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学位論文内容の要旨

博士の専攻分野の名称：　博　士（農学）　　　氏名　BALIMPONYA, Elias George

学位論文題名

Development of genetic management methods for rice varieties and prediction of spontaneous mutation frequencies using next-generation sequencing technology

（次世代シークエンシング技術を用いたイネ品種の遺伝的管理法の開発と自然突然変異の発生頻度の予測）

Plant breeding plays a vital role in advancing agriculture by improving existing crop varieties and developing new ones. The integrity of breeder seeds, particularly during the breeder seed stage, is crucial for ensuring the quality of subsequent seed grades. Next-Generation Sequencing (NGS) and Whole-Genome Sequencing (WGS) technologies have been instrumental in safeguarding breeder seeds against pre-existing mutations. In this context, the identification and removal of specific mutations in rice have been achieved using advanced genomic analysis techniques. This study focuses on the long-established Hinohikari variety, where a mutation responsible for albinism was identified and further explored to understand the impact of novel Heterozygous State of Mutations (HSMs) on the rice genome and phenotypes.

In Chapter 2, a detailed study on how to remove the spontaneous mutations from the population to maintain seed quality using albinism in rice as a case study is conducted. I propose the NGS-WGS approach of removing a deleterious allele within a population in a single season of one year by extracting the genomic differences of the whole genome sequenced pool of spontaneous albino mutants' plants' gDNA with the whole genome sequenced pool of a wild-type green plants' gDNA. In addition, sanger sequencing and mendelian inheritance pattern examination was done to verify whether the albinism was controlled by a single gene or many genes and whether its inheritance was in mendelian nature. In chapter 3, I look in detail on the rate of spontaneous HSM occurring per rice genome. Among of the procedures, I use three ways approaches to reduce false positive calling and these include use of two same copies of gDNA sequenced independently. I use same gDNA to determine the best mapping tool and variant calling combination and then use that combination to identify HSMs. I draw the conclusion on the rate of HSM in rice genome, the genomic effects on protein synthesis and locate the novel HSM in three different rice cultivars that were tested. I use simplified approaches for estimating the mutation rate per genome.

SWL1 gene was responsible for rice albinism in the "Hinohikari" variety. The albinism

occurrence was stable under the tested population due to a simple Mendelian inheritance mode. The mutation has been observed all over the population to a 1.36% frequency and so as seeds proliferate, the mutation proliferates as well. The *swl1-R332P* allele arose albinism as a recessive mutation and so became difficult to be removed from the population compared with dominant mutation. It took about one year to employ NGS analysis to find the target gene, and then the mutation allele was rapidly removed. I went through complementary procedures, and obtained an estimated heterozygous mutation rate of approximately 10^{-8} per genome in rice, a rate consistent with observations in other organisms. Importantly, this estimation could be accomplished within a single growing season, unlike previous studies that required multiple generations. Among the identified HSMs, only a limited number of them, specifically 9 out of 107 (8.41%), were found to be non-synonymous. As the total number of plant samples used were eight, then this makes an average of one non-synonymous HSM per plant. This finding offers warnings that the impact of these HSMs on the plant population is relatively higher as any single plant may be prone to non-synonymous HSM.

The utilization of advanced sequencing technologies and genomic analysis has enabled the identification and removal of specific mutations in rice, ensuring the integrity and quality of breeder seeds. Among the identified HSMs, certain proportion was non-synonymous, suggesting a considerable impact on the plant population. Their occurrence within genic regions, and their abundances compared to the normal distribution between genic and non-genic regions indicates a potential influence on the rice genome. If these mutations affect protein synthesis and render the gene non-functional, they could potentially impact the plant's phenotype. Thorough research on the beneficial mutations is necessary to advance crop breeding programs and capitalize on their potential for further improvement.