Intervarietal Difference in Radiosensitivity and Its Controlling Factors in Suspension Cultures of Rice Cells

Yasuo KOWYAMA* and Norio SHIBATA**

Introduction

Differences in radiosensitivity of higher plants have been reported among genera and species[28] and even among cultivated varieties in a given species[23,29]. SPARROW and his colleagues[18,19] revealed that DNA content per nucleus and interphase chromosome volume are major factors controlling cellular radiosensitivity of different plant species. As another biological factors, it has been shown that the level of polyploidy and the genome constitution also contribute to differential radiosensitivity in plants through genetic redundancy[6,12,22,30]. These factors, however, do not seem to play an important role in determining the varietal differences in radiosensitivity within a given plant species.

From genetic analysis of radiosensitivity in three crop plants, UKAI and YAMASHITA[29] showed that varietal differences in gamma- and X-rays sensitivity are controlled by different genetic systems, i.e., by polygenic systems in rice and barley, and by a single major gene in soybean. Although intervarietal variations of radiosensitivity have been investigated by many researchers[4,13,23], all the studies were carried out with either the seeds, seedlings or whole plants as experimental materials. While, little attention has been devoted to varietal differences in radiosensitivity of cultured cells in plants. Some cultured cells of plants are more or less heterogeneous cell population with various degrees of polyploidy[2,11,14,24,25]. In the cultured cells in contrast with an intact plant, therefore, high proportion of polyploid cells might modify the radiation effects.

The present experiments were made to reveal the varietal differences in radiosensitivity of callus tissue cultures derived from 6 rice varieties, and to evalutate the contribution of polyploid cells to the sensitivity. Based on the comparison of the radiosensitivities in the cultured cells with those of both the dry seeds and the seedlings, biological factors controlling the varietal differences in radiosensitivity are discussed.

Materials and Methods

Rice varieties and culture condition

Three rice varieties from Japonica type (Akamai, Oiran and Sensho) and three from Indica type (Zenith, Kumari and Tadukan) were picked up as experimental materials, because the prominent varietal differences in radiosensitivity had been observed among them in a preriminary experiment with the dry seeds.

Callus cells of the six varieties were separately induced and subcultured by the same culture conditions and nutrient medium as described in the previous paper[7] except that the subcultures were come to an end only for seven months. The growth of the callus cells in each variety was monitored by the sedimented cell volume (SCV) throughout subcultures.

Measurement of nuclear volume

Callus cells obtained from the above mentioned 6 varieties were sampled in the third subculture for estimating the ploidy levels of their nuclei. After fixation in glacial acetic acid, the cells were hydrolysed for

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

** A graduate student

50 minutes in 5N–HCl at 20°C, and then stained for 1 hr with the Feulgen reagent. The excess of stain was removed with three changes of SO_2 water each for 10 min. A drop of the stained cells was placed on a clean slide and then a coverslip was applied without any squashing. Using a screw micrometer eyepiece, two perpendicular diameters of the nuclei were measured in the 250 randomly chosen cells. The nuclear volume was calculated as an ellipsoid. For estimation of the diploid nuclear volume, root-tip cells of each variety were also prepared by the same procedures.

X-ray irradiation

The third subcultured callus cells of the 6 varieties were used for estimating the radiosensitivity. Based on the experimental results obtained in the previous paper[7], X-ray irradiation was made at the most sensitive growth phase, i.e., at mid log phase. The methods for irradiating and culturing the callus cells were followed by the same procedures as described in the previous paper[7]. Radiosensitivities of callus cells in the 6 varieties were compared by measuring the SCV at 35 days after irradiation.

For seed irradiation, respective seeds from the 6 varieties were adjusted to 11% moisture content by storing them in a desiccator with 75% glycerol for 7 weeks, and subjected to varying doses of X-rays (10–80 kR) at a dose rate of 336 R/min. The irradiated seeds were germinated and grown at 30°C for 5 days. For seedling experiments, 4-days-old potted seedlings were irradiated with 2.1 to 32.2 kR of X-rays at dose rate of 322 R/min. Radiosensitivities of the seeds and seedlings were estimated by measuring root length and seedling height at 5 days after irradiation, respectively. These experiments were carried out with two replications, in each of which 35 seeds and 40 seedlings were used for each dose in seed and seedling irradiation, respectively.

