

## Accelerator Applications Research Division

### Beam Mutagenesis Group

### Ion Beam Breeding Team

#### 1. Abstract

Ion beam breeding team studies various biological effects of fast heavy ions. It also develops new technique to breed plants and microbes by heavy-ion irradiations. Fast heavy ions can produce dense and localized ionizations in matters along their tracks, in contrast to photons (X rays and gamma rays) which produce randomly distributed isolated ionizations. These localized and dense ionization can cause double-strand breaks of DNA which are not easily repaired and result in mutation more effectively than singlestrand breaks. A unique feature of our experimental facility at the RIKEN Ring Cyclotron (RRC) is that we can irradiate living tissues in atmosphere since the delivered heavy-ion beams have energies high enough to penetrate deep in matter. This team utilizes a dedicated beam line (E5B) of the RRC to irradiate microbes, plants and animals with beams ranging from carbon to iron. Its research subjects cover study of ion-beam radiation mutagenesis, genome-wide analyses of mutation, and development of new plants and microbial varieties by heavy-ion irradiation. Thirty-five new varieties have already been brought to the market.

#### 2. Major Research Subjects

- (1) Study on the biological effects by heavy-ion irradiation
- (2) Study on the molecular nature of DNA alterations induced by heavy-ion irradiation
- (3) Innovative applications of heavy-ion beams

#### 3. Summary of Research Activity

We study biological effects of fast heavy ions from the RRC using  $^{135}\text{A}$  MeV C, N, Ne ions,  $^{95}\text{A}$  MeV Ar ions,  $^{90}\text{A}$  MeV Fe ions and from the IRC using  $^{160}\text{A}$  MeV Ar ions. We also develop breeding technology of microbes and plants. Main subjects are:

##### (1) Study on the biological effects by heavy-ion irradiation

Heavy-ion beam deposits a concentrated amount of dose at just before stop with severely changing the linear energy transfer (LET). The peak of LET is achieved at the stopping point and known at the Bragg peak (BP). Adjusting the BP to target malignant cells is well known to be effective for cancer therapy. On the other hand, a uniform dose distribution is a key to the systematic study for heavy-ion mutagenesis, thus to the improvement of the mutation efficiency. Plants and microbes therefore, are irradiated using ions with stable LET. We investigated the effect of LET ranging from 23 to  $640\text{ keV}/\mu\text{m}$ , on mutation induction using dry seeds of the model plants *Arabidopsis thaliana* and rice (*Oryza sativa* L.). The most effective LET (LETmax) was  $30\text{ keV}/\mu\text{m}$  in *Arabidopsis*. LETmax irradiations showed the same mutation rate as that by chemical mutagens, which typically cause high mutation rate. The LETmax was  $23\text{--}39\text{ keV}/\mu\text{m}$  in buckwheat,  $23\text{--}50\text{ keV}/\mu\text{m}$  in rice and  $50\text{--}70\text{ keV}/\mu\text{m}$  in wheat. By contrast, when LET was  $290\text{ keV}/\mu\text{m}$ , the mutation rate was low and the survival rate was greatly reduced in plants. In the case of microbe, filamentous fungus (*Neurospora crassa*), the Ar ions at  $290\text{ keV}/\mu\text{m}$  demonstrated higher mutagenic activity than the Fe-ions at  $640\text{ keV}/\mu\text{m}$ . Thus, the LET is an important factor to be considered in heavy-ion mutagenesis.

