vigor growth, completed with fruits and inflorescence, no of clumps with greeny branches colours were selected as mother plants (Figure 1). The plant morphological data were recorded (Table 1). The topographic information such as coordinates, altitudes and dates of assessment also were recorded (Table 2) for easy to trace back the mother plants if any misleading happened. In this study, stumps of *S. alata* from mother plants selected were dig out to enable easier handling and transporting back to FRIM kepong. Adequate care was applied during transportation to avoid mishandling of plants during loading and unloading. All of the stumps from plants selected were collected from four populations in Peninsular Malaysia i.e. i) Kuala Selangor, Selangor ii) Raub, Pahang and iii) Ketereh, Kelantan and iv) Kuala Pilah, Negeri Sembilan. All of them were packed with black plastic bag and were tagged properly using special code such as BG for Selangor, CG for Pahang, DG for Kelantan, and NSG for Negeri Sembilan. This part is important so that the stumps collected were not mixed-up after they were placed at Nursery.

**Figure 1. Selected mother plants of Senna alata from identified locations**

**Table 1. Morphological data of Senna alata mother plants from four populations selected**

| Criteria | 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 |

**Table 2. Topographic information of Senna alata mother plants from four populations**

| Mother Plants Code | GPS Points | Altitude |
|---|---|---|
| BG | N3 21’51.6 E101 19’21.5 | 9 m |
| CG | N3 56’28.2 E101 50’45.0 | 135 m |
| DG | N5 34’68.9 E102 13’89.3 | 48 m |
| NSG | N2 44’33.9 E102 08’88.7 | 115 m |

**Plant Maintenances**

Stumps from the four locations were potted in polybag of 10” x 10” with good growing media containing mixture of top soil, leaves compost and sand (Figure 2). All the stumps potted were arranged according their groups of population and placed under shading 70% to avoid direct sunlight. Sufficient watering was given during this stage to ensure optimum growth of the stumps.

Stumps at the Nursery took about 3 months until the new shoots produced and another 3 months required them to fully developed with many branches. Once stumps were at this stage, manuring with right amounts of NPK is important for the plants to ensure that they get sufficient nutrients to grow well.

However, one month prior to germplasm establishment, all developed plants were gradually placed under 50% shaded to enable them them to get direct sunlight. It is important so that the plants are getting tougher and survived after planting.

**Figure 2. Stumps of Senna alata were potted in a good growing media**

**Germplasm Establishment**

All the developed plants of Senna alata were transplanted at germplasm in FRIM Kepong, Selangor at a planting spacing of 1.5m x 1.5m (Figure 3). All plants planted were treated uniformly by applying rooting enhancer powder, Christmas Island Rock Phosphate (CIRP) and organic matter such a compost in each planting hole. Growth data such as survivality, height (cm), diameter (mm) and crown leaves (X and Y) were recorded up to one year after planting. The survived plants with good phenotypic characteristics were selected and tagged for future selection.

**Figure 3. Germpalsm layout of Senna alata at FRIM Kepong**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BG1 | BG2 | BG3 | BG4 | BG5 | DG1 | DG2 | DG3 | DG4 | DG5 |
| BG10 | BG9 | BG8 | BG7 | BG6 | DG10 | DG9 | DG8 | DG7 | DG6 |
| BG11 | BG12 | BG13 | BG14 | BG15 | DG11 | DG12 | DG13 | DG14 | DG15 |
| BG20 | BG19 | BG18 | BG17 | BG16 | DG20 | DG19 | DG18 | DG17 | DG16 |
| BG21 | BG22 | BG23 | BG24 | BG25 | DG21 | DG22 | DG23 | DG24 | DG25 |
| BG30 | BG29 | BG28 | BG27 | BG26 | DG30 | DG29 | DG28 | DG27 | DG26 |
| CG1 | CG2 | CG3 | CG4 | CG5 | NSG1 | NSG2 | NSG3 | NSG4 | NSG5 |
| CG10 | CG9 | CG8 | CG7 | CG6 | NSG10 | NSG9 | NSGG8 | NSG7 | NSG6 |
| CG11 | CG12 | CG13 | CG14 | CG15 | NSG11 | NSG12 | NSG13 | NSG14 | NSG15 |
| CG20 | CG19 | CG18 | CG17 | CG16 | NSG20 | NSG19 | NSG18 | NSG17 | NSG16 |
| CG21 | CG22 | CG23 | CG24 | CG25 | NSG21 | NSG22 | NSG23 | NSG24 | NSG25 |
| CG30 | CG29 | CG28 | CG27 | CG26 | NSG30 | NSG29 | NSG28 | NSG27 | NSG26 |

**Screening of superior plants of *Senna alata* using High Performance Liquid Chromatography (HPLC)**

A total of 0.5 g of sieved powder material (500 µm) from 12 superior plant leaves were prepared. These powders were mixed with 5 mL of methanol in the vial of 14mL. The mixture was ultra-sonicated for 15 minutes. The solution was filtered using 0.45 µm syringe filter prior to analysis. The samples were analysed by means of a HPLC system (Waters 600 quarternary gradient pumo, Waters 2707 Autosampler and Waters 2998 photodiode array detector). A Phenomenex Luna C18 column was used (4.6 mm i.d. x 250 mm) and for elution of the constituents, three solvents denoted as A, B and C were employed. A was 0.1% aqueous formic acid, B was acetonitrile and C was methanol. The flow rate used was 1.0 mL/min and the injection volume were 10 µL. The retention times and UV spectra of the targeted compounds were analysed at the wavelength of 220 nm and results and were compared with the standard chemical marker.

