Mutation Rate and Spectrum with Special Reference to Agronomical Traits Induced by X-rays in Dry Seeds of Rice, *Oryza sativa* L.

Yasuo KOWYAMA*, Yoshihiko YAMADA** and Tsuneo KAWASE*

Introduction

Dry seeds are the most suitable and the most widely used plant materials for radiobiological research and practical works on mutation breeding of crop plants. In fact, a large portion of new varieties released through induced mutations has been derived from dry seeds treated with physical or chemical mutagens[4]. This is due to the following versatile characteristics of dry seeds: (1) large populations can be readily handled, (2) normal viability often can be maintained under severe environmental conditions such as complete desiccation and vacuum, various gas atmospheres at high pressure and extreme low temperature, and (3) physical and chemical reactions with cellular macromolecules after irradiation can occur in the near absence of metabolism. From these points of view, mutagenic efficiency of dry seed treatment has also been employed as a standard criterion for comparing with the efficiencies of various treatments in different materials of plant parts.

Altering the mutation spectrum in a predictable manner and there-by achieving the directional mutagensis are an ultimate and important goal in mutation breeding of higher plants. The numerous attempts to control the direction of induced mutations have been carried out by treating dry seeds with different kind of mutagens and with their combinations, and have revealed some degrees of mutagen specificity[1,3,8,17]. Mutagenic treatments of dormant seeds, however, are confined to the cells at intrerphase of G_1 or G_2[2]. Besides these attempts, it has been reported in barley and rice that mutagenic treatments of the germinating seeds at different phases of cell cycle give rise to the specific mutation spectra for the respective phases[6,14,15,16]. These studies dealt with the spectrum concerning the various kinds of chlorophyll mutations, because of their ease in the detection and the frequent appearance after mutagenic treatment. However, it seems more important from practical point of view whether the directional mutations of agronomical traits could be induced by the mutagens. Therefore, further studies might be required for examining the cell cycle specificity for various agronomical traits. The present experiment was undertaken to examine the mutation frequencies and spectrum with special reference to agronomical traits in addition to chlorophyll mutations in M_2 generation after X-ray irradiation of dry seeds in order to compare with those obtained by the irradiations at different cell-cycle stages of mitosis and meiosis.

Materials and Methods

Three rice cultivars, 'Sensho', 'Aichi-Asahi' and 'Akamai' were chosen as experimental materials, because a preliminary experiment showed that the dry seeds of these varieties exhibited low, intermediate and high radiosensitivities, respectively. The dry seeds equilibrated to 11.3% water content were subjected to X-rays of 5 to 40 kR at dose rate of 215 R/min using a X-ray genertor operated at 280 kVp, 10 mA with a 1.0 mm aluminum filter. The number of serds of the respective variety used in each exposure was 500 to 1200 depending upon the estimated survival rates. The irradiated seeds were allowed to germinate at

Received June 5, 1985

* Plant Breeding Laboratory, Fac. Agri., Mie Univ., Tsu 514, Japan

** A graduate student

30°C on moistened blotter paper in Petri dishes for about 24 hr after exposure. Germinated seeds were then sown in a nursery bed and 46 days old seedlings were transplanted to the field. At maturity, three to five panicles were harvested from each M_1 plant. Radiosensitivity of each variety was estimated by measuring the root length, seedling height and survival rate on 4, 30 and 45 days after the seed sowing, respectively, and M_1 plant fertility at maturity.

In the following year, three panicle-progenies per M_1 plant were separately raised in a nursery bed as M_2 lines. At the seedling stage, numbers of chlorophyll mutants and normal plants were counted in each treatment of the three varieties. The M_2 lines derived only from the variety 'Aichi-Asahi' were transplanted to the field and grown to the maturity. Heading-date variants, morphological and sterile mutants were scored through the period from heading to maturity. When two or more segregants were detected in a line and their features were identical with one another, they were regarded as true mutants. Mutation frequency was estimated by the number of mutants per 1,000 M_2 plants.

