GUIDELINES FOR THE ESTABLISHMENT OF *Aquilaria malaccensis* ARBORETUM IN PENINSULAR MALAYSIA

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List of Acronyms and Abbreviations

CIRP	Christmas Island Rock Phosphate
cm	centimetre
CR	Critically Endangered
CTSP	CITES Tree Species Programme
DBH	Diameter at breast height
DRC	Diameter at the root collar
FRIM	Forest Research Institute Malaysia
g	gram
GPS	Global Positioning System
ha	hectare
IUCN	International Union for Conservation of Nature
KBG	Kepong Botanic Gardens
kg	kilogram
m	metre
RCBD	Randomized Complete Block Design
SSFD	Selangor State Forestry Department

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1.0 INTRODUCTION

Aquilaria malaccensis Lam. (Thymelaeaceae) is a plant species highly sought after for its resin content. Its distribution ranges from India, Myanmar, Sumatra, Peninsular Malaysia, Borneo and the Philippines (Hou 1960). Indiscriminate harvesting from the forests has depleted the wild populations and threatened the viability of populations. As a result, the genus of *Aquilaria* was placed under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in 2004 (CITES 2004). The species is categorized as Critically Endangered (CR) (Harvey-Brown 2018) in the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. Depletion of wild populations could slowly cause the loss of the species' genetic diversity, therefore establishment of arboreta or germplasms is a promising solution to safeguard the species' genetic resources.

According to Article 2, UNCED (1992), *ex situ* conservation is defined as the conservation of components of biological diversity outside their natural habitats. It is an approach normally used to protect populations that are threatened and need to be collected and conserved in gene banks or established arboreta. We proposed a strategy to conserve *A. malaccensis* in Peninsular Malaysia at the genetic level through the establishment of arboreta. An arboretum is an area set aside to grow and display different kinds of worthy ornamental trees, shrubs, vines and other plants. Arboretum has a scheduled maintenance plan and plants have proper record keeping and labels for reference (Wyman 1960). While it is possible to have as many plant species from each region, it does not necessarily have to include all, nor is it necessarily to have a formal planting arrangement in it. Richmond (1971) regarded an arboretum as "an institution which develops and administers collections of trees and shrubs, arranged in aesthetic harmony with the surrounding landscape, and which conduct programmes based upon these collections for the purposes of public service, education, and research".

The main objective in establishing *A. malaccensis* arboreta in Peninsular Malaysia is to conserve the genetic diversity of the species. In addition, the arboreta could also function as places to conduct research, education and seed sourcing activities. The guidelines were developed mainly based on the principles, results and recommendations that were outlined in the Conservation Action Plan for the Threatened Agarwood Species *Aquilaria malaccensis* (Thymelaeaceae) In Peninsular Malaysia (Chua *et al.* 2016). While the overall design of the arboretum is replicable, methods involving collecting sites and planting design need to be adhered strictly to ensure standardisation.

2.0 METHODOLOGY

2.1 Selection of collection sites

Past genetic studies conducted by the Forest Research Institute Malaysia (FRIM) based on 942 *A. malaccensis* samples collected from 35 natural populations in Peninsular Malaysia (Lee *et al.* 2022) inferred that these populations could be divided into two major genetic clusters, that is, Kedah-Perak Cluster and Kelantan-Johor Cluster. This was further narrowed to 16 populations based on the underlying genetic diversity and population health of these populations, and hence prioritised for *in situ* conservation, that is, Machinchang (Kedah), Penang National Park (Pulau Pinang), Bukit Kerajaan (Pulau Pinang), Gunung Jerai (Kedah), Bubu (Perak) and Universiti Teknologi PETRONAS (Perak) from the Kedah-Perak Cluster, and Chabang Tongkat (Kelantan), Gunung Tebu (Terengganu), Merchang (Terengganu), Berkelah (Pahang), Panti (Johor), Paya Rumput (Melaka), Sg. Udang (Melaka), Pasir Panjang (Negeri Sembilan), Mont Kiara (Selangor) and Bukit Lagong (Selangor) from the Kelantan-Johor Cluster (Figure 1). Ideally, all populations should be captured in the germplasm collection for *ex situ* conservation, because genetic diversity forms the basis of adaptive flexibility and evolutionary potential of the species.

