

The Second International Conference on Genetic Resources and Biotechnology

**Harnessing Technology for Conservation and Sustainable
Use of Genetic Resources for Food and Agriculture**

Bogor, Indonesia • 24–25 May 2021

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24th-25th of May 2021 virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Abstract. Oil palm is an important estate crop and Indonesia is the largest palm oil producer in the world. Since the average yield is relatively low, a more efficient breeding program that utilizes the latest technological developments in DNA analysis is necessary. The objectives of this study were to (1) characterize genomic variations of three Indonesian oil palm genotypes based on whole genome sequencing using next-generation sequencing (NGS) HiSeq; (2) provide genomic data resources for future oil palm breeding program. Whole genome sequencing was performed on three Indonesian oil palm genotypes (Dura, Pisifera, and *Elaeis oleifera* from Brazil). Sequence data were aligned with that of the *Eg5* genome reference sequences. The average sequence coverage depth for all loci was 34 and more than 95% of the genome was sequenced at least ten times. Comparison of the sequences revealed 3,735,311 sequence changes, an average of one sequence change per 197 bases. Among these changes, 3,371,088 were SNPs and 364,233 were insertions and deletions (indels). DNA variations found within exons total 329,087. Of these, 214,068 SNPs variations were capable of causing modification in the amino acid composition of the encoded proteins (nonsynonymous mutations). A genomic database was constructed to manage DNA variations resulted from this study. The SNPs collected from this study should be useful for constructing high density oil palm SNP chip to expedite future national oil palm breeding program.

INTRODUCTION

Oil palm (*Elaeis guineensis*) is the most important estate crop in Indonesia. Indonesia is the largest palm oil producer of the world, even though it is not a native plant of this country. However, oil palm annual average yield is still low. There is a huge gap between the theoretical oil palm yield potential of about 18 t/ha/year [1] compared to that of the Indonesian oil palm yield average of about 4 t/ha/year [2]. This yield gap should be narrowed down by developing high yielding oil palm variety by applying a modern breeding method, e.g. molecular breeding based on genomic data.

Oil palm genome reference sequences of two oil palm genotypes have been constructed and are publicly accessible [3, 4], i.e. the ones derived from *E. guineensis* and *E. oleifera*. These reference genome sequences are important fundamental resources to enable in identifying genetic variations across oil palm genetic resources to obtain the ones important for breeding purposes and to develop molecular markers as well as gene discovery useful for breeding purposes.

Recent development of biotechnology techniques that utilize DNA-based tools, such as marker-assisted selection and genomic selection, should be explored to improve the speed and efficiency of oil palm breeding programs. Next-generation sequencing (NGS) technology has capabilities to sequence whole complexed genomes such as oil palm that has genome size of about 1.8 GB in a shorter time with lower per unit data sequencing cost [5, 6]. This opens

the way to re-sequence every genotype of oil palm collection to comprehensively understand the genetic make-up of each oil palm genotype through resequencing the genome simultaneously. By resequencing, one will be able to discover the genetic variations of the targeted traits and identify the plants having the genes and alleles to be used in a breeding program of that particular trait. In summary, the use of NGS can assist the design of a large number of DNA markers, which would be useful for fine mapping, genome-wide association studies and genomic selection of important characters targeted for breeding purposes [7].

This study was intended to assist future oil breeding programs in Indonesia by characterizing the genomes of local oil palm genotypes. Whole genome sequencing was performed on three selected local genotypes in order to (1) identify genetic mutations across the three oil palm genotypes by comparing to the oil palm reference genome sequence *Eg5*, and (2) to identify allelic variation that changes amino acid sequences and the variations of which should be useful for the development of DNA markers and gene discovery for future oil palm breeding programs in Indonesia.

MATERIALS AND METHODS

Plant Materials

The plant materials consisted of three oil palm genotypes from Indonesia, namely Dura, Pisifera, and a genotype of *E. oleifera* (originated from Brazil). The three genotypes were selected based on several criteria, such as the presence of useful traits, their utilization in breeding programs, and genetic diversity according to a previous phylogenetic study using simple sequence repeat (SSR) markers [8–10]. The oil palm genotypes were obtained from the collection of the Indonesian Oil Palm Research Institute (IOPRI), Medan, Indonesia.

Genomic DNA Isolation and Library Construction

Genomic DNAs of the three oil palm genotypes used in this study were isolated from young leaf using CTAB buffer by following the method of Michiels *et al.* [11] with small modification as reported by Satyawati and Tasma [12]. DNA concentration and purity were then measured using a nanodrop spectrophotometer (Thermo Scientific, USA). DNA was also electrophoresed in 1% agarose gel [13] and the DNA bands were visualized with a ChemiDoc (Bio-Rad, USA). Genomic DNA library construction was conducted using the Illumina TruSeq DNA low throughput (LT) protocol (Illumina Inc., USA) using a recommended procedure [14, 15]. In brief, the procedure included DNA fragmentation, DNA end modification, adenylation of 3' end, adaptor ligation and purification, PCR amplification, and validation of the constructed oil palm genomic library. The library concentration and quality were measured using RT-PCR method.