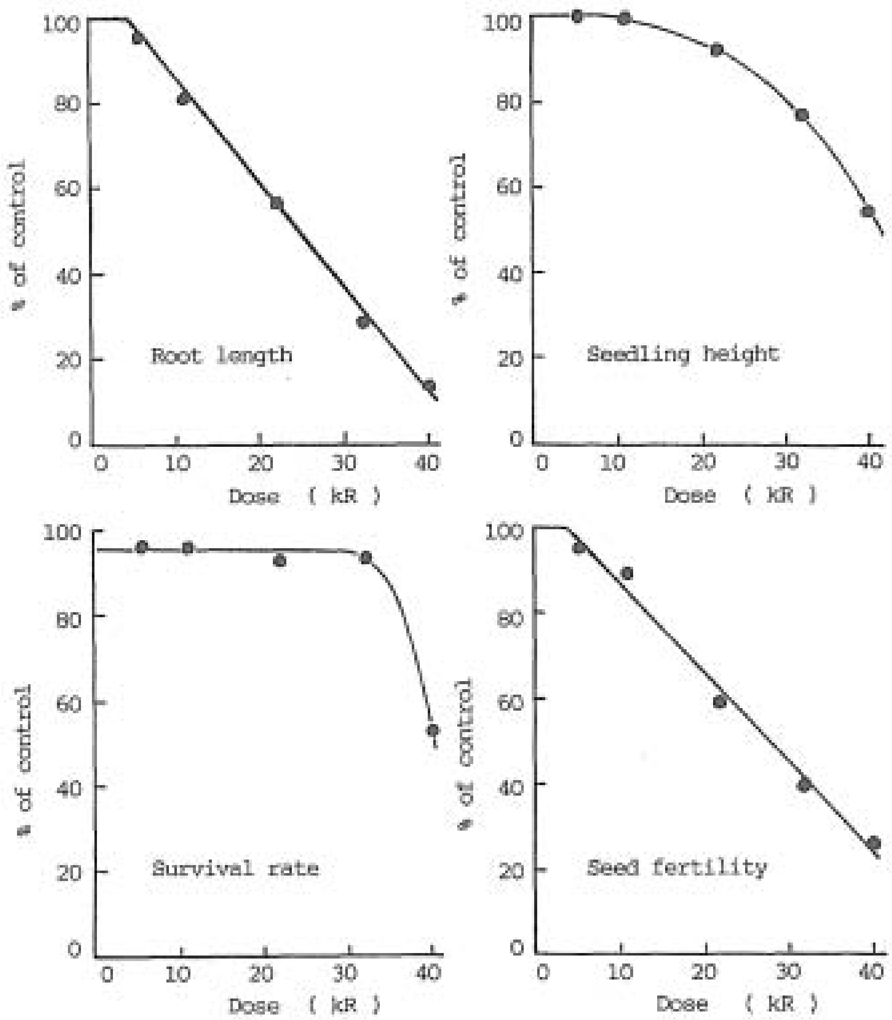

Fig. 1. X-ray dose responses of M_1 damages as measured by root length, seedling height, survival rate and seed fertility after seed irradiation of the rice variety 'Aichi-Asahi'.

Results

As shown in Fig. 1, root length and seed fertility of M_1 plant responded to X-ray doses in a similar manner, in which their reductions were almost linear with increasing doses. Half reduction doses (D_{50}) for root growth and seed fertility of the variety 'Aichi-Asahi' were about 25 kR and 27 kR, respectively. On the other hand, seedling height and survival rate showed not so clear reduction as the root length and the seed fertility. The similar trends of these four end points for estimating the radiosensitivity were also observed in the other two varieties.

The frequencies of chlorophyll mutation observed in M_2 generation of three different varieties are given in Table 1, and these results are presented graphically in Fig. 2. Among the various kinds of chlorophyll mutants, albina appeared most frequently with about half the proportion of total chlorophyll mutants, followed by viridis and xantha. Striata and other types of mutant including tigrina, maculata and alboviridis were found with very low frequency. The total chlorophyll mutation frequency which was pooled with different kinds of mutants, and albina frequency increased in a non-linear fashion with increasing X-ray dosage. From calculation of the best fitted parameters in the power-law equation $y=kD^n$, where y is chlorophyll mutation frequency per 1,000 M_2 plants, D is X-ray dose and k is a constant for each equation, it was revealed that the frequencies of total chlorophyll mutation and albina increased proportionately with dose to the power 1.42 and 1.83 respectively. Furthermore, no significant difference in these chlorophyll mutation frequencies was observed among three rice varieties over all the doses employed. The X-ray dose of 32 kR which caused sterility of about 60% in M_1 plant, gave 20 to 30 chlorophyll mutants per 1,000 M_2 plants.