For comparing the radiosensitivity among the varieties, half reduction doses (D_{50}) for cultured cells, seeds and seedlings of each variety were calculated as described in the previous paper[7].

Results

Radiosensitivity in cultured cells

Growth patterns of the 6 varieties in a culture period for 35 days are shown in Fig. 1. From this figure, it is clearly noticed that callus cells of Tadukan and Zenith proliferate more rapidly under the present culture conditions than those of the other four varieties. Callus cells at mid log phase which were decided based on the growth patterns of each variety were sampled and irradiated with 2.5–15 kR of X-rays. Figure 2 shows the growth curves of irradiated cells for 35 days after irradiation. In all the varieties, conspicuous depressions of cell growth occurred in exposure with the highest dose of X-rays, 15 kR. Half reduction doses for cell growth (D_{50}) in each variety were estimated from dose response curves as shown in Fig. 3, in which the SCV values were taken from the final measurements in the culture period for 35 days. As listed in Table 1, Tadukan was most sensitive (D_{50}; 8.0 kR) and Kumari was most resistant (16.2 kR). The other four varieties showed the intermediate D_{50} values.

Variation of nuclear volume in cultured cells

Figure 4 shows frequency distributions of nuclear volume in cultured cells sampled at mid log phase in 5th subculture. Kumari exhibited heterogeneous cell population with the widest distribution of the nuclear volume ranging from 129.4 μ^3 to 636.8 μ^3. On the other hand, all the varieties except Kumari consisted of considerably homogeneous cell population with bimodal distribution of the nuclear volume. Moreover, significant difference in the nuclear size was observed between Kumari and Tadukan, i.e., average nuclear volume being 233.0 μ^3 in Kumari and 98.6 μ^3 in Tadukan. From the measurements of nuclear volume in root-tip meristems of each variety, diploid, tetraploid and octaploid cell nuclei were estimated. Based on these estimation, proportions of polyploid cells in each cultured cell population were calculated, as shown in Table 2. In Kumari, again, high percentages of polyploid cells were observed; 36.0% tetraploid and

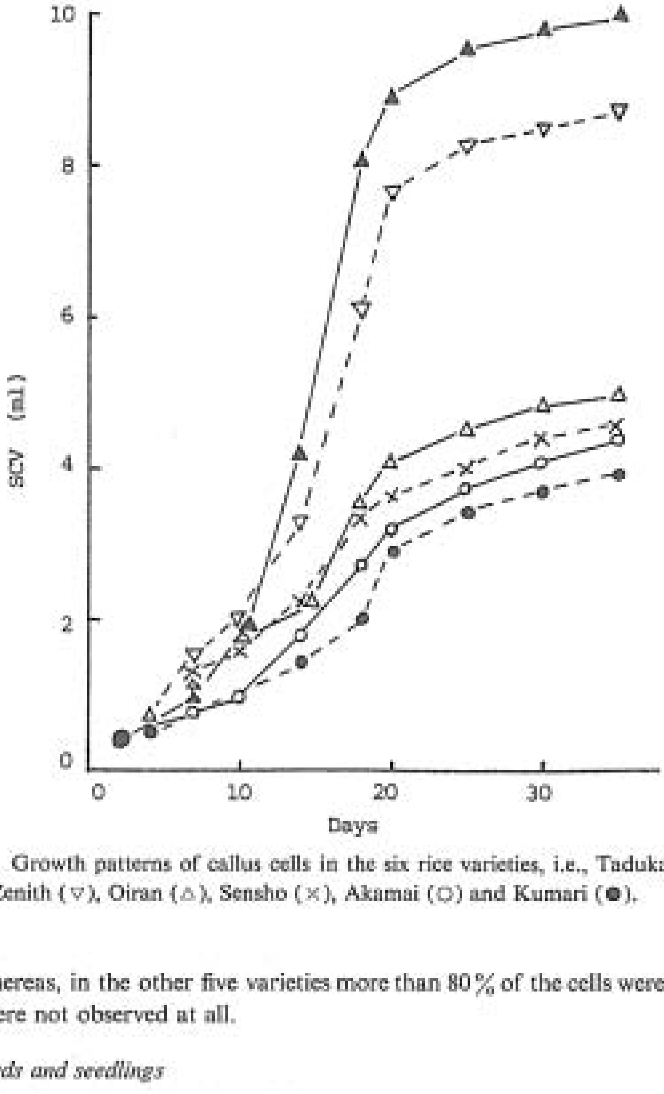

Fig. 1. Growth patterns of callus cells in the six rice varieties, i.e., Tadukan (▲), Zenith (▽), Oiran (△), Sensho (×), Akamai (○) and Kumari (●).

