Radiosensitivity in Plants

II. Relationship between Radiosensitivity and DNA Content per Nucleus of Diploid Species in *Oryza*

Taira KATAYAMA

Faculty of Agriculture, Kyushu University, Fukuoka

Synopsis. In diploid species or strains belonging to each genome constitution in *Oryza*, relationship between radiosensitivity and DNA content per nucleus was studied. From the results obtained, no clear relation between both of them was recognized as described by other investigators. The radiosensitivity in the genus *Oryza* may result from the interaction both of DNA content per nucleus and ability of recovery of DNA molecules injured by the radiation.

Since SPARROW and CHRISTENSEN (1953) proposed that plants with large chromosomes have a higher sensitivity to chronic gamma irradiation than do most plants with small chromosomes, a high correlation between nuclear volume (nuclear DNA content or ICV) and radiosensitivity was recognized by many workers (SPARROW & MIKCHE 1961b, SPARROW *et al.* 1964, YAMAGUCHI & TSUNODA 1969 *etc.*). There are few reports, however, on relationship between DNA content per nucleus and radiosensitivity in different species or varieties within a genus.

From the results of their experiments on the radiosensitivity of wild rice, FUJII and MATSUMURA (1959) and FUJII (1962) described that *O. sativa* f. *spontanea*, *O. perennis* and *O. glaberrima* were less sensitive than the other species including both of diploid and tetraploid species. On the other hand, the author already estimated DNA content per nucleus of diploid species in *Oryza*, and described the relative amounts of each genome (KATAYAMA 1967).

In order to make clear the relationship between DNA content per nucleus and radiosensitivity of species and varieties or strains belonging to different genomes in *Oryza*, the present experiment was carried out.

Materials and Methods

Seeds which were produced at the University farm in 1965 and which stored under 13% relative humidity at 15°C were used as materials for irradiation, and those in 1968 for

estimation of DNA content. Fifty hulled dry seeds from each species and strain or variety listed in Table 1 were exposed to the doses of 20, 36 and 45 kR of ⁶⁰Co source (3,000 Ci) at a dose rate of 19.74 kR/h. After irradiation, the hulled seeds were immediately sown on 0.6% agar gel leveled in plastic boxes and kept for three weeks in room temperature under same condition in July, 1966.

Radiosensitivity was decided on the basis of the germination rate, survival rate, and seedling height, especially of the last one.

For estimation of DNA content per nucleus, young (about 10 mm length) root tips of adult plants of *Oryza* species belonging to A, B and C genomes cultivated under same cultural condition were fixed with FARMER's fluid for 15 min in 1969. After dehydration in 1N HCl for 6 min at 60°C, the roots were stained with SHIFF's reagent for 2 hours at 20°C. In order to minimize the errors, the roots of each species or strain to be compared were dehydrated and stained under same condition, and a standard species or strain was smeared side by side with the species or strain to be compared on each cover slide.

After FEULGEN staining, the value for the relative absorption per nucleus was measured by two-wavelength method using Olympus Micro Spectrophotometer (MSP-IV), and calculated from MENDELSON's Table (MENDELSON 1958). The mean DNA content was mostly estimated from 30 telophase nuclei, 10 in each of 3 roots, in each species or strain.

Results

From the results of estimation of DNA

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content per nucleus, the author tentatively proposed a following quantitative interrelationship of DNA content with those various genome constitutions in diploid *Oryza* species: $F < B \approx A \approx C < E$ (KATAYAMA 1967). In the present experiment, DNA content was mainly measured on the materials belonging to the A, B and C genomes. DNA content per nucleus of different species within a genome of the

genus *Oryza* showed no significant difference in value (Table 1).

In the present experiments irradiated with γ -rays at dosages of 20, 36 and 45 kR, germination rate was not affected to any significant extent as compared with that of the control (Table 2). In *Oryza*, even in high radiosensitive species or strains, the seeds germinated after high dose irradiation, and soon the

