Investigation of seed longevity and viability and cutting propagation for *Aquilaria crassna*

Literature Review

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1 Introduction

Agarwood is an aromatic resin produced in the stems of tropical trees within the genera *Aquilaria* and *Gyrinops* (Thymelaeaceae). Agarwood is traded under various names (gaharu, agarwood, aloeswood, eaglewood) and is used for incense, (Qi and He 2005, Gianno and Kochumman 1981), perfumes (Chaudhari 1993), medicines, aromatherapy (Barden *et al*. 2000, LaFrankie 1994), and religious ceremonies (Qi and He 2005, LaFrankie 1994). Agarwood is highly valued by consumers in Asia and the Middle-East, including countries such as Saudi Arabia, the United Arab Emirates, Hong Kong, Japan and Taiwan for its distinctive fragrance. Indonesia and Malaysia were the leading exporter of agarwood from 1995 to 1997 with Singapore the main re-exporter in the same year (Barden *et al.* 2000). However, these authors also reported that little or no information was available from other agarwood producing or consuming countries.

Although agarwood species (*Aquilaria* and *Gyrinops*) are the focus of increasing conservation concern, information on their status and distribution is lacking in most countries in Southeast Asia including Papua New Guinea. Awareness and training programmes are required in many countries to assist local communities in harvesting agarwood on a sustainable manner and participate in its cultivation. The promotion and development of agarwood plantations would be an initiative to preserve natural *Aquilaria* and *Gyrinops* trees, as well as satisfy the high demand for agarwood in world market. To achieve this goal, greater research into propagation and silviculture is needed to safeguard the genetic resources from excessive exploitation.

Agarwood is classified as a non-wood forest product primarily from species in the genus *Aquilaria* (Thymelaeaceae). However, other genera such as *Gyrinops* and *Gonystylus* also contribute to the international agarwood supply. Agarwood is defined in this review as the fragrant resin produced in all species from the genera *Aquilaria* and *Gyrinops*. Agarwood provides distinctive ingredients in medicinal, aromatic and religious ceremonies and rituals in many east Asian and Arab countries (Chaudhari 1993, Barden *et al.* 2000, Than 2007). It is sold in the form of woodchips, wood pieces, powder, dust, oil, incense ingredients and perfume for several thousand US dollars per kilogram (LaFrankie 1994, Barden *et al*. 2000, Gunn *et al.* 2004a, Compton 2007). With such a high economic value, the rate of agarwood exploitation from natural populations has increased to meet the demand. This has resulted in the degradation of wild sources of *Aquilaria* in south-east Asian countries (Barden *et al.* 2000) including West Papua and Papua New Guinea (PNG) (Jensen 2007).

Consequently, all *Aquilaria* and other agarwood producing species are now listed under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix II (refer to Annex 2). CITES emerged in 1973 to address the threat posed by unsustainable international trade in wildlife and the current Appendix II regulates international trade through permits (Mandang and Wiyono 2002, CITES 2003, Compton 2007). The main purpose of listing agarwood species in Appendix II of the Convention (CITES) was to control and verify agarwood international trade for both trade and consumer countries on a legal and sustainable manner (Compton 2004). While Compton (2007) recognises that CITES is one of the world's most influential agreements on species conservation, he indicates that implementing trade permits for agarwood is challenging and more awareness and training by CITES Management Authority is required in each agarwood producing country.

The degradation of natural agarwood sources has led to an irregular product supply and quality. The cultivation of agarwood can potentially (i) increase the market supply for agarwood, (ii) provide a sustainable income source for local producers and (iii) reduce the harvest pressures on natural stands. Despite records of successful cultivation reported in Malaysia (Lok and Ahmed Zahaidi 1996) and Vietnam (Nakashima and Mai Thanh Thi 2005) no documented domestication activities for any agarwood species has been found for this review. All documented plantings were based on seeds or 'wildings' collected randomly from natural populations. Initiating domestication through the assessment of natural variation in characters important for cultivation can help to optimise the quality and production of agarwood from planted sources. Domestication activities are also dependant upon fundamental understanding of reproductive and seed biology, vegetative propagation (cuttings, grafting, tissue culture) and silvicultural management.

2 Taxonomy and Biology

Similarities in the 'general appearance, vegetative habit and structure' exist between all species of *Aquilaria* (Thawatchai 2007). The author further stated that the distinction between the species depends primarily on differences in floral and capsule characters.

Class:	MAGNOLIOPSIDA
Order:	MYRTALES
Family:	THYMELAEACEAE
Genus:	1. Aquilaria 2. Gyrinops
Species:	See Annex 1
Scientific synonyms:	See Annex 1
Common names:	See Annex 1

Annex 1: Taxonomy of the agarwood producing genera *Aquilaria* and *Gyrinops*. (Source: CITES 2004)

Given the morphological similarities between *Aquilaria* species the following description is based on *Aquilaria malaccensis* Lam. with a few characteristics of *A. crassna*. *A. malaccensis* is a tree of about 20-40 m in height and diameter at breast height (dbh) of 60cm (PROSEA 2005), whilst *A. crassna* is a medium-sized tree of 15-20 m in height and 40-50 cm diameter (dbh) at maturity (Hoang and Nguyen 2002). The wood of both species is typically white in colour, light in weight and soft in density. In contrast the resin rich wood is dark, heavy and hard (PROSEA 2005). The leaves of *A. malaccensis* are alternate and elliptic about 3-5 cm wide and 6-10 cm long with 12-16 pairs of veins. Its inflorescence is described as a terminal or axillary umbel, containing flowers of 5 mm long, yellowish green or white (PROSEA 2005).

The juvenile fruit is a green egg-shaped capsule 4 cm long and 2.5 cm wide, with a pubescent leathery exocarp. The mature fruits are blackish brown and can be collected directly from the tree, each containing two seeds (PROSEA 2005). The matured seed of *A. crassna* is also blackish with an oval shape and a diameter of 0.5-0.8 cm. (Fig. 1) (Hoang and Nguyen 2002). Based on the similarities in morphological appearance between *Aquilaria* species (Thawatchai 2007), there is need for further investigation of floral structure to help distinguish species among the two genera (*Aquilaria* and *Gyrinops*).

