Chapter 4
Development of Agarwood Induction Technology Using Endophytic Fungi

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Abstract Agarwood plays an important role in gaining foreign exchange and as a source of income for people living in, around, and inside the forest of Indonesia. Its production has declined rapidly due to the lack of proven technology for its induction. If no serious action is taken now, agarwood production will not be sustainable in the future. Agarwood formation is initiated by biotic and/or abiotic factors and is affected by host tree, microbe, and environment. Nonetheless, in cultivating agarwood on a large scale, a standard operating procedure is the principal factor that determines agarwood quantity and quality. Realizing the importance of a procedure for agarwood induction, we developed a technology using the biotic factor and its natural way in forming agarwood. We applied selected endophytic fungi directly on *Aquilaria* and *Gyrinops* trees. Because fungi are living organisms, naturally they help to spread the induction mechanism into other regions of the tree. Remarkably, the technology produced substantial amount of agarwood of high-grade quality. Although a better selection of fungal strains must be discovered and tested in field trials, this promising technology could be the answer to commercial agarwood production in Indonesia and could be adopted by other countries.

4.1 Introduction

For most of the forest communities in Indonesia, agarwood products have been known to retain social, cultural, and economic values (Donovan and Puri 2004). In other communities, it has been used as raw ingredients in fragrance, aromatherapy, pharmaceutical, and herbal medicines for centuries, such as in Buddhist, Hindu, and

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Islamic cultures (Heuveling van Beek et al. 1999; Barden et al. 2000). In China, the Chinese agarwood, *Aquilaria sinensis*, is used as a crude drug in traditional sedative, analgesic, and digestive medicine (Yang et al. 2013). The agarwood-producing tree species are widely distributed in Indonesia, including Sumatra, Borneo, Java, Nusa Tenggara, Sulawesi, Maluku, and Papua islands. High economic values and high demand have triggered overexploitation of the species in their natural habitats. The increasing rate of overexploitation is unfortunately not being replaced by proper cultivation technique; this highly threatens the species. Consequently, two important agarwood-producing genera, *Aquilaria* and *Gyrinops*, have been classified as threatened, which need proper regulation and restriction in their harvesting (CITES 2005).

A new trend in the agarwood industry termed as "one-stop service" has been on the rise in agarwood-producing countries. Based on this new scheme, the production chain of agarwood is managed comprehensively from upstream to downstream. Through this scheme, agarwood will not be sold exclusively as a raw material, but also processed into various kinds of agarwood-based products according to market demands like perfume, incense, soap, tea, and herbal medicine. Currently, the world's demand for agarwood products reaches 4500 tonnes per year and it is predicted to keep increasing year after year (Santoso 2015). To meet the demand, agarwood-producing countries in Indochina including Laos, Cambodia, Vietnam, and Thailand have started establishing their agarwood industry. Countries with long history of agarwood-bounded communities for many generations seem to be more equipped in managing comprehensive agarwood cultivation and production; they started from massive planting of the species to establishing research-based agarwood industry. Attention is given to every stage in the setting up of a comprehensive agarwood plantation including developing proper cultivation and inoculation techniques to maintain product quantity and quality. Generally, big agriculture companies, whose product segmentation had been correctly determined from the beginning, own such plantations. The companies will execute their management plans to fulfill their targets (Santoso 2015).

Many studies have been conducted on agarwood by researchers from many countries including those in Asia, Europe, and America. However, the results are scarce in the public domain and tend to stay as internal secrets, available only to limited communities. The high commercial value of agarwood and its derivatives had triggered such research to be patented and become inaccessible to the public. Many workshops, meetings, and seminars, both at international and national levels, mostly present the agarwood research in general terms and introduce only bits of information of their patented findings.