The criteria for selection of collection sites are based on:

i. Genetic information generated from previous molecular studies conducted by FRIM.
ii. Originated from natural populations in forest reserves.
iii. Healthy populations that produce ample seeds or seedlings with minimal anthropogenic disturbance.

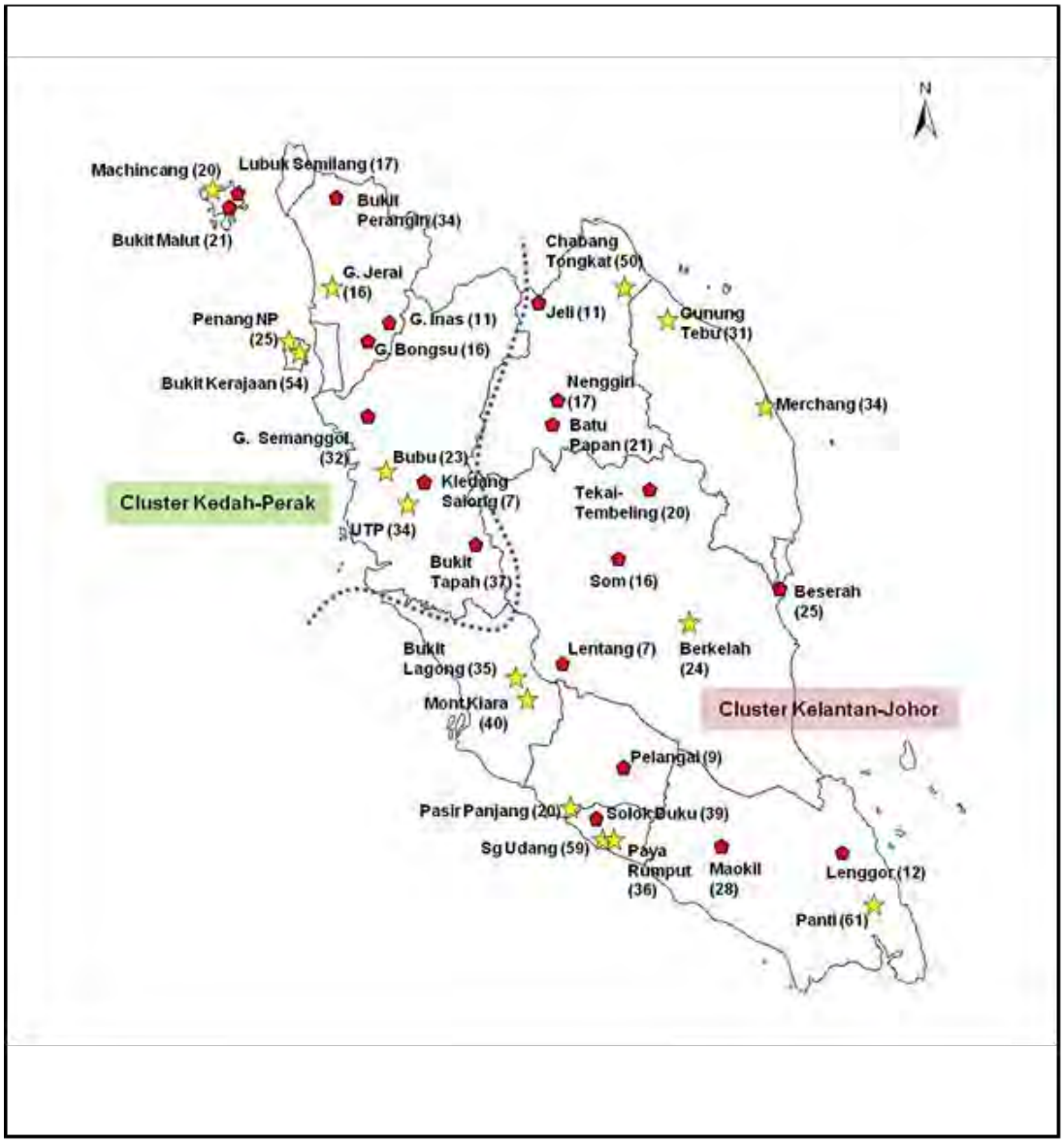

Figure 1. The two genetic clusters of *Aquilaria malaccensis* in Peninsular Malaysia as identified from population genetic studies (Image adapted from Chua *et al.* 2016).

