UTILIZATION OF HEAVY ION BEAM AS A MUTAGEN IN PLANTS

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Abstract

Radiation has been widely adopted in order to induce artificial mutations in various plant species easily and effectively. We found that the heavy ion beam is highly effective in causing mutagenesis of seed embryos at a particular stage during fertilization [1]. RIKEN and Suntory Flowers Ltd. have jointly developed a new variety of verbena that appeared on flower shop shelves in March 2002. It is the first success in the world to commercialise the new variety by using heavy ion beams. Thus heavy-ion irradiation is an alternative method for induction of mutations that were more stable and more varied than the ones induced using low- LET radiations.

INTRODUCTION

The RIKEN accelerator research facility (RARF) has been pursuing multidisciplinary research by the use of its highly energetic heavy ion beams (HIB) up to 135 MeV/u. The RARF covers a wide range of science not only for physics but also for engineering, chemistry and even for biology. The HIB of RARF is able to irradiate living tissues under ordinary atmosphere because of extremely high energy of ions that are expressed as moving distance of nucleon in water; for example 3.4 cm and 2.3 cm for ^{14}N ions (135 MeV/u) and for ^{20}Ne ions (135 MeV/u), respectively. Concepts and scopes of the HIB cancer therapy were investigated and developed by the collaboration of radiation oncologists, engineers, physicists, other physicians and biologists in the RARF. Above background encouraged us to test the RARF for finding a new method for plant mutagenesis.

At first we found that the ion beam is highly effective in causing mutagenesis of seed embryos at a particular stage during fertilization without damage to other plant tissues [1]. We isolated many types of mutants in tobacco including albino, periclinal chimera, sectorial chimera, herbicide-tolerant and salt-tolerant phenotypes [2]. It was suggested that ion-radiation would predominantly produce double-strand breaks, however it is still uncertain whether the repair systems for the genomic HIB lesions are inactivated, or unacceptable [3,4]. The HIB mutations at the molecular level have been most extensively studied in mammalian cells. Some experiments indicated that ion-irradiation gave higher frequencies of DNA deletion than γ-rays [5,6]. Another case showed that ion beams could induce a point-mutation in the haploid cells of yeast [7]. The case of Arabidopsis was rather complicated demonstrating that half of the mutants resulted by point-like mutations and the other half by large scale DNA alterations involving inversions, translocations and wide range deletions [8]. From these results, it can be concluded that ion irradiation-induced mutations show a broad spectrum and high frequency. During the fertilization cycle some plant species were treated with chemicals to induce mutagenesis, and the results indicate that there is an optimum time for treatment to obtain a high frequency of mutation [9,10].

In this report, tobacco embryos during the fertilization cycle were irradiated with HIB to obtain a high frequency mutation. And we applied the HIB irradiation method to cultivate new flowers and ornamental plants with high marketability.

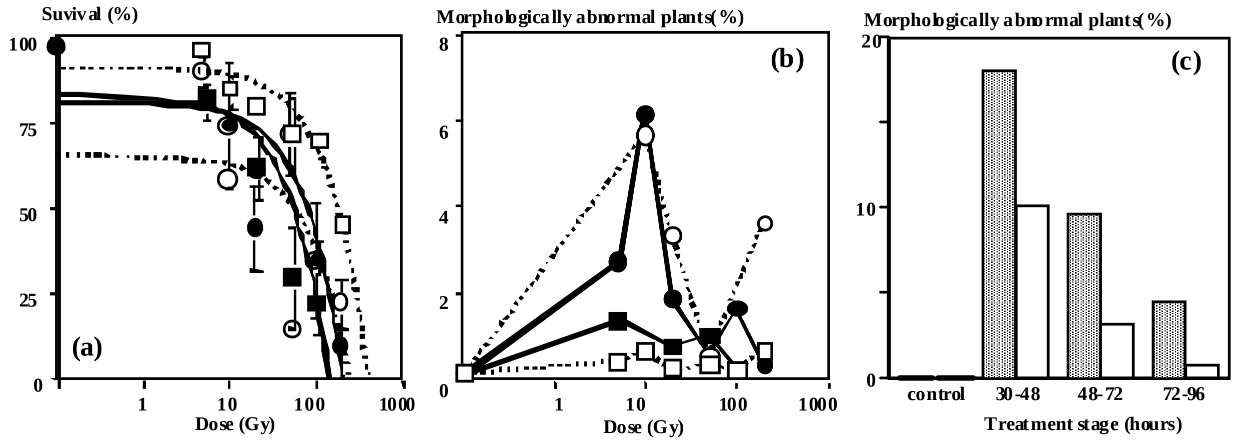

Figure 1: Effect of embryo treatment with HIB. (a) Survival percentage. ○ N ion for Xanthi; ● Ne ion for Xanthi; □ N ion for BY-4; ■ Ne ion for BY-4. (b) Frequency of morphologically abnormal plants. (c) Frequency of morphologically abnormal plants on different developmental stages. ▩ N ion for Xanthi; □ Ne ion for Xanthi.

