Journal of Agricultural Science; Vol. 8, No. 5; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Plant Mutation Breeding with Heavy Ion Irradiation at IMP

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Received: March 8, 2016 Accepted: April 3, 2016 Online Published: April 15, 2016

doi:10.5539/jas.v8n5p34 URL: http://dx.doi.org/10.5539/jas.v8n5p34

Abstract

The Heavy Ion Research Facility in Lanzhou (HIRFL) is one of the ion-beam acceleration facilities intensively used at IMP, founded as national laboratory and opened for user in world from 1992. Since then, a lot of experiments irradiated by heavy ion beam have been carried out in the HIRFL, including plant mutation breeding. In this review, the biological effects induced by heavy ions and their corresponding mechanisms were reported from the point of view of cytological, morphological and molecular levels. To date, a large number of mutants were isolated using heavy ion irradiation IMP, such as early maturity, flower color and shape, high yield and disease resistant. In conclusion, heavy ion beam irradiation is an efficient mutagen and has significant phenotypic variations in plant. Our research will be further focused on transformation of scientific and technological achievements and mutagenic mechanism of heavy ion beam on high plant at the molecular level in the recent future.

Keywords: plant breeding, heavy ion irradiation, biological effects, Mutants, IMP

1. Introduction

Since Muller (1927) and Stadler (1928) discovered that X-rays can induce mutations in drosophila and barley, respectively, the use of ionizing radiation such as X-rays and gamma rays to induce variation has become an established technology (Ahloowalia & Maluszynski, 2001). Ion beams have been applied as a nuclear technique since the late 1950s, especially in the field of surface modification on various materials (Cui & Luo, 1999). In 1986, Chinese scientists studied the biological effect of ion implantation and first applied it to rice breeding. Since then, a new research field Ion Beam Bioengineering has been established (Wu et al., 1999).The high linear energy transfer (LET) heavy-ion beam has recently been used on many plants, resulting fruitful achievements (Yu et al., 1991), such as the carnation (Okamura et al., 2003), chrysanthemum (Yamaguchi et al., 2010), wheat (Wei et al., 1998), buckwheat (Morishita et al., 2003) and Arabidopsis (Kazama et al., 2008), because of its higher mutation rate and wider mutation spectrum with lower damage to irradiated materials compared to the traditional low LET radiation methods, (Abe et al., 2000; Tanaka, 1999). Thus, more and more researchers pay attention to the application of heavy ion beam irradiation in plant mutation breeding.

The Institute of Modern Physics (IMP), affiliated with the Chinese Academy of Sciences, was founded in 1957 in Lanzhou, China. It is now an institute making important contributions to basic research as well as the applications of heavy-ion physics and nuclear techniques (Li, 2007; Wang, 2006). Aiming at the scientific front and strategy demand on modern agriculture, the researchers of IMP have been carrying out mutagenic improvement on higher plant since the middle of 1990s. In this review, we introduce IMP accelerators facility and focus on the plant breeding research with heavy ions at the IMP accelerators.

2. HIRFL—The Platform of Mutation Breeding Induced by Ion Beam at IMP

The Heavy Ion Research Facility in Lanzhou (HIRFL) is one of the ion-beam acceleration facilities intensively used in the past two decades at IMP (Sun et al., 1996). A lot of experiments irradiated by heavy ion beam have been carried out in the HIRFL.

The HIFRL was found as national laboratory and opened for user in world from 1992(Wei, 1989). Then, it was upgraded many times, especially, the new upgrade project of Cooling Storage Ring (CSR) at the beginning of the

21th century. Therefore, besides the researches on the nuclear physics, atomic physics, irradiative in material and biology, the researches on the cancer therapy by ion, hadron physics and high energy density are developing at HIRFL (Zhan et al., 2008). HIRFL-CSR is a multi-propose CSR system that consists of a main ring (CSRm), an experimental ring (CSRe), and a radioactive beam line (RIBLL2) to connect the two rings, shown in Fig.1 (Xia et al., 2002). The two existing cyclotrons SFC (K = 69) and SSC (K = 450) of the HIRFL will be used as its infector system. The heavy ion beams with the energy range of 8-30 MeV/μ from the HIRFL will be accumulated, cooled and accelerated to the high-energy range of 100-400 MeV/μ in the main ring, and then extracted fast to produced BIB or highly charged heavy ions. The secondary beams will be accepted and stored by the experimental ring for many internal-target experiments or high-precision spectroscopy with beam cooling. On the other hand, the beams with the energy range of 100-900 MeV/μ will also be extracted from CSRm using slow extraction or fast extraction for many external-target experiments. To realize heavy-ion cancer therapy with the intermediate energy ion beam provided by the HIRFL, a therapy terminal has been constructed underground the experimental hall of the HIRFL complex except the existing irradiation terminal for biomedical research, which equipped with magnetic scanning systems to generate uniform irradiation fields. Based on the biomedical terminal, Cellular and molecular biology experiments and even plant tissue culture can be routinely carried out in the laboratory. Thus, plant radiation breeding is available at IMP (Li, 2007).