2.2 Collection of planting materials

Planting materials should be derived from seeds and seedlings from mother trees. Regular phenological observation is critical to identify the reproductive state of trees in a population due to differences in flowering months of different populations. Leaf flush, floral budding, flowering and fruiting are important features that must be inspected with a pair of binoculars (Figure 2). A long-zoom-digital camera is also useful to quickly examine the condition of the canopy. The data should be kept in a database for analysis and used to plan collection trips. Generally, the onset of flowering to seed maturity is about four months.

Seeds and seedlings from a minimum of 25 mother trees per cluster were recommended to be used to establish an arboretum (Chua *et al.* 2016). On most occasions, however, this minimum number could not be reached due to the lack of fruiting mother trees, low germination rates, high mortality rates, small population sizes and inaccessibility. The collection of planting materials is therefore dependent on these factors. To mitigate this, the collection should be comprised of, as many as possible, seeds or seedlings of fecund mother trees found in a forest area. Each individual tree should be given a running number and tagged as AM*XXXX* (an acronym for *Aquilaria malaccensis*), for example, AM1234, at the very best, only seedlings that had germinated below the selected mother tree (having no overlapping canopy) should be collected. This is to ensure that their origin could be traced. The same approach was used to collect seeds using seed traps.

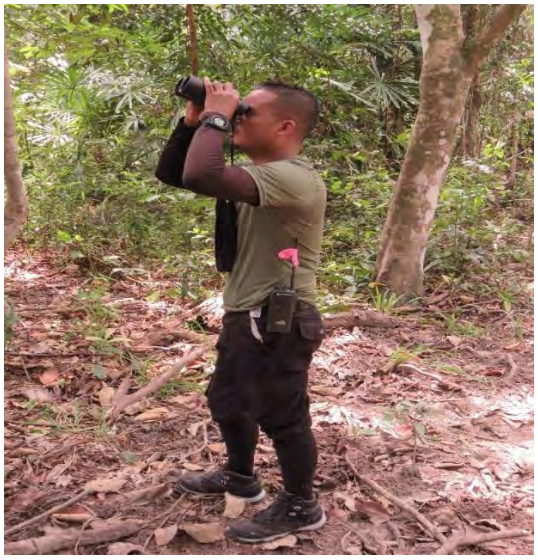

Figure 2. Binoculars are essential to observe the phenological state of trees.

Important points to adhere to when the collection involves seeds:

i. As *A. malaccensis* fruits mature on the tree, seeds should only be collected after the fruits are dehisced. The use of seed traps to collect the seeds is preferred to avoid ground contamination.
ii. Label each seed batch immediately to avoid mix up. The label should consist of collection date, location, mother tree number and quantity.
iii. Being recalcitrant, the seeds have to be sown as soon as possible to retain their viability.
iv. Seeds are scattered on sand medium, watered daily and will germinate in 15–20 days (Figure 3).
v. Seedlings with 4–5 leaves can be transferred into polybags.

Important points to adhere to when the collection involves seedlings:

i. Seedlings of less than 10 cm in height are preferred (Figure 4).
ii. Seedlings should only be collected from moist or wet forest ground to minimize root injuries. Only healthy seedlings without signs of disease and herbivory are chosen.
iii. Ensure the taproot is not injured when removing by gently loosening the surrounding soil with a short stick (Figure 5).
iv. Label each seed batch immediately to avoid mix up. The label should consist of collection date, location, mother tree number and quantity. (Figure 6).

v. To prepare seedlings for transport, they must be partially wrapped in moist tissue paper and placed in a sealed plastic bag (Figure 7). An adequately sealed plastic bag can retain the seedlings in good condition for at least seven days. Avoid keeping seedlings under direct sunlight. Seedlings should be planted into polybags as soon as possible.

Figure 3. Seeds are sown on sand medium and watered daily.

Figure 4. Seedlings of less than 10 cm in height.

Figure 5. A short stick is used to loosen the soil around the seedling.

Figure 6. Collected seedlings are labeled immediately to avoid mix up.

Figure 7. Seedlings are wrapped in moist tissue papers to retain humidity during transportation.

