Induced mutations in plant breeding and biological researches in Japan

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Abstract

Two hundred and forty two direct-use mutant varieties generated by using irradiation, chemical mutagenesis and somaclonal variations, have been registered in Japan. About 61% of these were induced by Gamma-ray irradiation, largely due to successful collaboration with the Institute of Radiation Breeding. This high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars. Some mutant cultivars of Japanese pear exhibiting resistance to diseases induced by Gamma-ray irradiation and development of a unique bioassay by using toxins of fungi was discussed. In addition, 228 indirect-use (hybrid) mutant varieties primarily generated in rice and soybean have found value as parental breeding germplasm resources in Japan. In 2005, two direct-use cultivars and 97 indirect-use cultivars of rice contributed approximately 12.4% of the total area of rice cultivation in Japan. The semi-dwarf gene (*sd-1*) generated in rice is perhaps one of the most significant contributions. For soybean, similar Gamma-ray induced mutants comprised nearly 9.4% of the total cultivation area of soybean in Japan. Molecular genetic studies focused on genome sequencing have become an extremely powerful tool for identifying the genes and for selecting mutants exhibiting specific phenotypes. It is anticipated that molecular genetic interaction will complement gains in mutation breeding on a dramatic scale. Chronic irradiation in the Gamma Field is also considered to be a useful tool for generating mutant resources for future molecular studies especially in rice, and expand its use into the other graminaceous crops which have genomic synteny to rice. There are interesting reports concerning mutations in rice, such as low glutelin content, in which the size and location of deletions and the mechanisms and phenotypes of low glutelin content were elucidated. Chronic irradiation in the Gamma Field is useful to generate mutant resources for molecular researches.

Introduction

After the construction of the Gamma Field, now considered the worlds largest radiation field (**Fig. 1**, 100m radius with an 88.8 TBq ^{60}Co source at the center), the Gamma Room and the Gamma Greenhouse in the Institute of Radiation Breeding (IRB) in 1960's, mutation breeding was accelerated by cooperative research with national and prefectural breeding laboratories, private companies and universities in Japan [1].

In *The New York Times* (*In* "Useful Mutants, Bred With Radiation" by William J. Broad, August 27, 2007), Dr. P. J. L. Lagoda of the Joint FAO/IAEA was quoted to say, "Spontaneous mutations are the motor of evolution. We are mimicking nature in this. We're concentrating time and space for the breeder so he can do the job in his lifetime. We concentrate on how often mutants appear - going through 10,000 to one million - to select just the right one."

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Figure 1 Gamma Field of IRB

The concept and objectives of the IRB's Gamma Field has the same goals for the plant breeder. The facility is used to artificially induce mutations at a higher frequency than it occurs in nature. The radiation dose at the nearest point of the field (10m from the center, ca. 2 Gy/day) is estimated to be about 300,000 times that of normal and natural background radiation. At the farthest point (100m from the center, ca. 0.01 Gy/day), the radiation dosage is about 2,000 times that of normal background radiation. This means that growing plants at the nearest point to the Gamma-ray sources are being treated to a 1,000 year's of accumulated normal background rates of radiation per day. Although we do not know all the genes or mechanism of mutations, radiation breeding has produced many useful mutant cultivars and contributed greatly to the farmers and industries of Japan.

In 1991, the Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan launched the Rice Genome Research Program (RGP), with the aim of fully decoding the rice genome in three phases over a 21-year period. With the cooperation of 10 participating countries [2], the genome sequencing of 12 rice chromosomes was completed in 2005 [3]. Following this achievement, molecular genetic studies based on the results of the genome sequencing project became the most powerful tool for selecting mutants of certain characteristics in rice. This is anticipated to revolutionize mutation breeding success in rice, and become applicable to a number of other important crop species.

In this report, the mutant cultivars developed mainly by Gamma-rays are discussed. In addition, their economic impacts in Japan, as well as molecular studies performed to elucidate the mutation at the DNA level are described.

Mutation breeding and released cultivars in Japan

In a 2007 search regarding the number of induced mutation varieties in the IAEA database, China is first in the number of described induced

mutation varieties at 638; India is second listing 272 varieties; and Japan is third with 233 varieties. The total number of mutant cultivars, including direct-use mutant cultivars and indirect-use cultivars, exceed these totals. A selection of mutant cultivars developed in Japan, including the economic impact of these cultivars, and their characteristics are reviewed here.