Agarwood is a scented product obtained after a pathological situation happened to the wood of the standing trees of the family Thymelaeaceae (most commonly the *Aquilaria* genus). More specifically, the condition could be initiated by an endophytic fungal invasion. Other fungi such as molds and decay fungi might play a role as well. Among the different fungal species associated with agarwood formation, few could exhibit pathogenesis, while others seem to be saprophytic (Tamuli et al. 2008). Indeed *Aquilaria* is naturally infected by a variety of fungi including

Aspergillus sp., *Botryodiplodia* sp., *Diplodia* sp., *Fusarium bulbiferum*, *F. laterium*, *F. oxysporum*, *F. solani*, *Penicillium* sp., and *Pythium* sp. (Sitepu et al. 2011). The involvement of these biotic agents perhaps explains the slow formation process of this chemically laden product in the natural habitat. In order to meet the demand for agarwood and protect the wild *Aquilaria* trees, many countries with *Aquilaria* plantations also include research on agarwood cultivation and inoculation techniques in their strategy.

In general, there are three approaches used to develop agarwood inoculation techniques: physical-mechanical, chemical, and biological. Chemical-based inoculation technology has been developed and practiced in Indonesia, Malaysia, Thailand, Cambodia, Vietnam, Laos, and China (Santoso 2015). However, some have no guarantee of yielding commercial agarwood product such as in a case reported in Indonesia, where farmers had used some chemical substances to stimulate the process of agarwood formation, only to find that the technique is ineffective. Unfortunately, they lost 6–7 years of their time in maintaining the trees before they learned that their efforts failed to produce agarwood. Agarwood cultivation should be developed based on its natural formation. The philosophy of agarwood formation stands as a triangle connecting the tree, microbes, and the environment. By understanding interaction of each unit in the triangle, it will be possible to apply endophytic fungi as inoculants into the host tree to yield commercial agarwood in the future. This chapter reviews and analyzes the utilization of artificial inoculation in the induction process of agarwood practiced by several countries in Asia with special reference to Indonesia.

4.2 Agarwood-Producing Species in Indonesia

Indonesia has a diverse collection of naturally growing agarwood-producing species when compared to other countries in Asia. Based on extensive ground surveys, farmers in the islands of Sumatra, Kalimantan, Java, Sulawesi, Nusa Tenggara, Moluccas, and Papua have been cultivating agarwood trees of at least seven different species. However, the plantation size is small, ranging from 10 to 5000 trees per farmer. A different trend is observed in neighboring countries such as Malaysia, Thailand, Vietnam, Laos, Cambodia, and China, where the plantation areas are of larger scale, starting as low as 40 hectares to more than 1000 hectares.

Among the species planted in Indonesia, *Aquilaria malaccensis* also known as “malakensis” is the most favorite species for mass cultivation in western Indonesia. Other species in the *Aquilaria* genus such as *A. microcarpa*, *A. beccariana*, and *A. hirta* have also gained popularity among farmers in Sumatra and Kalimantan. Another competitor of *Aquilaria*, also producing agarwood, is *Gyrinops*. Members of this genus are planted in the eastern part of Indonesia and are known by the trade name “filaria.” Generally, the name “filaria” is being loosely used on other species as well, such as on *Aquilaria filaria*, *Aquilaria cumingiana*, *Gyrinops versteegii*, and other *Gyrinops* species. The most important “filaria” species is *G. versteegii*. The species is planted in eastern Indonesia, covering several islands including the Lesser Sunda Islands (Lombok, Sumbawa, Flores, Sumba), North Celebes (Minahasa), and Papua. Although it is the most popular species in eastern Indonesia, *G. versteegii* is less favored compared to *A. malaccensis* when it comes to agarwood cultivation. Agarwood grouped under the trade names "filaria" and "malakensis" has no actual significance on the agarwood's quality. This grouping was applied merely to indicate the origins of the trees: "filaria" (eastern) and "malakensis" (western). In fact, both groups are included in CITES Appendix II for its regulation system in the international trade. To regulate agarwood trade, a DNA fingerprint database based on microsatellites has been set up for *Aquilaria crassna* to detect the geographic origins of traded wood and incense samples for forensic applications (Eurlings et al. 2010). This method could be adopted for detecting the geographic origins of *Aquilaria* and *Gyrinops* species in Indonesia.

