Name	Counterpart	Title
Bambang Irawan, Yabes God Anugrah Panjaitan	**B14**	De novo whole genome sequencing of Bulian (*Eusideroxylon zwageri* Binn & Teijsm) using millenial technology MinION Oxford Nanopore

Scientific Background and Method

Ironwood (*Eusideroxylon zwageri* Teijsm. et Binn., synonymous to *Bihania borneensis* Meissner and *Eusideroxylon lauriflora* Auct.) is known by several common names (e.g. bulian/ulin/belian/onglen) and belongs to the family of Lauraceae (tribe Cryptocaryeae, subtribe Eusideroxylineae) (Kostermans 1957). In nature it is distributed in Sumatra, Kalimantan and some small islands such as Tawi–Tawi (Philippines), Banka and Billiton.

E. zwageri is one of the most important species for construction wood in Indonesia. The wood is used to make furniture, windows and door frames, heavy constructions, roofs, bridges, railway sleepers, marine pillings, boat constructions, fence posts, heavy duty industrial flooring, shingles and vehicle body work. Seed extracts can be used as a skin medicine (Irawan 2005). The most valuable characteristic of *E. zwageri* is that it is not susceptible to termites and other ubiquitous tropical wood-eating insects and fungi. For this reason, the wood is in great demand for construction throughout Indonesia (Peluso 1992). Martawijaya *et al.* (1989) stated that the physical characteristics of *E. zwageri* are excellent. It has very high strength and durability (class one) and is very hard with a specific gravity of 0.88–1.19. In addition, Wong *et al.* (1996) found that *E. zwageri* is very resistant to fungi in a fungal decay test. Sampling of heartwood poles in Sarawak revealed only surface biodeterioration after 20 years of operation and ground-contact. Furthermore, although impermeable to preservatives, the wood can be used in highly decay-prone environments (burial in the ground).

Variability based on morphological structures (see Van Lijnden and Groll 1851; Teijsmann 1858; Teijsmann and Binnendijk 1863; Heyne 1927; Koopman and Verhoef 1938; De Wit 1949; Kostermans *et al.* 1994), as well as genetic variation based on DNA marker had been reported in several publications. Knowledge of genetic variation among *E. zwageri* varieties and populations is very important for the conservation and sustainable utilization of its genetic resources.

On the other hand, populations of *E. zwageri* are intensively decreasing in nature in parallel with the demand for this species. The decline in population is caused by overexploitation. Recently, *E. zwageri* has been excluded from the list of protected plant species in Indonesia, which may lead to further decline of natural population. The natural regeneration of *E. zwageri* in overlogged forests is limited. To date, the species has been planted on a small scale due to insufficient supply of seeds and seedlings and slow growth compared to more commonly planted trees (Oldfield *et al.* 1998). In Jambi, *E. zwageri* can be found in several remnant forest areas, namely Senami forest, Sengkati, Durian Luncuk I and II, and several small forest areas in Sungai Kandang, Batanghari (Harapan Rainforest), District VIII Conservation area of PT, Wirakarya Sakti, Mandiangin. Rimbo Bulian in Batanghari and some other regions in the eastern part of Jambi, namely Muaro Jambi in Kumpeh region and Muara Sabak, Tanjung Jabung Timur District. *E. zwageri* is also found in South Sumatra, including one stand of *E. zwageri* in PT REKI (Restorasi Ekosistem Indonesia). Considering the resilience of bulian, fundamental research is needed that requires sufficient genomic information to understand genetic diversity, mating systems, planting stock quality etc.

Samples were collected in Jambi Province in four villages, namely Bulian baru, Durian Luncuk, Belanti Jaya, and Muara Kilis. The method in general included (1) sample collection using tea bag method to preserve the leaf sample; (2) DNA isolation using modified CTAB method; (3) Quantity and quality check using electrophoresis and nanophotometer; (4) DNA sequencing using MinION Oxford Nanopore and associated softwares. In particular, for DNA isolation, modification was conducted on the grinding methods, namely i) DNA isolation with nitrogen in pestle, ii) sample grinding with CTAB in pre-chilled mortar and iii) sample grinding in tube with tips.

Objectives

The objective of this study is to sequence the whole genome of bulian as baseline information for subsequent genetic analysis using portable MinION ONT sequencer (Picture 1).

Approach

The samples were collected from Jambi Province which spread from four villages meanly Bulian baru, Durian Luncuk, Belanti Jaya, and Muara Kilis villages. The collected sample were obtained from leaves and preserved directly in the field using tea bag method by drying the leaves with silica gell (Picture 61). The DNA sequencing using MinION oxford nanopore as the data will be used to establish the whole genome sequence of bulian. Previously, the genetic study of bulian was conducted using RAPD marker, but this study did not provide whole genome information, but only focused on the genetic diversity of bulian. Whole genome sequencing information is important for analyzing SNP location, *Copy Number Variation* (CNVs), structural variation (SVs), SNP annotation and enrichment analysis of SNPs that has no synonym (Mei *et al*. 2016).

Picture 1. Collection of samples in the field.

Picture 2. Genetic analysis using portable MinION ONT sequencer.

Results Leaf sample collection

Leaves from a total of 38 individual trees were collected as presented in table 1.

DNA Isolation

The DNA extraction and electrophoresis results were presented in figure 1. Based on the results of figure 1, the modified grinding methods had an effect on the DNA yields. The results of the nanophotometer as shown in table 2 were generated using samples with clear DNA bands. In general, the results are still preliminary, and further analysis is necessary to ensure that the samples have passed the quality check before sequencing.

Table 1. Number and location of sample taken

No.	Location	Number of Sample (trees)
1	Muara Kilis Village	11
2	Bulian Baru Village	8
3	Durian Luncuk Village	10
4	Belanti Jaya Village	0
		38 (Total)

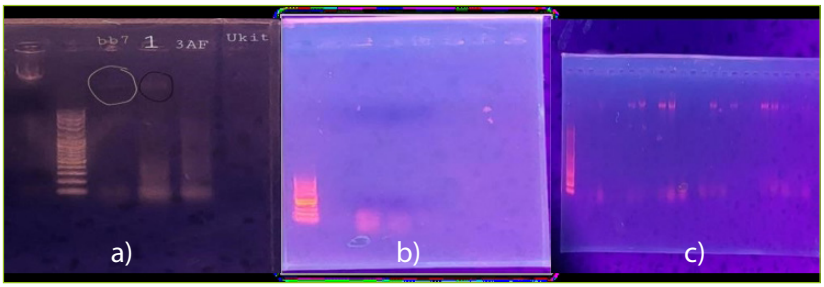

Figure 1. (a) DNA isolation with nitrogen in pestle (b) Sample ground withCTAB in pre-chilled mortar (c) sample ground in tube with tips.

Table 2. Nanophotometer results

No.	Name	Concentration (µg/ml)
1	D1	114,30
2	BB7	106,65
3	D10	145,15
4	BJ9	237,05
5	B3B5	86,400

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