

## 学 位 論 文 要 旨

Study on Genetic Variation and Physiological Disorder in Mangosteen (*Garcinia mangostana* L.)マンゴスチン (*Garcinia mangostana* L.)における遺伝的変異と生理障害に関する研究

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Mangosteen (*Garcinia mangostana* L.) is an important tropical fruit in Indonesia and has high economic value. However, research studies for genetics are still limited as well as a lack of genomic and transcriptomic data in mangosteen. The mangosteen is an apomictic fruit, and thereby the progeny is same genetic background. Therefore, plant improvement program is difficult to develop new variety for increasing fruit quality as well as health properties such as Xanthones as a powerful antioxidant. Besides, poor quality of fruits with a presence of two major physiological disorders are termed translucent flesh disorder (TFD) and gamboge disorder (GD) which is a serious problem for mangosteen marketing. In this thesis, we developed new microsatellite markers newly to analyze the genetic variation and relationship among mangosteen populations as well as carried out field experiment and transcriptome analysis for the physiological disorder.

The genetic variation and relationship of Mangosteen populations in Java were observed using twenty microsatellite loci from improved protocol, termed Selective Repeats from AFLP sequence by using a hybridized membrane. The microsatellite loci were evaluated using 78 genotypes from five populations including Leuwiliang, Wanayasa, Puspahiang, Kaligesing, and Trenggalek as well as successfully amplified four closely related *Garcinia* species, including *G. malaccensis*, *G. hombroniana*, *G. celebica*, and *G. porrecta*. Eight loci were monomorphic, and the other were polymorphic. Sixty-nine alleles were found, with 3.49 per locus on an average as well as genetic diversity (H) was calculated with average loci within populations ( $H_S$ ) as 0.39, average loci across many populations ( $H_T$ ) as 0.44, and genetic differentiation ( $F_{ST}$ ) as 0.14. Based on microsatellite loci, we clarified that mangosteen seems unlikely to be an apomictic plant with little genetic variation. In fact, mangosteen exhibits moderate genetic

variation within a population. The genetic variation in mangosteen could be arising from natural hybridization with multiple ancestors where genome asynchrony occurred. We proposed that rare interspecific hybridization may occur in a natural population of wild *Garcinia* species or those.

Comparative field experiment and transcriptome analysis of physiological disorders were investigated. The incidence of the physiological disorder in response to water availability was observed. In the present of the field experiment, the impact of physiological disorder in response to water availability was different among the treatments. The fruit characteristics and physiological disorder symptoms were observed. Fruit characteristic such as fruit weight and longitudinal diameter were different among the treatments as well as the percentage of TFD, and GD symptoms increased compared to control. The result of field experiment presents maintaining water supply is an important practical application to prevent incidences of physiological disorders in mangosteen during fruit development.

Transcriptome analysis was carried out using Ion Proton™ systems. The mRNA sequencing of thirty-six RNA populations produced a total of 520 million clean reads (after removal of adaptors, low-quality reads, and filtering of ribosomal RNA) from 760 million raw reads. The length and average of read ranged 35 – 230 bp and 100 bp, respectively. Pooled-clean reads were assembled de novo using Trinity program and produced a total of 211,005 transcripts with having an average, and N50 lengths were 649.89 bp and 722 bp, respectively. Further functional annotations of transcripts were searched against the public database such as NCBI and UniProtKB as well as Gene Ontology and Kyoto Encyclopedia Genes and Genomes (KEGG) also searched.

The downstream analysis of TFD and GD were performed using R package, edgeR program. Furthermore, the differentially expressed genes (DEGs) of TFD and GD were characterized, respectively and made a pairwise comparison, termed DCvsNC for disorder-affected fruits with normal fruit under control condition and DTvsNT for disorder-affected fruits with normal fruit for under treatment condition, respectively. Based on DEGs of TFD, a total of 5,120 (DCvsNC) and 1,694 (DTvsNT) transcripts were identified from each pairwise comparison with FDR threshold cut-off of 0.001. Whereas, DEGs of GD showed a total of 24 (DCvsNC) and 261 (DTvsNT) transcripts from each pairwise comparison with FDR threshold cut-off of 0.05. Overall, functional annotations of DEGs identified the most significant of gene ontology categories refers to “response to a stimulus, GO:0050896” for biological process, “organelle, GO:0043226” for the cellular compartment, “binding, GO:0005488” for molecular function, respectively. Moreover, KEGG enrichment analysis revealed some pathways such as carbohydrate metabolism, lipid metabolism, and plant hormone signaling. The combined results indicated the candidate genes associated with the ripening process including cell wall degradation/modification (expansin, pectinesterase, polygalacturonase) and sugar metabolisms ( $\beta$ -fructofuranosidase-insoluble isoenzyme, sucrose synthase, cellulose synthase) as well as response to water stress (NAC transcription factor and Pumilio homolog). These candidate genes represent correlation highly on the incidence of physiological disorder and will be useful for future validation studies.

Overall, the result of the present study provides a valuable resource for genetic and molecular studies as well as encourages further research in plant improvement program in mangosteen.