7.2% octaploid. Whereas, in the other five varieties more than 80% of the cells were identified as diploid, and octaploid cells were not observed at all.

Radiosensitivity in seeds and seedlings

Radiosensitivities in dry seeds of the six varieties were estimated from dose response curves for mean root length expressed as a ratio to the corresponding control value. As shown in Fig. 5, the higher sensitive varieties exhibited the steeper slope in the dose response. The lower sensitive varieties showed the distinctive feature with the more gentle slope and the wider sholder in their dose response curves. At 40 kR of X-rays, root growth in Akamai was almost completely depressed, whereas those in Oiran and Zenith were inhibited only by about 50% of the control. From comparison of the calculated D_{50} values, Akamai was approximately 3 times as sensitive as the other five varieties (Table 1).

Radiosensitivities in seedlings of the six varieties were similarly compared in dose response curves as shown in Fig. 6. After X-ray irradiation of 21.5 and 32.2 kR, no further growth of the seedling took place. The calculated D_{50} values in the seedling irradiation are listed in Table 1. From these data, it is clearly noticed that Sensho is most resistant to X-rays (D_{50}; 4.8 kR), and that both Tadukan and Akamai are most sensitive, D_{50} being 2.8 kR and 2.9 kR, respectively. Maximum varietal difference in radiosensitivity of seedlings, however, was only 1.7 times, i.e., smaller than 3.0 times in that of dry seeds.