The number of cultivars developed by mutation breeding

Figure 2 shows the number of direct-use and indirect-use (hybrid) mutant cultivars registered in Japan in each five-year period from 1960 to 2005. The number of direct-use cultivars had been rapidly increasing until 1995, when 67 cultivars were registered in five years (about 13 cultivars per year). This number fell from 2001 to 2005, with only 41 cultivars being registered (about 8 cultivars per year). The number of indirect-use cultivars primarily generated in rice has steadily increased over time and 68 cultivars were registered from 2001 to 2005. This number can be increased if agronomically useful, direct-use cultivars, such as "Reimei" with the *sd1* dwarf gene for rice are developed.

Two hundred and forty two direct-use mutant cultivars comprising 61 species generated through irradiation utilizing Gamma-ray, X-ray and ion beams, chemical mutagenesis and in vitro culture (somaclonal variation), have been registered and released in Japan (**Fig. 3**). More than 61% of these were induced by Gamma-ray irradiation and those induced by somaclonal variation and chemical mutagen, not including those with double chromosome numbers through colchicine treatment, are 15.7% and 6.6%, respectively. Recently, the development of mutant flower cultivars, generated by ion beam irradiation, has been a growing area of mutation induction in Japan.

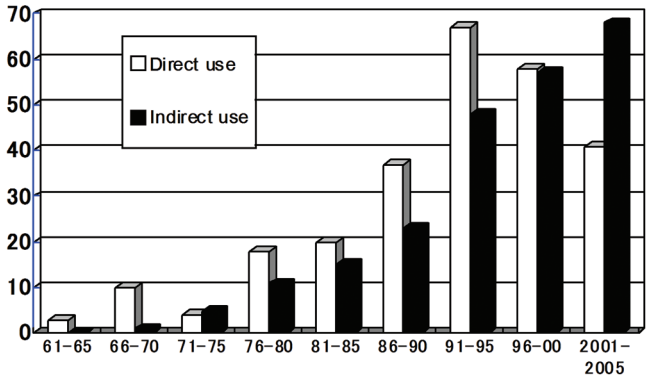


Figure 2 Number of cultivars developed by mutation breeding in each 5-year period from 1961-2005. Total number of direct use cultivars is 212 and that of indirect use cultivars is 230 [4].

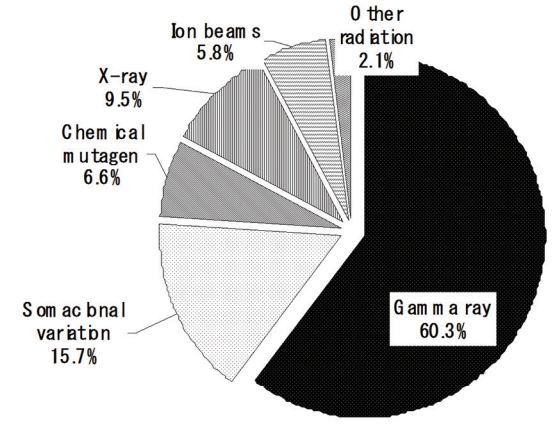


Figure 3 Percentage of total 242 cultivars developed by mutation breeding by using various kinds of methods in Japan (2008). Chemical mutagen does not include colchicine [4].

Table 1 shows the number of registered mutant cultivars of some crops developed by radiation, Gamma-rays, and those irradiated at the IRB, NIAS [4]. There are 50 mutant cultivars of chrysanthemum, 31 of rice, 16 of soybean, 10 of rose, etc. Among them, 100 cultivars have been generated at the IRB and these contributions of the IRB regarding the development and release of superior mutant induced cultivars has been extensive. This high percentage of Gamma-ray irradiated mutants indicates that mutation breeding via Gamma-ray irradiation is an effective and highly successful approach for the generation of commercial cultivars.

The first mutant rice cultivar is "Reimei," which means "dawn" in Japanese, was the first irradiation induced mutant cultivar that illustrated the potential of utilizing Gamma-rays for breeding improvements in Japan. Reduction of plant height, including dwarfism and semi-dwarfism is one of the characteristics that can be induced with high frequency by irradiation and can be easily detected in the field. "Reimei," registered in 1966 [5] was a successful case of an irradiation induced semi-dwarf mutant. This cultivar exhibits a mutation of the *sd-1* locus [6] and shows a culm 15cm shorter than the original cultivar "Fujiminori." The semi-dwarf is associated with the high-yielding ability and recorded the highest yield in Japan in 1967 [5].