4.3 Harvesting Natural Agarwood in Indonesia

Indonesian natural agarwood has a long history and is known worldwide. It was recorded as the main commodity bartered between the empires of China and the kingdoms of Indonesia since the Silk Road era. Traditionally, natural agarwood is gathered by cutting down the infected trees. Agarwood hunters usually have their own parameters in determining a suspected tree, among them are fallen yellow leaves and presence of black ants entering and exiting the tree trunk through small holes. For example, the indigenous people of East Kalimantan rely on several phenomena as external indicators of agarwood presence inside a tree. Symptoms of infection recognized by the local people include insect bore holes, knots, a hollow sound upon thumping, tumorlike growths, bark drop, and excessive leaf fall (Donovan and Puri 2004). To harvest the agarwood, hunters usually chipped all infected trunks, stems, and branches. Indonesia exported high-quality agarwood in small quantities before the 1990s. However, as the market demand keeps increasing, hunters tend to harvest natural agarwood in a speculative manner causing the depletion of agarwood-producing tree stocks in the nature. Hunters usually have their upper ordinate whose role is to support them financially. The fundraiser usually gives some amount of money as a disbursement to the hunters for their agarwood hunting trips in the forest, which can last between 1 and 3 months. By the time the hunters found the agarwood, the product price will be fully determined by their fundraiser. In most cases, there will be no bargaining position for the hunters. In this monopsony system, the hunters will not likely gain as much profits as the fundraiser (Siran 2013).

At the end of 1970s, demand for agarwood had increased significantly because the supply of high-quality resin wood from Cambodia and Vietnam diminished due to the political situation. At the same time, Saudi Arabia and the Gulf Emirates experienced the oil boom, which generated high income and consequently an increase in the demand for agarwood. Arab people from the neighboring Middle

Eastern countries also benefited from the oil boom and spent more on luxury products such as agarwood (Gunn et al. 2004). Between the 1980s and early 1990s, the "agarwood fever" had surfaced in East Kalimantan, where expeditions of professional collectors, sometimes dropped by helicopters and sponsored by bogus traders, were organized to hunt for agarwood. At the time, about 70 % of the collected agarwood was exported to the Middle East, while the rest to China, Hong Kong, Taiwan, and Japan. By 1995, traders stopped funding high-cost expeditions in Kalimantan and turned instead to the Papua island. There is a positive correlation between the numbers of felled trees from the wild and the rate of agarwood trade. Considering the high demand for agarwood products, the current population density of *A. malaccensis* in Indonesia has lowered to only 1–2 individuals per hectare. Although seed production was reportedly high, seed dispersal is limited and germination rate is low. Other factors that caused depletion of the agarwood tree populations in the wild include forest fires, illegal logging, forest conversion, and mining concession (Soehartono and Newton 2000, 2001a).

The formation of natural agarwood is a complex process (Santoso 2013). In the wild, it is usually triggered by wounding of twigs or branches due to friction between trees, or caused by wind, thunder, people, or wild animals. Borer insects and caterpillars could also make a hole in the tree and trigger the formation. Wounding tissues could easily be infected since fungi are present abundantly in nature in the form of spores or hyphae and are dispersed through water, wind, and soil. Suitable environment conditions such as high humidity and availability of carbon and energy sources can boost fungal growth. Fungi often secrete poisonous or toxic compounds during the infection process and the tree reciprocates by releasing chemical compounds as one of the defense mechanisms to counter the attack. The mechanism occurs continuously and this is believed to be the key process in agarwood formation. As happened in the wild, the process usually takes many years to produce high-quality natural agarwood, maybe even hundreds of years. The slow process explains why products from natural agarwood are highly valuable and fetch high prices in the market.

4.4 Establishment of Agarwood Plantation in Asia

Agarwood-producing trees are widely distributed in Asia, including India, Sri Lanka, Bangladesh, Bhutan, Myanmar, Laos, Vietnam, Cambodia, China, Brunei, Malaysia, Indonesia, Philippines, Thailand, and Papua New Guinea. There are more than 20 species within the genus *Aquilaria* and less than 10 species within the genus *Gyrinops*. All these species have been listed in CITES Appendix II since 2005 (COP 13). Among them, *A. malaccensis* and *G. versteegii* are most popular in Indonesia. These species, as well as *A. crassna* and *A. filaria*, are regarded as agarwood producers of commercial quality. Due to the economic value of these species, translocation or transplanting across different islands and countries became common. Such as the case with *A. crassna* of Indochina origin; this species has been planted widely across the regions in Sumatra.

