Ex-situ conservation effort for *Dipterocarpus spp* through the seedling collection and nursery management

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**Abstract.** *Dipterocarpus* or keruing belongs to member *Dipterocarpaceae*, known as commercial timber producer and oil for pharmacological purposes. Of 70 keruing species in the world, 38 species were found in Indonesia and listed in the IUCN Red List. Due to illegal logging, land conversion, low natural reproduction, and specific habitat requirements, the keruing population tend to decrease every year. Conservation effort was urgent for this species, one of which is an increasing number of species through conservation strategies using the conservation nursery concept. Nursery management in this research was conducted through keruing material field collection followed by sowing and acclimatization for seed and wildings, vegetative propagation using the Komatsu-FORDA Fog Cooling System cutting system, and nursing (watering, weeding, fertilizing, and soil change). The result of our research showed that using KOFFCO nursery application, 1226 individuals of fifteen dipterocarpus species seedlings originated from five provinces in Indonesia was conserved. All species are categorized as threatened based the IUCN red-list category and criteria. Hence, more than 60% of these threatened species were conserved and well-managed through the KoFCo nursery. Seedlings collection and KOFFCO nursery, therefore, play a vital role and support in ex-situ conservation effort and management of dipterocarps genetic resources.

1. **Introduction**

Dipterocarpus or locally known as keruing belongs to Dipterocarpaceae commonly found in Southeast Asia primary forest with various habitats up to an altitude of 1,500 m. asl [1]. There are 70 dipterocarpus species in the world [2]; 38 of them are found in Indonesia’s primary forests [3]. In the Malesia region, keruing is scattered distributed in the Malay Peninsula forests, Sumatra, Kalimantan, Philippines, Java, Bali, Lombok, and Sumbawa [4]. As a Dipterocarpaceae member, keruing has a strength class and a high durability class [5], which is used as the main construction. Keruing also produces oil and has long been traded, 20 species are known as oil producers [6]. Keruing oil is used as an aromatic raw material, waterproof coatings, and lithographic ink [7]. The chemical components of dipterocarpus consist of sesquiterpenes, triterpenes (resveratrol trimer, tetramer oligomer), and coumarin derivatives [8].

The keruing population, especially in Indonesia, has decreased drastically due to illegal hunting for...
timber and land conversion. As a result, 25 keruing species have been listed by International Union for Conservation of Nature (IUCN) various statuses with vulnerable (1 species), endangered (1 species), and critical endangered (23 species). The presence of distinctive habitat causes keruing also has a different phenology phase. As a result of this reproductive pattern, its natural regeneration becomes low. That could be a challenge in keruing conservation efforts regarding a continuous supply of planting stocks. Hence, vegetative propagation through cuttings needs to be considered to solve the regular availability of seed and its seed characteristic to prepare plant stock production.

The Komatsu-FORDA Fog Cooling System (KOFFCO) was developed by the Forest Research, Development, and Innovation Agency (FORDIA) in collaboration with Komatsu Ltd [9]. The system was implemented to allow massive vegetative propagation of dipterocarps by cutting. Series or nursery technology were developed to monitor environment factor conditions i.e., humidity (over 95 percent), light intensity (5,000-20,000 lux), and temperature (less than 30ºC) as the main factors in rooting ability [10]. At present, the KOFFCO system is not only produced for planting stocks of Shorea species but also for most of the dipterocarps species.

The decline of the dipterocarpus population and its habitat management influenced species genetic diversity [11,12]. One urgent effort to increase dipterocarpus diversity is to increase the species number through conservation strategies, both in-situ and ex-situ. Ex-situ conservation consists of maintaining activities and tree improvement of endangered species outside its natural habitat in partially or wholly manipulated conditions such as nurseries for decreasing environmental stresses [13]. Previous study conducted by [9] on D. cinereus (previously extinct reported) showed seedling handling were critical factor to determine survival rate of this species. Therefore, our research was conducted to find the best way for dipterocarpus conservation efforts of through the seedling collection and nursery management for its genetic conservation in Indonesia.

2. Materials and methods

2.1. Study site
The research was conducted in the KOFFCO nursery located in Forest Research and Development Center office, Bogor-Indonesia. The nursery area is 1.6 ha at 250 meters above sea level. The KOFFCO nursery is a conservation research nursery built in 1994 to support the rehabilitation project cooperation between FORDIA and KOMATSU Ltd. Japan. For this research, nine dipterocarpus species from different locations have been collected. The collection started from 2016 until now and have been successfully collected materials from seven sites from five provinces in Indonesia, namely Riau, Riau Island, North Sumatra, Lampung, and West Java. The material collection represented lowland mixed dipterocarps forest and sub-mountainous forest.

