THE ION-BEAM BREEDING MAKES GREAT SUCCESS IN PLANT BUSINESS

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Abstract

The ion-beam breeding developed by the Ring cyclotron of RARF is highly effective to create new flower cultivars within a short duration. A new type of garden plants "Temari" series (*Verbena hybrida*) keeps many flower clusters from spring until autumn, however "Coral Pink" of this series shows poor flower clusters. To improve "Coral Pink" using the ion-beam irradiation, sixty four single nodes were cultured in one plastic dish which was treated with 1-10 Gy of the N-ion beam at 135 MeV/u. Finally, four mutant lines with rich blooming were successfully selected. These mutants grew well compared to host plant, and kept many flower clusters even in autumn. The best mutant had larger number of flower clusters than the host plant in the pot-planting test so that it was released to the market in 2002 with a level of several hundred thousand pots. The development period of the new "Coral Pink" was only three years. The similar successful cases were demonstrated by the new Dahlia "World" (2002), the new Verbena "Sakura" (2003) and the new Petunia "Rose" (2003). Thus, we conclude that the ion beam irradiation is an excellent tool for mutation breeding to improve horticultural and agricultural crops with high efficiency.

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 $^{14}N^{+7}$ ions (135 MeV/u) and for $^{20}Ne^{+10}$ 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.

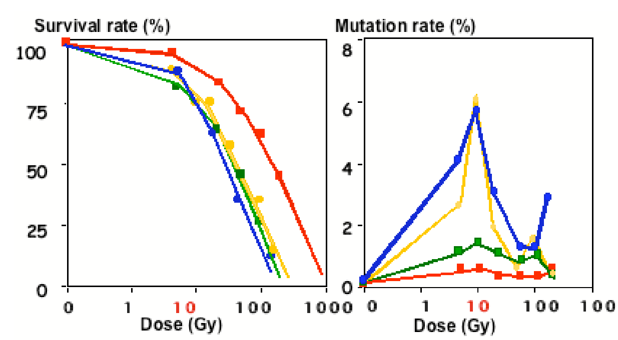

Fig. 1: Effects of tobacco embryo treatment with HIB. (a) Survival percentage. ● N ion for Xanthi; ● Ne ion for Xanthi; ■ N ion for BY-4; ■ Ne ion for BY-4. (b) Frequency of morphologically abnormal plants.

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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 N ions or 135 MeV/u Ne ions). Under this condition the Linear Energy Transfer (LET) values of the N and Ne ions were 31 and 63 keV/ m, 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 (Fig. 1). Dose amount of HIB irradiation affected on M_1 seeds as reduction of germination potential while the mutation rate showed an optimum peak around 10 gray (Gy) generating many mutants of morphologically abnormal shape and chlorophyll-deficient phenotypes (Fig. 2) [11,12]. 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. 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 [2]. Thus the preliminary experiment clearly indicated that very high efficiency is expected in HIB irradiation as a novel technology for plant breeding.

Fig. 2: Morphological mutants in Xanthi induced with HIB. (a) Wild type plant. (b) – (d) Morphological mutants. (e) Albino mutants.

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' and 'Sakura' of this series show sometimes the decrease in the number of flower clusters compared to other varieties. We concluded to isolate the sterile mutants of 'Coral Pink' and 'Sakura' 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 in 'Coral Pink' and less than 5 Gy in 'Sakura' were observed, and most of them showed normal morphology (Fig. 3). 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 in 'Coral Pink' and one in 'Sakura' mutant lines with stable sterility were successfully selected (Table 1). 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.

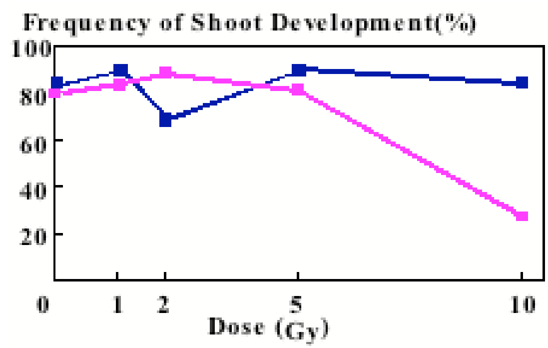

Fig. 3: Frequency of shoot development from nodal cultured tissue. ■ Coral Pink; ■ Sakura.