##### (2) Study on the molecular nature of DNA alterations induced by heavy-ion irradiation

A whole-genome analysis with high-throughput sequencing is a powerful tool used to characterize the nature of induced mutations. We have been using whole genome sequencing to analyze DNA mutations in *Arabidopsis* and rice genomes. C ions with LETmax mainly induced single nucleotide variants (SNVs) and small insertions and deletions (InDels), while the number of large deletions and chromosomal rearrangements was low. However,  $290\text{-keV}/\mu\text{m}$  Ar ions showed a different mutation spectrum: SNVs and number of small InDels was low, while the number of large deletions ( $\geq 100\text{ bp}$ ) and chromosomal rearrangements was high. Number of mutated gene induced by C-ion and Ar-ion irradiation is less than 10, relatively small, and often only 1 mutation is found near the mapped location. Thus, irradiation with these ions can efficiently generate knockout mutants of a target gene and can be applied to reverse genetics. Mutants of the causative gene of *Arabidopsis* induced by ion beam irradiation were compared at  $30\text{ keV}/\mu\text{m}$  and  $290\text{ keV}/\mu\text{m}$  with two typical LETs. The most mutations irradiated with C ion at  $30\text{ keV}/\mu\text{m}$  were small deletions ( $<100\text{ bp}$ ). Irradiation with  $290\text{-keV}/\mu\text{m}$  C-ion and Ar-ion resulted in the most large-deletions and chromosomal rearrangements, and decreased small deletions.

##### (3) Innovative application of heavy-ion beams

In 1999, we formed a consortium for ion-beam breeding consisting of 24 groups. In 2020, the consortium grew to 184 groups from Japan and 20 from overseas. Previously, the ion-beam breeding procedures were carried out using mainly flowers and ornamental plants. We have recently put a new non-pungent and tearless onion, 'Smile ball,' on the market along with 'Kiku Meigetsu,' an edible late flowering chrysanthemum. In addition, a new project was launched to expand the cultivation area of this variety of chrysanthemum in Yamagata prefecture. Beneficial variants have been grown for various plant species, such as high yield sea weeds, lipids hyperaccumulating unicellular alga, medicinal plant with high productivity of medicinal ingredient, peanuts without major allergens, oranges with delayed coloring and one-month late harvest, and lettuce with a low browning property as a cut vegetable. As a result of a collaborative study with the University of Hasanuddin, we have selected five useful aromatic rice mutants and increased the percentage of fertile grains from 32% control to over 52%. By broadening the target of heavy-ion breeding extending from flowers

to crops such as grains, the technology will contribute to solving the global problems of food shortage and environmental destruction.

## Members

### Team Leader

Tomoko ABE

### Research/Technical Scientists

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### Visiting Scientists

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Takuji YOSHIDA (Takii & Co., Ltd)

### Research Fellow

Hironari UCHIDA (Saitama Agricultural Tech. Res. Center)

## List of Publications & Presentations

### Publications

#### [Original Papers]

- T. Hirano, Y. Sato, K. Ichinose, M. Yamada, Y. Hayashi, N. Fukunishi, and T. Abe, "Mutant induction in gametophytes of *Undaria pinnatifida* (Phaeophyceae) by heavy ion beam irradiation," *Phycol. Res.* **68**, 63–69 (2019).
- N. Fukuda, N. Kitajim, Y. Terada, T. Abe, I. Nakai, and A. Hokura, "Visible cellular distribution of cadmium and zinc in the hyperaccumulator *Arabidopsis halleri* ssp. *gemmaifera* determined by 2-D X-ray fluorescence imaging using high-energy synchrotron radiation," *Metalomics* **12**, 193–203 (2019).
- 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.* (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).
- K. Kaya, Y. Kazama, T. Abe, and F. Shiraiishi, "Influence of medium components and pH on the production of odd-carbon fatty acids by *Aurantiochytrium* sp. SA-96," *J. Appl. Phycol.* **32**, 1597–1606 (2020).

- S. Muramatsu, K. Atsugi, K. Yamada, K. Ozasa, H. Suzuki, T. Takeuchi, Y. Hashimoto-Marukawa, Y. Kazama, T. Abe, K. Suzuki, and O. Iwata, "Isolation and characterization of a motility-defective mutant of *Euglena gracilis*," *Peer J* **8**, e10002 (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  $\beta$ -1,4-endoglucanase activity promote the seedling growth of *Pinus densiflora* in vitro," *Botany*, **99**(3), DOI:10.1139/cjb-2020-0076 (2020).