2.2. Methods

2.2.1. Collection of dipterocarpus species. Dipterocarpus species were collected from five provinces in Indonesia. The information on its distribution was gathered from some published articles and books [14,15]. Another information also gathered from local people before field collections activities to ensure the targeted species’ actual occurrence, the flowering/fruited season, seed availability, and seedlings occurrence. The exploration method and protocol of plant genetic resources collections were found in and followed [16-19].

There were three types of genetic materials to be collected in the field, i.e., fruit/seed, wildings/seedlings, and cutting materials, and ideally, all these materials should be obtained. When these genetic materials could not be found, collections were made from mature trees to get only cutting materials, but the survival rate was very low, around 30%. Hence, cutting materials were preferably made from the seedling which we had collected from the field. In most cases, collections were made from seedlings since they were available after its masting period. Seedling with a height less than 25 cm was the first targeted size and collected by carefully pulling out from the forest floor.
to avoid breakage of seedlings root. In abundant seedlings availability (>100 seedlings under one mother tree), at least 20 seedlings from each mother tree and seedlings from different mother trees of the same species were collected. The collected seedlings were recorded with unique specimen codes, including local name, scientific name (if known), mother trees’ coordinators, and individual numbers.

The seedlings should have been kept moist during the field exploration and apply regular watering when needed. During the time of transporting the seedling collections to the nursery, all the leaves should have been cut into half size to avoid excess evapotranspiration, reducing the survival rate. The seedlings were then placed in a bucket containing 1/3 part of the water in the moist room, covering the leaves or upper part by plastic bags before final packaging at the last exploration day.

2.2.2. Dubious species identification. During the field exploration, each mother trees of collected seedlings were identified directly. For the doubtful species, its mother tree's voucher specimens were collected and were brought to Herbarium Bogoriense (BO) to identify and decide its scientific name. The voucher specimens were prepared following by [20]. The species’ information was recorded in the labels contained its local name, name of the collector, genus, coordinate position, date and place of collection, and elevation. The additional information about habitus morphological characteristics and its environmental conditions were also recorded in tally sheet.

2.2.3. Sowing. Sowing was carried out when field collections were made from seeds, followed by weaning. The seeds were sowed in the nursery's germination bed with a size of 1 m x 6 m. The large-sized dipterocarpus seeds need a sowing distance of 10 cm, while the smaller seeds needed ca. 5 cm. Each bed will contain 300-800 seeds. Seedlings with two leaves were the ideal condition to be weaned. Weaning was carried out by pulling out the germination bed's seedlings carefully and planting them into a polybag containing topsoil and rice husk media at 2: 1 v/v. All weaned seedlings in polybag were then placed in shade nursery using two layers black netting roofs for controlling light intensity (reduce about 60-75% light intensity) for approximately 2-3 months before placing them in the more open nursery with only one shade of black netting roof to reduce 25-30% light intensity. For the large seed type, germination could take place directly in the polybag and keep them under the shade. In general, it took 6-8 months for seedlings to become a ready-to-plant individual or source of cutting materials.

2.2.4. Acclimatization. Acclimatization was carried out when genetic materials collected were seedlings. The acclimatization process was needed to allow the seedlings to adjust to the new environment. Wildlings were kept in a moist-shaded nursery chamber of 1-m high using the shade consisted of two layers of black netting roofs to reduce light intensity and keep it around 25% of light intensity. After maintaining the seedlings and wilding under the shade for 6-8 weeks on average, they were moved to a new shaded nursery chamber of 3 m high with one-layer black netting roofing that can reduce only 25% of the light intensity. This process was carried out to prepare plant materials ready to be planted in the real environment.

2.2.5. Vegetative propagation. When seedlings and fruit/seeds do not become available for certain species during exploration, cutting materials were taken both from juvenile or mature individuals. The cutting materials for vegetative propagation were managed using the KOFFCO technique that has been established for various species [9]. The KOFFCO technique (Figure 2) was aimed to provide the ideal environment condition such as humidity (above 95%), lighting (light intensity 5,000-20,000 lux), and temperature (less than 25ºC) that were required to optimize the rooting ability [10]. The process of vegetative propagation through the KOFFCO technique started by fogging activities that used a specific tool, namely, nozzle. The last activity was nursing, both at the rooting formation and adaptation stage.
2.2.6. Nursing. Nursing was a routine procedure for maintaining the optimum condition and growth of the plant materials collected from the field and for the cutting experiments' propagules. The nursing activities consist of watering, weeding, fertilizing, and changing the soil. This process was also essential to keep plant materials grow well in the nursery for years.