TOBACCO EMBRYOS DURING THE FERTILIZATION CYCLE

Morphological mutants

Ovaries of tobacco (*Nicotiana tabacum* L. cv. Xanthi and BY-4) were irradiated at the timing of 24 to 108 hrs after pollination with HIB (135 MeV/u ^{14}N ions or 135 MeV/u ^{20}Ne ions). Under this condition the Linear Energy Transfer (LET) values of the N and Ne ions were 31 and 63 keV/μm, respectively. Then treated plants were grown in a glass house in a regular way for over a month until harvesting M_1 seeds.

High irradiation dose caused a decrease in the survival percentage (Figure 1a). Dose amount of HIB irradiation affected on M_1 seeds as reduction of germination potential while the mutation rate showed an optimum peak around 5 or 10 gray (Gy) generating many mutants of morphologically abnormal shape and chlorophyll-deficient phenotypes (Figure 1b). Albino, variegation and pale green plants were counted as the chlorophyll-deficient population, which reached to nearly 20% of M_1 plants by the use of N ion for Xanthi at 30 to 48 hours of treatment after pollination (Figure 1c). On the contrary BY-4 showed dull response to the HIB dose. The HIB treatment induced abnormal cotyledon shapes in the M_1 seedling as well as fore-mentioned results of the chemical treatment. However, HIB irradiation also gave drastic shape variation with needle or wrinkle leaves that were not induced with chemical treatment. Thus the preliminary experiment clearly indicated that very high efficiency is expected in HIB irradiation as a novel technology for plant breeding.

Chlorophyll-deficient mutants in M_2 generation

A sectorial chimera mutant was obtained from a M_2 plant in Xanthi, which had been irradiated with Ne ions (Figure 2a). The calyces have white and green areas, the petals have white and pink areas, and the stigmata are green. The growth of this variegated plant was normal and it set seeds quite well. A self-cross with the parental line resulted in a ratio of green to variegated plants as 393:26 in the M_2 generation, which is consistent with a double recessive gene segregation ratio of 15:1 (χ^2=0.125, 0.7<p<0.8).

An unique phenotype isolated from a M_2 population of BY-4, which had been irradiated with N ions, exhibited green and white variegation on its leaves, stems, and calyces [11] (Figure 2b). Fluorescence microscopic observation and genetic analysis suggest that the mutant is a periclinal chimera with a White-White-Green histogenic composition.

An albino mutant (*ali*) was isolated from the M_2 population derived from a BY-4 plant, which had been irradiated with N ions [12] (Figure 2c). A self-cross with the parental line of *ali* resulted in a ratio of green to albino plants as 2588:587 in the M_2 generation, which is not consistent with a single recessive gene segregation ratio of 3:1 (χ^2=71.8, p<0.001). When either the parental line or wild type (WT) plants were used as pollen donors, all F_1 plants were green so as to conclude that the *ali* mutant should be resulted from a nuclear mutation. The plastid ultra structure in leaf mesophyll cells of *ali* showed that the leaves were devoid of developed chloroplasts, and stacked thylakoid membranes could not be detected in the plastids. The expression of plastid-encoded genes for the photosynthesis system (*rbcL*, *psbA*) was greatly reduced in *ali*, but at the transcript level, the expression of plastid-encoded genes for 23S rDNA and 16S rDNA was slightly reduced. Transcription of the nuclear-encoded *Lhc* and *rbcS* genes was almost the same as in WT plants. The *ali* mutant did not accumulate functional proteins of the photosynthesis system including Rubisco L and S units and PSII-D1 polypeptides. The number of chloroplasts per cell was almost the same in both *ali* and WT plants. These results suggested that *ali* should be a new type of albino mutant in which the chloroplast gene expression is affected. Mutants induced with HIB irradiation have potential for gene analysis which will facilitate the molecular understanding of the gene function.