Table 1. Number of registered mutant cultivars developed by radiation, Gamma-rays, and those irradiated in the Institute of Radiation Breeding, NIAS [4]

	Mutant cultivars[1]	Radiation	Gamma-rays	IRB[2]
61 Crops	242	188	146	100
Rice	31	14	12	11
Wheat	4	2	2	0
Barley	4	4	3	0
Soybean	16	16	15	9
Chrysanthemum	50	46	32	29
Rose	10	7	7	6
Sea pink (Limonium)	6	6	6	0
Cytisus	8	8	8	8
Apple	2	2	2	2
Japanese Pear	3	3	3	3
Others	108	80	56	32

[1] Total number of mutant cultivars developed by radiation (Gamma-ray, X-ray and ion beams), chemicals (Excluding colchicine treatment), somaclonal variation
[2] Number of mutant cultivars irradiated in the Institute of Radiation Breeding (IRB)

Table 2. Number of indirect use mutant cultivars in Japan (2008)

Rice	Wheat	Barley	Soybean	Tomato	Others	Total
198	3	7	9	3	7	228

In Japan, the total number of indirect-use mutant cultivars is 228, which includes 198 rice, 9 soybean, 7 barley, 3 wheat, 3 tomato, 4 lettuce, 1 eggplant, 1 Japanese lawngrass, 1 mat rush, and 1 mushroom cultivar in 2008 (**Table 2**). Interestingly, among the 198 indirect-use mutant cultivars in 2008, 89 cultivars (44.9%) were derived from the "Reimei" or its offspring. This suggests that agronomically useful mutations can be utilized as parental lines to develop new varieties with this characteristic and transferred efficiently to the farmers' field.

The Economic impact of mutant cultivars in Japan

Figure 4 shows the increase of mutant rice cultivars, which were derived from mutants generated by Gamma-rays, planted in farmers' fields in Japan since 1960. "Reimei" was first cultivated on 61,598 ha in 1968, (http,//ineweb.narcc.affrc.go.jp/). The number of mutant cultivars has

been increasing and 99 mutant cultivars (2 direct-use and 97 indirect-use cultivars) were in cultivation in 2005 [4].

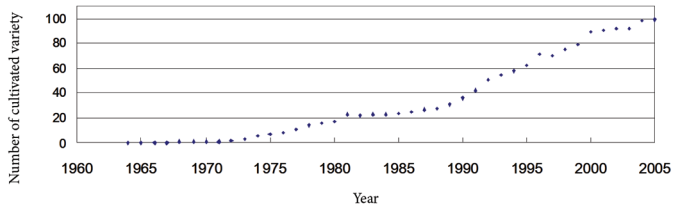


Figure 4 Total number of mutant rice cultivars, which are derived from mutants generated by Gamma-rays, cultivated in farmers' field from 1960 to 2005 in Japan [4].

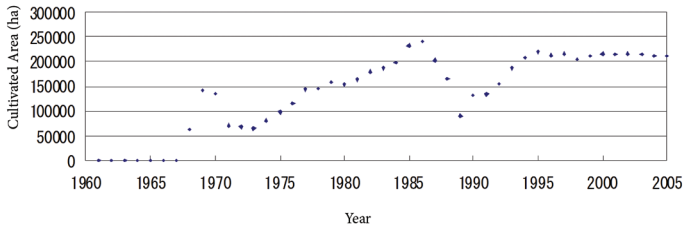


Figure 5 Total areas of mutant rice cultivars, which are derived from mutants generated by Gamma-rays, cultivated in farmers' field from 1960 to 2005 in Japan [4].

Figure 5 shows the total cultivated field of the mutant cultivars, which are derived from mutants generated by Gamma-rays, from 1961 to 2005. This increased after "Reimei" was released for cultivation in 1968. The peak use of mutant induced cultivars reached 250,000 ha in 1986 and was slightly more than 200,000 ha from 1994 to 2005. In 2005, the total cultivated area of mutant cultivars was 210,692 ha, which is 12.4% of total cultivated area of paddy rice (1,702,000 ha) in Japan [4].

The total crude income of farmers selling the brown rice of mutant cultivars also has been increasing as the increase of the cultivated area, although the price of the grain is different in each year. The amount of total income is estimated to be approximately 250 billion Yen (2.34 billion US dollars) in 2005 [4]). The mutant cultivars, which are derived from mutants generated by Gamma-rays and have been cultivated on more than 5,000 ha from 2001 to 2005, are the following 17 cultivars, "Kinuhikari (263,223ha)"; "Haenuki (219,734ha)"; "Tsugaru-roman (106,423ha)"; "Yume-akari (66,491ha)"; "Yume-tsukushi (58,893ha)"; "Aichi-no-kaori (53,697ha)"; "Asahi-no-yume (51,049ha)"; "Mutsuho-mare (46,959 ha)"; "Dontokoi (17,008ha)"; "Yume-shizuku (14,076ha)"; "Mine-asahi (10,698 ha)"; "Yume-hitachi (10,440ha)"; "Yume-minori (9,957ha)"; Aki-geshiki (7,510ha)"; "Aki-roman (7,450ha)"; "Miyama-nishiki (7,242 ha)"; and "Tsukushi-roman (5,533 ha)." The mutant cultivars, which have been cultivated in more than 100000ha of farmers' fields are the following 5 cultivars, "Akihikari (1,410,810ha)"; "Reimei (886,188ha)"; "Kinuhikari (263,223ha); "Haenuki (219,734ha); and "Tsugaru-roman (106,423ha)." Among them, "Reimei" is a direct-use mutation cultivar and the others are indirect-use cultivars [4].