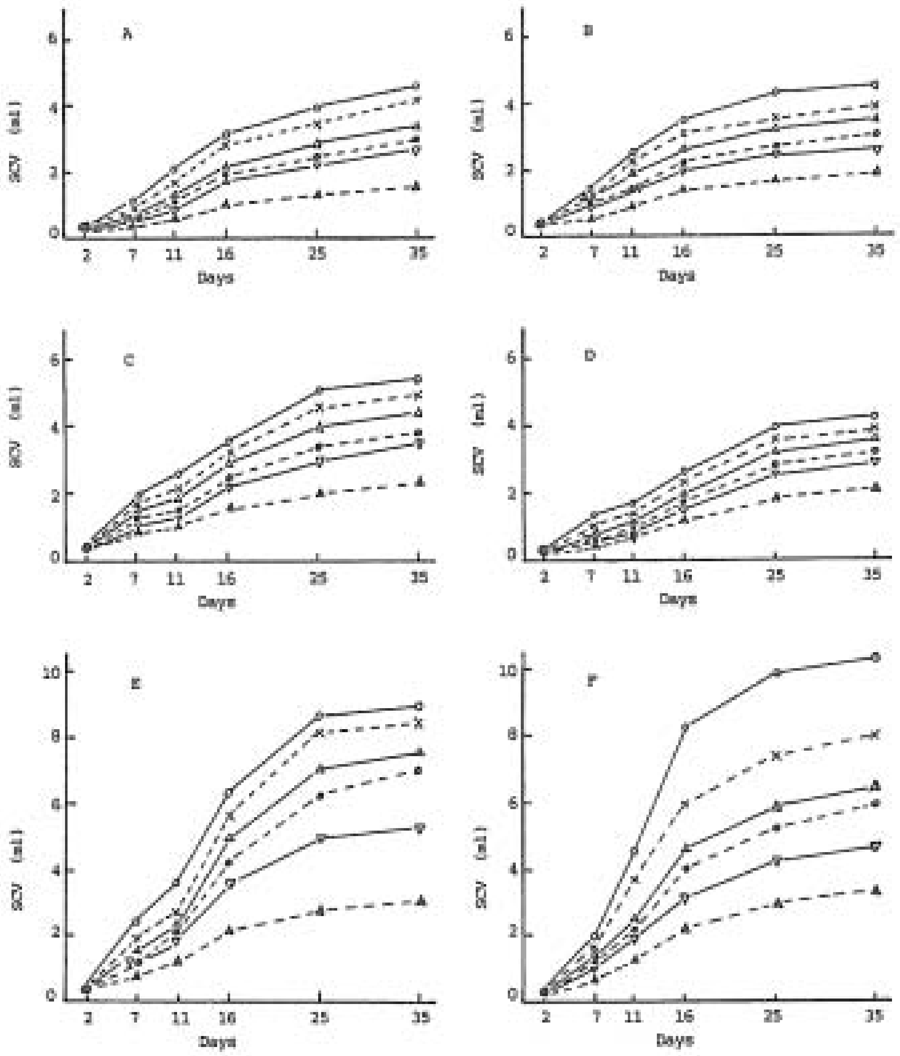

Fig. 2. Cell growth measured by the SCV after X-ray irradiations of 0 kR (cont., ○), 2.5 kR (×), 5.0 kR (△), 7.5 kR (●), 10 kR (▽) and 15 kR (▲) in callus cells of the six varieties, i.e., Akamai (A), Sensho (B), Oiran (C), Kumari (D), Zenith (E) and Tadukan (F).

Discussion

Among the cultured cells in the six rice varieties which include Japonica and Indica type, a two-fold difference in radiosensitivity and different proportions of polyploid nuclei in their cell populations were revealed in the present study. The radioresistance in the callus cells of Kumari coincided with a high percentage of polyploid cells. The callus cells of the other five varieties were composed of over 80% diploid nuclei and none of the octaploid in their cell population and showed higher radiosensitivity than those of Kumari. Maeda[9,10] observed the varietal difference in distribution of the nuclear size among callus tissues derived from seed embryos of six rice varieties. Polyploidy is one of the most common nuclear conditions in plant cells grown *in vitro*. The occurrence of polyploid cell *in vitro* is supposed

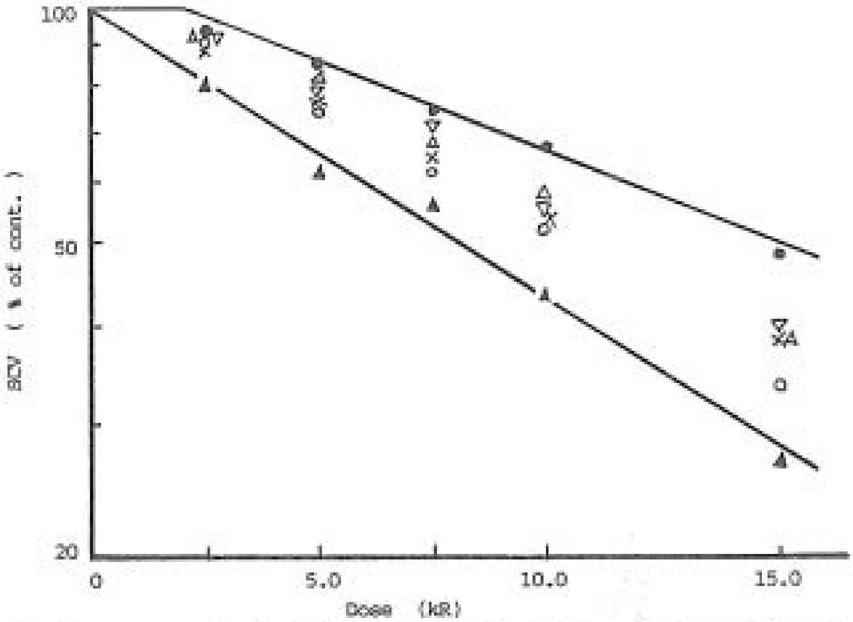

Fig. 3. Dose response of callus cells in six rice varieties, i.e., Kumari (●), Zenith (▽), Oiran (△), Sensho (×), Akamai (○) and Tadukan (▲).

Table 1. Radiosensitivity coefficient (k), constant (m) and D_{10} value in callus cells, seedlings and seeds of the six rice varieties.

