

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 heavy-ion 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) Study on the effect of chromosomal rearrangements on plant genomes and phenotypes
- (2) Genomics-based approach to revolutionize the mutagenesis in model and non-model plants and microbes

3. Summary of Research Activity

(1) Study on the effect of chromosomal rearrangements on plant genomes and phenotypes

We have established an efficient bioinformatics pipeline named AMAP, an automated mutation analysis pipeline, and have used it for genome-wide analysis of chromosome rearrangements and other mutations in various organisms. In *Arabidopsis*, we isolated a short-petiole mutant, Ar55-as1, from Ar-beam irradiated population at a dose of 50 Gy with an LET of 290 keV/ μm . We showed that this mutant has no typical base substitutions, deletions, and insertions that were linked to the mutant's phenotype, but has chromosomal rearrangements in the genome. Genetic linkage analysis showed that the short-petiole phenotype and the presence or absence of the inversion on chromosome 2 was completely linked in M3 generation, indicating that this inversion is responsible for the phenotype. The Ar55-as1 mutant will serve as a good model to investigate the effects on gene expressions by chromosomal rearrangements.

We previously isolated a high oil-production mutant of *Parachlorella kessleri*, a starch and oil-producing unicellular green algae, by C-beam irradiations. The effect on chromosomal rearrangements by Ar- and Fe-beams was quantitatively evaluated by cytological observations and high-throughput sequencing. As the result, Fe-beam irradiation at a dose of 75 Gy caused dramatic increase of fragmented chromosomes: 14 percent of the population had more than 30 chromosomes right after the irradiation and three percent of the population still remained more than 20 chromosomes after four consecutive cultures, although the wild-type *P. kessleri* cells have only seven A- and three B-chromosomes. We performed whole-genome sequencing in two mutants, and the results indicated that one mutant, Fe75-1-3H had chromosomal rearrangements with 10 junction regions, and the other, Ar75-1-2C, had a novel chromosomal terminus by the result from a translocation.

(2) 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. The semi-dwarf *L. japonicus* mutant is suitable for high-density cultivation in laboratory environment and may serve as an efficient model platform for legume-*Rhizobium* symbiosis.

Members

Team Leader

Tomoko ABE (~2020.09)

Hiroyuki ICHIDA (2020.10~)

Contract Researcher

Kotaro ISHII

Visiting Scientists

Ali FERJANI (Tokyo Gakugei Univ.)

Yusuke KAZAMA (Fukui Prefectural Univ.)

Part-time Worker

Yusaku NISHIMIYA (Administrative Part-time Worker II)

List of Publications & Presentations

Publications

[Original Papers]

- Y. Oono, H. Ichida, R. Morita, S. Nozawa, K. Satoh, A. Shimizu, T. Abe, H. Kato, and Y. Hase, “Genome sequencing of ion-beam-induced mutants facilitates detection of candidate genes responsible for phenotypes of mutants in rice,” *Mutat. Res. Fund. Mol. Mech. Mutagen.* **821**, 111691 (2020).
- R. Tabassum, T. Dosaka, H. Ichida, R. Morita, Y. Ding, T. Abe, and T. Katsube-Tanaka, “*FLOURY ENDOSPERM11-2* encodes plastid HSP70-2 involved with the temperature-dependent chalkiness of rice (*Oryza sativa* L.) grains,” *Plant J.* **103**, 604–616 (2020).
- 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 (2020). DOI:10.1139/cjb-2020-0076.

Presentations

[Domestic Conferences/Workshops]

- 市田裕之, 阿部知子, 「オープンソースな変異タイピング手法の開発とイネをモデルとした概念実証」, 日本育種学会 第 137 回講演会, 東京都文京区 (東京大学), 2020 年 3 月 28 日.
- 橋本佳澄, 西浦愛子, 上田純平, 風間裕介, 阿部知子, 市田裕之, 村井耕二, 「2 倍体ヒトツブコムギにおける重イオンビーム照射による超極早生変異体 *extra early-flowering 4* の原因遺伝子の同定」, 同上.
- 森田竜平, 市田裕之, 石井公太郎, 林依子, 安部弘, 白川侑希, 一瀬勝紀, 常泉和秀, 風間智彦, 鳥山欽哉, 佐藤雅志, 阿部知子, 「イネ長粒変異体 *lin1* の単離および原因遺伝子同定」, 同上.
- 石井公太郎, 大部澄江, 白川侑希, 阿部知子, 「全ゲノム変異解析による重イオンビームの高頻度な変異誘発線量区の推定」, 同上.
- Y. Ding (口頭発表), T. Katsube-Tanaka, H. Ichida, R. Morita, and T. Abe, “Studies on chalkiness of the rice cultivar ‘Kinmaze’ under heat stress,” 日本作物学会第 250 回講演会, オンライン開催, 2020 年 9 月 3–4 日.
- 蝶野真喜子 (ポスター発表), 藤田雅也, 神山紀子, 松中仁, 氷見英子, 市田裕之, 阿部知子, 川上直人, 「粒の赤みが弱い新規コムギ変異体の農業特性」, 日本育種学会 第 138 回講演会, オンライン開催, 2020 年 10 月 10–11 日.
- 藤田悠生 (ポスター発表), 市田裕之, 風間智彦, 阿部知子, 鳥山欽哉, 「インディカイネ品種 *Lebed* に由来する雄性不稔遺伝子とその抑制遺伝子の解析」, 同上.
- 石井公太郎 (口頭発表), 風間裕介, 浅野円花, 阿部知子, 河野重行, 「Ar・Fe イオンビーム照射によって生じるクロレラ染色体の断片化と染色体再編成」, 同上.
- 橋本佳澄 (ポスター発表), 西浦愛子, 風間裕介, 市田裕之, 阿部知子, 村井耕二, 「重イオンビーム照射によって作出された超極早生コムギ変異体 *extra early-flowering 4 (exe4)* の花成関連遺伝子の発現解析」, 第 15 回ムギ類研究会, オンライン開催, 2020 年 12 月 26 日.