PLANT BREEDING USING THE ION BEAM IRRADIATION IN RIKEN

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Abstract

Since 1986, RIBF has been the one of the biggest facilities capable of accelerating heavy ions world wide. Although nuclear physics is the primary theme investigated at the facility, plant scientists started our trials in plant breeding in 1993. Soon we found that the ion beam is highly effective for inducing mutagenesis of tobacco embryos during fertilization without damage to other plant tissue. We isolated many types of tobacco mutants including albino, periclinal chimera, sectorial chimera, herbicide-tolerant and salt-tolerant phenotypes. We have put 6 new flower cultivars on the market in Japan, USA, Canada and EU since 2002. The developmental period of these new cultivars was only three years. The ion beam irradiation technique induces a high mutation rate without severe growth inhibition at relatively low doses. Thus, we conclude that the ion beam is an excellent tool for mutation breeding to improve horticultural and agricultural crops with high efficiency.

INTRODUCTION

Since ion beams are produced by particle accelerators, our study strongly depends on the developmental history of such facilities. In fact, our predecessor started his studies when the accelerator facility in Lawrence Berkeley Laboratory (LBL), known as Bevalac was launched in the 1970's. Before Bevalac, there was no particle accelerator that could produce an ion beam with sufficient energy to pass thorough tissue. In these studies, it was well established that high-Linear Energy Transfer (LET) ion beams have higher biological effects than low-LET radiation such as gamma and X-rays.

Ion beam irradiation produces double-strand breaks. It is still not clear however whether the repair systems are inactivated, or merely that heavy-ion lesions are less repairable [1]. Mutations induced by ion beam irradiation at the molecular level have been most extensively studied in mammalian cells. It is reported that the frequency of deletion is higher for ion beam irradiation than for gamma rays [2]. However, ion beams could induce point-like mutants in the haploid cells of yeast [3]. And in the case of Arabidopsis, half of the mutants have point-like mutations and the other half have large DNA alterations such as inversions, translocations and large deletions [4]. From these results, it can be concluded that mutations induced by ion beam irradiation show a broad spectrum and high frequency.

The RIBF is not only for physics but pursuing multidisciplinary research utilizing energetic ion beams up to 135 MeV/nucleon (about 50% of speed of light). Research into cancer therapy was fostered at RIBF by the collaboration of radiation oncologists, physicists, and biologists starting in 1986. The experience of these researches then encouraged plant scientists to use RIBF for radiation biology research starting in 1989. Eventually, we started our trials in plant breeding about 10 years ago. Initially, we found that the ion beam is highly effective in inducing mutagenesis of seed embryos at a particular stage during fertilization without damage to other plant tissues [5]. We isolated many types of mutants in tobacco including albino, periclinal chimera, sectorial chimera, herbicide-tolerant and salt-tolerant phenotypes [6]. The sterile *Vervena* mutants, "Temari Bright Pink" became first flower marketed after being developed using the ion beam in 2002. The developmental period of the new cultivar was only three years. Similar successful cases were demonstrated by the new color *Dahlia* "World" (2002), the new sterile *Verbena* "Temari Sakura Pink" (2003) and "Temari Momo" (2006), the new color *Petunia* "Surfinia Rose Veined" (2003) and the new color *Torenia* "Summer Wave Pink" (2007). In this review we introduce our accelerator facility and summarize the experiments that applied the ion beam irradiation method to induce mutations in plants.

E5 BEAM LINE IN RIBF

Typical ions used for irradiation on biological samples are ^{12}C, ^{14}N, and ^{20}Ne at 135 MeV/nucleon, ^{40}Ar at 95 MeV/nucleon, and ^{56}Fe at 90 MeV/nucleon (Table 1). The sharp beam accelerated by an AVF cyclotron and a ring cyclotron is transformed into an approximately 8-cm-diameter beam with uniform intensity distribution with the wobbler scattering method originally developed at LBL [7](Fig. 1).

Table 1. Heavy ions for biological research in RIBF.

Ion	Energy MeV/u	Range in water* (cm)	LET keV/um	No. of ** particles
$^{12}C^{6+}$	135	3.9	23	277
$^{14}N^{7+}$	135	3.3	30	204
$^{20}Ne^{10+}$	135	2.2	63	101
$^{40}Ar^{17+}$	95	0.6	280	22
$^{56}Fe^{24+}$	90	0.3	624	10

*Surface on the samples, **10Gy in $(10\ um)^2$

J.F.Ziegler et al., The stopping and Range of Ions in Solids (Pergamon, New York, 1985)

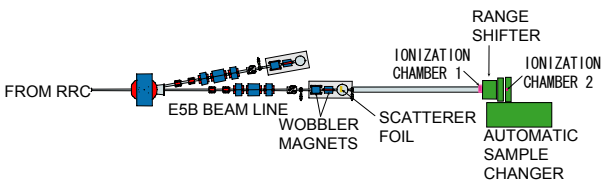

Figure 1: Schematic view of E5B beam line.