Table 2 shows average segregation ratios of the respective chlorophyll mutants in M_2 panicle-progeny at three different exposure doses. Four types of the mutant, i.e., albina, viridis, xantha and the other miscellaneous chlorophyll mutants, were segregated with a ratio of about 20% with considerably wide variance, indicating that the ratio was slightly lower than the expected ratio of 25% from recessive gene mutation at one locus. Striata, on the other hand, showed low segregation ratio, 4.1% on average. No

Table 1. Chlorophyll mutation frequency in M_2 generation after X-ray irradiation of dry seeds in three different varieties of rice.

Variety	Dose (kR)	Number of M_1 panicle used	Number of M_2 seedling observed	Number of chlorophyll mutant						Mutation frequency per 1000 M_2 plants
				Albina	Viridis	Xantha	Striata	Others	Total	
Aichi-Asahi	0	389	36607	0	0	0	0	0	0	0
	11.1	1058	79981	276	74	59	2	90	501	6.26
	22.2	1364	67771	348	129	174	3	114	768	11.33
	32.3	1899	62509	705	265	122	11	275	1378	22.04
Sensho	0	596	16353	0	0	0	0	0	0	0
	11.1	726	14969	22	65	2	9	3	101	6.75
	22.2	927	14540	67	79	23	20	39	228	15.68
	32.3	994	16223	220	98	15	112	65	510	31.44
Akamai	0	583	19912	0	*	0	0	0	0	0
	5.6	656	23711	9	*	12	0	23	44	1.86
	16.7	1212	36326	147	*	14	13	195	369	10.16
	32.3	1824	37419	492	171	17	10	288	978	26.14

* Viridis mutants were not counted, because they were not distinguishable from normal plants with pale green leaves at seedling stage.

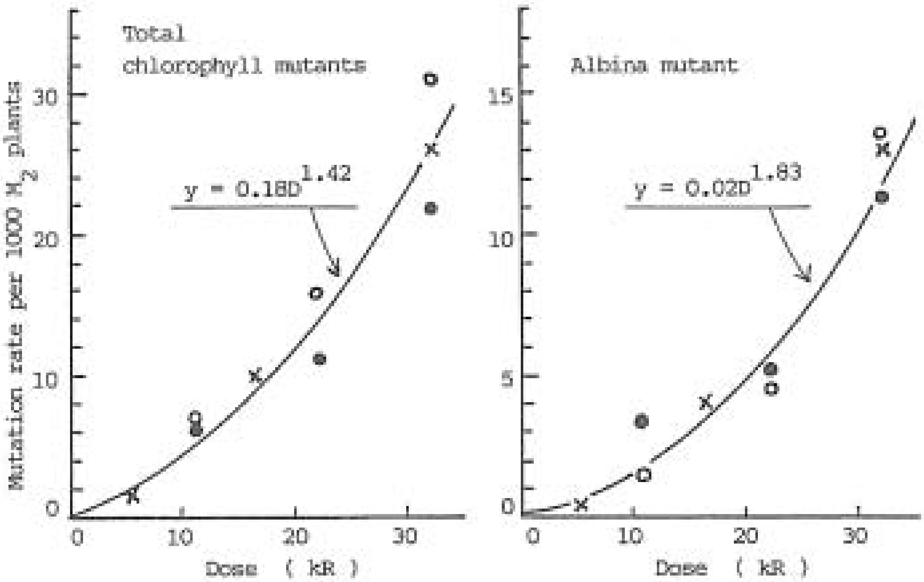

Fig. 2. Dose responses of total chlorophyll mutation frequency and albina frequency in three rice varieties, i.e., Aichi-Asahi (●), Sensho (○) and Akamai (×).

Table 2. Segregation ratios of chlorophyll mutant in M_2 generation after X-ray irradiation of dry seeds in the rice variety 'Aichi-Asahi'

Dose (kR)	Segregation ratio (%)				
	Albina	Viridis	Xantha	Striata	Others
11.1	19.9±11.3	18.1± 7.6	21.7± 6.5	1.4±0.1	20.3±3.6
22.2	17.1± 8.1	18.7± 9.1	16.9± 7.6	2.9±0.2	20.8±9.0
32.3	22.9±10.5	19.6±10.3	21.5±11.8	7.9±5.3	21.0±8.3
mean	20.0± 2.4	18.8± 0.6	20.0± 2.2	4.1±2.8	20.7±0.3

significant difference in these segregation ratios was found among the exposure doses of X-rays employed in the present experiment.