Table 1. Summary of diploid species used in the present experiment

Acc. no.	Species	Genome constitution	DNA contents per nucleus (arbitrary units)	Ratio of DNA content (1967)	
I-1	<i>O. sativa</i> (Kele)	AA	5.6871	ca. 1.0	
I-5	" (Blue Stick)	AA			
I-7	" (Hatishail)	AA			
I-8	" (Intan)	AA			
I-14	" (Gendjah Ratji)	AA	5.8272		ca. 1.0
I-17	" (Bhutmuri)	AA			
I-20	" (P. T. B. 10)	AA	5.3499		
Y-15	<i>O. sativa</i> f. <i>spontanea</i>	AA	5.6026		
Y-24	"	AA	5.3753		
Y-4	<i>O. sativa</i> var. <i>fatua</i> No. 9	AA	5.5830		
Y-8	<i>O. breviligulata</i>	AA	4.7730	ca. 1.0	
W0002	<i>O. officinalis</i>	CC			
Y-21	"	CC	6.0753		
W1514	<i>O. punctata</i>	BB	5.3296	ca. 1.0	
W008	<i>O. australiensis</i>	EE		ca. 1.3	
W023	<i>O. brachyantha</i>	FF		ca. 0.5	

Table 2. Radiosensitivity after irradiation by ^{60}Co in diploid *Oryza* species

Acc. no.	Total dose (kR)	Germin. rate (%)	Surviv. rate (%)	50% seedling height (kR)	Seedling height		Acc. no.	Total dose (kR)	Germin. rate (%)	Surviv. rate (%)	50% seedling height (kR)	Seedling height	
					(cm)	%						(cm)	%
I-1	0	98.0	100.0	65	15.3±0.9	100.0	Y-24	0	100.0	100.0	35	16.9±1.1	100.0
	20	100.0	100.0		13.6±3.0	88.6		20	100.0	100.0		13.9±1.4	81.9
	36	100.0	100.0		11.8±1.3	77.2		36	100.0	80.0		8.2±4.1	48.3
	43	100.0	100.0		9.8±1.6	63.6							
I-5	0	98.0	100.0	30	20.4±3.8	100.0	Y-4	0	100.0	100.0	35	12.1±2.5	100.0
	20	100.0	100.0		11.3±3.5	55.3		20	92.0	95.1		9.3±3.6	77.1
	36	100.0	100.0		9.2±2.6	44.8		36	92.0	73.2		5.8±1.6	47.7
I-7	0	100.0	100.0	65	14.4±2.4	100.0	W0002	0	82.0	80.5	10	7.8±2.2	100.0
	20	100.0	100.0		13.9±0.9	96.7		20	90.0	0.0		—	—
	36	100.0	100.0		11.3±2.3	78.7		36	92.0	0.0		—	—
I-8	0	100.0	100.0	30	14.3±1.6	100.0	Y-21	0	98.0	100.0	25	10.9±2.4	100.0
	20	100.0	100.0		12.1±1.1	84.7		20	100.0	100.0		8.0±1.9	73.6
	36	100.0	73.3		3.1±2.2	21.6		36	98.0	52.0		1.2±1.1	11.1
I-17	0	100.0	100.0	55	15.3±0.8	100.0	W1514	0	98.0	97.8	25	10.9±3.9	100.0
	20	100.0	100.0		14.0±1.2	91.4		20	100.0	86.0		8.4±2.8	77.2
	36	100.0	100.0		11.0±1.6	71.7		36	98.3	0.0		—	—
	45	100.0	100.0		9.2±2.9	59.9							
I-20	0	100.0	100.0	30	22.5±3.1	100.0	W008	0	98.0	100.0	12	24.8±1.6	100.0
	20	100.0	100.0		18.0±4.0	80.2		20	100.0	26.0		4.1±2.2	16.3
	36	98.4	88.9		5.3±3.0	23.8		36	98.0	0.0		—	—
Y-15	0	88.0	—	22	17.7±3.07	100.0	W023	0	78.0	79.4	40	17.6±4.9	100.0
	20	95.9	66.7		9.3±3.73	52.5		20	84.0	100.0		15.6±6.3	88.8
	36	84.0	60.0		3.2±1.92	18.1		36	84.0	97.6		12.3±6.1	69.7
								45	75.0	100.0		6.6±5.4	37.8

seedlings died.

Number of survival plants was counted 21 days after sowing. Survival rate was calculated on the basis of the number of germinated seedlings. As given in Table 2, it varied from species to species and also from strain to strain. *O. officinalis* (W0002) showed highest sensitivity and no survival seedling was obtained at 20 kR irradiation. *O. punctata* (W1514) completely died by irradiation at 36 kR. The radiosensitivity in *O. australiensis* (W008), having the largest amount of DNA per nucleus in diploid species of *Oryza*, was intermediate both of them. On the other hand, some strains of *O. sativa*, i.e., I-1, I-5, I-7 and I-17, showed the lowest sensitivity having survival rate of 100% at 36 or 45 kR, and followed *O. brachyantha* (W023) containing the smallest amounts of DNA per nucleus in *Oryza*.