Whole Genome Sequencing of Three Oil Palm Genotypes

Genome sequencing process of the constructed oil palm genomic libraries consisted of two steps, i.e. DNA clusterization in a DNA cluster platform (cBot) and sequencing of the clustered genomic DNA using an NGS HiSeq 2000. DNA cluster generation was conducted using the cluster generation protocol, reagents, and kits from Illumina (Illumina Inc., USA) [16]. The sequencing of the clustered libraries was performed using sequencing reagents and kit from Illumina (Illumina Inc., USA) according to the sequencing procedure as previously reported [15, 17]. A paired-end genome deep sequencing method was accomplished for the three oil palm genotypes using a total of 200 cycles for both sequencing reads.

Detection of DNA Variations across Oil Palm Genome

The quality of sequence data resulted from this study were analyzed and the good quality sequence data were aligned to that of the *Eg5* oil palm genome reference sequence [3]. The reference sequence was downloaded from Phytozome (www.phytozome.net). The sequence alignment was done by using Bowtie2 [18]. The sequence alignments were followed by single-nucleotide polymorphism (SNP) calling using the program mpileup in Samtools [19]. Annotation of the locations and predicted effects of the SNPs was performed using SNPEff [20]. Phylogenetic analysis and tree construction were carried out using DarWin [21]. Tree drawing was generated in Dendroscope [22].

RESULTS AND DISCUSSIONS

Sequencing Depth

The average sequence coverage depth for all loci was 34, and more than 95% of the genome was sequenced at least ten times. This average depth exceeding 30 times was the *de facto* standard [23] and the type of genome sequence coverage was classified as a deep sequencing data [24]. The coverage obtained from this study is very excellent to be used to obtain high quality SNPs and insertions and deletions (indels) as the more sequence overlapping would be obtained within the identified SNP or indel that increase SNP/indel discovery accuracy. The identified SNPs/indels, therefore, should be more reliable for further analysis and future breeding application purposes.

Analysis of Genomic DNA Variations

In total, we identified 3,735,311 sequence differences from all three genome sequences of Dura, Pisifera, and Brazilian *E. oleifera* with an average of one sequence change per 197 bases (Table 1). Among these variations, 3,371,088 were SNPs and 364,233 were indels. The number of variations varied in different parts across oil palm chromosomes. The DNA variations were observed in different regions across the oil palm genomes. The majority of the genome variations were discovered outside protein coding regions (exons). These included the intergenic region (upstream region, downstream region, and intron) (Table 2). DNA variations located within the gene sequences (exon, intron, and untranslated regions) were 19.39% (Table 2, Fig. 1). Among the genic SNPs discovered, only 329,087 (8.25%) sequence changes were located in exons (Table 2).

TABLE 1. The number of DNA changes and the rate of DNA changes detected in each of the oil palm chromosomes after comparison three genome sequences of Dura, Pisifera, and Brazilian *Elaeis oleifera*.

Chromosome	Length	Changes	Change rate
EG5_Chr1	68,432,966	325,213	210
EG5_Chr2	65,556,141	347,672	188
EG5_Chr3	60,058,032	300,326	199
EG5_Chr4	57,248,047	291,200	196
EG5_Chr5	51,953,839	282,277	184
EG5_Chr6	44,354,769	200,964	220
EG5_Chr7	43,453,266	211,879	205
EG5_Chr8	40,192,799	198,996	201
EG5_Chr9	38,054,796	182,000	209
EG5_Chr10	31,889,635	147,495	216
EG5_Chr11	30,067,610	157,672	190
EG5_Chr12	28,799,275	169,641	169
EG5_Chr13	27,816,170	147,132	189
EG5_Chr14	24,378,543	139,278	175
EG5_Chr15	24,313,565	125,175	194
EG5_Chr16	21,370,583	108,391	197
Total/average	657,940,036	3,735,311	197

TABLE 2. Number of DNA variations observed across chromosomal regions.

Chromosome region	Number of DNA variations observed	%
Upstream	584,063	13.06
Downstream	551,772	12.34
Intergenic	2,782,751	54.31
Intron	492,316	11.01
Exon	329,087	8.25
Not identified*	4,234	0.11
Splice site acceptor	84	0.01
Splice site donor	85	0.01

*The location of DNA variations in the chromosome cannot be determined.