The taxonomy of *Gyrinops* species in PNG and West Papua is poorly known (Bangai 2007, Braden *et al*. 2000). Differentiating *Gyrinops* from *Aquilaria* in PNG is based on the number of stamens, with *Aquilaria* possessing 10 and *Gyrinops* 5 (Gunn *et al*. 2004a, Bangai 2007). The first *Gyrinops* species was confirmed in PNG in 1997. There are two main species of *Gyrinops* in PNG. *G. ledermanii* is found in the northern region and *G. caudata* is found in the southern region of PNG. (Gunn *et al.* 2004a, Bangai 2007). There is however, very little morphological difference between these species. At maturity *G. ledermanii* is a medium size tree of 20-30 m in height and diameter at breast height of 19-27 cm. *G.caudata* is a large tree of 35 cm in height and 20-65 cm in diameter (Gunn *et al.* 2004a, Bangai 2007). Leaves of both species have an alternate arrangement and the underside is pubescent while the upper side is glabrous. The inflorences are elliptic and axillary umbel containing flowers of 1-2 cm long, yellowish green or white. The hairy juvenile fruit is green, oval shaped with a pointed end, containing two seeds per fruit. Matured seeds are blackish in colour with a pointed end (Kipiro pers. comm. 2008).

Figure 1. *Aquilaria crassna* flowers and seeds. Photo taken from Cambodian Tree Species, CTSP, FA, DANIDA, 2004.

2.1 Biogeography

The various species of *Aquilaria* and *Gyrinops* are well distributed in the tropics and subtropics in south-east Asia, mainly in India, Bhutan, Peninsular Malaysia, Indonesia, Myanma, Cambodia, Vietnam, China, Thailand, Lao Peoples' Democratic Republic, and New Guinea (Fig. 2) (Barden *et al*. 2000). The genera is well adapted to various natural habitats from lowland rainforest to montane forests at an altitude of 800-1500 m.a.s.l (CITES 2004, Thawatachai 2007).

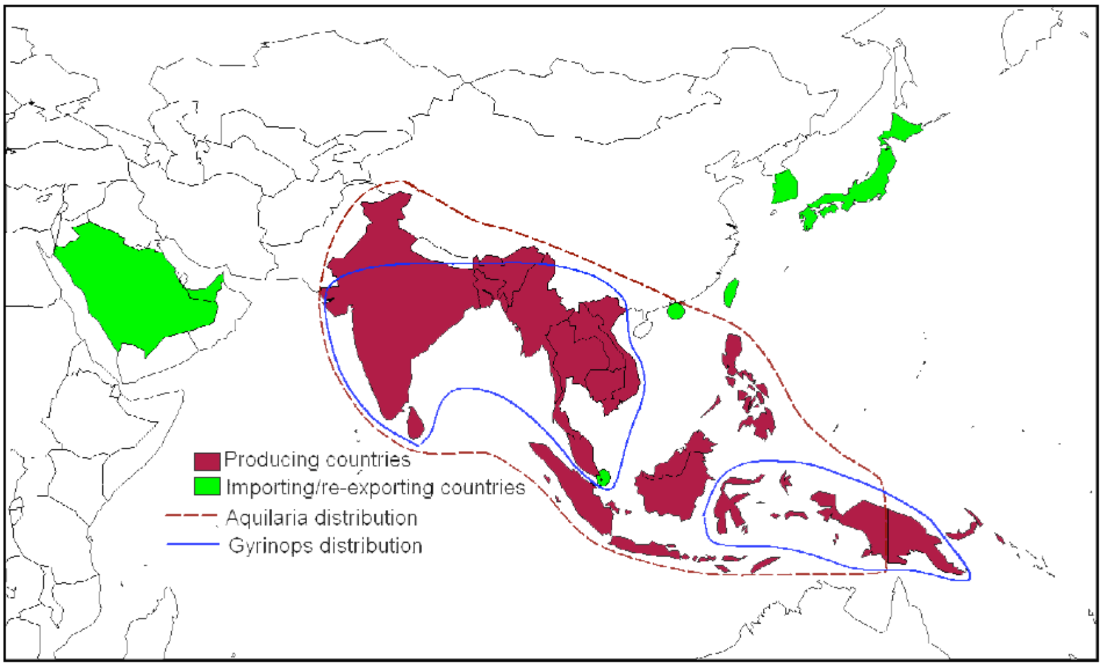

Figure 2. Distribution map of agarwood and importing/re-exporting countries (information source: Barden *et al*. (2000).

The genus *Aquilaria* consists of species that are adapted to rocky, sandy or calcareous, well-drained slopes and ridges and near swamps (Barden *et al*. 2000). In PNG, *A. falaria* and *G.caudata* occurs on ridges and lowland rainforest including areas adjacent to swamps in the southern region (Kipiro pers. comm. 2008). Soehartono and Newton (2000) reported that reliable distribution information is lacking in many countries like Indonesia, Malaysia and Thailand due to difficult access to National Herbarium databases in south-east Asia, and rudimentary inventory and trading pattern surveys.

Approximately 15-20 agarwood producing species are well distributed in south and south-east Asia to the Pacific (Barden *et al*. 2000, Donovan and Puri 2004, Thawatachai 2007). The number of agarwood producing species may be expected to increase if more inventories are conducted in countries like PNG (Gunn *et al*. 2004a, Bangai 2007). Like other tropical tree species, they can be found in single dominant stands, but are predominantly scattered throughout the habitat (Barden *et al.* 2000). Nevertheless, there is a need for more research regarding the distribution pattern in most countries to understand the magnitude of its limits and occurrences.

3 Overexploitation of agarwood

Excessive exploitation of natural sources of agarwood has been reported in Laos People Democratic Republic (Jensen 2007) China (Wang 2007), Myanmar (Than 2007) and Indonesia (Newton and Soehartono 2001). In Malaysia, *A. malacensis*, the main agarwood producing species is classified as endangered resulting from overexploitation of its natural populations for the international trade (Ibrahim *et al.* 2007). The depletion of agarwood populations in India was reported as early as 1907 with no significant amount of agarwood available. In response to this, agarwood plantations were established in India in 1930s and 1940's, primarily by private growers across Assam (Quavi 2007).

Indonesia was a major exporter of high quality agarwood (*A. malacensis*) but there is little knowledge on the extent and status of remnant wild stands (Soehartono 2003). Most remaining *Aquilaria* stands are decreasing rapidly particularly in the forests of Sumatra, Kalimantan and Borneo due to excessive exploitation and illegal logging operations, gold mining operations and clearing of huge forest areas for agricultural purposes (Barden *et al.* 2000, Soehartono 2003, CITES 2004, Donovan and Puri, 2004). PNG has been regarded as one of the last significant sources of natural agarwood. It is likely that illegal harvesting and trading of agarwood occurred well before *Gyrinops ledermanii* was confirmed in 1997 as an agarwood producing species (Gunn *et al.* 2004a; Bangai 2007). Illegal harvesting is still increasing, mostly in the northern region of PNG (Gunn *et al*. 2004a; Bangai 2007). Barden *et al.* (2000) reported that the illegal trade of agarwood from PNG occurred primarily through the township of Vanimo to the Irian Jaya Province of Indonesia.