	Callus cells			Seedlings			Seeds		
	k	m	D_{10}	k	m	D_{10}	k	m	D_{10}
Akamai	0.0733	1.110	10.46	0.2639	1.102	2.89	0.0756	1.352	12.08
Sensho	0.0652	1.058	11.24	0.1838	1.297	4.80	0.0396	2.006	31.07
Oiran	0.0631	1.117	12.22	0.1921	1.108	3.99	0.0346	1.950	34.88
Zenith	0.0614	1.115	12.54	0.2239	1.183	3.63	0.0382	2.416	36.35
Kumari	0.0463	1.082	16.17	0.2130	1.200	3.87	0.0711	3.445	23.94
Tadukan	0.0867	1.005	8.04	0.2745	1.105	2.78	0.0473	2.627	30.90

either to be a consequece of its appearance in original explants *in vivo*, or to be a result of its induction and selection during culture period[14-29]. Furthermore, it was found in the present experiment that D_{10} value in the callus cells of Sensho were 11.2 kR, indicating more radioresistant than the long-term subcultured cells of the variety described in the previous paper[7] in which the D_{10} values were in the dose range from 2.2 to 6.9 kR depending upon the growth phases at time of X-ray irradiation. The long-term subcultured cell population holded more than 90% diploid nuclei, whereas in the present callus cells of Sensho which were subcultured only three time, 18% tetraploid nuclei were observed (Table 2). It has been reported in some plant species that after a preliminary period with large variability in chromosome number or in DNA content of the nuclei, there is a progressive tendency towards stability within one or two ploidy levels[1,18,24-29]. Cell suspension cultures of *Haplopappus gracilis* were stabilized in the diploid condition[17]. On the other hand, tetraploid cells were predominant in *Nicotiana tabacum* callus