The mutation frequencies of agronomic traits detected in the field are summarized in Table 3. Sterile mutants which include both partial and complete sterilities were observed with the highest frequency, and amounted to about 10% of M_2 plants at 32 kR X-ray exposure. Short-culm and dwarf mutants were also induced with a considerably high frequency, whereas long-awned mutants and the other type of morphological mutants were found with very low frequency. Total frequency of sterile and morphological mutants increased carvilinearlly against the X-ray dose.

Mutation spectra which were calculated from the respective mutation frequencies at three different doses are presented in Fig. 3. It is noticeable from this figure that there is no significant difference in the spectra among the three doses. Sterile mutants accounted for 55.6% of all the mutants detected in M_2 plant population, short-culm mutants for 27.1% and chlorophyll mutants for 10.4%, on average respectively. Dwarf and long-awned mutants were 3.7 and 1.4% respectively. From a practical point of view, it is noteworthy that a number of agronomically promising mutants were induced by the present experiment, for instance, five M_2 lines of early headings, two lines of short-culm with normal length of the panicale, and one line of dense grain and of glabrous mutant.

Table 3. Frequency of sterile and morphological mutations in M_2 generation after X-ray irradiation of dry seeds in the rice variety 'Aichi-Asahi'

Dose (kR)	Number of M_1 panicle strain	Number of M_2 plant observed	Number of mutant						Mutation frequency per 1000 M_2 plants
			Sterile	Short culm	Dwarf	Awned	Others	Total	
0	101	2626	17	6	0	0	0	23	8.76
11.1	313	8093	397	170	13	2	7	589	72.78
22.2	367	9274	465	324	28	16	27	860	92.73
32.3	388	9578	954	355	104	36	28	1477	154.21

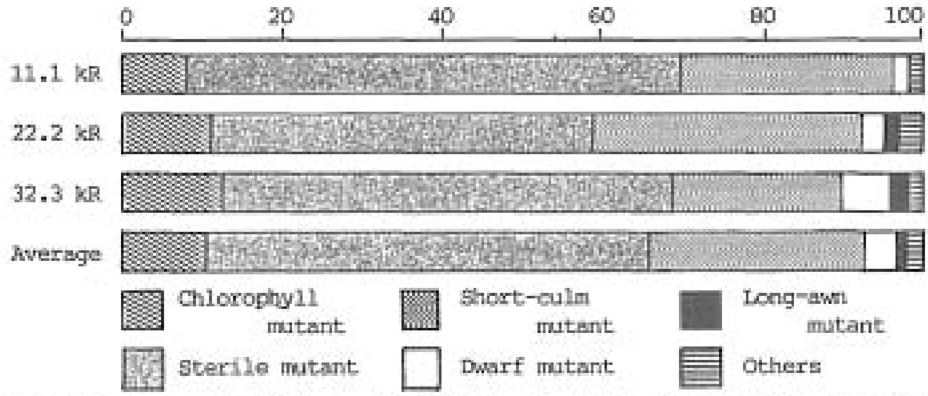

Fig. 3. Mutation spectra in M_2 generation after X-ray irradiation of dry seeds in the rice variety 'Aichi-Asahi'.

Discussion

Although radiation-induced reduction in root growth and M_1 plant fertility after irradiation of rice seeds are originated from the different radiation injuries, viz., from mitotic inhibition and chromosomal aberrations respectively[4,10,11], the dose response of M_1 plant fertility was similar to that of root length in the rice variety 'Aichi-Asahi'. This implies that an approximate half-reduction dose (D_{50}) for M_1 plant fertility could be estimated from the root growth reduction at the seedling stage of a rice variety with intermediate radiosensitivity.

UKAI[10] examined the varietal difference in chlorophyll mutation frequencies among seven rice varieties having different radiosensitivities measured by the reduction in root length after X-ray irradiation of dry seeds, and pointed out that there was no significant correlation between chlorophyll mutation frequencies and the root-length radiosensitivities, though his data on the mutation rate were based on a single dose exposure to X-rays. Also in the present experiment, three varieties with different root-length radiosensitivity exhibited no varietal difference in dose response of chrlorophyll mutation frequency. These results indicate that mitotic inhibition, which is a radiation-induced damage leading to varietal difference in the root-length radiosensitivity, has no relationship with occurrence of chlorophyll mutation after X-ray irradiation of rice seeds. Furthermore, the present experiment showed that frequency of total chlorophyll mutation increased as dose to the power 1.42 and albina frequency had a dose exponent of 1.83 in the power-law equation. The exponential increase in the chlorophyll mutation frequency with X-ray dosage suggests that chlorophyll mutation, in particular albina mutation occurs from two-hit event of radiation, viz., from a minute interstitial deletion of chromosome following X-ray irradiation of dry seeds. Therefore, chlorophyll mutations as well as the reduction of M_1 plant fertility after X-ray exposure of rice seeds seem to be ascribed not to the point mutation but to the induced minute chromosomal aberrations.