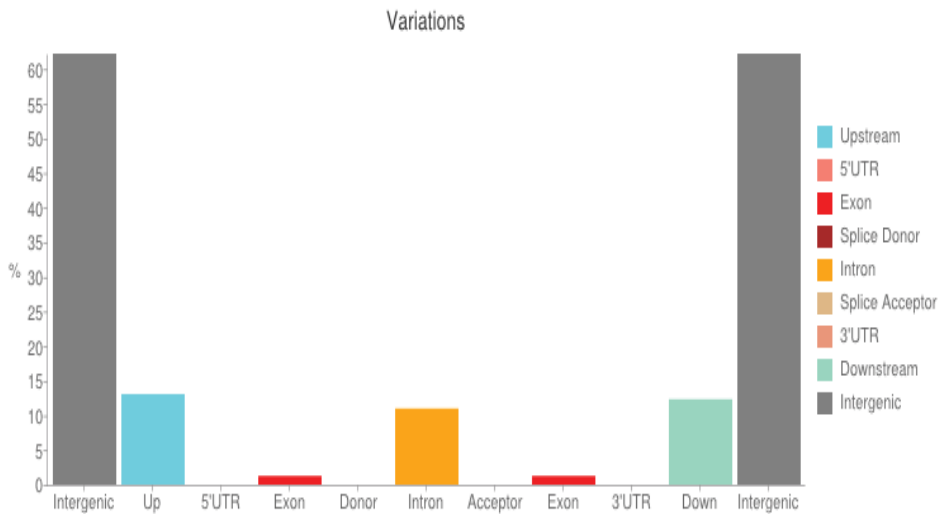


FIGURE 1. Distribution of genomic variation observed across the oil palm chromosomes based on next-gen re-sequence data of three Indonesian oil palm genotypes.

Observation of Exon-Based SNP Variations

To assist future research in fine mapping and gene identification using quantitative trait loci (QTL) mapping and association studies, sequence variations in exon regions were further characterized (Table 3). For breeding and gene discovery purposes, characterization of DNA variations located within the genic regions should be of utmost interest.

Out of 832,783 genic SNPs identified from this study, 322,788 SNPs were located within the exon (Table 3). Of those, 108,720 SNPs were synonymous (i.e. do not change amino acid composition of the encoded proteins) and thus did not affect gene function. The other 214,068 SNPs were nonsynonymous (i.e. cause the changes of amino acid composition of the proteins encoded by the genes containing the SNPs). This later type of SNPs certainly affects the protein composition, and might, therefore, affect the gene function. The majority of the nonsynonymous SNPs (188,949) were missense mutations (mutations that change amino acid) and the rest caused either change in stop and start codons or splice sites (Table 3) during RNA processing. These latest SNP types also significantly affect gene function through the loss of functions of the genes containing such types of SNPs.

A lower number of genic indels were also observed. Among 219,126 identified, only 6,299 indels were located within the exons. The remaining majority was located within introns that were spliced out during RNA processing. The majority of the exon indels caused protein frameshift that might cause those proteins not to function properly. Table 3 also categorizes the non-silent mutations according to their effect on mRNA/protein composition and lists the number of mutations of each type. This information is important for further analysis of such mutations for the ones who are interested in functional genomics of such gene mutations.

TABLE 3. Number and types of DNA sequence variations detected based the sequence data of the three Indonesian oil palm genotypes.

Types of DNA variation	Number of DNA variations observed
Number of total variants	
SNP	3,371,088
Indel	364,233
Genic SNPs	
In introns	509,995
In exon	322,788
Synonymous	108,720
Nonsynonymous	214,068
Missense	108,499
Start codons gained	8,133
Start codons lost	616
Stop codons gained	10,078
Stop codons lost	1,355
Splice site acceptor	2,536
Splice site donor	2,401
Genic indels	
In introns	212,827
In exon	6,299
Frameshift	5,359
Codon change plus codon deletion	173
Codon change plus codon insertion	304
Codon deletion	260
Codon insertion	203

Genome Database of the Oil Palm Genomic Variation

A genomic database termed as The Indonesian Center for Agricultural Genomics (ICAG) (<http://genome.pertanian.go.id>) was constructed to house the genomic data of eight Indonesian important crops including oil palm and an animal species (cow) [25, 26]. The database contents have been regularly updated to incorporate new developed data into the database. These include the incorporation of 96 SNP markers into the database resulted from an IAARD oil palm SNP marker development project [26]. The genomic data may be accessible to the scientific community interested in genetic data such as that for DNA marker development, gene discovery, as well as for searching other types of DNA variations that might be useful resources for genetic studies as well as for supporting future oil palm cultivar development.

CONCLUSION

A total of three Indonesian oil palm genotypes (Dura, Pisifera, and *E. oleifera* from Brazil) were sequenced their whole genomes using NGS HiSeq. The average sequence coverage depth was 34 and more than 95% of the genome was sequenced at least ten times. Comparison of the sequences to that of the *Eg5* oil palm reference genome sequence revealed 3,735,311 sequence variations with an average of one sequence change per 197 bases. A total of 3,371,088 SNPs and 364,233 indels were discovered from this study. Among the SNPs discovered a total of 329,087 were variations within the exon and 214,068 SNPs of the DNA variations were capable of causing mutations that would modify the amino acid composition of the encoded proteins. A genomic database was constructed to manage DNA variations resulted from this study. The SNPs and indels collected from this study should be useful for future oil palm breeding program.

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