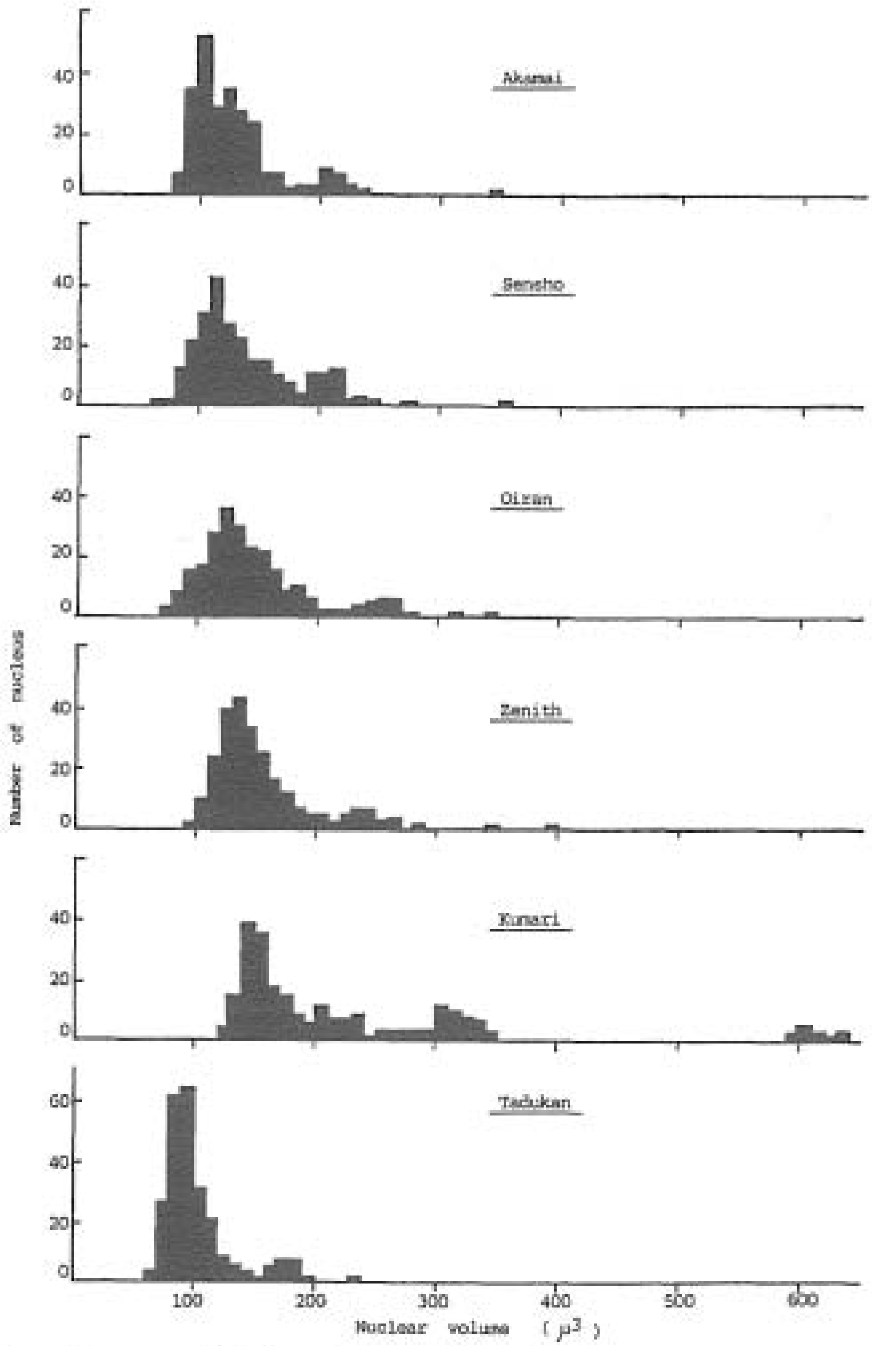

Fig. 4. Frequency distributions of nuclear volume in 5th subcultured callus cells of the six rice varieties.

Table 2. Proportions of polyploid cells in 5th subcultured calluses of the six rice varieties.

	Diploid		Tetraploid		Octaploid	
	range (μ^2)	%	range (μ^2)	%	range (μ^2)	%
Akamai	<180	89.6	181–360	10.4	361<	0
Sensho	<180	82.0	181–360	18.0	361<	0
Oiran	<210	89.2	211–420	10.8	421<	0
Zenith	<210	88.4	211–420	11.6	421<	0
Kumari	<200	56.8	201–400	36.0	401<	7.2
Tadukan	<150	90.8	151–300	9.2	301<	0

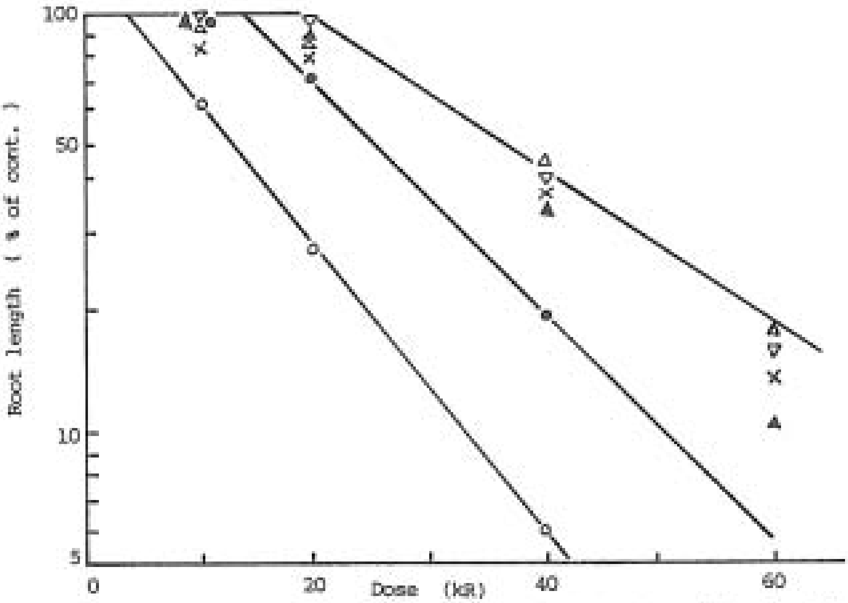

Fig. 5. Dose response of dry seeds after X-ray irradiation in six rice varieties, i.e., Oiran (△), Zenith (▽), Sensho (×), Tadukan (▲), Kumari (●) and Akamai (○).