2.3 Seedling management in the nursery

The medium used in the polybag should be a mixture of a 3:2:1 ratio of soil, sand and compost, respectively. A deep small hole is made when potting to reduce damage to the taproot. Potted seedlings are placed under 70% shade and watered once a day (Figure 8). Watering should be reduced to once in two days during the rainy season to avoid pest and disease outbreaks. All polybags should be labeled with a living collection number and grouped in batches (Figure 9). Pesticides should be applied only during serious pest outbreaks (Figure 10). Seedlings in the nursery are susceptible to infestation from the larvae of *Heortia vitessoides*, *Pitama hermesalis*, scale insects and whiteflies, and diseases like Anthracnose, blight and fungus infection (Mohd Farid *et al.* 2015).

Figure 8. Seedlings are placed under 70% shade and watered once a day.

Figure 9. Seedlings are grouped into batches for better management.

Figure 10. Pesticides are applied only during serious pests' outbreaks.

2.4 Planting stock production

Planting materials used for the arboretum establishment originated from the selected sources as described in 2.1 above, collected in either seeds or germinated wildings. Germinated wildings would be potted in individual polybag while germinated seeds would only be transferred to polybags once the seedling has produced 4 to 5 leaves. Hereafter, these planting materials would undergo nursing protocols as described in 2.3 above. Only seedling that has reached a minimum height of 60 cm is selected for out-planting. Taller seedlings are even preferred to ensure a higher survival rate in the field (Figure 11). The selected seedlings should be morphologically sturdy, and free from disease. Diseased and unhealthy seedlings will continue to be nursed and used as replacement seedlings if improvement is observed. In order to allow seedlings to adapt quickly during the hardening process, these seedlings are gradually moved from shades of 70%, 50% and 0% (under open-air) (Aminah 2011). This stage is very important to ensure the seedlings would not be 'shocked' after being planted. The best period for out-planting is the beginning of rainy season and the planting is done in the morning until noon (Ahmad Zuhaidi *et al.* 2015).

Figure 11. Seedlings of at least 60 cm in height are used for out-planting.

2.5 Planting design and layout

An example of a planting design and layout for a nine-population set-up is shown in Figure 12. Colour codes and P*X* (an acronym for *population*), for example P1, P2, etc., can be used to represent the origin of the seedling. Similarly, the word 'B' stands for buffer seedling (see 2.6 below). Seedlings placement can be generated randomly using the 'Generate Random Numbers' in Microsoft Office Excel, for example. The orientation can be altered depending on site suitability. The arboretum should be cleared from other trees including small vegetation to ensure all seedlings received the same amount of sunlight.

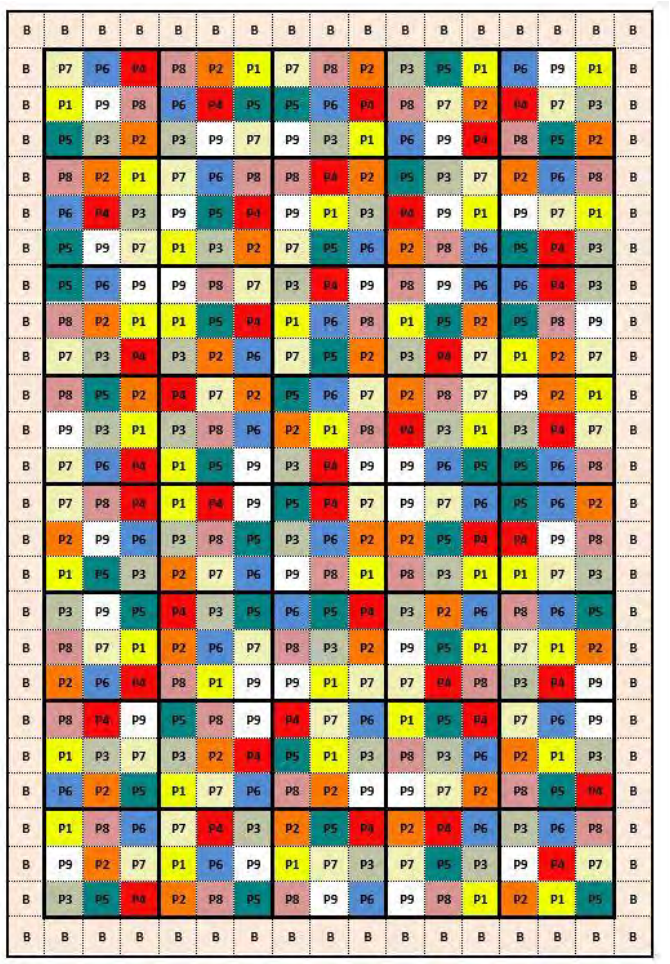

Figure 12. An example of a planting design and layout for the arboretum establishment.