#### [Proceedings]

- A. M. Okasa, M. Riadi, K. Toriyama, K. Ishii, Y. Hayashi, T. Sato, T. Abe, Trisnawaty, N. J. Panga, and R. Sjahril, "Mutation breeding for improvement of aromatic rice mutant by using ion beam irradiation," *IOP Conf. Ser. Earth Environ. Sci.* **486**, 012091 (2020).

### Presentations

#### [International Conferences/Workshops]

- T. Kashiwabara, N. Kitajima, R. Onuma, N. Fukuda, S. Endo, Y. Terada, T. Abe, A. Hokura, and I. Nakai, "Synchrotron micro-X-ray fluorescence imaging of arsenic in frozen-hydrated sections of a root of *Pteris vittata*," *Goldschmidt2020: Global Virtual Conference*, online, June 21–26, (2020).
- T. Abe (invited), "Development for ion-beam breeding technology over the last two decades in Japan," *Virtual Meeting on FAO/IAEA Mutation Breeding Network (MBN) for Asia Pacific Region*, online, November 12–13, (2020).

#### [Domestic Conferences/Workshops]

- 常泉和秀, 山田美恵子, 一瀬勝紀, 市田裕之, 金禧珍, 萩原篤志, 川田実季, 片山貴士, 崎山一孝, 手塚信弘, 小磯雅彦, 阿部知子, 「シオミズツボワムシの高増殖・大型変異系統の樹立」, 日本農芸化学会 2020 年度大会, 福岡県福岡市 (九州大学), 2020 年 3 月 26 日.
- 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.
- 渡邊遙, 大部澄江, 阿部知子, 風間裕介, 「ゲノム編集によるシロイヌナズナへの 758 kb の逆位の導入」, 同上.
- A. M. Okasa, M. Riadi, 佐藤雅志, 鳥山欽哉, 石井公太郎, 林依子, 阿部知子, R. Sjahril, "An approach toward isolating early-heading mutants from Tana Toraja local aromatic rice 'Pare Bau' irradiated with heavy ion-beam," 同上.
- 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 倍体ヒトツボ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.
- 村井耕二, 風間裕介, 阿部知子, 「2 倍体ヒトツボコムギにおける重イオンビーム突然変異体作出のための最適処理条件」, 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.
- 南壮次郎 (口頭発表), 渡邊遙, 大部澄江, 阿部知子, 風間裕介, 「ゲノム編集を用いたシロイヌナズナへの染色体再編成の導入」, 同上.
- 野村文希 (ポスター発表), 風間裕介, 阿部知子, 村井耕二, 「イオンビーム照射により作出された Ppd-1 欠失『農林 61 号』変異系統の解析」, 第 15 回ムギ類研究会, オンライン開催, 2020 年 12 月 26 日.
- 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」, 同上.

### Awards

- 佐藤陽一, 斎藤大輔, 最上谷美穂, 令和 2 年度 (第 21 回) 民間部門農林水産研究開発功績者表彰 (農林水産技術会議会長賞民間部門), 「わかめ養殖種苗の優良系統開発と生産条件最適化による普及実用化」, 農林水産会議, 2020 年 11 月 12 日.

### Patents

- 石井重久, 阿部知子, 林依子, サクラ「真和」, 品種登録出願番号 34356, 出願日 2019 年 11 月 27 日.
- 石井重久, 阿部知子, 林依子, サクラ「真理」, 品種登録出願番号 34356, 出願日 2019 年 11 月 27 日.
- 石井重久, 阿部知子, 林依子, サクラ「真星」, 品種登録出願番号 34356, 出願日 2020 年 4 月 14 日.

### Outreach Activities

- We established the "Asagao (morning glory) club" to deepen the understanding of our technology of mutation breeding. The club distributes the morning glory seeds irradiated with C-ion on request, and collects and compiles the observation reports of their growth.