**Germination of superior plants of *Senna alata***

In order to produce good planting materials and to maintain the stocks of superior plants, seed from matured fruits of superior *S. alata* were collected for germination purpose. A total of 100 *S. alata* seeds from 12 superior plants selected were sown in a germination tray containing 100% sand medium. The tray was placed at FRIM nursery under 50% shade and equipped with complete irrigation system. The mist sprinkler was set up for 1 minute three times per day. Data on germinated seeds were collected every two days.

**Propagation of superior plants of *Senna alata***

Eventhough *Senna alata* can be easily produced by seeds, it is impossible to maintain and conserve the plants using this technique because of their irregular fruiting. Another reason is that the plants produced by seeds are not always identical to the selected parent plant. So, another alternative method of propagation is by propagating the 12 superior plants of *S. alata* using air layering technique so that the plants are genetically parallel and uniform for all the superior plants selected.

Simple air layering technique for *S. alata* is as bellow:

a) Healthy green stem or branches of superior *S. alata* one year old were selected as planting materials
b) Complete bark of stem or branches were cut out using stem-girdling method.
c) The area of the stem was applied with rooting hormone indole-3-butyric acid (IBA).
d) Then, the girdled region was covered with mixture soil (top soil and coco peat) and isolated with a PVC plastic film.

**Figure 4. Air layering technique for *Senna alata***


## RESULT AND DISCUSSIONS

This is the first germplasm establishment for potential medicinal plants such as *S. alata* conducted in Malaysia. After one year planting at FRIM Kepong, Selangor, our data showed the survivality of S. *alata* was below 50%. This may be due to the factor of planting environments at this location that have to be study later.

However, the survived plants still showing a good growth for further selection of their superior characteristics, determination of their chemical profiling and determining the targeted active compound.

Data after one-year growth of *S. alata* for four populations is as shown in Table 3. From the data, *S. alata* from Selangor (BG) population has a good growth in term of their height, diameter, and crown. They achieved their maximum height of 1.63 m with a diameter of 6.8 cm. It is followed by *S. alata* from Kelantan with their maximum height of 1.13 m and diameter of 5.4 cm. Whereas, value data for the growth of *S. alata* from Pahang and Negeri Sembilan is approximately similar to one another. Crown x and y for *S. alata* from Selangor were also larger than the others. The variance of the growth is due to plants planted have different viability due to different genotype and even phenotype (Zobel and Talbert, 1984).

**Table 3. Population Descriptive on *Senna alata* growth after one year planting at FRIM Kepong germplasm**

| Pop | Height (m) | Diameter (cm) | Crown X (cm) | Crown Y (cm) |
|---|---|---|---|---|
| BG | 0.38-1.63 | 1.2-6.8 | 28.0-127.0 | 20.0-113.0 |
| CG | 0.30-0.96 | 0.7-7.5 | 19.0-64.0 | 17.0-57.0 |
| DG | 0.17-1.13 | 1.0-5.4 | 15.0-86.0 | 23.0-69.0 |
| NSG | 0.18-0.70 | 1.0-4.0 | 10.0-50.0 | 9.0-47.0 |

Based on data evaluated, 12 superior plants of *S. alata* from four populations planted at germplasm were selected as they have a good growth characteristic in terms of height, diameter, no of clumps, leaf length and leaf width.