There are 16 direct-use mutant cultivars of soybean registered in Japan since "Raiden" and "Raikou" were developed by Gamma-ray irradiation in 1960. The improved characteristics were early-maturity and late-maturity, yellow hilum, seed-coat color, short-stem, and the number of pods/stem, lipoxygenase-free, low allergen etc. Among them, one cultivar was induced by X-ray and the other 15 were induced by Gamma-rays. The number of indirect-use cultivars is 10. The total cultivated area of mutant cultivars cultivated in the farmers' fields came to 13,283 ha (9.4% of total cultivated area (142,000ha) of soybean in Japan in 2005) and total farmers' crude income was 5.56 billion Yen (ca. 52 million US dollars) [4]. As a result, economic impact of mutant cultivars is huge in Japan.

Some useful mutant varieties by using various screening methods.

Rice

Although rice is not a high protein grain crop, the protein content is ca. 7% when the white rice is cooked. A mutant line with a low content of gulutelin was obtained from the ethyleneimine (EI) treatment to "Nihonmasari." The "LGC-1" was developed from back-crossing this mutant with the original "Nihonmasari" to eliminate undesirable characteristics, such as semi-sterility and semi-dwarfism [7]. The seed protein of the "LGC-1" is composed of mainly of a low amount of digestible glutelin and high amount of indigestible prolamine. This construction of protein is disadvantageous for the digestion of rice grains in humans, though the total amount of protein is mostly similar to the original cultivar. As a result, the "LGC-1" is useful as "low protein rice," and some clinical trials on patients with kidney disease indicate that the variety is a useful and effective daily food for such patients [8]. The defect of the "LGC-1" is its eating quality, and there are the other loci that control the biosynthesis of digestible protein, such as globulin. Therefore, Nishimura, *et al.* [9] induced a mutant named "89WPKG30-433" with a deficiency in globulin from the leading Japanese cultivar "Koshihikari" through Gamma-ray irradiation. They hybridized it with the "LGC-1" and selected "LGC-Katsu" and "LGC-Jun" from the hybrids, whose globulin content was as low as the "LGC-1," where the globulin content is zero. The total digestible protein content tested to about 30% of ordinary rice. As the eating quality is highly improved and digestible protein content is lower than "LGC-1," these two cultivars will greatly help in the dietary management of proteins with chronic renal failure.

Soybean

Takagi [10] identified two major genes, which control radio-sensitivity, in some soybean varieties. When the 50% reduction rate (RD_{50}) of root length was determined with acute irradiation to the seeds or the chronic irradiation to the plants for the entire growth period, radio-sensitivity of a sensitive cultivar, "Shinmejiro," is more than twice that of the resistant variety, "Tachisuzunari." The differences in radio-sensitivity between the varieties to the chronic irradiation in the Gamma Field were controlled by a single recessive gene, *rs1*. Besides, the second recessive gene rs2, which was discovered in "Goishishirobana," whose activity is only expressed following acute seed radiation.

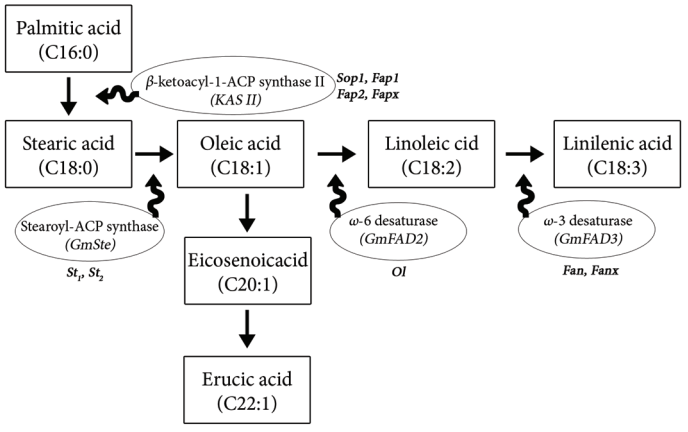


Figure 6 Metabolic pathway and key genes of fatty acid in soybean [13] (courtesy of Prof. Y. Takagi).