Unsustainable exploitation of agarwood across all producing countries has prompted CITES to list *Aquilaria* and *Gyrinops* species (or other agarwood-producing species yet to be described in many countries) on the CITES Appendix II. This listing requires regulation of international trade of agarwood 'specimens' under legal instruments. However, the effective implementation of these regulatory guidelines can be problematic (Compton 2007). Therefore initiatives such as establishing cultivated agarwood resources can help to satisfy international demand and relieve pressure on natural sources.

3.1 International trade of agarwood

The earliest records for the international trade of agarwood occurred in India during the thirteen century, which was a source of agarwood for foreign markets (Donovan and Puri 2004, Jensen 2007, Quavi 2007). Early agarwood trading from India and China passed through the Arabia Peninsula to Persia, Mesopotamia, Egypt, the Roman Empire and Greece (Sultan Al-Salem 2007). Agarwood is now traded among an increasing number of countries as the resinous wood products sell for several thousand US dollars per kilogram (LaFrankie 1994, Barden *et al*. 2000, Gunn *et al.* 2004a, Compton 2007). In Papua New Guinea, 'Super A grade' agarwood is valued at about US$ 560 per kilogram (Gunn, *et al.* 2004a). In Thailand, retail value of high grade agarwood approaches $2,000 per kilogram and the distilled agarwood resin US$15,000 per litre (Page pers.com. 2007).

The market price for agarwood varies considerably according to its quality, but there is no standardised international classification for the quality of agarwood products. At present, each agarwood-producing country has its own grading system for agarwood, which is typically based on the concentration of resin within the wood (Fig. 3). Without a standardised classification there is a potential for exploitation of both resource owners and consumers by agarwood traders and merchants.

Figure 3: Grades of agarwood harvested from Sarawak (Malaysia). Grade A has the darkest colour, highest concentration of resin and highest price. (Photo: J. Dawend 2007)

Masataka (2007) and Compton (2004) indicate that a rise in fake agarwood products such as wood impregnated with artificial oil ('Black Magic Wood' or BMW) might be expected as the global demand increases and supply decreases. Such products are also traded under CITES regulation (Masataka 2007) because the base wood originated from *Aquilaria* or *Gyrinops* species. Masataka (2007) asserts that CITES needs to ensure *Aquilaria*-based fake products are certified under permits distinct from the traditional agarwood products to maintain consumer confidence in product quality.

3.2 Agarwood production in natural populations

The harvesting of agarwood requires the felling of the tree to obtain the internal infected portions of the trunk and main branches (Barden *et al*. 2000, Gunn *et al*. 2004a). Agarwood is produced within the tree in response to a generalised pathological infection (Donovan and Puri 2004). This process was first identified in the 18th century, with reported cases of fungi studies in the 1930s (Donovan and Puri 2004). Although the whole tree is felled during harvesting, only a small proportion of the tree contains agarwood, which is extracted from the low value 'white wood'. Furthermore, not all *Aquilaria* or *Gyrinops* trees necessarily contain agarwood. Donovan and Puri (2004) indicate that many traditional collectors in south-east Asia can discriminate between resin-producing trees from those without agarwood. The visual symptoms for agarwood production include insect bore holes, dry knots, a hollow sound upon thumping, tumour-like growths on the tree, and excessive leaf fall

(Donovan and Puri 2004). However, these visual indicators can be subtle and difficult to detect in very large trees and unskilled harvesters of agarwood may indiscriminately fell trees in search of the valuable resinous wood.

4 Cultivation

The preservation of natural *Aquilaria* populations to increase the supply of agarwood in the world market can be assisted through cultivation of *Aquilaria* species. In recognition of this, agarwood plantations have been established in Indonesia, Cambodia, Thailand, Vietnam and other countries (Fig. 4) (Barden *et al.* 2000). Cultivation of agarwood was also reported in Malaysia (Lok and Ahmed Zahaidi 1996, Lok and Chang 1999). Given the low level of documentation the present status of the plantation resource in these countries is unknown. Agarwood cultivation in Vietnam is based on seedlings collected from the wild with many plantings now being used as seed stands to support the establishment of further plantations (Nakashima and Mai Thanh Thi 2005). In Bangladesh the earliest record of agarwood cultivation was in 1925 with artificial inoculations on remaining trees conducted 55 years later (Rahman and Basak 1980, Rahman and Khisa 1984). The findings from these early inoculations studies were not published. Even though plantations were reported to exist in these countries no written reports on propagation, silviculture, production or sales of agarwood from these plantations could be located.

Figure 4. A cultivated *A.crassna* plantation in Cambodia. Photo taken from Cambodian Tree Species, CTSP, FA, DANIDA, 2004.

4.1 Formation of agarwood resin

The fungal infection of *Gyrinops* or *Aquilaria* trees may occur after physical injuring of a stem. This allows fungal species such as *Cytosphaera mangiferae* or *Zeuzera conferta* to enter the plant through the hollow inside the stem, which serves the initial sites of infections (Deodhar 2002). The infection then spreads slowly to all sides and gradually a larger wood volume gets infected. Accumulation of oleoresins is associated with the increased infection and 'ageing' of the initial infection. The oleoresin rich areas spreading from the infection site form dark-coloured scented wood called 'agar'. Rao and Dayal (1992) compared the anatomy of scented and unscented wood in *A. agallocha* by microscopic examination and found that in the scented wood the aromatic oleoresin was mainly concentrated in the included phloem. The intensity of the colour of infected wood becomes prominent as more oleoresins are deposited. Donovan and Puri (2004) reported that in a natural population about 25 to 30% of the trees may be infected and produce commercial agarwood.