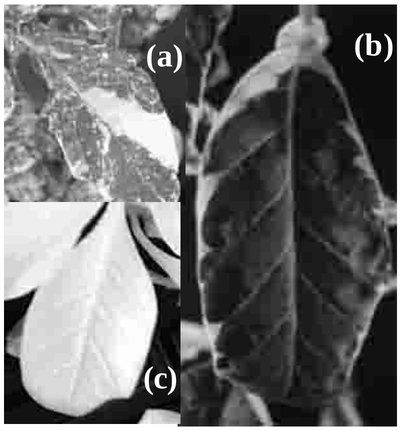

Figure 2: Leaves of Chlorophyll-deficient mutants induced with HIB. (a) Sectorial chimera. (b) Periclinal chimera. (c) Albino mutant.

FLOWERS AND ORNAMENTAL PLANTS

Verbena

Long blooming period with large number of flowers is the important characteristics of floricultural crops. A new type of Verbena cultivar ‘Temari’ series (*Verbena hybrida*) cultivars keep blooming with large number of flower clusters from spring until autumn in temperate zone area. However, ‘Coral Pink’ of this series shows sometimes the decrease in the number of flower clusters compared to other varieties. We concluded to isolate the sterile mutants of ‘Coral Pink’ using HIB irradiation [13]. Sixty four single nodes containing two lateral meristems at each base of two opposite leaves were cultured in one plastic dish, and irradiated with 1-10 Gy of the N-ion beam at 135 MeV/u. All shoots developed from lateral meristems were planted in soil, and grown in a green house. About 80 % shoots formation among all doses irradiations were observed, and most of them showed normal morphology. Some branches of flower clusters containing all sterile flowers were selectively propagated in several times by cutting. These plants were grown for flowering and the sterile strains were selected again. Finally, four mutant lines with stable sterility were successfully selected. These sterile mutants continuously grew well compared to host plant, and especially in the end of blooming season, autumn, when the host plant start senescence probably due to continuous reproductive state with seed setting. It was observed in the pot-planting test for three months that the sterile mutant always had larger number of flower clusters, finally over three times, than the host plant. Finally, we conclude that HIB irradiation is an excellent tool for sterile mutational breeding with high frequency. And it could shorten the breeding period, when HIB irradiation was combined with the tissue culture technique.

Dahlia

Dahlia is one of the important crop for cut flower industry during winter time in Horoshima City. However, the number of the varieties, which are adapted for winter production, is quite limited. The irradiation of HIB is a new method for the induction of dahlia mutations. Irradiation treatments were conducted on the shoots (ca.1 cm in length) of pink flower dahlia cv. 'Miharu', which were grown in the modified MS medium, in dose range of 5-20Gy for N-ion at 135 MeV/u. Plants treated with 10Gy grew vigorously in vitro as well as in the experimental field, and showed highest frequency of mutant induction. The results under the cultivation of experimental field are as follows; with the increase in exposure dose, 1) decrease in frequencies of anthesis, 2) increase in the variation of flower diameter, 3) increase in the variation of flower colours (Figure 3), 4) increase of the malformation of flower [14]. As for floral diameter, those of 3-12 cm have been observed. The mutants, such as petals in darker or paler colours or with white tip petal were observed in the present experiment and these were observed in our previous experiment with γ-ray irradiation. Mutants with darker floral colours were more commonly observed by the irradiation of N ion than γ-ray. Frequency of mutants with darker flower colours is higher than paler ones. The analysis for the colour of the flowers with chroma meter, showed that the mutations of floral colours after 5Gy and 10Gy were not continuous variation but marked two peaks. More than 10 clones with flowers of pink and white tip petal, with pale colours, and with darker colours are selected as promising strains for marketability.

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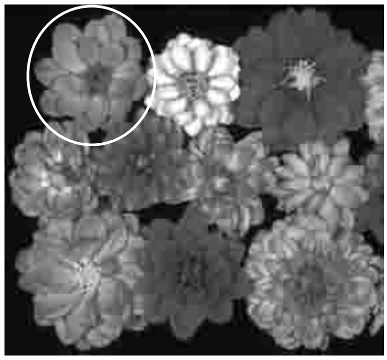

Figure 3: Variation of mutations. ○ indicates a wild-type flower of 'Miharu'.

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