Soybean is the most widely used source of edible oil for human con-

sumption. Fatty acids of soybean seeds consist of palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid (**Fig. 6**). Altered unsaturated fatty acid content (elevated oleic acid and reduced linolenic acid) increase the oxidative stability that provides health benefits and improvement of fatty acid contents. This has been one of the most important breeding objectives of soybean. As natural genetic diversity in soybean is limited, mutation induction is one effective approach to induce modification. Through the use of X-rays or chemical mutagens, mutants with different fatty acid compositions, such as reduced and elevated palmitic acid, elevated stearic acid, elevated oleic acid (50%) , and reduced linolenic acid (3%) content were isolated and found to be controlled by major genes (**Fig. 6**; [11-13]).

Soybean seed has three lipoxygenases called *L-1*, *L-2* and *L-3*, respectively [14]. The lipoxygenases are the main factors of the grassy-beany flavor of the products. Soybean lines lacking each of the three genes have been developed. However, no line lacking all three genes has been obtained because of tight linkage between *L-1* and *L-2* [15]. The F_2 seeds derived from a cross between a line without *L-1*, *L-3* and a line without *L-2*, *L-3* were irradiated with Gamma-rays. After surveying 1,813 M3 seeds by using SDS/PAGE, one mutant seed lacking all *L-1*, *L-2*, and *L-3* was selected [16]. A new cultivar "Ichihime" with this unique characteristic was registered and released in 1994 [17].

Italian ryegrass

Mutation breeding has been mainly established in seed propagated, self-pollinated species. Although several methods have been widely used for the screening of mutants in self-pollinated species by the single-seed descent approach [18,19] and by single seed descent (one-plant-one-grain method, Yoshida [20]), these methods have not been applied to cross-pollinated species. Ukai [21] developed a new method for obtaining mutants of cross-pollinated species efficiently in a temperate forage grass, Italian ryegrass (*Lolium multiflorum* L.). The method was called the "Crossing-within-Spike-Progenies Method." This method is composed of 1) taking seeds separately from each spike from a population of plants irradiated with Gamma-rays, 2) sowing the seeds in a hill plot as a spike-progeny, 3) isolating each hill from others at the time of flowering and allowing the open-pollination of plants within hills, and 4) taking seeds from each of the hills and sowing the seeds in hill progenies for the screening of mutants. This procedure is repeated each year. When 300 Gy of Gamma-ray was irradiated to the seed, the frequency of chlorophyll mutations was approximately 70.6% per hill progeny and 1.87% per plant. In contrast, open-pollinated populations exhibited that only 10% per progeny and 0.12% per plant, respectively. This method will be applied to the other wind- or insect-pollinating outcrossing crop species.

Chrysanthemum

In general, it is very difficult to isolate mutants from mutation sectors in vegetatively propagated crops although the maintenance of mutant genotypes is easier than the seed-propagated species. It has been shown that the combined method of chronic Gamma-ray irradiation and tissue culture is very effective in solving this problem. By tissue culturing the floral organs of chrysanthemum (*Chrysanthemum morifolium* Ramat.) plants chronically irradiated in the Gamma Field from the seedling to the flowering stages, many non-chimeric mutants, with various flower colors and shapes, are obtained [22]. From these mutant lines, 10 cultivars were registered. The technology, given the term "radiobiotechnology," is not only effective in obtaining non-chimeric mutants but also effective in producing high mutation frequencies. The method has been utilized to induce mutations in various vegetatively propagated crops and many mutant cultivars have been registered.

Japanese pear and apple resistant to Alternaria disease

A popular cultivar of Japanese pear (*Pyrus serotina* Rehd. var. *culta* Rehd.), "Nijisseiki," which was a leading variety, occupied 28% of the total cultivated area of Japanese pear in 1990 in Japan. The cultivar, however, is highly susceptible to the black spot disease, *Alternaria alternata* (Fr.) Keissier (= *Alternaria kikuchiana* Tanaka), one of the most serious diseases of pear [23]. Growers are required to spray fungicides several times during the growing season to counter the disease. To induce mutations resistant to the disease by Gamma-ray irradiation, small plants of the cv. "Nijisseiki" were planted at every 4 meters from 37 m to 63 m from the ^{60}Co source in 1962 and chronic Gamma-ray irradiation was applied (30 x 10-2 Gy - 4 x 10-2 Gy/day) in the Gamma Field [24]. In 1981, nearly 20 years after the planting, a twig without the symptom of the disease was found in a plant planted at a distance of 53 m from the irradiation source. As it was ascertained that there was no difference in other agronomic characteristics between the mutant and the original variety except for the resistance to the black spot disease, it was registered and released in 1991 with the name "Gold Nijisseiki"[24]. It was registered as the same name in Australia in 2004 (Certificate Number 2533).

