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 single-strand 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-nine 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 irradiation

3. Summary of Research Activity

We study biological effects of fast heavy ions from the RRC using 135 MeV/nucleon C, N, Ne ions, 95 MeV/nucleon Ar ions, 90 MeV/nucleon Fe ions and from the IRC using 160 MeV/nucleon 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 keV/μm, on mutation induction using dry seeds of the model plants Arabidopsis thaliana and rice (*Oryza sativa* L.). The most effective LET (LETmax) was 30 keV/μm in *Arabidopsis*. LETmax irradiations showed the same mutation rate as that by chemical mutagens, which typically cause high mutation rate. The LETmax was 23–39 keV/μm in buckwheat, 23–50 keV/m in rice and 50–70 keV/μm in wheat. By contrast, when LET was 290 keV/μm, 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 keV/μm demonstrated higher mutagenic activity than the Fe-ions at 640 keV/μm. 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 in *Arabidopsis*. However, 290 keV/μm Ar ions showed a different mutation spectrum: SNVs and number of small InDels was low, while the number of large deletions ($\geqq$100 bp) and chromosomal rearrangements was high. Number of mutated gene induced by C-ion and Ar-ion irradiation is less than 10, relatively small, and often only 1 mutation is found near the mapped location. Thus, irradiation with these ions can efficiently generate knockout mutants of a target gene and can be applied to reverse genetics. Rice mutants of the causative gene induced by ion-beam irradiation were compared at 23–50 keV/μm and 290 keV/μm with typical LETs. The most mutations irradiated with C ion at 23–50 keV/μm were small deletions ($<$ 100 bp). Irradiation with 290 keV/μm Ar-ion resulted in the highest number of large deletions and decreased small deletions. In rice, as in *Arabidopsis*, the LETmax with high mutagenic effects was dominated by SNVs and small deletion mutations, while large deletions and chromosomal rearrangement mutations dominated LETs with high lethal effects.

(3) Innovative application of heavy-ion irradiation

In 1999, we formed a consortium for ion-beam breeding consisting of 24 groups. In 2020, the consortium grew to 183 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 Shizuoka prefecture, we have created a new variety of 'Haru Shizuka,' which is late coloring. The harvest time of the Satsuma mandarin has tended to come earlier due to global warming, 'Haru Shizuka' is harvested about a month later than the original variety, but the fruits are sour when harvested. 'Haru Shizuka' is suitable for long-term storage and the fruits turn into good quality sweet oranges during storage till April. By broadening the target of heavy-ion breeding extending from flowers to crops, the technology will contribute to solving the global problems of food shortage and environmental destruction.

Members

Team Leader

Tomoko ABE

Research/Technical Scientists

Tokihiro IKEDA (Senior Research Scientist)
Masako IZUMI (Senior Research Scientist)
Ryouhei MORITA (Technical Scientist)
Teruyo TSUKADA (Senior Research Scientist)
Kazuhide TSUNEIZUMI (Senior Research Scientist)

Technical Staffs

Yoriko HAYASHI
Yuki SHIRAKAWA
Sumie OHBU
Mieko YAMADA

Special Temporary Technical Scientists

Hiroshi ABE
Katsunori ICHINOSE

Research Consultant

Masahiro MII (Chiba Univ.)

Visiting Scientists

Ayumi DEGUCHI (Chiba Univ.)
Makoto FUJIWARA (Sophia Univ.)
Eitaro FUKATSU (Forestry and Forest Products Res. Inst.)
Tomonari HIRANO (Univ. of Miyazaki)
Akiko HOKURA (Tokyo Denki Univ.)
Kotaro ISHII (QST)
Yutaka MIYAZAWA (Yamagata Univ.)
Kazumitsu MIYOSHI (Chiba Univ.)
Koji MURAI (Fukui Prefectural Univ.)
Kyosuke NIWA (Tokyo Univ. of Marine Sci. and Tech.)
Tadashi SATO (Tohoku Univ.)
Yoichi SATO (Riken Food Co., Ltd.)
Kenichi SUZUKI (Suntory Flowers Co., Ltd.)
Hinako TAKEHISA (Nat'l Agriculture and Food Res. Org.)
Masao WATANABE (Tohoku Univ.)