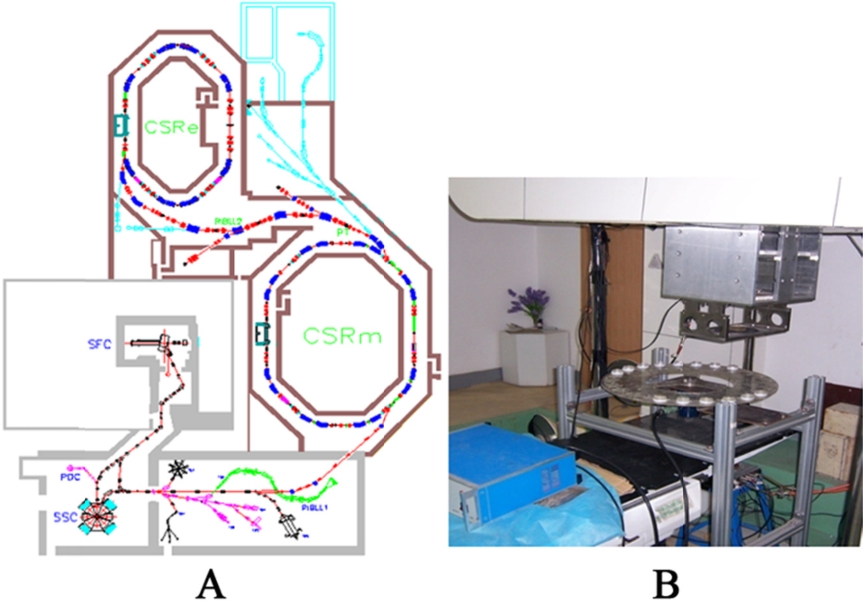

Figure 1. Platform of plant mutation breeding has been established at IMP. A is overall layout of the HIRFL-CSR complex, B is biological irradiation terminal

3. Biological Effects of Heavy Ion Beam on Plant Materials

Ionizing radiation is known to have several effects on plant growth and development, ranging from stimulatory effects at very low doses, harmful consequences at intermediate levels and pronounced detrimental outcomes at high doses (Cucinotta & Durante, 2006; Durante & Cucinotta, 2008; Veronica et al., 2001). The severity of the effects is dependent upon several factors including species, cultivars, plant age, physiology and morphology as well as plant genome organization (Holst & Nagel, 1997). The basic research on plant mutation breeding is paid more attention to the biological effects induced by heavy ions and their corresponding mechanisms from the point of view of chromosomal aberrations, plant growth and mutated genes at IMP (Table 1) (Chen et al., 2008; Dong et al., 2008; Hou et al., 2008, 2008; Li et al., 1996; Liu et al., 2006, 2012, 2014; Mei et al., 1994; Qian et al., 2007; Wang et al., 1992, 2012; Wang & Zhang, 2007; Wei et al., 2003; Xie et al., 1998; Zhang et al., 2008; Zhou et al., 2006).

Firstly, the biological effects of wheat seeds irradiated by 48 MeV/μ and 20 MeV/μ $^{14}N^{7+}$ beams were studied, respectively (Li et al., 1996). The results revealed that heavy ion irradiation could induce a lot of free radicals and inhibit seedling growth of wheat seeds, leading to a great variety of chromosomal aberrations in root tip cells of irradiated seeds and high aberration frequencies compared to the corresponding control. In order to analyze the relationship between plant growth and cytological effects, wheat dry seeds were exposed to various doses of $^{12}C^{6+}$ beams and the biological endpoints reflecting plants growth and root apical meristem activities were investigated (Liu et al., 2013). The results showed that the plant survival rate descended at higher doses and various types of chromosome aberrations were observed in the mitotic cells. The frequencies of mitotic cells with lagging chromosomes and these with anaphase bridges reached the peak around 60 Gy, while the frequencies of fragments increased as the irradiation doses increased up to 200 Gy. Thus, the total frequencies of mitotic cells with chromosome aberrations induced by irradiation increased significantly with the increasing doses. To further investigate the mutagenic mechanism, Liu et al. (2012) comparatively studied the differences in pollen viability and pollen mother cells meiosis between KFJT-1 and KFJT-CK, which was isolated from KFJT-CK after carbon ion beam irradiation in sweet sorghum. The results showed that the total number of pollens and pollen viability of KFJT-1 were more than that of KFJT-CK. Triad, unequal separation of chromosome and asychronization of chromosomes segregation were found in KFJT-1 meiosis, but the most of chromosomal aberrations induced by carbon ions were repaired, the aberration rate of KFJT-1 being only 4.5%.