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SAMPLES AND AUTOMATIC SAMPLE CHANGER

The automatic sample changer, which consists of a movable table and six stages for cassettes, carries more than 500 sample containers filled with biological samples and automatically places each sample at the beam position operating together with the range shifter. Typical sample containers are plastic box (5cm X 7.5cm X 1.25cm) for dry seeds and scions, plastic Petri dishes (3, 6 and 9cm diameter) for imbibed seed and tissue culture material and plant box for cultured plantlets (Fig. 2). More than 500 sample containers can be automatically irradiated in a single procedure [8].

IRRADIATION OF SAMPLES

The range shifter decreases the energy of the beam. Decreasing the beam energy not only produces a shorter beam range, but also controls the LET of the beam. The apparatus consists of twelve 12-cm-diameter energy absorbers attached to air cylinders. The energy absorbers are comprised of aluminum foils and plates ranging from approximately 0.02 to 20 mm in thickness. The total thickness of the range shifter is obtained by selecting a combination of absorbers [9]. For example, LET values can be adjusted with energy absorbers from 22 to 285 keV/um in C ion, from 60 to 700 keV/um in Ne ion (135MeV/u) irradiation. Lethal effects on buckwheat were measured with various LET beams of C, Ne, Ar and Fe ions. The RBE (relative biological effectiveness) was obtained from each D_{37} (mean lethal dose) divided by the given D_{37} value of the gamma ray. The RBE reached the maximum about 20 at 305 keV/um [10].

Figure 2: Plant samples set in cassettes.

The Bragg peak (BP) characteristics deliver a greater dose to the stopped cells and considerably lower dose to the normal tissues penetrated by the beam. It is well know to be beneficial for cancer treatment to adjust the BP to target malignant cells. However, a uniform dose distribution is a key to systematic study, and thus to the improvement of mutation efficiency. We selected a sufficiently higher beam energy to avoid the effect of BP to realize a uniform dose distribution. Plant materials are irradiated to the ion beam for a few seconds or minutes, triggering various genetic changes in the plants. The sensitivity of plant organs decreases in the following order: embryos during the fertilization cycle [6] and stem nodes, imbibed seeds and scions, dry seeds (Table 2).

Table 2. Mutant lines developed in various crops using RIBF

Mutant phenotype	Plant material	Ion/Dose(Gy)	Survival(%)/Mutation(%)	Collaboration	Ref
Sterile					
Verbena	Stem	N / 10	84/2.8	Suntry Flowers Ltd	[12]
Cyclamen	Tuber	C /12	50/13	Hokko Chem,Ind. Co Ltd	[13]
Flower color and shape					
Dahlia	Shoot	N / 5	NE / 20.3	Hiroshima City Agri.Forest.Prom,Cen.	[14]
Rose	Dormant scion	Ne / 15	70 / 51.7	Kanagawa	[15]
		N / 30	90 / 43.1	Pref.Agri.Cent	Fig. 4
Chrysanthemum	Stem	C / 10	94 / 14	Plant Biotech. Inst. Ibaraki Agri. Cen.	[16]
Torenia	Leaf and stem	N / 50	NE / 1.9	Suntry Flowers Ltd	[17]
		Ne /20	NE / 1.6		
Variegation					
Petunia	Stem	N / 5	ND	Suntry Flowers Ltd	[18]
Semi-dwarf					
Barley	Dry seed	N / 50	ND / 2.6	Natl.Agr.Res.Cen.Min.	[19]
	Imbibed seed	N / 5	ND / 0.9	Agr.Forest.Fish	
Sweet pepper	Dry seed	Ne / 10	80 / 1.3	Natl. Inst. Veget. And Tea Sci.	[11]
Buckwheat	Dry seed	C / 40	NE / 0.9	Natl.Inst.Agr.Sci.	[10]
		Ar / 20	NE / 1.0		[20]
		Fe / 30	70 / 4.0		
Salt-tolerance					
Rice	Imbibed seed	C / 40	40 / 1.1	Tohoku Univ.	[21]
Waxy					
Rice	Dry seed	N / 200	NE / 2.2	Chiba Pref. Agri. Res. Cen.	[22]