Visiting Technicians

Yukiko KANAZAWA (Nippon Beet Sugar Manufacturing Co., Ltd.)
Norihide KISHINO (Sanwa Norin Co. Ltd.)
Miho MOGAMIYA (Riken Food Co., Ltd.)
Daisuke SAITO (Riken Food Co., Ltd.)

Research Fellow

Hironari UCHIDA (Saitama Agricultural Tech. Res. Center)

Student Trainee

Yuzo MARUYAMA (Tokyo Denki Univ.)

List of Publications & Presentations

Publications

[Original Papers]

R. Tabassum, T. Dosaka, 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 (2020).

S. Muramatsu, K. Atsuji, K. Yamada, K. Ozawa, 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*," PeerJ **8**, e10002 (2020).

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).

H. Tojo, A. Nakamura, A. Ferjani, Y. Kazama, T. Abe, and H. Iida, "A method enabling comprehensive isolation of arabidopsis mutants exhibiting unusual root mechanical behavior," Front. Plant Sci. **12**, 646404 (2021).

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 vittate*," Metallomics **13**, mfab009 (2021).

A. Sanjaya, Y. Kazama, K. Ishii, R. Muramatsu, K. Kanamaru, S. Ohbu, T. Abe, and M. T. Fujiwara, "An argon-ion-induced pale green mutant of arabidopsis exhibiting rapid disassembly of mesophyll chloroplast grana," Plants **10**, 848 (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).

Y. Sato, H. Nagae, G. N. Nishihara, and R. Terada, "The photosynthetic response of cultivated juvenile and mature *Undaria pinnatifida* (Laminariales) sporophytes to light," J. Appl. Phycol. **33**, 3437–3448 (2021).

A. Sanjaya, R. Muramatsu, S. Sato, M. Suzuki, S. Sasaki, H. Ishikawa, Y. Fujii, M. Asano, R. D. Itoh, K. Kanamaru, S. Ohbu, T. Abe, Y. Kazama, and M. T. Fujiwara, "Arabidopsis EGY1 is critical for chloroplast development in leaf epidermal guard cells," Plants **10**, 1254 (2021).

R. D. Itoh, K. P. Nakajima, S. Sasaki, H. Ishikawa, Y. Kazama, T. Abe, and M. T. Fujiwara, "TGD5 is required for normal morphogenesis of non-mesophyll plastids, but not mesophyll chloroplasts, in arabidopsis," Plant J. **107**, 237–255 (2021).

A. M. Okasa, R. Sjahril, M. Riadi, M. Mahendradatta, T. Sato, K. Toriyama, K. Ishii, Y. Hayashi, and T. Abe, "Evaluation of Toraja (Indonesia) local aromatic rice mutant developed using heavy-ion beam irradiation," Biodiversitas **22**, 3474–3481 (2021).

V. Q. Nhat, Y. Kazama, K. Ishii, S. Ohbu, H. Kunitake, T. Abe, and T. Hirano, "Double mutant analysis with the large flower mutant, ohbana1, to explore the regulatory network controlling the flower and seed sizes in *Arabidopsis thaliana*," Plants **10**, 1881 (2021).

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. Ishii, S. Kawano, and T. Abe, "Creation of green innovation and functional gene analyses using heavy-ion beam breeding," Cytologia (Tokyo) **84**, 273–274 (2021).

T. Takesita, K. Takita, K. Ishii, Y. Kazama, T. Abe, and S. Kawano, "Robust mutants isolated through heavy-ion beam irradiation and endurance screening in the green *Alga Haematococcus pluvialis*," Cytologia (Tokyo) **84**, 283–289 (2021).

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K. Hashimoto, Y. Kazama, H. Ichida, T. Abe, and K. Murai, "Eincorn wheat (*Triticum monococcum*) mutant *extra-early flowering 4*, generated by heavy-ion beam irradiation, has a deletion of the *LIGHT-REGULATED WD1* homolog," Cytologia (Tokyo) **84**, 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 (Tokyo) **84**, 303–309 (2021).