Dr. Sanada, one of the breeders of this cultivar, mentioned, "The situation of mutation breeding on fruit trees has been severely criticized because there have been no successful results." Although it took them nearly 20 years to identify a useful mutation and 30 years for the registration, the release of "Gold Nijisseiki" is a monumental achievement for the Gamma Field.

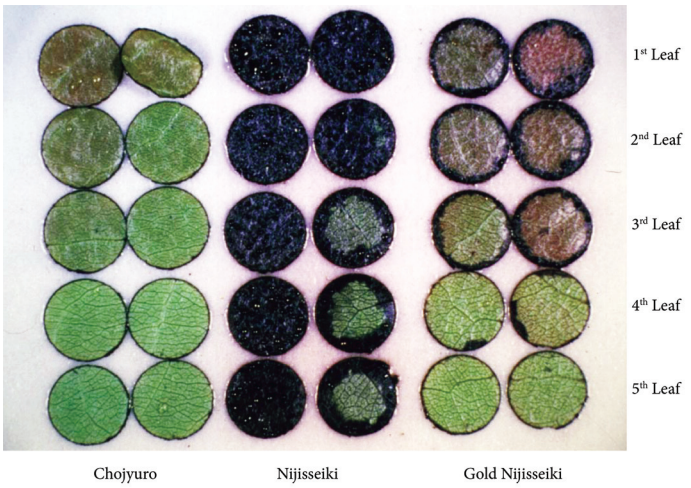


Figure 7 Bioassay of resistant to the black spot disease by using the AK-toxin obtained from the culture of the fungus. Upper to lower leaf disc(1 – 5) means 1 (young) to 5 (older) leaf; cv."Chojyuro", highly resistant; cv. "Nijisseiki", highly susceptible; cv. "Gold Nijisseiki", resistant

At the same time an easy and effective method for the screening of resistance to the fungus has been developed by treating leaf discs (7 mm in diameter) by the AK-toxin produced by the fungus [25]. It was coincidental and lucky for the breeders that Nakashima, *et al.* [26,27] isolated and identified the chemical structure of the toxin named "AK-toxin" produced by the fungus of black spot disease and generating the symptom of black spots on leaves at the same time. As a consequence, the breeding group entered into a cooperative research program with the chemistry group and established this unique method. When the leaf discs are placed on the filter paper soaked with AK-toxin obtained either from the extract of the fungal body or artificial synthesis in a Petri dish, and kept for two or three days, susceptible leaves turned to black and resistant leaves stayed green (**Fig. 7**). After the development of this method, two new mutant varieties, "Osa-Gold [28,29]" and "Kotobuki Shinsui [30]" were developed in a short period of time by using this screening method. The economic effect of this research has been great [4].

These researches suggest that the breeding of fruit trees requires pa-

tience and that development of easy and precise screening methods is a very important addition to the development of methods for mutation induction.

Achievement of biological researches on mutations induced by Gamma-ray irradiation

Deletion size generated by Gamma-ray

Naito, *et al.* [31] studied the deletion sizes of transmissible and non-transmissible mutations induced with Gamma-ray and carbon ion beam irradiation by the sophisticated pollen-irradiation methods in *Arabidopsis*. It has been revealed that most mutants induced with these ionizing irradiations possess extremely large deletions (more than 6 Mbp), most of which are not transmittable to the next generation, as well as small deletions (1 or 4 bp), which are normally transmissible.

In rice, the same tendency was observed in transmissible mutants. Morita (unpublished) researched the frequency of transmission of different mutations possessing different deletion sizes as obtained with Gamma-ray irradiation in rice. Among 11 Gamma-ray induced mutants, one *GluA2* mutant exhibited 1 base pair (bp) substitution, and among 10 mutants with a deletion, the deletion size of 6 mutants, which include *CAO* (*chlorophyllide-a oxygenase*), *GA3os* (*GA3-beta-hydroxylase*), *GluA1* (*glutelin A1*), and *GluA2* (*glutelin A2*) are 1 bp deletion, and those of the other *CAO mutants* and *PLA1* (*Plastochron1*) are 3 and 5 bp deletions, respectively. Those of *GluB4/5* (*Glutelin B4/5*), two α-globulin mutants are more than 10 kbp, 15 kbp, and 90 kbp, respectively. It is very interesting that the Gamma-ray induced mutations transmittable to the next generation are primarily classified into 2 groups, the one with extremely a large deletion and the other with small deletions (1 to 5 bp). We are not sure whether or not it is very difficult to obtain mutants with medium deletion size by Gamma-ray irradiations. However, we are accumulating data to elucidate it.