Table 1. Effects of heavy ion beam on chromosome aberrations, growth of different irradiated materials

Ion type	Energy (MeV/μ)	Species	Treated material	Biological endpoint	References
$^{12}C^{6+}$	87.5	Arabidopsis	Seed	Homologous recombination	Wang et al., 2012
$^{12}C^{6+}$	90.4	Wheat	Dry seed	Growth and survival	Zhang et al., 2008
$^{14}N^{7+}$	20,48	Wheat	Seed	Growth and chromosomal aberrations	Li et al., 1996
$^{14}N^{7+}$, $^{14}N^{1+}$	157,72	Wheat	Seed	Micronuclei and chromosomal aberrations	Wei et al., 2003
$^{12}C^{6+}$, $^{14}N^{7+}$, $^{16}O^{6-}$, $^{40}Ar^{15+}$	46.6,45.8,7,21	Wheat	Seed	Chromosomal aberrations	Xie et al., 1998
$^{12}C^{6+}$	46.6	*Lycium barbarum*	Dry seed	Growth and chromosomal aberrations	Wang et al., 1992
$^{12}C^{6+}$, $^{16}O^{8+}$	3.2,8,16,75	*Z.mays*, Wheat	Dry seed	Mutations and chromosomal aberrations	Liu et al., 2006
$^{12}C^{6+}$	60	*Allium fistulosum*	Dry seed	Micronuclei and chromosomal aberrations	Qian et al., 2007
$^{12}C^{6+}$	80	Tomato	Seed	Chromosomal aberrations	Wang & Zhang, 2007
$^{12}C^{6+}$	80.55	Brassica napus	Dry seed	Chromosomal aberrations	Hou et al., 2008
$^{12}C^{6+}$, $^{36}Ar^{18+}$	80.55,82.55	*Z.mays*	Dry seed	Mutations	Chen et al., 2008
$^{12}C^{6+}$	80.55	*Linum usitatissmum*	Seed	Pollen viability	Hou et al., 2008
$^{12}C^{6+}$	100	Sweet sorghum	Seed	Chromosomal aberrations and pollen mother cells	Liu et al., 2012
$^{40}Ar^{15+}$, $^{56}Fe^{24+}$	400	Rice	Seed	Seedling height, morphology	Mei et al., 1994
$^{12}C^{6+}$	960	*S.ionahta*	Leaf explant	Survival and malformations	Zhou et al., 2006
$^{12}C^{6+}$+	100	Lavender	Dry seed	Survival and growth	Liu et al., 2014
$^{12}C^{6+}$	100	Sweet sorghum	Seed	Survival and growth	Dong et al., 2008