T. Hirano, Y. Matsuyama, A. Hanada, Y. Hayashi, T. Abe, and H. Kunitake, "DNA damage response of *Cyrtanthus mackenii* male gametes following argon ion beam irradiation," Cytologia (Tokyo) **84**, 311–315 (2021).

A. Matsuta, T. Mayuzumi, H. Katano, M. Hatashita, K. Takagi, Y. Hayashi, T. Abe, K. Murai, and Y. Kazama, "The effect of heavy-ion beams with high linear energy transfer on mutant production in M_1 generation of *Torenia Fournieri*," Cytologia (Tokyo) **84**, 317–322 (2021).

T. Kobayashi, M. Takahashi, R. Nishijima, R. Sugiyama, K. Ishii, S. Kawano, and Y. Kazama, "Effective chromosomal preparation protocol for the dioecious plant *Silene Latifolia*," Cytologia (Tokyo) **84**, 323–328 (2021).

W. Aonuma, H. Kawamoto, Y. Kazama, K. Ishii, T. Abe, and S. Kawano, "Male/female trade-Off in hermaphroditic Y-chromosome deletion mutants of the dioecious plant *Silene Latifolia*," Cytologia (Tokyo) **84**, 329–338 (2021).

[Review Articles]

常泉和秀, 阿部知子, 「重イオンビームで魚養殖餌料に適した大型ワムシを創る」, バイオサイエンスとインダストリー **79**, 284–287 (2021).

T. Abe, "Ion beam mutagenesis for crop improvement," Plant Breeding & Genetics Newsletter **47**, 17–19 (2021).

K. Murai and T. Abe, "Development of early-flowering durum wheat suitable for the climate conditions of the Hokuriku region of Japan through conventional breeding aided by heavy ion beam mutagenesis," Plant Breeding & Genetics Newsletter **47**, 20–22 (2021).

[Book]

T. Abe, H. Ichida, Y. Hayashi, R. Morita, Y. Shirakawa, K. Ishii, T. Sato, H. Saito, and Y. Okumoto, "Ion beam mutagenesis—An innovative and effective method for plant breeding and gene discovery," in S. Sivasankar, N. Ellis, L. Jankuloski, and I. Ngelbrecht (eds.), Mutation Breeding, Genetic Diversity and Crop Adaptation to Climate Change, The Joint FAO/IAEA Programme, CABI, Oxfordshire UK, pp. 411–423 (2021).

Presentations

[International Conferences/Workshops]

T. Abe (invited), "New varieties developed in various crops using ion-beam irradiation in Japan," The Contribution of Innovative Nuclear Technology to Sustainable Agriculture, VCDNP (Vienna Center for Disarmament and Non-proliferation), Online, April 1, 2021.

T. Abe (invited), "New mutagen-ion beam-development for ion-beam breeding technology over the last two decades in Japan," Consultants meeting on radiation-induced crop genetic diversity and functional genomics to accelerate variety development for tolerance to climate extremes, Joint FAO/IAEA Center of Nuclear Techniques in Food and Agriculture, Online, August 2–6, 2021.

J. G. Pablo, M. Lindley, A. Isozaki, K. Hiramatsu, W. Peterson, K. Ishii, T. Abe, and K. Goda, "High-throughput Raman flow cytometry

for directed evolution," The Great Scientific Exchange 2021, Providence RI, USA, September 26–October 1, 2021.

H. Park, Y. Narasako, T. Abe, H. Kunitake, and T. Hirano, "Construction of mutant library by heavy-ion beam irradiation: toward efficient sweet potato breeding," JKTC Extra Edition 2021 International Student Online Seminar, "The future of plant, environment, and human with/after COVID-19," Online, November 22, 2021.