4.1.1 Artificial inoculation

In order to increase the production of agarwood in the international market, 'agarwood induction' a number of studies has been conducted using wild trees. No statistical data has been published from any of these early induction studies using the wild trees. Research on artificial inoculation on 6-8 year old plantation trees is on-going in Vietnam, parts of Indonesia and Thailand (Barden *et al.* 2000). Jalaluddin (1997) reported that when the uninfected wood of *A. agallocha* was inoculated with *Cytosphaera mangiferae* fungus a substantial portion of agarwood was produced. Deodhar (2002) isolated *Paecilomyces varioti* fungus from *A. agallocha* and showed that it had the potential to illicit reactions such as reduction of cyclic terpenoid and aromatic aldehyde. The author however, did not extend the use of the fungus in artificial inoculation experiments. Blanchette (2007) conducted agarwood inducement trials in Bhutan, Thailand, PNG and Vietnam across a range of geographical areas with varying environmental conditions. While there is a range of anecdotal evidence that agarwood is induced by artificial inoculation more replicated experiments are required to confirm that these can be carried out successfully in commercial plantations to increase the volume of agarwood.

4.2 Seed handling and research

Aquilaria appears to flower and fruit sporadically at different times of the year (Gunn *et al*. 2004a), for instance selected trees in West Kalimantan flowered and fruited in June and July while trees in East Kalimantan flowered and fruited during September to October (Soehartono and Newton 2001). The authors also stated that flowering and fruiting of *A. beccariana*, *A. malaccensis* and *A. microcarpa* in the Bogor Botanical Garden occurred between September and December. While, flowering and fruiting of *A. crassna, A. malaccensis* and *A. microcarpa* in plantation areas occurred from April to December. A study in Vietnam revealed that *A. crassna* trees commence flowering at an age of 6-8 years, between the months of March and April with fruiting taking place between June and July (Koskela *et al*. 2002). It is apparent that phenology patterns seem to be variable with little evidence of a distinct pattern over a range of agarwood producing countries.

4.2.1 *Fruit and seed of Aquilaria crassna*

The fresh green fruits of *A. crassna* are thick with soft pericarp and when collected fresh must be dried under the shade for 4-5 days (Luu *et al*. 2001). As the fruits mature, they change from green to brown and open on the tree to allow the seeds to fall (Jensen 2000). The brownish-black seed is an oval shape and a diameter of 0.5-0.8 cm (Koskela *et al*. 2002). The fully matured seeds can stay on the tree for up to 2 weeks (Jensen 2000). The author reported that the moisture content of a fresh seed is 49% (recalcitrant) and will desiccate further to 25% with germination of 33%. The optimum storage condition with seeds dried to 25% mc can be stored at 8 ˚C with germination of 22% after two months storage (Luu *et al.* 2001).

Luu *et al*. (2001) also revealed that the 2-3 cm ‘peduncle / pedicel’ of the seeds aid in maintaining the moisture content in the stored seeds. They conducted a storage test of seeds with and without ‘peduncle / pedicel’, where the moisture content of both treatment and control was measured following complete decay of the ‘peduncle / pedicel’. Moisture content of 39.1% was measured for those seeds with ‘peduncle / pedicel’ and 34.5% for those without ‘peduncle / pedicel’ over a period of 2 weeks. This study indicates that ‘peduncle / pedicel’ had an effect on the moisture content in the seed, but it was only indicative given the trial was not conducted under a statistically replicated design.

5 Vegetative propagation

Vegetative propagation and clonal selection offers a means to enhance yield and quality of forest products from commercial planting in the tropics (Leakey, 1987). The domestication of forest trees through breeding commenced in the 1950s with *Pinus* specie (Barnes and Simons 1994). Ever since there was an ultimate interest for higher yields and better products has come to the domestication of forest trees. A number of approaches were applied including grafting, stem cuttings, hardwood cuttings marcotting (air-layering), suckering and in vitro techniques (meristem proliferation, organogenesis and somatic embryogenesis) (Macdonald 1986, Leakey 1985, Hartmann and Kester 1983). Apart from these propagation techniques stem cuttings is becoming a common propagation method in forestry and agroforestry (Leakey *et al.* 1990).

However, this can be costly in developing countries with the use of electricity and a piped water supply. This relates to mist propagation systems for research and large-scale commercial projects. The stem cuttings require an appropriate environment for root initiation that would minimize physiological stress in the cuttings (Leakey *et al.* 1987). In a broader term, providing shade to lower the air temperature, providing high humidity, and reduce transpiration losses. Importantly, ensuring the vapour pressure of the atmosphere surrounding the cutting is maintained close to that in the intercellular space of its leaf (Leakey *et al.* 1990). Many propagation systems were used in commercial horticulture. These are either based on spraying mist, fogging or enclosing the cutting in polythene. However, recent improvement on the design of non-mist propagators for use with wide range of timber and multi-purpose tree species in both tropical moist forest and semi-arid area being so success (Leaky and Longman 1988).

In forestry, vegetative propagation is used for the production of fast growing trees that produce high quality timber. Clonal approach to plantation improvement through cutting propagation of ‘plus trees’ plays an important role in increasing productivity, uniformity and quality in forest plantations. This propagation methods (stem cuttings) plays a vital role in capturing existing genetic traits that can be used a basis of a genetic variety or cultivar alternative for tree improvement programmes.

5.1 Stem cuttings

Stem cuttings can be classified as leafy stem cuttings from young shoots (softwood) and leafless hardwood cuttings from older shoots (Leaky 2004, Danehloueipour *et al*. 2006). However, in this review most focus is on the softwood cuttings. In a propagation bed leafy cuttings photosynthesize that the favorable factors are met. The factors necessary for the development of adventitious roots are: propagation environment, postseverance, cutting origin and environment and preseverance stockplant environment (Leaky *et al.* 1994). The physiological response to cutting propagation will vary for different species (Hartmann *et al.* 2002), and therefore modifications to their treatment and management will be required to achieve successful propagation. Therefore, having a good physiological perception of the factors influencing rooting stem cuttings is an important factor to achieve best rooting of cuttings.

5.1.1 Propagation systems and environment

As indicated by Leakey (2004), there is numerous propagation system applied through out the world for stem cuttings. The most common are: (1) fogging systems, (2) intermittent mist and (3) airtight, watertight, high-humidity, nonmist propagators. All are effective but vary in cost and operation. Non-mist propagators will be the main focused in this review which has a low cost, simple to use compare to electricity and water supply fogging and intermittent systems.

In most of these propagation environments the main aim is to promote physiological activity (photosynthesis and transpiration) in the cuttings leaves to minimise as possible physiological stresses from the tissues, from transpiration and respiration while encouraging meristematic activity (mitosis and cell differentiation) in the stem (Hartmann *et al.* 1997, Leakey *et al.* 1982). Considerably, the transportation of assimilates and nutrients from the leaves to the base of the cuttings and water from the base of the stem to the leaves in the propagation environment is so important.