In Asia, the establishment of an agarwood plantation usually originates from generative propagation by collecting seeds and germinating them in the nursery. Normally, the fruiting season happens twice a year. Trees that fruit during the months of July to August will mature in November to December, while those that fruit between March and April will mature in July to August. Most farmers in Indonesia are still managing their small-scale plantation traditionally. They are dependent on the wildings to supply their planting stocks. New micropropagation or tissue culture technique to fulfill the need for planting stocks is still untried. Thailand and Laos are among the few countries that use micropropagation as their propagation technique for setting up commercial plantations. Seedlings of *A. crassna* propagated by tissue culture in Thailand have been exported to northern Australia. However, such technique is more expensive when compared to collecting wildings. Moreover, tissue culture requires an investment for laboratory facilities and skilled human resources. Regardless of its high cost, mass vegetative propagation by means of tissue culture should be considered when superior mother trees are available as explant sources.

Field visits to several locations in different Asian countries revealed that agarwood-producing plantations have been practicing standard silviculture technique in many aspects, starting from nursery management to field planting and post-harvesting. In Vietnam, the agarwood association maintained that they have planted more than 20 million agarwood-producing trees in established plantations. In Cambodia, an agarwood farmer can plant as many as 2 million trees. Most companies or private farmers usually plant species that are already well known for their superior genetic characteristics such as with *A. malaccensis* and *A. crassna*. For the same reason, these two species are widely planted in many countries. In the near future, breeding strategies of such species will be in high demand to produce quality-planting materials that can yield agarwood of "super" or "double super" quality.

4.5 Agarwood Induction Technology

Generally, it is agreed that this aromatic resin is produced as the tree sap thickens in response to wounding and fungal infection (Donovan and Puri 2004). The resinous agarwood acts as a chemical barrier to attacks by fungi and insects, which otherwise is not formed by the tree (Paoli et al. 2001). Agarwood formation takes place in different woody organs like in the stem, branch, and roots, with one condition, they must be infected. Due to the importance of infection in inducing agarwood, induction techniques have been developed and are discussed below.

4.5.1 Physical-Mechanical Inoculation

Physical-mechanical inoculation has been practiced since long time ago. Agarwood farmers in different Asian countries have tried several wounding methods to produce agarwood, including ax chopping, nailing, and holing. These methods often take a long time, with generally poor yield and low quality in the agarwood they produced (Liu et al. 2013). In Indonesia, these methods are widely practiced in Riau-Sumatra, East Kalimantan, and Lombok. The initial process is tree wounding, usually performed by a blade, knife, cleaver, and spikes. Naturally, the wounded tissue will be infected by fungi, and this leads to the gradual formation of agarwood. This technique is highly risky and very speculative because the amount of agarwood produced is unpredictable, often in small amounts.

Another type of mechanical inoculation is by imbedding nails into the tree, lengthwise. In average, a mature wild tree supposedly will need around 20 kg of nails if the nail is to be imbedded every 10 cm along the length of the tree. Farmers believed that trees treated by this practice can be harvested at most 2 years after the treatment. However, the produced agarwood has been found of low quality and is frequently tainted by blue stains. Furthermore, the scent is inferior due to contamination from the smell of rusty nails. The use of nails is only applicable for a small number of trees as it will be less efficient and cost ineffective if applied in a big company plantation, especially when labor wages in Southeast Asian countries are starting to rise. At several locations in Sumatra and Kalimantan, heated nails have also been used. Similar mechanical inoculation technique is also being practiced in Bangladesh on *A. malaccensis* and in Yunnan Province (P.R. China) on *A. sinensis* and *Aquilaria yunnanensis*. Similar to those in Indonesia, this inoculation technique produces small amounts of agarwood with low-grade quality. Other examples of mechanical inoculation are by scratching the tree trunk with sharp tools such as a machete or a cleaver, or by debarking and sawing the stem. Nonetheless, the quantity and quality of agarwood produced through these techniques are still without certainty.