Average segregation ratios in different kinds of chlorophyll mutatns were 19.88% in the M_2 panicle-progeny. The same range of the segregation ratio has been reported in M_2 generation after irradiation of dormant rice seeds, e.g., to be 19.75% for various kinds of chrolophyll mutants[15] and 20.50% for albina[16]. The fact that the segregation ratios in M_2 progenies are significantly less than the Mendelian expectation of 25% has been explained as contribution of more than one initial cells in the corpus to chimaeric formation in an M_1 panicle[7]. On the other hand, striata showed very low segregation ratio of 4.1%, suggesting that this mutation is ascribed not to a recessive gene mutation. FUKUI[3] studied the inheritance and morphogenesis of striata mutant in rice, and suggested that the striata mutant originates from a cytoplasmically inherited chloroplast mutation.

The frequency of mutation with regard to agronomical traits increased with increasing dosage of X-rays, though its dose response was not so clear because of smaller number of M_2 plants observed, than that of seedlings used for counting of chlorophyll mutants. From the comparison of mutation spectra at three different doses of X-rays, it was revealed that sterile mutants amounted more than 50% of all mutants detected at both the seedling stage and maturity, irrespective of the exposure doses. YAMAGATA and SYAKUDO[12] also reported that partially or completely sterile mutants which were induced by gamma-ray irradiation of rice seeds, occupied the most part of mutated M_2 plants over all the dosages employed. This report agrees well with the results presented here. These experimental results indicate that sterile mutations are readily induced by radiation exposure of dry seeds, and that the main genetical effect of radiation on dormant seeds is due to the chromosomal aberrations. From practical point of view, it is desirable to reduce the induction of chromosomal aberration as low as possible, either by any pre- or post-irradiation treatment of dry seeds or by a treatment of any other plant part than dormant seed.

Summary

Dry seeds of three rice varieties with different radiosensitivity were subjected to X-rays of 5 to 40 kR, and the mutation frequencies and spectra were examined in the M_2 panicle-progeny, with the aim of comparing mutagenic efficiencies of the irradiation at different stages of mitosis and meiosis.

Chlorophyll mutation frequency and, in particular, albina frequency increased exponentially with increasing dosage of X-rays, suggesting that the chlorophyll mutations are originated from minute interstitial chromosome deletions induced by two-hit events of the radiations. There was no varietal difference in the chlorophyll mutation frequencies among the three varieties, irrespective of their radiosensitivities measured by root-length reduction of the irradiated seeds. Of all the mutants detected at the seedling stage and maturity, more than 50% were sterile mutants, most of which seems to be due to chromosomal aberrations. The present results imply that chromosomal aberrations are predominantly induced by X-ray irradiation of dormant rice seeds and bring about high proportion of sterile and chlorophyll deficient mutations in the M_2 generation.

References

1) BHAN, A. K. and KAUL, M. L. H. (1976) Frequency and spectrum of chlorophyll-deficient mutations in rice after treatment with radiation and alkylating agents. Mutat. Res. **36**: 311–318.
2) D'AMATO, F. (1977) Nuclear cytology in relation to development. Cambridge University Press, Cambridge. pp. 48–54.
3) FUKUI, K. (1985) Genetic and developmental studies of the chlorophyll mutant, striata in rice (*Oryza sativa* L.) Bull. Natl. Inst. Agri. Sci. Ser. D **36**: 219–245.
4) KOWYAMA, Y. (1986) High correlation of varietal differences in radiosensitivity after gamma-ray and thermal neutron exposures in dry seeds of rice. Envir. Exp. Bot. (Under submitting).

