Development of thickened aleurone layer mutant lines in rice (Oryza sativa L.)

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Title : Development of thickened aleurone layer mutant lines in rice (*Oryza sativa* L.)
(イネにおける厚糊粉層突然変異系統の開発)

Category : Kou

Thesis Summary

Aleurone layers in rice grains store various nutritional components: dietary fiber, vitamins, phytochemicals, minerals, and lipids, compared to starchy endosperm. Recently, there has been increasing interest in thickening the aleurone layer to enhance rice quality and market value of brown rice and rice bran oil. The objective of this study is to develop new thickened aleurone layer mutant lines with higher lipid contents.

The introduction of mutation in crops has impressively increased recently in crop production improvement. Chemical mutated agent *N*-methyl-*N*-nitrosourea (MNU) is prevalently used. I developed a new method for visualizing aleurone layers and incorporated it in screening more than 2,200 MNU-mutated lines for aleurone layers. A mutant at M_2 generation whose aleurone layer increased at least 50% thickness compared to wild type (WT) Mizuhochikara was isolated. After isolation, the mutant was crossed with WT to produce a population for identifying causal single nucleotide polymorphisms (SNPs) using a MutMap, a rapid method in identifying candidate SNPs. Four candidate SNPs alternation were found on chromosomes 4 and 12. SNPs on chromosome 12 were at the intron region. Of three SNPs on chromosome 4, there were 2 SNPs at the intron region, whereas the remain at nucleotide position 34,272,675 corresponded to *Os04g0671800,* which encode Zinc finger, CCCH-TYPE domain-containing protein.

The M_2 candidate mutant was subjected to self-pollination and continuously grew up to the sixth generation (M_6 generation). M_6 and WT plants were grown at different transplanting times. M_6 plants showed the same as WT 's agronomical characteristics, whereas fertility, thousand-kernel weight, and grain yield of M_6 were lower than those of WT. However, aleurone thickness and aleurone area of M_6 grains were higher than those of WT grains at any transplanting time. The percentage of fat weight /grain weight of M_6 grains increased by 30% compared to WT grains. In fatty acid compositions, the portion of oleic over the total fatty acid was also increased in M_6 grains

In this study, the primary goal of selecting a mutant with thickened aleurone layers was accomplished. The mutant showed the adaptability the same as wild type Mizuhochikara, increasing in aleurone layer thickness, aleurone area, and improved fatty acid content. In addition, the discovery of the SNPs responsible for the increase of the aleurone layer can support further studies in isolating the genes relating to aleurone thickness.