Different size and location of deletion generates different kinds of phenotypes

In the course of plant evolution, genes are often duplicated in tandem, resulting in a functional redundancy. The analysis of function of these genes by developing double mutants might be difficult because they would be very tightly linked. Mutants of such tandem duplicated genes were investigated for their genotypes and phenotypes. There are reversely repeated two loci, which both codes for mRNA of glutelin production. There are various mutants that exhibit low glutelin contents isolated by SDS-PAGE [7, 32]. The mechanisms of low glutelin contents of mutants that have been studied suggest that the size and the position of deletions generate different characteristics of mutations. Some act as dominant genes or recessive genes, and those relationships between genotypes and phenotypes, etc. are provided as example below.

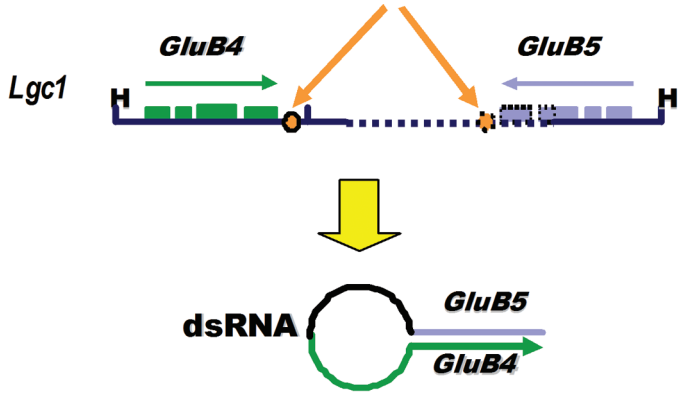


Figure 8 Mechanism of low glutelin in LGC-1 through a deletion at the transcription termination signal and produced double-stranded RNA suppress the glutelin synthesis by RNAi [33] (by courtesy of Prof. M. Kusaba, Hiroshima University).

Mechanism of low glutelin content in the "LGC-1" mutant

The *Low glutelin content* (*Lgc-1*) is a dominant mutation that reduces glutelin content in the rice grain. Glutelin is a major digestible seed storage protein encoded by a multigene family. Kusaba, *et al.* [33] reported that in *Lgc-1* homozygotes, there is a 3.5 kbp deletion between two highly similar glutelin genes that forms a tail-to-tail inverted repeat, that might produce a double stranded RNA molecule, a potent inducer of RNA silencing (**Fig. 8**). As a result, glutelin synthesis is suppressed and the glutelin content is lowered. The *Lgc-1* provides an interesting example of RNA silencing occurring among genes that exhibit various levels of similarity to an RNA-silencing- inducing gene. This was the first report that shows the mechanism of a mutation was RNAi.

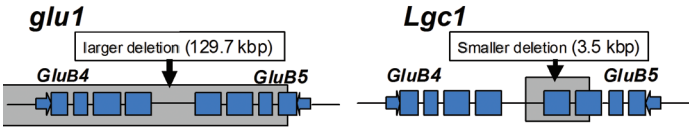
Mechanism of low glutelin content in the "glu1" mutant

The "*glu1*" is a gamma-ray-induced rice mutant, which lacks an acidic subunit of glutelin, a major seed storage protein. Morita, *et al.* [34] elucidated that the *glu1* harbors a 129.7 kbp deletion involving two highly similar and tandem repeated glutelin genes, *GluB5* and *GluB4*. The deletion eliminated the entire *GluB5* and *GluB4* gene except half of the first exon of *GluB5*. As a result, the phenotype of the *glu1* gene is a complete lack of the acidic subunit of glutelin and acts as a recessive gene for low glutelin content in rice grains (**Fig. 9**).

Conclusion

The above examples illustrate that the position and the size of deletions in the same loci have the capacity to dramatically alter the phenotype of mutants through the process of transcription and translation. The *glu1*, which has a large 129.7 kbp deletion, acts as a recessive gene, while the *LGC1*, which has 3.5 kbp deletion including probably a terminal signal of the transcript region acts as a dominant gene.

