Accelerator Applications Research Division Beam Mutagenesis Group Plant Genome Evolution Research Team

# 1. Abstract

Established in May 2018 and succeed in October 2020, the plant genome evolution research team studies the effect of heavyion induced mutations on plant and microbial phenotypes. Chromosome rearrangements including translocations, inversions, and deletions are thought to play an important role in evolution and have a greater potential to achieve large phenotypic changes. However, this potential has not been fully investigated due to the lack of an effective method to induce and analyze complexed mutations. We employ the population genomics approach with robust molecular biology and bioinformatics techniques to characterize genomic mutations in model and non-model plant and microbial species, and create the future of mutation breeding.

#### 2. Major Research Subjects

- (1) Genomics-based approach to revolutionize the mutagenesis in model and non-model plants and microbes
- (2) De novo genome sequencing and assembly of cultivars and isolates, and its application to evolution and breeding studies

### 3. Summary of Research Activity

#### (1) Genomics-based approach to revolutionize the mutagenesis in model and non-model plants and microbes

Recent advances in genome sequencing and bioinformatics technology enabled us to obtain a genome-wide view of the induced mutations in unselected populations. We chose baker's yeast and a legume *Lotus japonicus* as model systems, irradiated different doses of carbon-ion beams to these organisms, and determined the dose-survival correlation. We isolated a semi-dwarf *L. japonicus* mutant, identified the responsible mutation by whole-genome sequencing, and started to characterize the phenotypic traits of this mutant. Phenotyping analysis indicated that the isolated semi-dwarf mutant shows significantly shortened internode length while still maintaining good seed production equivalent to the wild-type plants. The semi-dwarf *L. japonicus* mutant is suitable for high-density cultivation in the laboratory environment and may serve as an efficient model platform for legume-*Rhizobium* symbiosis.

# (2) De novo genome sequencing and assembly of new cultivars and isolates, and its application to evolution and breeding studies

We apply the latest *de novo* genome sequencing technology to elucidate genome sequences in different cultivars and isolates and successfully created some high-quality draft genome sequences. The determined and assembled draft genome sequences are subjected to gene predictions and functional annotations, then shared in a searchable data visualization and analysis system accessible through the internet. With these fundamental genome information resources, we and our collaborators work together to achieve new findings that will achieve major advances in both science and practical applications. These resources as well as the overall experimental techniques and the analysis pipeline are essential to further expand our genome-wide mutation and evolution approach to previously uncharacterized non-model species.

#### Members

Team Leader Hiroyuki ICHIDA

#### Postdoctoral Researcher Ni LEI

Visiting Scientist Yusuke KAZAMA (Fukui Prefectural Univ.)

**Student Trainee** Alvin SANJAYA (Sophia Univ.)

#### Administrative Part-time Workers

Yusaku NISHIMIYA (Administrative Part-time Worker II)

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# List of Publications & Presentations

#### **Publications**

# [Original Papers]

Y. Sato, T. Hirano, H. Ichida, N. Fukunishi, T. Abe, and S. Kawano, "Extending the cultivation period of *Undaria pinnatifida* by using regional strains with phenotypic differentiation along the Sanriku coast in northern Japan," Phycology **1**, 129–142 (2021).

K. Hashimoto, Y. Kazama, H. Ichida, T. Abe, and K. Murai, "Einkorn wheat (*Triticum monococcum*) mutant *extra-early flowering 4*, generated by heavy-ion beam irradiation, has a deletion of the *LIGHT-REGULATED WD1* homolog," Cytologia **86**, 297–302 (2021).

- R. Morita, H. Ichida, Y. Hayashi, K. Ishii, Y. Shirakawa, S. Usuda-Kogure, K. Ichinose, M. Hatashita, K. Takagi, K. Miura, M. Kusajima, H. Nakashita, T. Endo, Y. Tojo, Y. Okumoto, T. Sato, K. Toriyama, and T. Abe, "Responsible gene analysis of phenotypic mutants revealed the linear energy transfer (LET)-dependent mutation spectrum in rice," Cytologia 86, 303–309 (2021).
- H. Udagawa, H. Ichida, T. Takeuchi, T. Abe, and Y. Takakura, "Highly efficient and comprehensive identification of ethyl methanesulfonate-induced mutations in *Nicotiana tabacum* L. by whole-genome and whole-exome sequencing," Front. Plant Sci. 12, 671598 (2021).
- H. Murata, S. Nakano, T. Yamanaka, T. Shimokawa, T. Abe, H. Ichida, Y. Hayashi, and K. Tahara, "Argon-ion beam induced mutants of the ectomycorrhizal agaricomycete *Tricholoma matsutake* defective in β-1,4 endoglucanase activity promote the seedling growth of *Pinus densiflora* in vitro," Botany **99**, 139–149 (2021).
- R. Tabassum, T. Dosaka, R. Morita, H. Ichida, Y. Ding, T. Abe, and T. Katsube-Tanaka, "The conditional chalky grain mutant '*flo11-2*' of rice (*Oryza sativa* L.) is sensitive to high temperature and useful for studies on chalkiness," Plant Prod. Sci. 24, 230–243 (2021).
- K. Tsuneizumi, M. Yamada, H. J. Kim, H. Ichida, K. Ichinose, Y. Sakakura, K. Suga, A. Hagiwara, M. Kawata, T. Katayama, N. Tezuka, T. Kobayashi, M. Koiso, and T. Abe, "Application of heavy-ion-beam irradiation to breeding large rotifer," Biosci. Biotechnol. Biochem. 85, 703–713 (2021).

#### Presentations

#### [International Conference/Workshop]

T. Abe (invited), Y. Hayashi, R. Morita, Y. Shirakawa, and H. Ichida, "Ion-beam mutagenesis for creation of new varieties and discovery of genes", Bangabandhu International Conference on Sustainable Agriculture through Nuclear and Frontier Research, Online, January 2022.

#### [Domestic Conferences/Workshops]

- H. Ichida (invited), "Heavy-ion beam mutagenesis and its molecular characteristics in plants", RIKEN-OIST Joint Symposium, Series 1: Green and Blue Planet How Can Ecological Research Shape Our Future?, Okinawa (OIST), April 2021.
- 森田竜平, 市田裕之, 林依子, 石井公太郎, 白川侑希, 臼田祥子, 一瀬勝紀, 畑下昌範, 高城啓一, 三浦孝太郎, 草島美幸, 仲下英雄, 遠藤 貴司, 奥本裕, 佐藤雅志, 鳥山欽哉, 阿部知子, 「重イオンビームの LET がイネ変異体の原因遺伝子に与える影響」, 日本育種学会 第 140 回講演会, オンライン, 2021 年 9 月 24 日.
- 市田裕之, 宇田川久史, 竹内貴規, 阿部知子, 高倉由光, 「Whole-exome sequencing を用いたタバコ EMS 変異体における網羅的変異 検出法の開発」, 日本育種学会 第 140 回講演会, オンライン, 2021 年 9 月 25 日.
- 武田信哉, 市田裕之, 阿部知子, 有村慎一, 陳孫禄, 金岡義高, 貴島祐治, 烏山欽哉, 「台中 65 号の細胞質を持つ Oryza glaberrima の CMS 関連遺伝子の解析」, 日本育種学会 第 140 回講演会, オンライン, 2021 年 9 月 25 日.
- N. Lei, T. Abe, and H. Ichida, "Massive genome sequencing analysis of accelerated carbon-ion-induced mutations in *Saccharomyces cerevisiae*", RIKEN-OIST Joint Symposium, Series 2: Kinds of Minds—what is thinking?, Online, October 2021.