4.5.2 Chemical Induction

Chemical induction technique has been applied in many sourcing countries. Jasmonic acid, sulfuric acid, acetic acid, and alcohol are among the common chemical compounds applied to initiate agarwood formation. Jasmonic acid has been proven in Vietnam; it induced agarwood formation to the thickness of 2–3 mm. The activation of agarwood tree secondary to metabolism by jasmonates can be divided into two modes: (1) the augmentation of biosynthetic activities constitutively observed in plants and (2) the induction of latent biosynthetic abilities that occur only during unusual physiological processes such as mechanical wounding and microbial infection (Kenmotsu et al. 2013). Sulfuric and acetic acids have also been practiced in several countries; however, the results were not so successful, and in some cases, the trunks were broken.

Some chemicals can be extremely toxic to human. Therefore, the choice of chemical is of paramount importance especially when the agarwood is intended for use as raw materials in making perfumes, incenses, tea, and medicines. However, agarwood formed by this technique is not completely useless because after elimination of the toxic compounds, the product can still be used for other purposes such as decoration materials and accessories. When compared to mechanical induction, chemical induction appears to be a faster process, whereby the wood color changes from white to blackish in matters of days to weeks. Delivery methods of the chemicals have also been tested. The most popular method uses a needleless syringe that is attached to a tubing that connects to a reservoir containing the chemical solution. The chemical solution is then taken up from the point of syringe insertion, usually at the bottom of the stem, to the upper parts of the tree. After some time, the wood color will change from whitish to dark brown. Other delivery techniques are like the bamboo sticks, which are soaked into the chemical solution and then spiked into the trunks and branches. This practice was conducted by several groups of farmers in Indonesia; however, it is now becoming unpopular due to its side effect. The chemicals injected into the trees could be released back to the environment and cause water and soil pollution.

4.5.3 Biological Inoculation

Many researchers believe that microbes have an important role in agarwood formation (Tamuli et al. 2005; Mohamed et al. 2010). Since early 1990s, biological inoculation using specific fungi as inoculants has been practiced. However, wounding has to precede inoculation because it is the entry mode for the fungi to infect the host and initiate agarwood formation. Wounding can occur naturally from the act of animal or other physical interactions between the tree and its environment. Introduction of fungal propagules such as spores into the wounded tissue causes the host tree to secrete a combination of metabolic compounds. Major metabolic compounds reported from infected woods of *A. malaccensis* are chromones, aromatic compounds, sesquiterpenes, monoterpenes, sterols, and fatty acid methyl esters. Endophytic fungi have been associated to important agarwood compounds when used as inoculants and can be detected as early as 6 months after inoculation (Jong et al. 2014). However, not all fungi can trigger agarwood formation on the host tree. Some endophytic fungi are able to change the color of the wood fiber and cause the presence of the fragrant resin on the host tree. *Acremonium* sp., for example, could change the color of wood from white to dark brown, but the scent of agarwood appears to be inconsistent. More efforts have to be carried out to determine the most suitable agarwood-inducing endophytic fungi. As reported by the local Penan people in East Kalimantan, an insect may be implicated in the etiology of agarwood formation. Insect often bores through the cracks and crevices in the bark, while carrying fungal propagules to the underlying wood (Donovan and Puri 2004). Using insects as the vector for transmitting endophytic fungi (e.g., weevils, termites) could also be applied to accelerate agarwood formation. However, control on insect population should be determined strictly to prevent outbreak that can negatively affect the trees.