Furthermore, the *GluB5* and the *GluB4* have the same amino acid sequence in their acidic subunit, suggesting that only the mutation involving both *GluB5* and *GluB4* result in the resultant phenotype. That is the lack of the glutelin acidic subunit deleted in the "glu1" mutant. It probably is very difficult to knock out both loci by chemical treatment or transposon techniques. Sequenced plant genomes exhibited more that 14% of the genes formed tandem array [3, 35]. This finding, however, suggests that Gamma-rays can be an effective mutagen to generate knock-out mutants of both loci and to analyze tandem repeated and functionally redundant genes.



	Phenotype	Dominant or Recessive	Mechanism
glu1	Deletion of 1 acidic subunit	Recessive	Knock-out of 2 genes
Lgc1	Total glutelin contents lower	Dominant	Suppresses glutelin genes via **RNAi.**

Figure 9 Comparison of phenotype, mode of inheritance and mechanism of mutation character between glu1 and Lgc1 mutation with different size and place of deletion in the same region of 2 loci, GluB4 and GluB5 (by courtesy of Dr. R. Morita, IRB, NIAS). glu1,Morita *et al.* [34] ; Lgc1, Kusaba *et al.* [33]

Genetic studies by the useful mutations induced with Gamma-ray chronic irradiation

As the history has shown, spontaneous and induced mutation resources have played an important role not only for mutation breeding but also genetic studies and the elucidation of gene functions.

Phytochrome

Takano, *et al.* [36] have isolated *phytochrome B* (*phyB*) and *phy C* mutants from rice and have produced all combinations of double mutants. Seedlings of *phy B* and *phyB phyC* mutants exhibited a partial loss of sensitivity to continuous red light but still showed significant deetiolation responses. The responses to red light were completely canceled in *phyA phyB* double mutants. These results indicate that *phyA* and *phyB* act in a highly redundant manner to control deetiolation under red light. They also found that mutations in either *phyB* or *phyC* locus causes moderate early flowering under a long-day photoperiod, while monogenic *phyA* mutations had little effect on flowering time. The *phyA* mutation, however, in combination with *phyB* or *phyC* mutation caused dramatic early flowering. Early flowering mutants were generated by chronic Gamma-ray irradiation with dose rates ranging between 3 and 6 Gy/day [36].

Aluminum tolerance

Ma, *et al.* [37] isolated a mutant with highly sensitivity to aluminum concentration from cv. Koshihikari of japonica rice, which has an aluminum resistance [38]. The mutant was induced with chronic Gamma-ray irradiation and exhibited the same phenotype to the wild type with the absence of aluminum. That is, M_1 plants were irradiated in the Gamma Field from seven days before heading to two days after heading under 20 Gy/day for eight days. The root elongation of the mutant, however, was highly inhibited in the presence of 10 μM Al. The mutant also exhibited poorer root growth in acid soil. Genetic analysis showed that the high sensitivity to Al is controlled by a single recessive gene. The gene was mapped to the long arm of chromosome 6.

Conclusion

The Gamma Phytotron was established in Korea in 2005 and the Gamma Greenhouse, approximately doubled the size of the Gamma Greenhouse located at the IRB, Japan, was established in Malaysia in 2008. Both facilities are focused on the induction of mutation by chronic Gamma-ray irradiation to growing plants of important crop species. As described earlier in this report, chronic irradiation is a useful tool for the generation of mutant genome resources that have application toward molecular analysis as well as conventional breeding.

Conclusions

A. M. van Harten [39] describes in "Mutation Breeding -Theory and practical application,"

"An explanation for the decreasing interest in mutation breeding, at least in most "developed" countries, may be that during the past two decades attention has become more and more directed towards studying the possibilities offered to plant breeding by various new molecular technologies…As a result of these developments mutation breeding seems to have lost part of its previous attraction for young researchers."

It is not necessary to mention, however, that mutation breeding is still a very interesting and useful technology for isolating genes and for elucidating gene mechanisms and metabolic pathways in various crops.

The record has also shown that mutation induction is a very useful conventional breeding tool for developing superior cultivars. Today, site-directed mutagenesis *in vivo* or *in vitro* cell can be envisioned and many researchers are conducting programs in this direction.

New fields of science and technologies were developed on the basis of achievements of traditional or classic methods. It is highly desirable that the IRB continues their work while incorporating the new knowledge and technologies. The IRB is well equipped with appropriate facilities and equipment that will contribute to the future mutation breeding developments and be a contributor in solving the problems mentioned in this review.

ACKNOWLEDGEMENTS

Author would like to express his sincere thanks to Dr. Bryan Kindiger, USDA, ARS-SR, Grazinglands Research Laboratory for his critical reading of the manuscript.

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