5) MacKey, J. (1954) The biological action of mustard on dormant seeds of barley and wheat. Acta. Agr. Scand. **4**: 419–429.
6) Nishimura, S. and Futsuhara, Y. (1976) Studies on the induction of mutations in rice by ethylene imine III. Mutagenic activity of ethylene imine on the pre-soaked seeds. Japan. J. Breed. **26**: 99–109.
7) Osone, K. (1963) Studies on the developmental mechanism of mutated cells induced in irradiated rice seeds. Japan. J. Breed. **13**: 1–13.
8) Seetharami Reddi, T. V. V. and Reddi, V. R. (1984) Frequency and spectrum of chlorophyll mutants induced in rice by chemical mutagens. Theor. Appl. Genet. **67**: 231–233.
9) Sigurbjornsson, B. and Micke, A. (1969) Progress in mutation breeding. In "Induced Mutations in Plants" IAEA, Vienna, pp. 673–698.
10) Ukai, Y. (1968) Studies on varietal differences in radiosensitivity in rice. II The radiosensitivities with respect to the reduction in seedling height, coleoptile length, pollen fertility and seed fertility, and to the frequency of chlorophyll mutation. Japan. J. Breed. **18**: 127–138.
11) Ukai, Y. (1968) Studies on varietal differences in radiosensitivity in rice. III. Radiosensitivities with respect to the reduction in the number of dividing cells and the occurrence of chromosome bridges. Japan. J. Breed. **18**: 221–228.
12) Yamagata, H. and Syakudo, K. (1960) Studies on the utility of artificial mutations in plant breeding. I. γ-ray sensitivity of rice and induced aberrant plants in the X_2 generation. Japan. J. Breed. **10**: 153–162.
13) Yamaguchi, H. (1962) The chimaeric formation in an X_1 panicle after irradiation of dormant rice seed. Radiat. Bot. **2**: 71–77.
14) Yamaguchi, H. (1972) The production of mutations by ionizing radiations and chemical agents in relation to the duplication of chromosomes. Gamma Field Symp. **11**: 29–42.
15) Yamaguchi, H. (1976) Mutations induced in presoaked barley seeds by diethyl sulfate and 5-bromodeoxyuridine. Environ. Exp. Bot. **16**: 145–149.
16) Yamaguchi, H. and Matsubayashi, I. (1973) Mutational response of rice seeds to ethyl methanesulfonate and busulfan in relation to chromosome duplication. Mutat. Res. **17**: 191–197.
17) Yamashita, A., Ukai, Y. and Yamaguchi, I. (1972) Comparison of genetic effects of gamma-ray irradiation and treatments of chemical mutagens in a six-rowed barley. Gamma Field Symp. **11**: 73–92.

摘　　要

イネ乾燥種子のX線照射後代における農業形質に関する突然変異率とそのスペクトラム

神山　康夫・山田　善彦・川瀬　恒男

体細胞分裂周期や減数分裂期の種々のステージにおける突然変異誘発効果とその突然変異スペクトラムを比較することを目的として，本研究では突然変異体誘発に最もよく用いられている乾燥種子について調査した。

放射線感受性が顕著に異なる水稲の3品種について，乾燥種子に 5〜40 kR のX線照射を行ない，M_2 世代において葉緑素突然変異体，不稔変異体および形態的変異体の頻度を調べた。その結果，葉緑素突然変異体とくにアルビノ変異体の頻度は照射線量に対して指数関数的に増加した事から，これら葉緑素突然変異はX線照射による2ヒット型事象に基づく染色体の中間部欠失から由来するものと考えられる。さらに葉緑素突然変異率に関して3品種間で有意な差異が認められなかった事から，発芽時の根長で顕著に認められる放射線感受性の品種間差異をもたらす要因として染色体異常の誘発が直接的に関与していないことを示唆している。

M_2 世代において可視的に検出されたすべての突然変異体のうち55.6%が部分不稔個体や完全不稔個体を分離する不稔変異体であり，これらの大半は相互転座や逆位などの染色体異常に由来するものと考えられる。さらに突然変異スペクトラムの内訳を見ると，短稈変異体が27.1%，葉緑変異体が10.4%でその他矮性や出穂期変異体および長芒変異体などが数%認められた。

以上の結果から，イネの乾燥種子に対する放射線照射においては染色体異常が最も誘発されやすく，照射後代にお

いて染色体異常による不稔変異体や葉緑素突然変異体が高い割合で生ずるものと考えられる。従って種実生産を目的とする作物の突然変異育種においては染色体異常を伴なわない遺伝子突然変異体の効率的な誘発が望まれることから，種子照射よりもむしろ減数分裂期照射や受精卵細胞の分裂周期別照射などのように植物体の照射ステージを考慮することが重要であると考えられる。