イオンビーム育種の意義と期待

誌名	Gamma field symposia
ISSN	04351096
巻/号	40
掲載ページ	p. 15-20
発行年月	2003年2月

Gamma Field Symposia, No. 40, 2001 Institute of Radiation Breeding
NIAS, Japan

SIGNIFICANCE AND EXPECTATIONS OF ION BEAM BREEDING

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Introduction

"Ion beam breeding" is one of mutation breeding using ion beams as a new mutagen instead of X-rays, γ-rays and neutrons that have been predominantly used for the radiation breeding. We prefer to use the term "ion beam breeding", which might make a modern impression, although the term "heavy particles" is generally applied for medicine.

It was already known in 1950's that the ion beams, as its linear energy transfer (LET) increases, induce higher biological effects such as lethality, chromosomal aberration, etc. compared with X-rays and γ-rays of low LET radiation. In 1960's, fundamental studies on ion beam-induced mutation of plants were vigorously performed in USA. However, the use of ion beams was declined without any discovery of special features of ion beams. The late Dr.T.C. Yang still continued the ion beam study in 1970's. In 1980's basic studies were systematically carried out for the heavy particle therapy by using large accelerators that had mainly been utilized for nuclear physics. These studies allowed further understanding of ion beam-induced biological effects. Nevertheless ion beam application for the plant breeding was hardly progressed. The research program for the advanced radiation technology was planned in 1987 by JAERI taking a leading part, and Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) was completed in 1993 as a first facility in the world for the exclusive use for materials science and biotechnology. This led really basic research of the ion beam application for the plant breeding on the basis of collaboration between universities and national institutes. From these studies, the characteristic of ion beams as a new mutagen has been gradually elucidated, and novel mutants that could be never induced by other means have been isolated. It might be premature to say, but here I dare to address the characteristics of ion beam breeding and its expectation for the future.

1. *Characteristics of ion beam-induced mutation*

In order to figure out a special feature of ion beams as an effective mutagen, it is necessary to compare the mutation spectrum induced by ion beams with that by the low LET radiation such as γ-rays, but these studies have never been carried out. Nagatomi et al. (1995) first showed the most important data, i.e. the mutation spectrum of flower color by using chrysanthemum cv. Taihei (pink color) (Table1 and Fig.1A). The explants of leaf and floral petals incubated in agar medium were irradiated with 220 MeV carbon ions. After irradiation, the culture was transferred to a new medium to induce callus. The mutation rate of the regenerated plants from the callus was compared to that from γ-ray irradiation. The flower color mutation frequency induced by carbon ions was approximately half of those induced by γ-rays. The flower color mutants induced by carbon ions, however, showed complex and stripe types that were never obtained by γ-rays, other than single

Table 1. Comparison of flower color mutation spectrum on chrysanthemum

Mutagen	Frequency (%)					
	White	Light pink	Dark pink	Orange	Yellow	Complex/stripe
Control	0	0.3	0	0	0	0
γ-rays	0	27.3	2.1	0	0	0
C ions	0.3	4.6	0.3	0.3	0.3	10.2

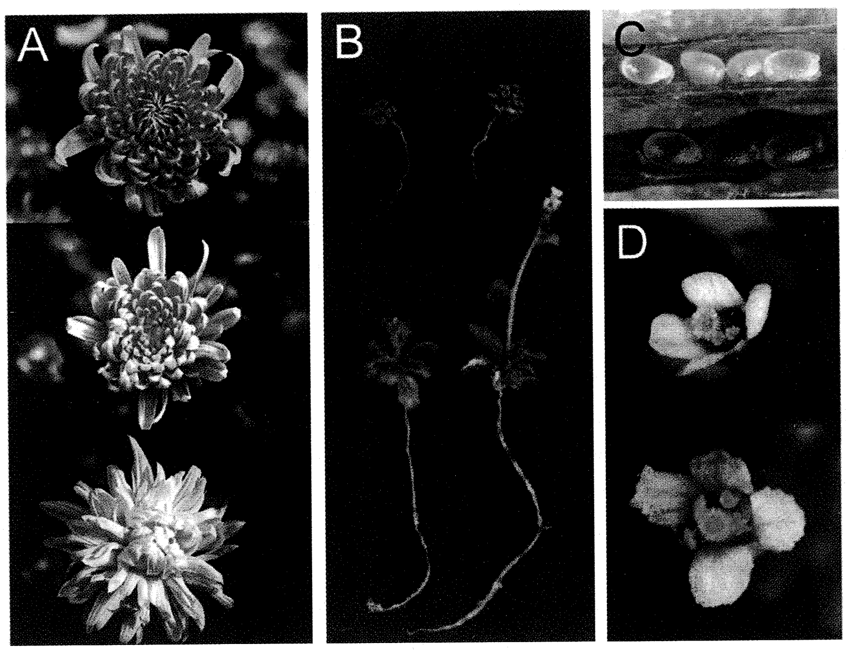

Fig. 1. Representative novel mutants induced by ion beams. A: Flower of chrysanthemum cultivar Taihei (top) and its mutants of complex color (middle) and striped color (bottom). B: One-month-old Arabidopsis plants of wild type (top) and *uvi4* (bottom) under high UV-B (11~13 kJ/m^2/day) irradiation. C: Six DAF immature Arabidopsis seeds of wild type (top) and *anthocyanin spotted testa* mutant (bottom). D: Flower of Arabidosis wild type (top) and *frill* mutant (bottom).