2.6 Site preparation

Site boundary and planting holes are determined using mapping equipment (Figure 13 and Figure 14) to ensure precision. The planting layout uses a *Randomized Complete Block Design* (RCBD) so that all seedlings in an experimental unit would receive the same treatment. The planting interval is 4 m × 4 m, hence an area of approximately 0.7 ha (including buffer plants) is required to establish an arboretum consisting of nine populations with 40 seedlings from each population. Every experimental unit is composed of one seedling per population, and the entire planting design is comprised of 40 experimental units (replicates). This resulted in 360 seedlings being used. Apart from that, another 82 seedlings (which can be derived from the same populations or different populations of the 360 individuals) will be planted as a buffer along the perimeter of the plot. The buffer acts as a separator from the surrounding vegetation.

The design uses a monoculture approach where only *A. malaccensis* is planted without inter-cropping.

Figure 13. Mapping equipment used to determine site boundary and planting holes. 1. Angle encoder. 2. Laser range finder. 3. Tandem compass. 4. Data controller 5. Diameter tape. 6. Measuring tape 7. Handheld laser range finder. 8. Prism reflector.

Figure 14. A temporary base station is put up as a starting point to align the boundary and planting holes.

2.7 Planting operations

Seedlings to be planted must undergo a hardening process at the planting site for at least seven days. A temporary shelter should be constructed for this purpose. During the hardening process, the same level of care must be given to the seedlings to prevent mortality, dehydration, pests and disease infestation. Wooden poles of 1 m height are used to mark the planting holes and each is coloured to distinguish different populations (Figure 15 and Figure 16). For example, nine different colours were used to represent the nine populations. To ensure accuracy during distribution to each of the planting holes, the same colour code has to be painted on the side of the polybag accordingly. The planting hole has a dimension of 30 cm (width) × 30 cm (depth). Approximately 1 kg of compost soil and 50 g of Christmas Island Rock Phosphate (CIRP) can be added to the planting holes to enhance the soil quality. It is of

utmost importance to ensure that the soil encapsulating the root ball is intact at all times during planting when the polybag is opened, and carefully put into the planting hole. Seedlings must be watered after planting.

Figure 15. Coloured wooden poles were used to mark the planting holes.

Figure 16. Seedlings were planted into assigned planting holes.

2.8 Data management and seedling arrangement in the arboretum

Documentation in the arboretum is very important. Each seedling carries a unique living collection number and has an aluminium tag attached (Figure 17). The arboretum should be divided into smaller grids for ease of position reference. For example, the 360 seedling positions are assigned with a grid number starting from 1–1, 1–2, 1–3, 2–1, 2–2, 2–3…15–24. Under each number is the unique living collection number, for example, 1–1 corresponds with KBG 2015–0007, 1–2 corresponds with KBG 2015–0001, etc. For buffer, no grid number is used and seedlings can be planted randomly. The living collection number is a unique running number assigned to the living collection deposited in the Kepong Botanic Gardens (KBG), FRIM. The following data is collected and managed in an Excel data worksheet (Figure 18).

i. Date/Month/Year of seed/seedling collection, sown in the nursery and planted in the arboretum.
ii. Collectors' names.
iii. Origin (name of forest reserve, Global Positioning System (GPS) position, living collection number, herbarium specimen number (where applicable).

iv. Type of material collected, whether seed or seedling.
v. Number of seeds/seedlings sown in the nursery.
vi. Height, diameter at the root collar (DRC), diameter at breast height (DBH).
vii. Other relevant information.

Figure 17. An aluminium tag on each seedling.