In the case of phenotypic effects induced by heavy ion beam irradiation, *Saintpaulia ionahta* (Zhou et al., 2006), sweet sorghum (Dong & Li, 2012; Dong et al., 2015) and *Arabidopsis thaliana* (Du et al., 2014) were studied at IMP, respectively. For *Saintpaulia ionahta*, in vitro leaf explants samples were irradiated with carbon ion beams with linear energy transfer (LET) values in the range of 31-151 keV/um at different doses. It was found that the fresh weight increased and surviving fraction decreased dramatically with increasing LET at the same doses. In addition, malformed shoots, including curliness, carniation, nicks and chlorophyll deficiency, occurred in carbon ion beam. Eventually, a chlorophyll-deficent mutant and other malformations were obtained. For sweet sorghum, the carbon ions had a marked stimulatory effect on the survival rate at low doses, with the value at 30 Gy being 86%, higher than that of non-irradiated seeds. The carbon ion irradiation resulted in physiological damage of the KFJT-CK seeds and the surviving fraction in the field decreased as the dose increased above 30 Gy. The results demonstrated that disappearance of the growth point, curing of the leaf tip, plant withering and etiolated seedlings were observed in some mutants. Other mutants showed stalk thickening, spikes and larger grain. However, an early-maturity mutant, KFJT-1, was isolated at 80 Gy, which the growth period was shortened by about 20 days compared to the wild type KFJT-CK. In addition, the seed coat color of KFJT-1 was dark yellow brown than that of KFJT-CK. Physiological analysis showed that the proline content of KFJT-1 was increased by 11.05% while the malondialdehyde content was significantly lower than that of KFJT-CK. In terms of *Arabidopsis thaliana*, a total of 1363 lines of plants from 28062 M2 populations displayed alterations in the leaf, stem, flower, or life cycle, with abnormal leaves and a premature life cycle as the main phenotypic variations. The total mutation rate was 4.77%.In the 1363 mutant lines, 64% were displaying one conspicuous phenotype, 13.3% displayed two conspicuous phenotypes, and 13.1% showed at least three mutant traits.

The reason heavy ions are more suitable for induced mutagenesis is well explained by Liu et al. (2008) and Tanaka et al. (2010). However, mutation mechanism for heavy ion irradiation remains unknown at molecular levels. In recent years, preliminary studies were carried out to reveal the molecular nature of phenotype variation in higher plants after heavy ion irradiation. In the previous research, Hd1, a homologue of CO, was found to be a major determinant of photoperiod response (Laurie et al., 2004) while Hd3a was shown to be a homologue of FT (Yano et al., 2001). Therefore, we analyzed the Hd1 and Hd3a genes between early-maturity mutant and wild type in sweet sorghum (Dong et al., 2015), which indicated that there was no difference for the Hd1 gene, but 2 bp insertions were clearly observed in early-maturity mutant compared to wild type in the case of the Hd3a gene. This finding might provide the first insight into the mechanism of the photoperiodic control of flowering time in an early-maturity mutant induced by the heavy ion beam irradiation. In the post-genomic era, researchers not only identify the mutated genes but also explore the function and the molecular regulation of those genes. At IMP, five mutants with transmittable phenotypes were roughly mapped based on the positional cloning technology in Arabidopsis thaliana (Du et al., 2014), which we discovered that mutants #164, #60 and #352 correspond to previously described genes, while mutants #197 and #357 correspond to genes that have not been reported before. In addition, there are two mutational sites in the #197 genome. Above researches indicated that the heavy-ion irradiation induced more complicated mutations. Further verification and detailed molecular analysis will be carried out using gene expression methods and RNA sequencing technology in the near future.

4. Some Typical Mutants from Plant Breeding with Heavy Ions at IMP

So far, some typical mutants were obtained from wheat, maize, sweet sorghum, dahlia and Arabidopsis thaliana, respectively (Table 2, Figure 2) (Dong et al., 2007; Dong & Li, 2012; Du et al., 2014; He et al., 2011; Wang & Zhang, 2007; Wu et al., 2009; Xie et al., 2007, 2008; Yu et al., 2014; Zhao et al., 2006; Zhou et al., 2006).

Table 2. The mutant types induced by heavy ion beam irradiation at IMP

Mutant phenotype	Plant material	Ion/Dose (Gy)	Energy (MeV/μ)	References
Early maturity				
sweet sorghum	Dry seed	C/80	100	Dong, 2012
Arabidopsis thaliana	Dry seed	C/200	43	Du et al., 2014
Flower color and shape				
Wandering Jew	stem	C/20	95.8	He et al., 2011
Dahlia	shoot	C/8	80	Dong et al., 2007
S. splendens	Dry seed	C/100	80.55	Wu et al., 2009
Saintpaulia ionahta	shoot	C/20	960	Zhou et al., 2006
High yield				
Wheat	Dry seed	O/24	75	Zhao et al., 2006
Potato	Micro-tuber	Ar/60	55	Xie et al., 2008
Disease-resistant				
Angelica	Dry seed	Ar/25	55	Xie et al., 2007
Maize	Imbibed seed	Ar/20	82.55	Chen et al., 2008
	Dry seed	C/30	80.55	
Seed coat color				
Soybean	Dry seed	C/100	80	Yu et al., 2014