5.1.2 Stock plant Management

Pruning, fertilizer application and light management would enhance rooting ability in the latter stages (Leakey 2004). Managing hedge plants under nitrogen-fixing species like *Leucaena leucocephala* would be beneficial. Rooting of cuttings taken from such practice does not affect rooting ability if shade providing species is manageable (cutback to a required height). Stock plants managed as seedling and clonal hedges, and coppice or root sprouts can be used as the source material for cutting collection.

Stock plant physiology will be important to the successful propagation of cuttings and this can be controlled, to some degree, by stock plant management (Hoad and Leakey 1994). The physiology of the stock plant will be influenced by the plant genotype and environmental conditions such as water, temperature, light, carbon dioxide and nutrition (Hartmann *et al.* 2002). Therefore a basic understanding of stock plant physiology and its management to maintain its health, vigour and longevity will help to maximise adventitious root formation in its cuttings.

5.1.2.1 Water

Stocks plants under extreme conditions such as drought may have an effect on the rooting ability of its cuttings due to moisture limitations (Hartmann *et al.* 2002). Therefore it is crucial to take cutting materials from stock plants that have little to no drought stress, which can be ensured by regular stockplant irrigation. Collection of cuttings in the early morning when plant material is in a turgid condition is also recommended to minimise any local water deficits (Leakey *et al.* 1992). Adventitious root induction is substantially reduced in drought stressed cuttings compared with those with adequate cell water potential (Hartmann *et al.* 2002). Lebude *et al.* (2004) reported that loblolly pine (*Pinus taeda* L.) optimum cutting results would be achieved with moderate cutting water potentials (-0.5 to -1.2 MPa) during 4-5weeks of the rooting period. In plants under drought, stomata are closed limiting the exchange of gases as well as the reduction of photosynthesis, which can continue in the cuttings once severed thus limiting carbohydrate availability for adventitious root induction and growth.

5.1.2.2 Temperature

Little is known about the effect of temperature on stock plants (Hartmann *et al.* 2002). But, providing shade to nursery plants or planting nitrogen-fixing species to provide shade for hedge plants must be considered to prevent dehydration from extreme temperatures. In general, temperature exceeding 60 ˚C will kill the roots of all plants and above 40 ˚C reduces the root growth for most species (Handreck and Black 1984). The dead roots may provide a favourable condition for pathogens to invade the stockplant. The optimum temperature in the medium for plant growth (roots and shoots) should be in the range 15-30 ˚C (Handreck and Black 1984). This range in temperature may not be suitable for most plants because they vary with plant species and variety, moisture in the medium, air temperature, light intensity and nutrient content in the media or soil.

5.1.2.3 Preseverance of stockplant (Light and Nutrients)

Nutrients and light is considered to be the main effects as preconditioning agents on rooting ability. As study showed that in *T.scleroxylon* the interactive effects of nutrients and quantity and quality of light had an effect on photosynthesis and the carbohydrate status of cuttings (Leaky 2004). It is understood that active photosynthesis is associated with best rooting. This may link to amount of low irradiance and low red-to-far-red ratios that believe to independently enhance rooting ability (Leakey 1983).

Cuttings taken from under-shade *Eucalyptus grandis* stockplant tends to have longer internodes, larger leaf area, codominance between shoots lower rates of perseverance net photosynthesis, lower chlorophyll concentration, but higher rates of net photosynthesis per unit of chlorophyll (Leakey 2004). These physiological changes thus relate to shoots response to its surrounding resulting in the differences in stem and leaf morphology. Light duration (*photoperiod*) and spectral quality (*wave length*) can influence the stock plant condition and subsequent rooting of cutting (Felker 2008, Hartmann *et al.* 2002). Therefore managing light for stock plants at the nurseries would be critical for the success of rot cuttings.

5.1.2.4 CO2

Level of carbon dioxide (CO_2) in the propagator (non-mist propagator) can be reduced during propagation, limiting photosynthesis and growth in the cuttings (Hartmann *et al.* 2002). This can happen if the media used is saturated, in which no exchange of gases between the roots and the atmosphere. The plant roots can eventually die, as they no able to effectively respire in the medium. It was reported that photosynthesise could be also limited to low daytime CO2 concentrations in the propagator (Leaky *et al.* 1990). Thus further research in future can experiment on enhancing CO2 diffusion into the propagator for successful rooting of various tree species.

5.1.3 Branch or shoot position

The origin of a stem cutting within a shoot and the position of the shoot within the donor plant influence the formation of adventitious roots (Longman and Leakey 1995). Rosier *et al*. (2004) conducted an experiment using cuttings taken from 3- and 4-year-old stock plants of Virginia pine (*Pinus virginiana* Mill.) and reported 46% rooting percentage of cutting taken from semi-wood cuttings compared to 33% rooting percentage of cuttings taken from softwood cuttings. The difference in the rooting of cuttings from different shoots can be affected by the level of competition between shoots for light (Longman and Leakey 1995). These authors reported that irradiance and its spectral composition influence leaf and stem physiology in the donor plant, which affects adventitious root formation in the cutting. Therefore, understanding the physiological factors beforehand would help in collecting the best cutting materials to propagate.

5.1.4 Seasonal effects

In many species the development of adventitious roots in cuttings can be affected by the timing of shoot collection during the year (Hartmann *et al.* 2002). Such seasonal effects are typically due to the variable physiological condition of the stockplant throughout the year as it responds to changing climatic conditions. The authors also stated that some species, such as privet, can be rooted at any time of the year but the rooting capacity of others like creeping fig (*Ficus pumila*) were influenced by seasonal changes. Plants vary in response to rooting depending on the period of the year cuttings are collected. This reflects on the physiology of the stock plant and environmental condition to determine the optimum rooting success.

5.1.5 Ontogenetic age

Ontogenetic and physiological aging is an important aspect which can have effect on the stockplant physiology pertaining to rooting ability that is still unresolved (Leakey 2004). As trees grow to reach reproductive maturity (ontogenetic aging), they develop new shoots and produce flowers and fruits, while those below the forest floor are still juvenile. The transition is term as phase change (juvenility to maturity). There were ultimate numbers of reports in the literature that cuttings from mature shoots are very difficult to root than those from juvenile shoots and was attributed this to phase changes. Considerably, there were reports that mature old trees propagated by cuttings were successful, especially in the early spring (Leakey 2004). The reasons behind this success are yet to be fully understood.