color such as white, light pink, dark pink, orange and yellow. On the contrary, the most of flower color mutants induced by γ-rays were light pink and a few were dark pink color. Thus, mutation spectrum of flower color induced by ion beams is wide and novel mutation phenotypes can be inducible. Recently, Okamura et al. (2001) also investigated mutation spectrum of flower color and form with explants of carnation. In carnation variety Vital (spray type, cherry pink flowers with frilly petals) tested, flower color mutants such as pink, white and red were obtained by X-ray irradiation, whereas the color spectrum of the mutants obtained by carbon ion irradiation was wide such as pink, light pink, salmon, red, complex and striped types. The difference of mutation spectrum between ion beams and low LET radiation, which was remained unclear for almost forty years long, was elucidated for the first time.

In order to estimate mutation frequency of a gene locus induced by ion beams, we used Arabidopsis three mutant phenotypes, i.e., transparent testa (*tt*) which seed coat is transparent because of pigmentless, glaborous (*gl*) which has no hair on their leaves and stems, and long hypocotyl (*hy*) which hypocotyl is longer than wild type in the light condition. Mutation frequencies per locus per diploid cell per dose for carbon-ion induced *tt*, *gl*, and *hy* were 2.6×10^{-6}, 1.9×10^{-6}, and 2.3×10^{-6}, respectively, and for electron induced *tt*, *gl*, and *hy* were 0.08×10^{-6}, 0.25×10^{-6}, and 0.14×10^{-6}, respectively (unpublished data). Thus, mutation frequencies by carbon ions (LET= 113 keV/μm) were 8-fold to 33-fold higher than those by electrons (LET= 0.2 keV/μm). Furthermore, new *tt* loci (tentatively named as *ttA* and *ttB*) were unexpectedly found from complementation analysis, even though many *tt* loci (*tt1*~*tt16* and *ttg1*~*ttg2*) had ever been found by several research groups.

In an attempt to isolate novel mutants by means of ion beams, UV-B resistant Arabidopsis plants were screened. Because many UV-B sensitive mutants have been isolated in Arabidopsis by chemical mutagen, X-rays, etc., but there has been no report of mutants in any higher plant that is resistant to UV light by gain of repair ability. Four kinds of UV-B resistant mutants, *uvi1*~*uvi4*, have been found successfully in 5,100 M2 families derived from 1,280 M1 irradiated seeds (Fig.1B). The fresh weight of *uvi1*, *uvi2* and *uvi4* after one month under UV-B radiation was 2-fold higher than that of wild type, and 1.5-fold higher in the *uvi3*. From DNA repair analysis, *uvi1* showed very high abilities of both photoreactivation and dark repair (Tanaka et al., 2002). Other than UV-B resistant mutants, several novel mutants such as *anthocyanin spotted testa* mutant (Fig.1C) that accumulates anthocyanin in testa (Tanaka et al, 1997), *frill* mutant (Fig.1D) that has serrated petals and sepals (Hase et al, 2000), and so on have been isolated from 1,488 M1 seeds irradiated with carbon ions. These facts indicate the distinctive effectiveness of ion beams as a new mutagen.

It is mentioned that lethality and mutation efficiency become maximum at the LET around 100 keV/μm in the experiments using mammalian cells. In contrast, these biological endpoints showed maximum at about 200~400 keV/μm of LET in Arabidopsis, expecting that the mutation frequency will still become several-fold higher at the best condition. It is not clear why maximum values of biological effectiveness shifts to higher LET in plants, but this feature should give an advantage over plant breeding.