Table 1: Isolation of sterile mutants of 'Coral Pink' and 'Sakura' of the Temari series.

Cultivars	Dose (Gy)	No. of Material	No. of Shoot	No. of Sterile Plant
CORAL PINK'	0	15	25	0
	1	64	115	0
	2	64	88	0
	5	64	115	1
	10	64	108	3
'SAKURA'	0	15	24	0
	1	64	107	0
	2	64	113	0
	5	64	104	1
	10	64	35	0

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 ions 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 (Table 2), 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. 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.

Table 2: Effects of ^{14}N ion beam irradiation on dahlia.

Photo	Flower color		Dose (Gy)			
	ground color	edge	0	1	5	10
3-1	white	-	0.0	0.0	0.0	0.2
3-2	whitish pink	-	0.0	0.2	1.6	11.6
3-3	pale pink	-	2.9	5.9	10.0	15.9
	pale pink	white	0.1	0.0	0.2	0.0
FD	standard (pink)	-	96.2	93.6	79.7	49.9
3-4	pink	white	0.8	0.0	0.6	0.8
3-5	pink	pink	0.0	0.2	0.1	0.0
3-6	red	-	0.0	0.1	7.6	21.6
	Mutation rate		3.8	6.4	20.3	50.1

ACKNOWLEDGEMENTS

We are grateful to Ms.M.Hamatani in Hiroshima City Agriculture and Forestry Promotion Center for cooperation.

REFERENCES

[1] ABE T., YOSHIDA S., SAKAMOTO T., KAMEYA T., KITAYAMA S., 5INABE N., KASE M., GOTO A. and YANO Y. (1995). Modification of gene expression and non-Mendelian inheritance, edited by OONO and TAKAIWA, NIAR, Japan, PP 469-477.
[2] ABE T., BAE C.H.,OZAKI T., WANG J.M. and YOSHIDA S (2000) Gamma Field Symposia 39:45-56
[3] GOODHEAD D.T. (1994). Int.J.Radiat.Biol.65: 7- 17.
[4] RYDBERG B. (1996). Radiat. Res. 145: 200-209.
[5] THACKER J. (1986). Mutat. Res. 160: 267-275.
[6]SUSUKI M., WATANABE M., KANAI T., KASE Y., YATAGAI F., KATO T., and MATSUBARA S. (1996). Adv. Space Res. 18: 127-136
[7] YOSHIMASU M., ABE T., YOSHIDA S., LING F., and SHIBATA T. (2000). RIKEN Accel. Prog. Rep. 33:139.
[8] TANAKA A. (1999) Gamma Field Symposia 38:19-28.
[9] LEE H.Y., and KAMEYA T. (1991). Theor Appl Genet 82:405-408.
[10] KANG K.K., and KAMEYA T. (1993). Euphytica 69:95-101.
[11] BAE C.H., ABE T., NAGATA N., FUKUNISHI N., MATSUYAMA T., NAKANO T., and YOSHIDA S. (2000). Plant Sci. 151: 93-101
[12] BAE C.H., ABE T., MATSUYAMA T., FUKUNISHI N., NAGATA N., NAKANO T., KANEKO Y., MIYOSHI K., MATSUSHIMA H. and YOSHIDA S. (2001). Ann.Bot. 88, 545-554.
[13] SUZUKI K., YOMO Y., ABE T., KATSUMOTO Y., MIYAZAKI K., YOSHIDA S. and KUSUMI T. (2002). RIKEN Accel. Prog. Rep. 35:129.
[14] HAMATANI M., IITSUKAO Y., ABE T., MIYOSHI K., YAMAMOTO M. and YOSHIDA S. (2001). RIKEN Accel. Prog. Rep. 34:169-17