Furthermore, poor rooting ability of mature shoots is understood to be attributed to physiological aging rather than to ontogenetic aging. Study on mature *Prunus avium* cuttings indicated that in comparison with leafy juvenile shoot cuttings, rooting capacity of mature softwood cuttings were limited by the availability of stored reserves (Dick and Leaky 2006). Propagating mature trees by vegetative means can be difficult and a constraint in tree-improvement programmes. In juvenile shoot materials, rooting capacity is affected by number of interacting morphological (i.e., leaf area, leaf thickness, stem length, stem diameter) and physiological (i.e., photosynthetic capacity, leaf water potential, leaf abscission) characteristics of cuttings (Dick *et al.* 2004). Therefore understanding the effects of physiological and morphological of mature or juvenile cutting materials would resolve some difficulties, especially for mature materials and that would enhance opportunities for mass production of selected mature trees of proven superiority in an orchard or plantation.

5.1.6 Auxin applications

Root-promoting growth-regulatory substances (auxins) (indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthalene acetic acid (NAA)) are the most common treatment to enhance rooting in stem cuttings (Leakey *et al.* 1982, Tchoundjeu *et al.* 2002, Hartmann *et al.* 2002). They effects on cell differentiation, promotes starch hydrolysis and the mobilization of sugar and nutrients to the cutting base (Leakey 2004, Hartmann *et al*. 2002). However, cutting dry mass will not relatively increase in respect to increase auxin concentration (Mesen *et al.* 1997). Considerations as to be taken into account the auxin applications and interaction with cutting materials and the propagation environmental variables that may affect the rooting capacity of cuttings.

Auxin treated cuttings root more quickly and produce more roots, with higher percentage of cutting rooted. Usually, indole-3-butyric acid (IBA) is found to be the most effective root-promoting auxin, but occasionally naphthalene acetic acid (NAA) can be effective, as in *Pausinystalia johimbe* (Tchoundjeu *et al*. 2004). However, tree species and even clones can respond differently to individual and mixed applications of auxin at different concentrations, even when many other factors are constant. For instance, clones of *T. sceroxylon*, which appeared to have different dose-response curves, all rooted equally well at 40µg auxin per cutting (Dick *et al.* 2004, Leakey 2004). This demonstrate that cuttings of various tree species can response differently of auxin types and concentrations, so finding out the optimum auxin concentration for important tree species would be research worth for latter mass propagation for further research projects or commercial scale planting programmes.

5.1.7 Rooting medium for stem cuttings

Ideally, rooting medium will vary for different species as it needs to provide (i) physical support, (ii) moisture, (iii) air and (iv) reduce light penetration to the stem cutting (Hartmann *et al.* 2002). The typical rooting media to encourage the above requirements are: organic requirements - tree bark, sawdust, coconut coir, peat moss and coarse mineral requirements – coarse sand, gravel pumice, perlite and vermiculite. The organic requirements should increase the water-holding capacity while the mineral requirements should increase the air-filled porosity (Hartmann *et al.* 2002). Experimenting different combination of these medium is required as rooting capacity of various species can be varied in response to moisture content and air pores in the media.

Furthermore, a cutting medium of fine gravel (2-3) diameter will require additional rotted sawdust (50% by volume) for water-holding capacity (Leakey *et al*. 1990). Other materials can be used (e.g., coconut coir) depending on the availability of materials. The rooting results will differ among each medium used. As reported by Tchoundjeu *et al*. (2002) that rooting success of juvenile cuttings of *Prunus africana* (a medicinal plant in Africa) was significantly greater ($P < 0.05$) in sawdust (80%) compared with sand alone (72%) and combination of the two (71%). Understanding the purposes of each combination of materials used for cutting media is very important in promoting moisture retention and porosity to achieve best rooting success.

5.1.8 Leaf area

Rooting of leafy stem cuttings is dependent on the presence of a leaf and ideally the physiological process of this leaf (Leaky 2004). Practically, leafless cuttings will die quickly resulting in rotting. The causes of the failure may due to use of unsuitable tissues, photosynthetically inactive, water-stressed, diseased, pest-infected, or to an undesirable rooting environment (too hot, too wet, too dry) (Leakey 2004). An infected or inactive cutting can show signs of leaf shedding, leaf rot and stem rot in the propagator.

Leaf play a role in the cutting rooting ability if the severed cutting is photosynthetically active and producing assimilates for the development and elongation of the root primordial (Brady and Weil 2002), and when the leaf is not suffering water stress (Leakey 2004). Optimum leaf area should have a best balance between photosynthesis and transpiration. This can be vary between species and clones, attributing to leaf area (leaf thickness), stomatal density, leaf morphology (waxiness etc) and the age of the leaf (Leakey 2004, Salisbury and Ross 1992).

Therefore balancing photosynthesis and transpiration is crucial for cuttings in the propagation environment. Water loss from a cutting can be tempered by placing it within a high humidity propagator so its leaf area is an important character that influences the balance between photosynthesis and transpiration. In considering this fact, leaf has to be reduced by trimming to give an optimum balance (Longman and Leaky 1995). This would allow the cutting to quickly root and develop a sink for assimilates in the propagator (Corress *et al*. 2005, Leakey 2004). The trimming off leaf will very much depend on the size that can be varied among species.

Cuttings with excessive leaf frequently loss water and come under stress, therefore closing their stomata and limiting their capacity to photosynthesize. The number of stomata on a cutting will be directly proportional to its leaf surface area and therefore leaf area will influence its potential water loss during photosynthesis. Leaf area is a vital variable in relation to level of irradiance in the propagation environment.

5.1.9 Cutting length

Uniformity of stem cutting length can be achieved but can be varied in the number nodes present. Depending on the morphology of the shoot (length of internodes, leaf size etc,) decision has to be made as this would reflect on the rooting success. The best option would be to prefer constant length for ultimate rooting success (Leaky 2004) as a practical measure. In terms of research, minimize variation as to be considered. Ideally, it is appropriate to use a fixed number of nodes with varying internode length within a stem. Leakey (2004) stated that internode length varies down a stem and it is not considered as an independent variable.

5.1.10 Temperature and light effect on cuttings

The differences in temperature and light-intensity in a propagation environment can affect rooting success. Test conducted in ITE glasshouses reported an air temperature of 20˚C while in non-mist propagators rose to a mid-day peak about 34 ˚C during bright, sunny, mid-summer weather. This rise temperature was associated with a decrease in relative humidity from about 95% to about 75% (Leakey *et al*. 1990). The authors further stated that this substantial increase in the saturation vapour pressure deficit (SVPD) of air from 0.02kPa to 1.37 kPa.