4.6 Endophytic Fungi-Based Inoculation

Agarwood is formed in the main stem, branches, and roots where natural wounding usually occurs. Agarwood-producing trees can be exemplified as a warehouse for various microbes, commonly endophytic fungi. Endophytes are microorganisms that maintain endosymbiotic relationship within plants at least in one stage of their life cycle. Both fungi and plants will receive ecological benefit from this relationship. Some genera of known endophytic fungi include *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, *Phaeoacremonium*, and *Trichoderma*. In the process of infection, fungi secrete several pathogenesis-related enzymes such as polyphenol oxidase, peroxidase, pectinase, and cellulase. These enzymes have been detected in naturally infected and inoculated *A. malaccensis*. Samples infected naturally with *Chaetomium globosum* and *F. oxysporum* exhibit higher activity of all the enzymes when compared to healthy samples (Tamuli et al. 2008). Changes in the activity of various enzymes in naturally infected tree indicate that they may be involved in the infection process and development of disease symptoms in agarwood trees. Many scientists believe that wounding causes the tree to weaken and then become vulnerable to attacks by a pathogenic fungus. Other factors such as tree age, genetic background, and seasonal and environmental variation may be important in agarwood formation (Ng et al. 1997). The complex process of natural agarwood formation has attracted many scientists to conduct extensive research on this phenomenal natural product. Scientists believe that the triangle factors, host tree, endophytic fungi, and environment, are important players of the formation process (Fig. 4.1).

4.6.1 Host Tree

The host tree is confined to the family Thymelaeaceae. There are six genera in this family: *Aquilaria*, *Gyrinops*, *Enkleia*, *Gonystylus*, *Wikstroemia*, and *Aetoxylon*. Some of the species found in Indonesia are listed here; however, not all have been examined for their agarwood-producing abilities: *A. hirta*, *A. filaria*, *A. malaccensis*,

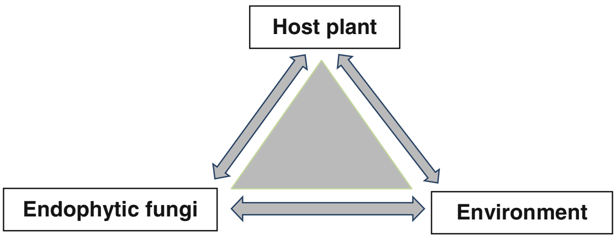

Fig. 4.1 Agarwood is formed under the influence of the plant disease triangle paradigm (genetic of the host tree, pathogenic endophytic fungi, and conducive environment), which affects the amount and quality of agarwood produced in a host tree (Santoso 2013)

A. microcarpa, *A. beccariana*, *A. cumingiana*, *G. versteegii*, *G. moluccana*, *G. decipiens*, *G. ledermannii*, *G. salicifolia*, *G. podocarpa*, *Enkleia malaccensis*, *Gonystylus bancanus*, *Wikstroemia polyantha*, *W. tenuriamis*, *W. androsaemofilia*, and *Aetoxylon sympetalum* (Sitepu et al. 2011). Because the taxonomy and systematics of this family have not been resolved, several of the names listed here could be synonyms or misidentified cases. A comprehensive study is still needed to classify the species found in Indonesia. Nevertheless, among the Thymelaeaceae members, *Aquilaria* and *Gyrinops* are the most well-known high-grade agarwood-producing genera in the world.

4.6.2 Environmental Factors

There are several environmental factors that influence agarwood formation. They include soil fertility, temperature, humidity, light intensity, and pests and diseases (Pratiwi et al. 2011; Purnomo and Turjaman 2011). Indeed, many defense strategies have evolved to fight off these numerous factors (Wong et al. 2013). For example, in a field study at Bangka Belitung province, Indonesia, trees grown on poor soil happened to yield high-grade agarwood when compared to trees grown on rich soil (Pratiwi et al. 2011). Agarwood hunters in Gunung Palung (West Kalimantan) observed that the occurrence of agarwood is more frequent in trees at high elevations and on poor soils, and trees growing under stressful conditions are more vulnerable to infection (Paoli et al. 2001). The occurrence of pests and diseases on a tree also influences inoculation procedure. Trees that are heavily under attack by pests (e.g., *Heortia vitessoides*) are not recommended for endophytic fungal inoculation. This is because the trees are already sick and inoculation with an endophytic fungi will only worsen the trees' condition possibly leading to death. Previously attacked trees can be inoculated after the trees are allowed some times to recover from the attack. Planting essential oil-producing species such as *Citronella* could help in reducing the occurrence of pests and diseases. *Citronella* has some aromatic attractants that can protect agarwood-producing trees from the attacking females of *H. vitessoides*.