ND: no data, NE: no effect

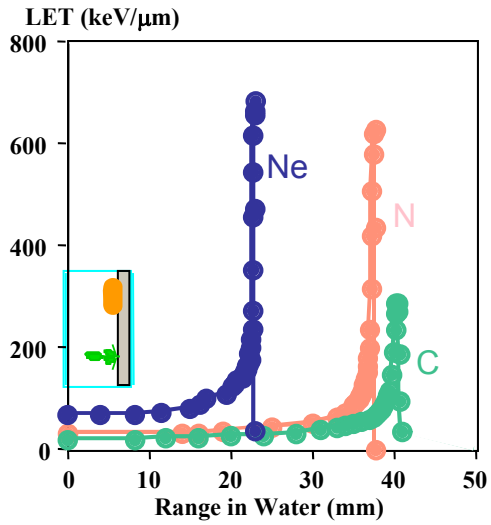

Figure 3: Schematic diagram of ion beam irradiation for a plantlet and plant tissue. A beam with highly sufficient energy penetrates a plantlet and/or plant tissue with rather low and uniform LET.

SUMMARY

The ion beam irradiation technique shows a high mutation rate without severe growth inhibition at relatively low doses. Irradiation of each plant material for only seconds or a few minutes is sufficient to induce mutation using RIBF. Thus, we conclude that the ion beam is an excellent tool for mutation breeding to improve horticultural and agricultural crops with high efficiency. Our research consortium includes 120 user groups, agricultural experimental stations, universities, seed companies and flower companies. Recently, we have shown a variety of results in plant physiology, genetics, botany, and agricultural science through our extensive studies using mutants of many species in the plant kingdom. We isolated three mutants of sweet pepper: two dwarf plants and a yellow pepper [11]. Figure 3: Schematic diagram of ion beam irradiation for a plantlet and plant tissue. A beam with highly sufficient energy penetrates a plantlet and/or plant tissue with rather low and uniform LET.

These mutant appearances (or phenotypes) are recessive and due to changes in single genes (or monogenic). The isolation of monogenic homozygous recessive mutants in M_1 irradiated generation have not normally been described in other mutational studies using many plant species. The ion beam can induce homozygous recessive mutations in nuclear genes that allow detection of mutants even in M_1 plants. It has taken a very short time for plants to develop new cultivars.

REFERENCES

[1] Goodhead D.T. Int.J.Radiat.Biol.**65**, 7(1994).
[2] Thacker J. Mutat. Res. **160**, 267(1986).
[3] Yoshimasu M.et.al. RIKEN Accel. Prog. Rep., **33**, 139(2000).
[4] Shikazono N. et.al. J.Exp.Bot. **56**, 587 (2005).
[5] Abe T. et.al. Proc.of the US-Japanese Joint Meeting, p.469(1995).
[6] Abe T. et.al. Gamma Field Symp. **39**, (2000).
[7] Chu, W.T. et.al. IEEE Trans. Nucl. Sci. NS-32: 3321(1985).
[8] Ryuto H. et.al. J. Biomed.Nanotech. **2**, 88 (2006).
[9] Akiyoshi H. et.al. RIKEN Accel. Prog. Rep., **29**, 291(2003).
[10] Morishita T. et.al. Nucl. Instrum. Methods Phys. Res. B **206**, 565 (2003).
[11] Honda I. et.al. Euphytica **152**, 61(2006).
[12] Suzuki K. et.al. RIKEN Accel. Prog. Rep. **35**, 129 (2002).
[13] Sugiyama M. et.al. RIKEN Accel. Prog. Rep. **40**, 261 (2006).
[14] Hamatani M. et.al. RIKEN Accel. Prog. Rep. **34**, 169 (2001).
[15] Hara Y. et.al. RIKEN Accel. Prog. Rep. **36**, 135 (2003).
[16] Suzuki K. et.al. RIKEN Accel. Prog. Rep. **37**, 152 (2004).
[17] Miyazaki K. et.al. Plant Biotech. **23**, 163 (2006).
[18] Miyazaki K. et.al. RIKEN Accel. Prog. Rep, **35**, 130 (2002).
[19] Honda I. et al. RIKEN Accel. Prog. Rep. **34**, 171 (2001).
[20] Morishita T. et.al. RIKEN Accel. Prog. Rep. **40**, 255 (2006).
[21] Hayashi Y. et.al. RIKEN Accel. Prog. Rep. **40**, 253 (2006).
[22] Ohkoshi K. et.al. RIKEN Accel. Prog. Rep. **39**, 138 (2005).

Figure 4: An example of mutant flowers of rose.