[Domestic Conferences/Workshops]

T. Abe (口頭発表), 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, and M. Koiso, "Application of heavy-ion-beam irradiation to breeding large rotifer," 第一回沖縄科学技術大学院大学—理研連携シンポジウム, 沖縄県国頭郡恩納村 (沖縄科学技術大学院大学), 2021 年 4 月 6–7 日.

T. Abe (口頭発表) and H. Ichida, "Heavy-ion beam mutagenesis and its molecular characteristics in plants," ibid.

T. Abe (ポスター発表), R. Morita, H. Ichida, Y. Hayashi, Y. Shirakawa, and K. Ichinose, "Heavy ion mutagenesis in rice (*Oryza sativa* L.)," ibid.

常泉和秀 (口頭発表), 「重イオンビーム照射による有用系統の作出」, 理化学研究所—水産研究・教育機構連携ワークショップ, オンライン, 2021 年 4 月 27 日.

阿部知子（招待講演), 「イオンビーム育種技術による特産品の創成」, 先端技術セミナー兼福井イオンビーム育種研究会高エネルギービーム利活用に向けた展望〜イオンビームを用いた育種と社会実装に向けた研究〜, オンライン, 2021 年 9 月 15 日.

鳥山欽哉 (口頭発表), 武田信哉, 市田裕之, 阿部知子, 有村慎一, 風間智彦, 陳孫禄, 金岡義高, 貴島祐治, 「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 23–25 日.

阿部知子 (口頭発表), 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, 「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」, 同上.

高倉由光 (口頭発表), 市田裕之, 宇田川久史, 竹内貴規, 阿部知子, 「Whole-exome sequencing を用いたタバコの EMS 変異体における網羅的変異検出法の開発」, 同上.

風間裕介 (ポスター発表), 黛隆宏, 松田彩花, 畑下昌範, 高城啓一, 阿部知子, 村井耕二, 「重イオンビームを用いたトレニア変異系統の作出」, 同上.

村井耕二 (ポスター発表), 橋本佳澄, 風間裕介, 阿部知子, 「CO-like 遺伝子の日周期発現パターンシフトが超極早生コムギ変異体 extra early-flowering 4 (EXE4) における早生性」, 同上.

阿部知子 (ポスター発表), 田中朋之, 小林麻子, 「地球温暖化とお米の未来/福井のいろいろなお米」, ふるさと環境フェア 2021, 福井県福井市 (福井県産業会館), 2021 年 11 月 23 日.

阿部知子 (口頭発表), 橋口太亮, 飯山光太郎, 長井純一, 藤川和博, 「サツマイモ『シロユタカ』と外観識別可能な『こなみずき』欠刻葉系統の作出」, 第 84 回（令和 3 年度）九州農業研究発表会, 作物部会, オンライン, 2021 年 12 月 7–8 日.

村井耕二 (ポスター発表), 橋本佳澄, 風間裕介, 阿部知子, 「WD リピートタンパク質遺伝子（WWDR1）を欠失した変異体 exe4 はどうしてこんなに早生なのか？」, 第 16 回ムギ類研究会, オンライン, 2021 年 12 月 25 日.

Award

T. Abe, Women in plant mutation breeding award, Joint FAO/IAEA Center, September 20, 2021.

Press Release

「赤ちゃんマグロの餌, ワムシの大型化に成功—重イオンビームで 『メガワムシ』が誕生—」, 理化学研究所, 水産研究・教育機構, 長崎大学, 2021 年 1 月 15 日.

Patents

久村麻子, 諸岡淳司, 阿部知子, 林依子, 平野智也, 石井公太郎, キク 「長崎 SYC1」, 品種登録出願番号 35321, 出願日 2021 年 3 月 26 日.

中村茂和, 中嶌輝子, 平井実季, 小林康志, 加々美裕, 澤野郁夫, 稲葉元良, 小野章子, 寺岡毅, 馬場明子, 荒木勇二, 青島加奈子, 吉川公規, 土田友香, 阿部知子, 福西暢尚, 龍頭啓充, ウンシュウミカン 「春しずか」, 品種登録出願番号 35533, 出願日 2021 年 6 月 28 日.

Outreach Activity

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.