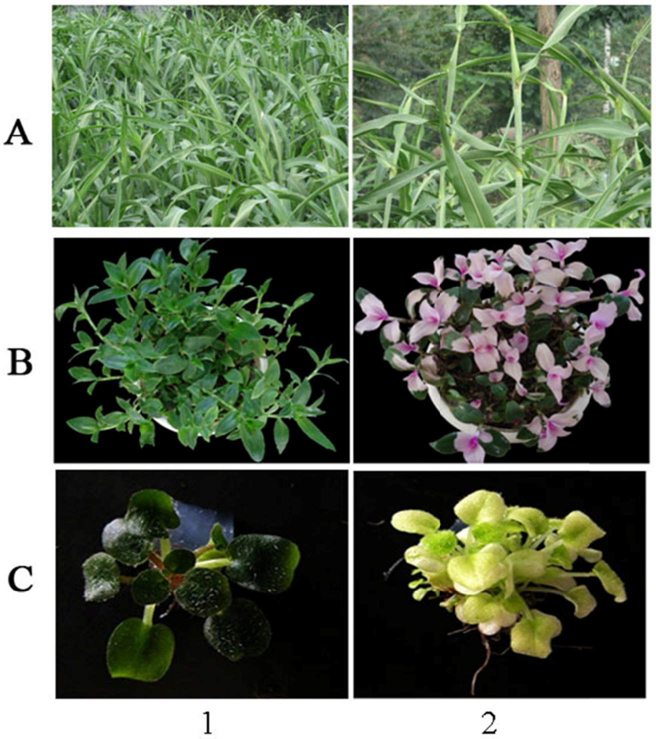

Figure 2. The mutants isolated by heavy ion beam irradiation at IMP

Note. A is sweet sorghum, B is *Wandering Jew*, C is *Saintpaulia ionahta*. The first columns are wild type plant, and the second columns are mutant plant, respectively.

Furthermore, the mutants of wheat, angelica, sweet sorghum and *Wandering Jew* have been identified by the Gansu provincial variety Approval Committee after regional trial and production test for several years, resulted in achieving economic benefits for local governments and enterprises. At present, Longfu No. 2, the new variety of spring wheat, was successfully bred by heavy ion beam mutation breeding techniques at IMP, which has extended the planting filed of 5.5×10^5 ha during the past several years with excellent agronomic characteristics, resulted in increasing the yield of 9.9×10^4 t. White-flowered *Wandering Jew* is a perennial evergreen herb. However, the mutant induced by carbon ion irradiation has been seasonally exhibited green or pink-variegated leaf and maintained by vegetative propagation since 2007. Sweet sorghum is a potential useful energy crop characterized by a high photosynthetic efficiency and a high biomass- and sugar- yielding crop. After irradiation by heavy ion beam, an early-maturity mutant was acquired and the growth period had stably shortened for around 20 days in sweet sorghum, which is expected to solve the difficult problem of earlier frost during industrialization plant for bio-ethanol production using sweet sorghum as feedstock in the northwest region of China. To date, IMP has established industrial chain based on circular economic to develop yeast products and good fodder for sweet sorghum.

5. Conclusions and Perspectives

Ion beam is an excellent tool for mutation breeding to improve horticultural and agricultural crops with high efficiency, which showed a high mutation rate without severe growth inhibition at relatively low doses (Abe et al., 2007). At IMP, we have carried out mutation breeding of many plants using various ion types, including $^{12}C^{6+}$, $^{14}N^{7+}$,$^{16}O^{6-}$, $^{40}Ar^{15+}$, and so forth (Table 1). Many biological endpoints were investigated ranging from growth and survival, chromosomal aberrations to homologous recombination. The results revealed that different biological effects were discovered from various plant materials, such as model plant, energy plant and medicinal plant. As shown in Table 2, a lot of mutants were obtained to enhance economic benefits and elucidate mechanism of plant mutation breeding by heavy ion beam. In addition, a large number of mutants were also isolated from maize, cotton, castor and pelargonium induced by heavy ion beam irradiation, respectively. In the future, our research will further focus on transformation of scientific and technological achievements and mutagenic mechanism of heavy ion beam on high plant at the molecular level.

Acknowledgements

The authors thank the accelerator crew of the HIRFL at IMP and our colleagues in the Radiobiology Department for their helpful suggestions and unreserved cooperation. This work was supported by the STS project (KFJ-EW-STS-086), Western Light Co-scholar (29Y406020) Program and the item of Xingjiang Province supported by Science and technology (2BY40604) of the Chinese Academy of Sciences.

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