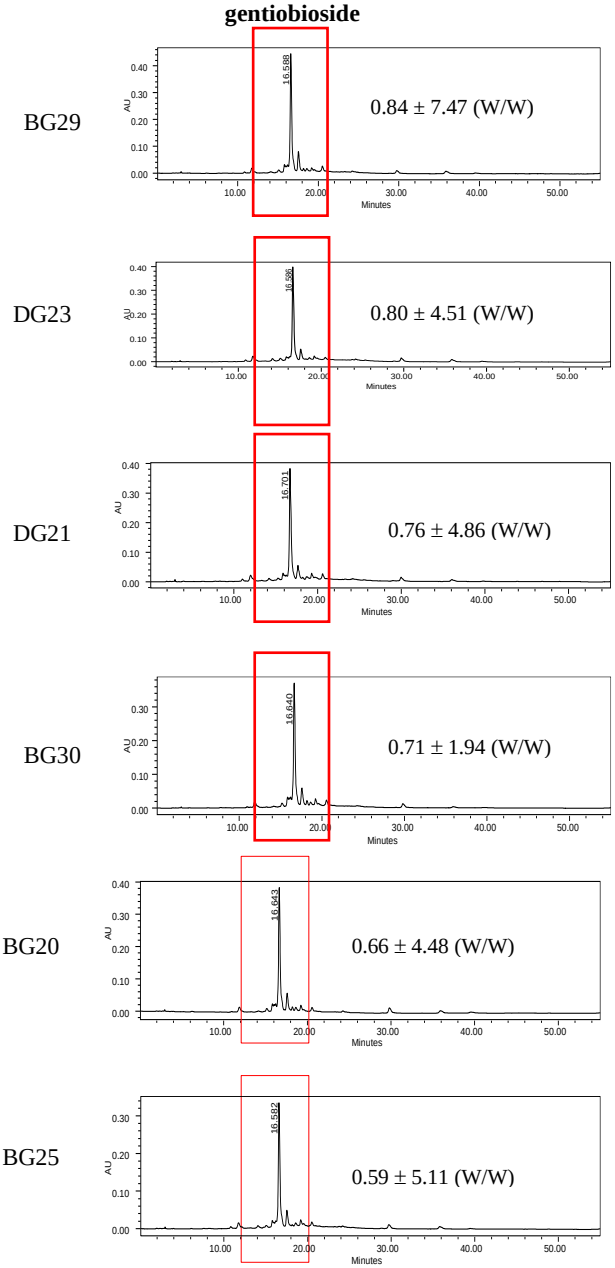
After one year, it was observed that most of these plants recorded height of more than 1.0 m with diameter range of between 1.2 to 5.8 m. In terms of leaf characteristics, the plants recorded more than 10.0 cm of leaf length and more than 5.0 m for leaf width. The growth performance for selected superior plants were shown in Table 4. Twelve selected plants mostly come from the populations of Selangor (BG20, BG24, BG25, BG29, BG30, BG6, BG17), the followed by population Pahang (CG30, and CG32) and two plants from population Kelantan (DG23, and DG21).

**Table 4: Growth Performances of 12 Superior Plants after One Year Planted at FRIM Kepong**

| Mother Plants Code | 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 | 1 | 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 |

From these 12 selected superior plants, quantitative analysis using HPLC were further conducted for screening their targeted chemical compounds. Results showed only six superior plants were selected as high yielding accessions with high concentration compound of kaempferol 3-O-gentiobioside (Figure 5). This compound was present at minutes of 16.

**Figure 5. HPLC Chromatogram of six selected high yielding of *Senna alata* with high concentration kaempferol 3-O-gentiobioside**



Compound found in *S. alata* from FRIM Kepong germplasm were similar as found in other studies done by Moriyama et al. (2003), Pham et al. (2017) and herbal monograph in Globinmed (Feb, 2021).

After screening of their chemical compounds and good growth, mutiplication of these superior plants were conducted by germination test and asexually techchnique. Based on evaluation, BG29 gave maximum germination rate (>90%) compared to others superior seeds after two days of germination. The number of germinated seeds increased drastically from 22 % during day two to 90% after day four germination. It is in line with the study done by Parede et al. (2019), where seeds from family Fabaceae is easy to to be germinated and has a potential to achieve 100% germination rate if some treatments are given.

However, other seeds such as DG23, DG21, BG30, BG20, BG25, BG6, BG24, BG17, and NSG12 have lower germination rate below than 40%. Whereas, CG30 and CG32 have lowest germination rate below than 5%. This may be due to less viability of the seeds itself if compared to the seeds from *S. alata* BG29. It showed that BG29 has a good characteristics and has potential to be selected as good planting material for future breeding programme. In addition, according to Neerja et al. (2001), seed characteristics such as seed length of different provenance is an 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.

Other than that, propagation by air layering technique on superior plants of *S. alata* shows that BG 29, BG 30 and CG 30 has a successfull rooting. The rooting just takes about one months. All the rooted stems were cut and being transplanted in a polybag containing mixture of top soil, leaf compost and sand. All the propagules are placed at the nursery under 70% shade and were growing well for future breeding program (Figure 6) .

**Figure 6. Plants grow under 70% shade at Nursery**

## CONCLUSION

This study shows that 50% of *S. alata* plants from four populations can survived under specific germplasm location. The good growth trait from 12 selected *S. alata* shows a good result in germination seed and by air layering technique. It means this plant can be propagated from both techniques in order to produce planting stocks. All 12 selected *S. alata* also presence of targeted compound of kaempferol 3-O-gentiobioside and only six of them were selected to have a high concentration of kaempferol 3-O-gentiobioside after narrow screening. In future, six selected *S. alata* will be propagate to produce uniform planting stocks, tested again in different location before it be released as cultivar.

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