2. *DNA damage and mutation induction by ion beams*

It is obvious that double-strand DNA breaks induced by ion beams are less reparable than those by γ-ray irradiation. Because ion beams deposit high energy on a target densely and locally as opposed to low LET radiation, it is well suggested that ion beams predominantly induce complicated damage (clustered damage) including double-strand DNA breaks with damaged end groups whose reparability would be low. Therefore it is possible that ion beams could cause lethality and mutation at higher frequency compared with low LET radiation. However, the molecular nature of mutation induced by ion beams has never revealed. In order to investigate the mutation at the molecular level, a PCR analysis and a sequence analysis using carbon ion-induced Arabidopsis *tt* and *gl* mutants were carried out. According to the PCR analysis of 26 mutants, DNA fragments were successfully amplified in all combinations of primers in the 13 mutants, indicating that large structural alterations were not generated in these mutations. In the rest of the mutants, however, DNA fragments were not completely amplified in all or some combinations of primers, indicating that intergenic large mutation such as inversion, translocation or deletion were generated. Thus, the PCR analysis indicates that a characteristic of the carbon ion-induced mutations is that large structural alterations are induced with similar frequency to point-like mutations. The sequence analysis of three carbon ion-induced mutations (*gl1-3*, *tt4*(C1) and *ttg1-21*) revealed that inversions or translocations were accompanied with short deletions at the breakpoints (Shikazono et al., 2001). Moreover, the inversion or translocation was found to be originated from recombinations between short homologous ends, suggesting that the nonhomologous end-joining pathway operates after plant cells are exposed to ion beams. It was also found that most of the carbon ion-induced point-like mutations contained a short deletion of 1 to 100 bp. From this study, it is hypothesized that the characteristics of the mutation induced by ion beams are not only high mutation rate but also high frequency of inducing null mutations at a single gene.

3. *Expectations of the ion-beam breeding in plants*

Ion beams as a new mutagen is expected for making new commercial varieties even in plants for which recombinant DNA techniques can not be applied. Plant breeding using recombinant DNA technique is predominantly carried out in USA at present. The recombinant DNA technique and radiation breeding technique should be complementary to each other, but we might depend on radiation for practical breeding, because of repulsion of consumers for the recombinant DNA crops. Ion beam breeding, as an original technique of our country, is useful for activation of domestic breeding and strengthening of international competitive power. On the other hand, the 21st century is a time that many problems generated in the former century such as global warming and environmental pollution should be resolved, and also a time that a food crisis by population growth should be feared. Therefore, ion beam breeding in plants is strongly expected for conservation of global environment and security of food resources.

Apart from plant breeding, the ion beam-induced mutant is expected to be used for the isolation and functional analysis of the corresponding gene in the post-genomic age. Although new technique of direct gene isolation from ion beam-induced mutant is not still developed, it will be possible to isolate quickly the mutant gene from total genome by the combination with microarray technique on the basis of the characteristic that ion beams can easily induce deletion type of mutation. Thus, Ion beams are expected to become a useful tool for both the practical breeding and the basic gene analysis.

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イオンビーム育種の意義と期待

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イオンビームのような高 LET 放射線では，致死効果や染色体異常などの生物効果が γ 線のような低 LET 放射線よりも大きくなることが 1950 年代にすでに知られていた。しかし，1993 年に世界初の材料・バイオ研究専用施設である TIARA が完成し，学官の協力のもとに本格的な基礎研究が開始されるまで，変異原としてのイオンビームの特徴は不明だった。この 40 年を超す沈黙を破ったのは，永冨らによるキクの花色変異スペクトルの比較解析研究と田中らによるシロイヌナズナでの新規突然変異体の作出研究であった。これらの研究によって，新しい変異原としてのイオンビームの特徴が明らかになり，「イオンビーム育種」とも呼ぶべき新しい育種技術が誕生した。この育種技術の特徴は，γ 線よりも突然変異率が 1 桁以上高く，突然変異スペクトルが格段に広いために，これまで作出することが困難であった変異体を得ることが出来ることである。キクにおいては γ 線では作ることが困難な複色や条斑の変異体が高効率で誘発されることが分かった。また，シロイヌナズナでは新規の tt 変異体や ast 変異体などが発見され，色素合成系に係る新たな遺伝子の発見につながった。それればかりでなく花弁の形態異常である frill 変異体が発見され，形態形成に係る貴重な遺伝子の同定につながった。更に，意図的に行った紫外線耐性変異体の作出の試みでも，見事に性質の異なる 4 株の uvi 変異体を作出することに成功した。このようにスペクトルが広いことに加えて，イオンビーム照射で得られた変異体には，付随する不要な変異がほとんど見られないという特徴がある。戻し交雑を繰返す必要がないという点でこの特徴は実用的な品種を確立する上で大きなメリットとなる。このようなイオンビームで起こる突然変異の特徴は，イオンビームが DNA に与える損傷の数は少ないけれども，欠失，転座，逆位などの形で高い確率で変異を誘起することによるのであろう。この推定を支持する DNA 解析データが蓄積されつつある。

イオンビーム育種に対する期待は，遺伝子組換え技術が使えない植物にも利用できることである。しかし，遺伝子組換え作物に対する消費者の強い反発を受けて，遺伝子組換え技術が停滞を余儀なくされている現状では，実用的な育種は放射線に頼らざるを得ないだろう。我が国の独自技術であるイオンビーム育種技術は，遺伝子組換え技術に代わって，国内のアグリバイオ産業の活性化や国際競争力の強化に役立つことが期待される。また，イオンビームでは欠失型の変異体が誘発されやすいという特徴があるから，それを活かしてポストゲノムの遺伝子機能解析など学術的な分野においても大いに貢献することが出来るだろう。