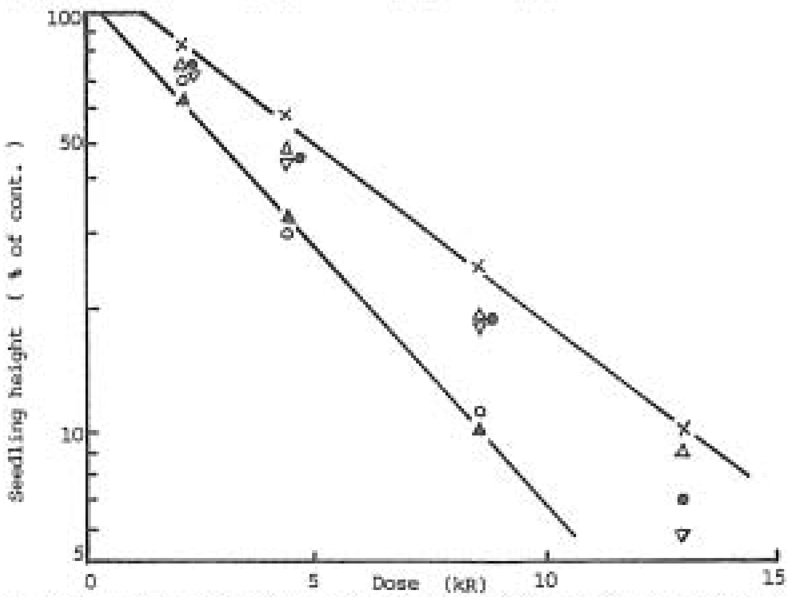

Fig. 6. Dose response of seedlings after X-ray irradiation in six rice varieties, i.e., Sensho (×), Oiran (△), Kumari (●), Zenith (▽), Akamai (○) and Tadukan (▲).

cultures[11]. Mixoploid composition in the short-term subcultured cell population seems to contribute to the lower radiosensitivities as shown in the present experiment. The proportion of polyploid cells in a callus cell population can be influenced by some factors like the composition of the medium, in particular the auxin and cytokinin concentrations in the medium, the duration of subcultures and genertical traits in a parental plant used as experimental material. From these considerations, it seems reasonable to point out that the polyploid cell composition and their ploidy levels play an important role in modifying the radiation effect on a cell population cultured *in vitro*.

In the present experiment, the ploidy levels of the callus cells were roughly estimated by measuring their nuclear volumes. Although the counting of chromosome number is the most reliable method for determination of polyploidy in a cell nucleus, metaphase plates are too rarely observed in plant cells cultured *in vitro* to permit a systematic analysis in the cell population. While, cytophotometrical measurement of nuclear DNA contents make it possible to deal with a large number of the cell nuclei and to evaluate their ploidy condition and the frequency distribution of polyploid cells[19]. Further experiment with the aid of the cytophotometry, therefore, might be required for accurate estimation of polyploidy found in the present experimental materials.

The maximum intervarietal difference in radiosensitivity after X-ray irradiation of callus cells was 2.0 times among the six varieties used in the present study. While a 3.1-fold difference in radiosensitivity is revealed among different growth phases of callus cells during a culture period, as shown in the previous paper[7]. These results suggest that nuclear and/or cytological factors more decisively take part in controlling the radiosensitivity of plant cells cultured *in vitro* than genetical factors inherent in each rice variety. This suggestion was also confirmed by the comparative study on radiosensitivities in callus cells, seedlings and seeds of the six varieties after X-ray irradiation. Figure 7 shows the correlations of D_{50} value in callus cells with those in the seedlings and seeds. It is clearly noticed that there exist no correlation between D_{50} for callus cells and that for seeds ($r=0.001$, $P>0.05$) and slight correlation between callus cells and seedlings irradiations ($r=0.470$) which are, however, statistically not significant ($P>0.05$). Takagi[23] found the positive correlation of radiosensitivity between dormant seed and seedling irradiations among 21 varieties of *Glycine max*, and revealed that the large varietal difference in radiosensitivity is controlled by two major genes, Rs_1 and Rs_2 which exert their actions throughout life cycle of the plant. On the other hand, it has been pointed out in many crop plants that a marked varietal difference in radiosensitivity observed in dry

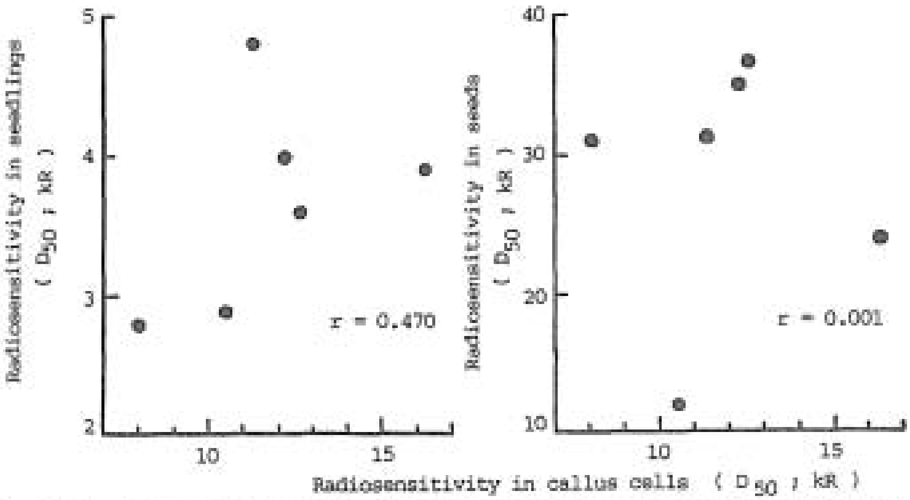

Fig. 7. Correlation of radiosensitivity in callus cells with those in seedlings and seeds among six rice varieties.