It is therefore so important to keep the lid of the non-mist propagator tightly closed at all times. By doing so, may decrease in relative humidity if open up to five minutes at midday (Leakey *et al*. 1990). However, the relative humidity can increase rapidly if the lid is closed back. The increase in temperature of cutting medium can also aid in callus formation and development of cutting.

Many temperate species will root rapidly when the medium is held at 27-30˚C while tropical plants is at 24-27˚C (Handreck and Black 1984). For instance, study on cutting medium of *Schlumbergera* ‘Russian Dancer’ indicated that optimum temperature for root development was between 21.3˚C and 24.7˚C, whereas increasing temperature above 24.7˚C would promote bud growth to occur before roots were developed (Kristiansen *et al.* 2005). Controlling the temperature in a propagator is very important for the induction of root, shoot development and maintaining humidity to maximise overall rooting capacity.

5.1.11 Cutting origin and environment

There are two source of variation in stockplants that are influential by the stockplant environment. The with-in shoot factors and between-shoots factors (Leakey 2004). There are numerous variations with-in a shoot which is associated with the age of the shoot as it develops. For instance, age of leaf, leaf water potential, leaf carbon balance, leaf senescence, internode length, internode diameter, stem lignification, nutrient and stem carbohydrates content, and respiration (Leaky 2004, Hartmann *et al*. 2002).

These variables can have effect of the rooting ability therefore physiological and morphological status of a shoot must be considered prior to setting cuttings. For example, most influential factor on rooting ability was found to be between cutting length and node position with-in shoot variation (Leakey *et al*. 1982). Sprouting shoots from a cut back stockplant may vary in between-shoot factors. It was reported that most rooting success was from upper shoots than those below from a cutback stockplant (Leaky 2004). Nevertheless, the lower shoots can be reoriented or apply pruning to expose these shoots to light so rooting success can be achieved same as the upper shoots. It is therefore very important to be aware of the morphological and physiological variation with-in or between shoots in order to achieve higher percentage rooting.

5.1.12 Unsuccessful rooting

There are numerous causes that can cause the cuttings not to root and must pay attention to. Some of these could be leaf symptoms (leaf decolourisation, leaf portion attack by insects, and showing sign of wilting) that must be avoided during material collection. As reported by Leakey (2004) that signs of leaves dying were attributed to leaf abscission which associated to water stress, photoinhibition, anoxia and negative carbon balance. The cuttings fail to root would obvious die or rot. It is ideally important to be aware of these aspects to prevent unsuccessful rooting of cuttings.

5.1.13 Leafy stem cutting of agarwood species

There could be very limited research on the vegetative propagation of genera *Aquilaria* and *Gyrinops*. As very little information were retrieved for this review. A report from Vietnam by Koskela *et al*. (2002) stated that *A. crassna* had showed a rooting success of 90%, with a mean of 10.9 roots per cutting and 2.9 cm in length. However, details on stockplant management, cutting origin and environment, temperature and light effects on cuttings, cutting length and other variables were not reported.

A study of stem cuttings of *Gyrinops ledermanii* was conducted at the PNG Forest Research Institute (PNGFRI) recently which reported that *Gyrinops* can be propagated easily by stem cuttings (Gunn *et al.* 2004b). Further study had revealed that adventitious roots were produced within 6-7 weeks with 4-5 roots per cutting and root length of 1-10 cm (Lata 2006, *unpublished)*.

Figure 5. *Gyrinops ledermanii* root cuttings 6-7weeks and a month old clones. Photo by Lata A. (2006)

Furthermore, cuttings treated with 0.8 mg/L IBA produced roots more rapidly than those treated with 0.3 mg/L IBA. The overall rooting success of 0.8 mg/L IBA treated cuttings was 78% while those treated with 0.3 mg/L IBA was 58%. Rooting percentage was greater in coarse sand (72.8%), fine sand (35%) and forest soil (15%) after 6 weeks under mist propagation (Lata 2006, *unpublished)*. Rooted cuttings produced an average of 2 pairs of leaves and fibrous root system 4-weeks after transplanting into pots (Fig. 5). These studies indicate that *Aquilaria* and *Gyrinops* species can produce adventitious roots as stem cutting, but more research is needed to optimise the management and physiology of the stock plant (ortet), the morphology of the cutting and the environmental conditions during propagation.

5.1.13.1 Stockplant management of Gyrinops PNGFRI

The potted plants of *Gyrinops ledermanii* consisting of 15 plus families were kept in a transparent roof shed. Slow release fertilizer (osmocote) was applied quarterly or as required. The plants were cut back when the plants were more than 45 cm to promote new shoots for cutting experiments. The lower bushy branches were also pruned regularly to allow light penetration and water to reach the potting media and not prevented by the leaves. It took 5-6 months to have a new shoot ready to take cutting materials from. Plants were watered manually twice a day during dry season and once a day during wet season.

5.1.13.2 Cutting variables and propagation environment

The variables being investigated in this trial of *Gyrinops* stem cuttings were cutting medium and IBA concentration to examine the end result of rooting percentages to quantify on the variables being tested. This was just a beginning therefore more variables just as cutting length, leaf area, branch or shoot position, different auxin application, various medium, cutting origin and environment, temperature and light effect on cuttings and genetic variation have to be statistically investigated given the 15 families being propagated for research purposes.

As these results may seem to be promising, further research is still required to cover all the points being discussed in section 5 of this review to fully understand all the aspects of propagating *Aquilaria* or *Gyrionops* species vegetatively by means of stem cuttings for further research trials or demonstrations plots to initiate the domestication process of this valuable tree species.

6 Conclusion

The genus *Aquilaria* (Thymelaeceae) is well known for the production of the highly valued agarwood. The wild sources of many *Aquilaria* species are decreasing rapidly and becoming endangered through uncontrolled harvesting and discriminate felling of natural population. This may lead to local scarcity of these tree species in some of the agarwood producing countries. As a consequence, there is increasing concern that the natural stocks of *Aquilaria* trees are not sufficient to meet the international demand for agarwood. Therefore, there is a need for domestication and cultivation of this tree species to relieve pressure on the harvest of natural population.

Efforts to domesticate and cultivate agarwood are dependent upon knowledge related to seed biology and vegetative propagation. Like other tropical trees, *Aquilaria* seed supply from the wild is limited for planting and research programs. Vegetative propagation through stem cuttings may therefore be appropriate to accelerate the establishment of the planting stocks, thereby promoting their conservation and sustaining the production of agarwood. It is therefore worthwhile to undertake applied scientific research to determine the amenability of *Aquilaria* species to vegetative propagation and techniques to optimise its success.

