Ion beam breeding – An efficient synergic link between agricultural science and accelerator physics –

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We have developed a unique technology for mutation induction using energetic heavy-ion beams at the RI Beam Factory. This development was achieved through an efficient synergic link between agricultural science and accelerator physics. At relatively low doses, ion beams induce mutations at a high rate without severely inhibiting growth. The irradiation treatment can be given to various plant materials and quick, lasting between seconds and a few minutes are enough to induce mutation. The ion beam is thus a highly efficient tool for improving crops through mutation breeding. This technology has developed in three stages.

Demonstration as a mutagen in a model plant

In 1993, we started with the seed embryos at a particular stage during fertilization in tobacco (*Nicotiana tabacum* L.) plant, which generates a large number of seeds per ovary, as a model to investigate mutagenesis by heavy-ion beams. When irradiated by heavy-ion beam immediately after fertilization, an embryo should produce a plant composed of mutant cells. The highest percentage of morphologically abnormal plants geminated seeds at 30–48 hrs of treatment stage after pollination was 18%.[1)] Morphological (e.g., variegated strains, albinos, and flower color mutants) and physiological (e.g., heavy metal, salt, and herbicide tolerance) mutants were successfully selected, suggesting the effectiveness of heavy-ion beams as a mutagen.

Fig. 1. A flower bed in front of RIBF building. These flowers were created by ion beam breeding. "Safinia Rose Veind", "Temari Sakura", and "Temari Momo" from front.

Trial use for flower plant breeding

We have collaborated with flower companies and public agricultural experiment stations since 1996 on the potential of practical breeding using heavy-ion beams. As a result, two new cultivars of plants were generated from irradiated materials in 1998. Specifically, *dahlias* "World" with large flowers and a new color was successfully generated, and have been marketed in Hiroshima City since the autumn of 2001. We successfully bred sterile *verbena* "Temari Bright Pink", which generates no seeds with a prolonged lifespan of the flower. It has been marketed since the spring of 2002. The similar successful cases were demonstrated by the new color flowers, *Petunia* "Safinia Rose Veined" (2003), "Safinia Pure White" (2012), *Torenia* "Summer Wave Pink" (2007), *Dianthous* "Olivea pure white" (2008), Cherry blossom "Nishina Zao" (2008) and many new cultivars.

Discovery of LET_{max} in a model plant

The effect of LET on mutation frequencies is important to determine the most effective irradiation condition in mutagenesis. We investigated the effect of LET on mutation induction using the model plant *Arabidopsis thaliana*. The most effective LET (LET_{max}) for mutation induction was 30 keV/μm.[2)] Subsequently, we have reported detailed analyses on the molecular nature of DNA alterations induced by heavy-ion irradiation with LET_{max} using morphological mutants. The most mutations were deletions ranging from several to several tens of bp.[3)] Irradiation at LET_{max} is effective for breeding because of its very high mutation rate. Since most mutations are small deletion, these are sufficient to disrupt a single gene.

Perspective

Recently, beneficial variants have been grown for various plant species, such as salt resistant rice, low polyphenol oxidase lettuce, low allergen peanut, high yield wakame (edible seaweed), and high oil content algae. We have found that high LET ions such as iron are effective for breeding microbes. And we worked

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with SAITC to create “Saitama F yeast”, that is used by 20 breweries in Saitama Pref. to ferment flavored rice wine. One brand of them is collectively known as “Nishina-Homare” (in honor of Nishina), named after Yoshio Nishina. We built a new beam line, called WACAME to increase available ion species with higher LET and longer range. The beams are accelerated by the AVF, RRC, and IRC to 160 MeV/nucleon and sent to E5 biological experiment room. We would like to contribute to advances in these fields, examine the effects of physical factors (e.g., ion species, LET, and dose) on DNA-mutated regions, and elucidate the mechanism of mutagenesis with heavy-ion beams. And the discovery of genes using the combination of mutants and genome sequencing technology may lead to new field in biology, “mutagenomics”.

References

1) T. Abe et al., Gamma Field Symposia **39**, 45 (2000).
2) Y. Kazama et al., Plant Biotech. **25**, 113 (2008).
3) Y. Kazama et al., BMC Plant Bio. **11**, 161 (2011).