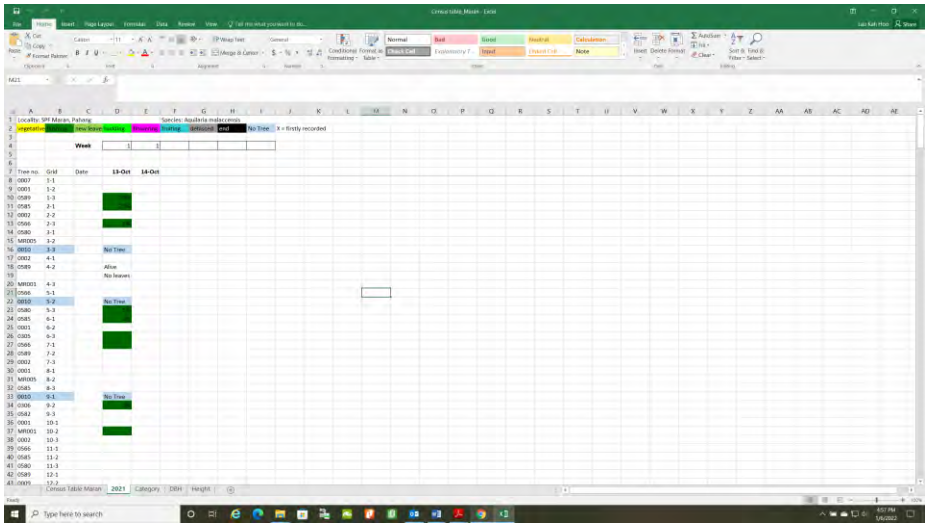

Figure 18. A sample from a sheet of the database used to manage data collected from an arboretum.

2.9 Growth performance and mortality monitoring

A full inventory should be carried out one month after planting to check for seedling mortality. Dead seedlings can be replaced within three years after first planting. For growth performance, height, DRC and DBH of the seedlings are to be measured once every two months after the first full inventory (Figure 19). Height is measured to the highest point (Figure 20) while DRC is measured just above the natural ground line (Figure 21). For juveniles, DBH at 1.3 m from the ground is used (Manokaran *et al.* 1990).

To avoid growth performance bias, planted seedlings are not fertilized, pruned or manipulated in any manner. Weed removal is required. Under a plantation environment, *A. malaccensis* is susceptible to diseases such as felt fungus (Figure 22) and white rot. The same group of pests that occur in the nursery can also present in plantation environments (Figure 23 and Figure 24), including other pests such as *Zeuzera* species, borer and termites (Mohd Farid *et al.* 2015).

Figure 19. Height, diameter at the root collar and diameter at breast height are once measured every two months.

Figure 20. Seedling height is measured to its highest point.

Figure 21. Diameter at the root collar is measured just above the natural ground line.

Figure 22. Felt fungus can attack both treelets as well as juvenile seedlings.

Figure 23. Scale insects can be fatal to seedlings as they weaken the whole plant system.

Figure 24. Larvae of *Heortia vitessoides* seen here consuming young leaves of a seedling.

2.10 Maintenance and protection

Replacement for seedlings can be carried out up to three years after first planting. Planted seedlings are not to be pruned or thinned such as in other normal plantation environments for them to be fully grown. No fertilizers are to be used as the arboretum also serves as a growth performance experimental plot for different populations of seedlings. Pesticides are not to be applied regularly except when there is a pest/disease outbreak. Animals could be deterred from entering the arboretum by erecting fences along the perimeter. No open burning and smoking are allowed in or near the arboretum area as a fire prevention measure.

3.0 CONCLUSION

The guidelines are meant to provide information to researchers and planters on how an *A. malaccensis* arboretum could be established for the purpose of conservation and future research. The arboretum holds the genetic composition of the species from Peninsular Malaysia that could be optimized for conservation, management and sustainable utilisation of the species. Mature and established arboreta can also function as seed sources to reduce seed harvesting from natural populations.

In this regard, the guidelines have been used to establish two *A. malaccensis* arboreta in Peninsular Malaysia under the CITES Tree Species Programme (CTSP), namely, one each in the states of Pahang and Selangor, where a report on the "Establishment of *Aquilaria malaccensis* arboreta in Pahang and Selangor, Peninsular Malaysia" has been produced and uploaded to the CTSP website.

In recent years, the idea of arboreta and open gardens as a place for recreational and health purposes have grown in popularity, and hence, the established arboreta could one day become a recreational facility with proper administration and management procedures. With the advancement of research and development, the established arboreta, together with further research from population genetic study of the natural populations, could provide ample information to forest managers in preparing management and conservation strategies.

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