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Annex 1. Scientific names, synonyms and common names of *Aquilaria* and *Gyrinops* (taken from CITES 2004).

No.	Scientific name	Synonyms	Common names
1	*Aquilaria beccariana* van Tiegh.	*Aquilaria cumingiana* (Decne) *Ridley var. parviflora* Airy Shaw; *Aquilaria grandiflora* Domke; *Gyrinpsis grandifolia* Quis.	Agarwood; garu tanduk (Kalimantan); mengkaras putih (Sumatra); Gaharu, gumbil, njabak (Malaysia)
2	*Aquilaria hirta* Ridl.	*Aquilaria moszkowskii* Gilg.	Chamdan, audate, kayu chamdan, sahare (Madura)
3	*Aquilaria macrocarpa* Baill.	*Aquilaria microcara* van Teigh; *Aquilaria borneensis* van Teigh; *Aquilaria norneensis* Boerl	Tendkaras (Madura); hepang (Bangka); engkaras (Dayak); karas or sigi-sigi (Bugis); Kumbil, garu, tulang (Madura)
4	*Aquilaria cumingiana* (Decne) Ridle.	*Gyrinops cumingiana* Decne; *Dacaisnella cumingiana* O.K.; *Gyrinopsis cumingiana* var. *pubescens* Elm.; *Gyrinops decemcostata* Hall.f.; *Gyrinopsis pubifolia* Quis.	Alahan, maga-an, palisan (Tagalog); bago (Mbo), binukat (Ak. Bis.); butlo (Neg.); dalakit (S.L. Bis.); magwalen (Sub.); pamaluian (Bag.); giba kalo (Halmahera)
5	*Aquilaria audate* (Oken) Merr.	*Gyrinopsis brachyantha* Merr., *Cortes filarius* Rumph., *Pittosporum ferrugineum* var. *filarium* DC., *Pittosporum filarium* Oken., *Aquilaria tomentose* Gilg, *Gyrinopsis bracyantha* Merr. *Gyrinopsis acuminate* Merr., *A. audate* e Quis.J.	Age (Sorong), bokuin (Morotai), Iason (Ceram), kasjik (Tehid), malowassi (Uliansers)
6	*Aquilaria brachyantha* (Merr.) Hall.f.	*Gyrinopsis brachyantha*	-
7	*Aquilaria urdanetensis*	*Gyrinopsis urdanetensis*	Mangod, makolan (Mbo)

8	*Aquilaria citrinaesarpa* (Elmer) Hall.f	*Gyrinop citrinaecarpa* Elmer	Agododan (Mbo)
9	*Aquilaria apiculate* Elmer	-	-
10	*Aquilaria parvifolia* (Quis.) Ding Hou	-	-
11	*Aquilaria rostrate* Ridl.	-	-
12	*Aquilaria crassna* Pierre ex Lecomte	-	-
13	*Aquilaria banaense* Phamhoang Ho	-	-
14	Aquilaria khasiana H. Hall	-	-
15	*Aquilaria subintegra* Ding Hou	-	-
16	*Aquilaria grandiflora* Bth.	-	-
17	*Aquilaria secundana* D.C.	-	-
18	*Aquilaria moszkowskii* Gilg	-	-
19	*Aquilaria tomentose* Gilg	-	-
20	*Aquilaria bailonii* Pierre ex Lecomte	-	-
21	*Aquilaria sinensis* Merr.	-	-
22	*Aquilaria apiculate* Merr.	-	-
23	*Aquilaria acuminate* (Merr.) Quis.	-	-
24	*Aquilaria yunnanensis* S.C. Huang	-	-
25	*Gyrinops verteegii* (Gilg) Domke	*Gyrinops wala* (non Gaertn.) Koord.; *Brachythalamus versteegii* Gilg; *Aquilaria versteegii* Hall.	Ketermun (Lombok); ruhu wama (Sumba); seke (Flores)
26	*Gyrinops moluccana* (Miq.) Baill.	*Lachnolepsis moluccana* Miq.; *Aquilaria moluccana* Hall.f.	-
27	*Gyrinops decipiens* Ding Hou	-	-
28	*Gyrinops ledermanii* Domke	-	-
29	*Gyrinops salicifolia* Rodl.	-	-
30	*Gyrinops audate* (Gilg) Domke	*Brachythalamus versteegii* Gilg; *Aquilaria versteegii* Hall.f.	Niwawur
31	*Gyrinops podocarpus* (Gilg) Domke	*Brachythalamus podocarpus* Gilg; *Aquilaria podicarpus* Hall.f.; *Gyrinops ledermanii* (non Donke) Merr & Perry	Kokkoree (Asmat)

Annex 2: Definition of CITES Appendices I, II and III

CITES (the *Convention on International Trade in Endangered Species of Wild Fauna and Flora*) is an international agreement between Governments purposely to set measures in controlling the international trade in specimens of wild animals and plants does not threaten their existence or survival (Compton 2004).

Appendices I, II and III to the Convention are lists of species that are protection from over-exploitation by issue of permits to eligible to trade the species. Threatened flora or fauna may be added or eliminated from Appendix I and II, or moved between them, by the discretion of the Parties at their Conventional meetings. In comparison, species in Appendix III may be added or eliminated at any time by the any Party (Compton 2004).

As report by Compton 2004, a total of 25 000 plant species and 5000 animal species were listed by the Convention as follows:

- Appendix I: about 6oo animal species and 300 plant species;
- Appendix II: about 1400 animals and 25 000 plant species; and
- Appendix III: about 270 animal species and 30 plant species.

Appendix 1 comprised CITES most listed endangered animals and plants. These are threatened with extinction due to overexploitation that is prohibited from International commercial trade in specimens of these species. They can be only allowed under exceptional circumstances, for instance, for scientific research which may be authorised by the granting of both an export permit and an import permit.

Appendix II lists of species not necessarily threatened with extinction but are likely to there should be control measures in placed. This involves species that are said to ‘look-alike species in terms of specimens in trade similar to most species listed for conservation reasons. Specimens of species in this Appendix when traded internationally have to be granted and export permit or re-export certificate; no import is permitted. But, the granting of permit to relevant authorities depends of the satisfactory and conditions that are met to ensure there is no threat to the existence of the species in the natural habitat.

Appendix III are lists of fauna and flora species already traded but need assistance from Party member countries to prevent unsustainable or illegal exploitations.