3. Results and discussion

3.1. Dipterocarpus seedling field collection
Conserving genetic resources in the form of the seedling collection had not been common practice compared to botanical gardens and or arboretum. Establishing such a collection needed proper technique during field exploration and also nursing period. When explorations were conducted in the forest near the nursery, a long and far transportation scheme could be avoided. Furthermore, there would be no necessity to have bare-root seedlings due to the absence of quarantine check and other legal permission between sites and or islands of origin. However, most of the explorations were carried out far from conservation nursery that would require a long period of bare-root transportation. Thus, following and establishing a standard proper collection and nursing technique was a must to guarantee the seedlings' survivorship since transporting bare-root seedlings would be more complicated than those of seedlings with the medium. For those collected using the non-standard proper collection, the survivorship rate was only 35% compared to those collected using standard appropriate seedlings collection, which reached 95% of the survivorship rate. Our result showed that dipterocapus seedling from forest have different survival rate and acclimatization time (table 1). With the same procedure and same transportation time *D.kerii* have the lowest survival rate compared to other.

| Species       | Origin (Province) | Transportation time (days) | Number of seedling collection | Number of survival seedling | Survivor percentage (%) | Acclimatization time (month) |
|---------------|-------------------|-----------------------------|-------------------------------|----------------------------|--------------------------|-------------------------------|
| D. cinereus   | North Sumatra     | 6                           | 200                           | 99                         | 49.5                     | 24                            |
| D. humeratus  | Lampung           | 6                           | 100                           | 60                         | 60                       | 4                             |
| D. kunstleri  | Lampung           | 6                           | 40                            | 17                         | 42.5                     | 4                             |
| D. haseltii   | West Java         | 6                           | 200                           | 200                        | 100                      | 4                             |
| D. haseltii   | West Java         | 6                           | 1                             | 1                          | 100                      | 4                             |
| D. baudii     | Riau              | 6                           | 1                             | 1                          | 100                      | 4                             |
| D. kerii      | Riau              | 6                           | 3                             | 1                          | 33.3                     | 4                             |
| D. crinitus   | Riau              | 6                           | 2                             | 1                          | 50.0                     | 4                             |
| D. gracilis   | Riau              | 6                           | 2                             | 1                          | 50.0                     | 4                             |
| D. rigidus    | Riau              | 6                           | 45                            | 40                         | 88.9                     | 4                             |
| D. palembanicus | Riau           | 6                           | 42                            | 10                         | 71.4                     | 4                             |

Seedling growth quality after transportation depends upon physiological vigor and dormancy. Physiological vigor must be managed and maximized in the nursery. The risk of lower physiological quality is substantially higher in bare-root than in containerized planting stock. Several factors influence bare-root transplant success. Drought conditions are responsible for many establishment failures, the timing of lifting and transplanting, and any stress factors that damage bare-root stock during the interval between lifting and planting [21]. Temperature and duration of storage and relative humidity and packing are primary environmental parameters that need to be optimized for storage and transport. These parameters are crop-specific (tropical, subtropical, etc.), as plants have different tolerances and show different sensitivities to storage conditions [21]. Therefore, seedling handling and
transportation were critical steps for collecting plant species from the forests. The age of seedlings at transplanting is also an important contributor to better performance on the acclimatization process. Acclimatization is a crucial process in which plant materials adjust to environmental changes such as temperature, humidity, photoperiod, and pH [22]. When seedlings were available in various sizes, selecting seedlings under 25 cm in height would be a better choice. Smaller seedlings will deliver higher survival rates during the acclimatization stage because of their mild stress compared to bigger ones. The exploration series's experiences determined that seedlings in 10-20 cm height with 2-4 leaves would be the ideal condition that gives more than 80% of survival rate. In comparison, seedling at 30-50 cm showed a lower survival rate and only gave off 40%-<80%. When the seedlings were only available to the size of >50cm, it was almost certain that the highest survival rate after acclimatization would be less than 40%. In *S. album*, [23] found that seedlings transplanted at six weeks after sowing tended to give slightly higher survival than eight weeks old seedlings.

As exploration usually takes more than 8 hours/day, it will be necessary to keep the leaves of collected seedlings moist. During collection activities, seedlings are exposed and vulnerable to environmental stress. Exposure of leaves and root to drying conditions significantly decrease seedling survival. Maintaining a seedling out of the ground is complex activities, whereas improper care and handling translate into mortality. Many planting failures thought to be caused by receiving damage or death stock from the field can be traced to inadequate handling procedures between the area and the collection site. When all activities are conducted through proper procedures, the mortality percentage will be decreased.