seeds completely disappears in irradiations of germinating seeds and seedlings, resulting in no correlation of radiosensitivity between dry seeds and germinating seeds or seedlings[21,26,28]. In these crop plants, for example, *Arachis hypogaea*[5], *Lycopersicon esculentum*[3] and *Secale cereale*[8], polygenic controls of radiosensitivity were demonstrated. UKAI[27] and UKAI and YAMASHITA[28] also revealed from genetic analysis on diallel cross among some varieties in *Oryza sativa* and *Hordeum vulgare* that the major part of the varietal difference in radiosensitivity of the dry seeds could be ascribed to additive and dominance action of polygenes. The varietal variations of radiosensitivity revealed in the present study seems also to be controlled by not a major genic but a polygenic genetical system, and therefore the radiosensitivity could be readily modified by different physiological and environmental factors in different cellular conditions like callus cells, seedlings and dry seeds.

Summary

The present study was carried out to examine the varietal difference in radiosensitivity of callus cells derived from root-tip segments of six rice varieties. The induced callus cells which were subcultured through three transfers were subjected to X-rays of 2.5 to 15 kR at mid log phase of each variety. In all the varieties used, the highest dose of X-rays brought about conspicuous depressions of cell growth measured by the sedimented cell volume (SCV) at 35 days after irradiation. From the comparison of D_{50} values estimated from X-ray dose response curves of the SCV, Kumari was 2 times as radioresistnt as the most sensitive variety, Tadukan. The radioresistance in Kumari coincided with a high proportion of polyploid nuclei in its callus cell population. The present results suggest that polyploid cell composition and their ploidy levels might play an important role in modifying the radiosensitivity of a plant cell population cultured *in vitro*.

The varietal differences in radiosensitivity were also compared among the six rice varieties after X-rays irradiations in the callus cells, seedlings and dry seeds. There was no correlation between D_{50} for callus cells and either those for seedlings or dry seeds, suggesting that the varietal differences of radiosensitivities in different cellular conditions of rice plant are controlled by a polygenic genetical system.

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摘　　要

イネの培養細胞における放射線感受性の品種間差異とその支配要因

神山　康夫・柴田　憲男

イネにおける放射線感受性の品種間差異とその支配要因を明らかにするために，日本型およびインド型イネの6品種を供試し培養カルス細胞，乾燥種子および幼苗についてそれぞれ放射線感受性を調査した。

まず各品種の根端から誘導したカルス細胞を3回継代培養し，対数増殖中期の細胞に 2.5～15 kR のX線照射を行なった。照射後35日間培養した後，細胞増殖量に対する線量反応曲線から各品種における培養細胞の増殖量半減線量 (D_{50}) を求めた。その結果，Kumari (D_{50} = 16.2 kR) は最も抵抗性であり，感受性の最も高い Tadukan (D_{50} = 8.0 kR) に比べ約2倍の品種間差異が認められた。各品種の対数増殖中期における細胞集団について核体積を測定し倍数性細胞の頻度を推定した結果，Kumari の細胞集団では倍数性細胞の頻度が最も高く43.2%であったが，他の品種では9～18%であった。この事は，植物の培養細胞における放射線感受性の支配要因として細胞集団における倍数性細胞の頻度やその倍数性レベルが主に関与している事を示している。

次に各品種の乾燥種子および幼苗に対してX線照射を行ない，線量反応曲線からそれぞれの生育半減線量 (D_{50}) を求めた。その結果，各品種の培養細胞における D_{50} 値と乾燥種子および幼苗における D_{50} 値との間ではいづれも有意な相関は認められなかった。従ってイネにおいては，放射線感受性の品種間差異をもたらす遺伝的要因としてポリジーンが関与しており，乾燥細胞系や培養細胞系などの細胞をとりまく環境要因によって感受性が容易に変更されるものと考えられる。