4.6.3 Endophytic Fungi

The wounded trunk of an agarwood-producing tree in the natural environment harbors multiple fungal taxa that exist in a complex system as a whole or in succession leading to agarwood production in the tree trunk (Mohamed et al. 2010, 2014). Isolation of many endophytic fungi from agarwood trees have been carried out extensively around the world since 1934 (Sitepu et al. 2011). It is unlikely that a single fungal species could be responsible for agarwood. More than 20 fungal species have been identified in the aromatic wood and the number keeps increasing. Fungi of several genera have been determined as agents in agarwood formation including *Torula* sp., *Aspergillus* sp., *Fusarium* sp., *Lasiodiplodia* sp., *Penicillium* sp., and many more. Research carried out by a team at the Environment and Forestry, Research, Development, and Innovation Agency (FORDA), Ministry of Environment and Forestry, Indonesia, identified *Fusarium solani* as one of the most effective agents in agarwood formation.

The capability to induce agarwood is different for each fungal species. Some are slow to infect and induce agarwood, while others are more aggressive. Thus, isolation and screening for appropriate fungi are necessary to assure successful induction. Being aware of the importance of endophytic fungi to the Indonesian agarwood industry, FORDA has taken the initiative to study and characterize fungal isolates collected from all over Indonesia (Table 4.1) (Sitepu et al. 2011). The cultures are deposited at the Indonesian Tropical Forest Culture Collection (INTROF-CC) to ensure their viability and for future use.

Agarwood cultivation requires a quite fair amount of capital due to its complex process. Firstly, wild agarwood seedlings must be planted and maintained for 6–7 years. Then, trees that are big enough (>20 cm in diameter) require artificial induction to produce agarwood. Following inoculation is monitoring the trees until the time to harvest. The optimal time for harvesting is 3 years after inoculation upon which the agarwood usually has reached maturity. Finally, the cleaning and carving process take place to separate the agarwood from non-agarwood parts. This process is done manually hence it is labor intensive (Mucharommah 2011).

Our research series determined that the endophytic fungi application that we developed at FORDA is a promising technology that can yield high-grade agarwood product (Santoso 2015). The inoculation procedure is easy to conduct by forest communities. The procedure begins by selecting a tree with diameter at breast height (dbh) of more than 15–20 cm. Then holes of 3 mm in diameter are made in a spiraling pattern along the stem starting with the first hole at the trunk base (point 1) (Fig. 4.2a). Then the second hole is made 7–10 cm in the horizontal direction and 12–20 cm in the vertical direction (point 2). This pattern is repeated until the highest reachable shoot tip. Each tree of 15–20 cm dbh has about 100–200 holes. The maximum depth a wound can be drilled is one-third of the stem diameter. Once ready, each hole is inoculated with one cc of the inoculum, which is in liquid form (Fig. 4.2b). The inoculation procedure requires a skilled and courageous personnel, who is physically fit to work in the field. After 2–3 months, agarwood formation can be detected and evaluated. Using this technology, we have inoculated many trees in different provinces in Indonesia and the results are very promising (Fig. 4.3a–c). An *A. malaccensis* tree in Sanggau (West Kalimantan) with 15 cm dbh yielded 4.5 kg of dried weight (dw) agarwood, with the selling price of about USD 200 per kg. In Kandangan (South Kalimantan), another *A. malaccensis* (40 cm dbh) yielded 13 kg (dw) of agarwood, 18 months after inoculation with *F. solani* (Turjaman and Santoso 2012). With advancement in the inoculation technology, it is not surprising that within the next 5 years, cultivated agarwood of various qualities will be released into the market. This trend will help to ease the tension on natural agarwood exploitation.

