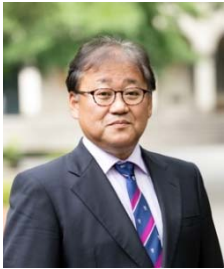


【Grant-in-Aid for Scientific Research (S)】

Broad Section F



Title of Project : Establishment of the basis for plant mitochondrial genome breeding

TSUTSUMI Nobuhiro

(The University of Tokyo, Graduate School of Agricultural and Life Sciences, Professor)

Research Project Number: 20H05680 Researcher Number : 00202185

Keyword : Plant mitochondria, Genome editing, Mitochondria breeding

【Purpose and Background of the Research】

Mitochondria have their own genome of which transformation was impossible and still difficult. We recently achieved the first example of the target gene disruption of plant mitochondrial genomes using artificial nuclease TALEN with mitochondria targeting signals (mitoTALENs, Figure 1). Plant mitochondrial genomes encode genes for energy-producing oxidative phosphorylation complexes, for their expression/translation, and agriculturally highly-used cytoplasmic male sterility (CMS). Therefore, mutations and modifications of plant mitochondrial genomes should have high breeding potential. This research project has three objects for establishing mitochondrial genome breeding, 1) basic research, molecular genetics of plant mitochondrial genomes using mitoTALENs, 2) improvements and assessments of mitoTALENs, and 3) molecular studies of different systems of cytoplasmic male sterility of three plants. We try to make the base for the future breeding of unassessed plant mitochondrial genomes during the project.

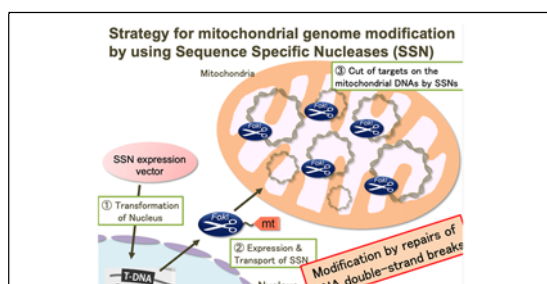


Figure 1 mitoTALEN

【Research Methods】

Mitochondrial genomes modified by mitoTALENs are good specifically-labeled genomes to trace them inside the cells, mitochondria, individuals during development, and to progenies as genetics. We use such special plants with mitochondrial-specifically tagged plants to dissect unknown transmission, replication, and maintenances of plant mitochondrial genomes.

mitoTALENs caused the targeted gene disruption with a large deletion including the target and novel connections of the sequence, resulting in new genomic structures. Such disturbance of the genomic structure might be suitable for novel mutagenesis of the genomes bringing new chimeric

genes. They would be useful for a new breeding system. We will assess the phenotypes of many mutants by mitoTALENs for seeing the potential for the important agricultural traits. Improvements of mitoTALENs and their effects on the mitochondrial genomes will also be analyzed. In addition to the model plants, agriculturally important materials with CMS will be analyzed to identify the responsible genes and dissect the molecular mechanisms.

【Expected Research Achievements and Scientific Significance】

Identifying genes for CMSs in three plants and unveiling the CMS's molecular mechanisms in each plant would have significant impacts on basic research and agricultural breeding. Assessments of mutant panels of plant mitochondrial genomes would be the first step essential information for the future of mitochondrial breeding.

Plant mitochondrial genomes are multi-copy in each cell, and heterogeneous state of them in each cell was still hard to be analyzed. Our new approach dissecting basic mechanisms for maintaining the mitochondrial genome in each mitochondrion, cell, organ, and individuals to progenies will give us further insight into basic science and applied breeding systems.

【Publications Relevant to the Project】

- **Kazama T**, Okuno M, Watari Y, Yanase S, Koizuka C, Tsuruta Y, Sugaya H, Toyoda A, Itoh T, **Tsutsumi N**, Toriyama K, Koizuka N and **Arimura S** (2019) Curing cytoplasmic male sterility via TALEN-mediated mitochondrial genome editing. *Nature Plants*, 5: 722-730.
- Method for editing plant mitochondrial genome. Arimura S, Kazama T, Katayama K, Hidaka T, Toriyama K, Tsutsumi N. JP patent-pending and US patent.

【Term of Project】 FY2020-2024

【Budget Allocation】 152,600 Thousand Yen

【Homepage Address and Other Contact Information】

<http://park.itc.u-tokyo.ac.jp/pmg/>