3.2. *Dipterocarpus* species collection in KOFFCO nursery

Ex-situ conservation can involve some approaches of preservation of whole plants, seed, tissue or cell cultures, vegetative propagules, and pollen [24-28]. On the other hand, ex-situ conservation may be developed through selection and breeding, storage, and use (seed, seedlings, pollen, DNA) and establishment of ex-situ conservation stand [29]. Ex-situ conservation through seedling conservation defines as a technique referring to the conservation of young seedlings arrested in their development by storage at low temperature and under low light intensity.

**Table 2. Dipterocarpus species collection in KOFFCO nursery.**

| Species                  | IUCN Status           | Origin (Province)                | Source of materials   |
|--------------------------|-----------------------|----------------------------------|-----------------------|
| *Dipterocarpus retusus*  | VU A1cd+2cd           | West Java, North Sumatra         | Seedling, cutting     |
| *Dipterocarpus kunstlerii* | CR A1cd+2cd           | Bangka Belitung, and North Sumatra | Seedling              |
| *Dipterocarpus cinereus* | EX                    | North Sumatra                    | Seedling, cutting     |
| *Dipterocarpus elongatus* | CR A1cd+2cd           | Riau Islands, and North Sumatra  | Seedling and seed     |
| *Dipterocarpus grandiflorus* | CR A1cd+2cd          | North Sumatra, Riau, Riau Islands | Seedling, cutting     |
| *Dipterocarpus crinitus* | VU A2cd ver 3.1       | North and South Sumatra          | Seedling              |
| *Dipterocarpus humeratus* | NA                    | South Sumatra, West, and East Kalimantan | Seedling              |
| *Dipterocarpus rigidus*  | CR A1cd+2cd           | Riau Island, Riau                | Seedling              |
| *Dipterocarpus baudii*   | CR A1cd+2cd           | Riau, North Sumatra              | Seedling              |
| *Dipterocarpus gracilis* | CR A1cd+2cd           | Riau, West Java                  | Seedling              |
| *Dipterocarpus palembanicus* | NA                   | Riau                             | Seedling              |
| *Dipterocarpus costulatus* | CR A1cd+2cd           | Riau, Riau Islands               | Seedling              |
The KOFFCO nursery may take a position as ex-situ conservation in the form of whole living plant collection. There are 7500 samples of 85 species from 9 genera of Dipterocarpaceae collected in the KoFCo nursery consisted of samples collected from the field and samples derived from the cutting experiment. Using KoFCo nursery, 1226 individuals of fifteen dipterocarpus species seedlings originated from five provinces in Indonesia was conserved (table 2). Of these, all species are categorized as threatened based on the IUCN red-list category and criteria.

| Species                  | IUCN Status     | Origin (Province) | Source of materials |
|--------------------------|-----------------|-------------------|---------------------|
| Dipterocarpus coriaceus  | CR A1cd+2cd, B1+2c ver 2.3 | Riau Islands      | Seed                |
| Dipterocarpus hasseltii  | EN A2cd ver 3.1 | West Java         | Seedling            |
| Dipterocarpus kerii      | EN A2cd ver 3.1 | Riau              | Seedling            |

The living collection in the KoFCo nursery represented floristic regions, endemic, and various ecosystems where Dipterocarps species occurred in Indonesia. Shorea selanica is an endemic species from Maluku and Hopea gregaria representing species in the eastern Wallace region. Shorea belangeran represents peat swamp species. The Bornean endemic, such as Upuna borneensis and several Shorea species section Pachycarpae (S. pinanga, S. stenoptera, and S. macrophylla) are Tengkawang groups that are endemic to West Borneo. Thus, the KOFFCO nursery collection is considered the most extensive ex-situ collection of dipterocarps in Indonesia (figure 1).

![Figure 1. Wilding collection (a), seed extraction (b), seed germination (c), cutting propagation (d), seedling collection in KOFFCO Nursery (e, f)](image)

Those of endemic and habitat-specific species were collected during exploration activities. Also, widely distributed dipterocarp species were collected from several locations or islands (i.e., Shorea parvifolia, Shorea leprosula, Shorea ovalis, Shorea johorensis, etc.). Therefore, during collecting samples for KOFFCO nursery, the centrality of provenance as the unit of genetic resources was fully recognized as those suggested by [30]. With this concept, it is expected that this conservation nursery can meet the objectives for the conservation of genetic resources. On the other hand, seedlings from the different populations were also partitioned to differentiate genetic variation considering samples' numbers and size. In a practical sense, the concept of provenance often has no clear definition;
however, it has important practical implications and should be considered in documenting collections of seedlings [31].

4. Conclusions
In conclusion, through this study, KOFFCO nursery as an option for conserving species through seedling collection could be proved. In this study, many seedlings of dipterocarpus species have been collected for supporting dipterocarps genetic resources conservation and management. KOFFCO nursery could be developed as a place for collecting both dipterocarps and non-dipterocarps species, which could save the space and collected more individuals.

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