Table 4.1 A list of 36 strains of fungal endophytes associated to agarwood identified through molecular methods

No.	Isolate number	Origin (province)	Molecular identification
1.	FORDA-CC 506	North Sumatra	*Fusarium solani*
2.	FORDA-CC 509	Gorontalo	*Fusarium solani*
3.	FORDA-CC 503	West Sumatra	*Fusarium solani*
4.	FORDA-CC 512	Papua	*Fusarium solani*
5.	FORDA-CC 500	Jambi	*Fusarium solani*
6.	FORDA-CC 501	West Sumatra	*Fusarium solani*
7.	FORDA-CC 510	Molluca	*Fusarium solani*
8.	FORDA-CC 497	Central Kalimantan	*Fusarium solani*
9.	FORDA-CC 499	West Kalimantan	*Fusarium solani*
10.	FORDA-CC 2372	East Nusa Tenggara	*Fusarium solani*
11.	FORDA-CC 504	Riau	*Fusarium solani*
12.	FORDA-CC 514	Papua	*Fusarium solani*
13.	FORDA-CC 502	West Sumatra	*Fusarium ambrosium*
14.	FORDA-CC 515	East Nusa Tenggara	*Fusarium* sp.
15.	FORDA-CC 2379	Molluca	*Fusarium solani*
16.	FORDA-CC 511	West Nusa Tenggara	*Fusarium solani*
17.	FORDA-CC 2370	Bangka Belitung	*Fusarium solani*
18.	FORDA-CC 517	Bangka Belitung	*Fusarium solani*
19.	FORDA-CC 513	Papua	*Fusarium solani*
20.	FORDA-CC 519	West Java	*Fusarium falciforme*
21.	FORDA-CC 2375	East Kalimantan	*Fusarium oxysporum*
22.	FORDA-CC 520	West Java	*Fusarium solani* f. batatas
23.	FORDA-CC 518	Bangka Belitung	*Fusarium solani* f. batatas
24.	FORDA-CC 2371	Bangka Belitung	*Fusarium solani*
25.	FORDA-CC 2377	West Java	*Fusarium solani*
26.	FORDA-CC 507	Lampung	*Fusarium solani* f. batatas
27.	FORDA-CC 498	Central Kalimantan	*Fusarium solani*
28.	FORDA-CC 2369	West Sumatra	*Fusarium ambrosium*
29.	FORDA-CC 495	South Kalimantan	*Fusarium solani*
30.	FORDA-CC 2373	West Nusa Tenggara	*Fusarium solani* f. batatas
31.	FORDA-CC 2374	East Kalimantan	*Fusarium solani*
32.	FORDA-CC 508	Bengkulu	*Fusarium* sp.
33.	FORDA-CC 505	North Sumatra	*Fusarium solani*
34.	FORDA-CC 496	South Kalimantan	*Fusarium solani* f. batatas
35.	FORDA-CC 516	Bangka Belitung	*Fusarium solani*
36.	FORDA-CC 2378	West Java	*Fusarium solani*

The isolates were collected from 17 provinces in Indonesia (Sitepu et al. 2011)

Fig. 4.2 Application of the fungal inoculation technology on an agarwood-producing tree at a community forest. (**a**) A hole is made using a 3 mm drill bit, and (**b**) a total of 1 cc liquid inoculum is applied into the hole

Fig. 4.3 Agarwood was successfully produced using the endophytic fungal inoculation technology developed at FORDA. Some examples of the produced agarwood: (**a**) after 24 months from South Sumatra, and (**b**) and (**c**) after 15 months from West Kalimantan

4.7 Concluding Remarks

Agarwood bioinduction technology by means of endophytic fungi is proven to be a promising technique for agarwood formation. This technique forms agarwood at a faster rate compared to customary techniques, is more environmentally friendly compared to chemical inducers, and is widely acceptable from health and safety perspectives compared to physical or chemical methods. This method was developed following natural agarwood formation mechanism, whereby wounds were first initiated by making holes on the trunk followed by inoculating a specific fungal endophyte into the "wound." Triangular factors, namely, the host tree, microbes, and environment, are the important elements in determining a successful formation of agarwood. Selecting appropriate tree species, inoculating a suitable fungus, and establishing supporting environmental factors will yield agarwood of commercial value. Applying an accurate and proven inoculation technique in the field should become a serious concern to support the success of any agarwood cultivation program. Our comprehensive inoculation technique, developed since 1984, has proved that establishing a manmade agarwood plantation is a prospect that could materialize very soon. Nowadays, many procedures that employ endophytic fungi as inoculants are being tested on *Aquilaria* and *Gyrinops*. It is conceivable that one day the inoculation technique will become more efficient, produces agarwood of super quality, and is cost effective.

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