Seedling height was measured 10 days after sowing. The results were given in Table 2. As shown in Table 2, decrease in seedling height varied from species to species and also from strain to strain. In some species and strains in *Oryza*, i.e., *O. sativa* (I-8 and I-20), *O. sativa* f. *O. spontanea* (Y-15) and *O. officinalis* (Y-21), the seedling height at 36 kR

irradiation was decreased to about 11–24% as compared with the non-irradiated control, while in the other strains belonging to *O. sativa*, i.e., I-1, I-7 and I-17, that at the same dosage corresponded to about 72–79% of control (Table 2). The relationship between DNA content per nucleus and dosage of 50% seedling growth inhibition was showed in Fig. 1. From Fig. 1, correlation between both them was not clear because of the highest resistance for γ -ray irradiation of *O. sativa*.

Discussion

The radiosensitivity of dormant dry seeds or growing plants in rice was investigated by various workers (KAWAI 1962, FUJII and MATSUMURA 1959, NAGAMATSU and KATAYAMA 1959, FUJII 1962). NAGAMATSU and KATAYAMA (1959), YAMAGUCHI and KOBAYASHI (1961) and FUJII (1962) described that the sensitivity differ from variety to variety, especially resistance in *indica* type is higher than that in *japonica* type of rice plants. FUJII and MATSUMURA (1959) and FUJII (1962) investigated the sensitivity of some species in *Oryza* belonging to different genomes, and reported that *O. sativa* f. *spontanea* and *O. glaberrima* were most resistant to radiation.

The reports in which the inherent radiosensitivity of different species of higher plants is determined by size and number of the genetic targets and by average length of the mitotic cycle were described by SPARROW and his coworkers (1961a and b, 1962, 1963, 1965). Namely, in diploid species it was reported that nuclear volumes and interphase chromosome volumes, particularly the latter was more effective to the sensitivity. And also, the high correlation found between DNA content and nuclear volume leads to the conclusion that radiosensitivity is also correlated with DNA content per nucleus. Moreover, a better correlation exists between sensitivity and DNA content per chromosome. These results suggest that it is not a constant percentage of the DNA or nucleoprotein per cell or per chromosome injured by the radiation that is critical, but rather the absolute amount injured (SPARROW *et al.* 1964).

On the other hand, BROWEN (1962) obtained a less satisfactory correlation between DNA per cell and LD-50 or between cell weight

Fig. 1. Relationship between DNA content and radiosensitivity in diploid *Oryza* species

and LD-50 for forty species of higher plant. For this results, SPARROW *et al.* (1964) assumed that the part of the somewhat unsatisfactory correlation may have been caused by mixing polyploid and diploid. Based on his results obtained from three *Sorghum* species, NIRULA (1963) concluded as follow: i) no direct relationship may exist between chromosome length and DNA content is species characterized by chromosomes having distinct eu- and hetero-chromatic segments, ii) no well defined relationship may exist between nuclear volume, chromatic length and DNA content. Nuclear volume alone, therefore, may not always be a reliable index of radiation sensitivity.

And also there were some reports on the genetical control of radiosensitivity (SMITH 1942, in *Triticum*; DAVIES 1962a and b, in *Lycopersicum* and *Melandrium*; TAKAGI 1969, in *Glycine*). From their data obtained after irradiation with neutron and γ -rays for the F_1 seeds which were produced by reciprocal crosses between high and low radiosensitive varieties, YAMAGUCHI and KOBAYASHI (1961) supposed that radiosensitivity in rice plants was affected by both genetic and plasmic influences.

In the present experiments, *O. brachyantha* (FF) contained the minimum amount of DNA per nucleus in diploid species of *Oryza*, while *O. australiensis* (EE) showed the maximum amount. DNA content per nucleus in *O. punctata*(BB), *O. sativa*(AA) and *O. officinalis* (CC) showed roughly same (KATAYAMA 1967 and Table 1). As to radiosensitivity, however, the maximum resistance to radiation showed in some strains of *O. sativa* (I-1, I-5 and I-7), and followed by *O. brachyantha* (W023). On the other hand, as tabulated in Tabel 2, *O. officinalis* (W0002) showed no germination even at 20 kR irradiation. And also, in spite of the fact that the different species within a genome had significantly no difference in DNA content per nucleus, they showed remarkable difference of radiosensitivity. From these facts, the author assumed that the radiosensitivity of the various species in *Oryza* is affected not only by the amount of DNA per nucleus but also by recovery of DNA molecules injured by the radiation. This may be the reason why it does not show clear

correlation between radiosensitivity and DNA content per nucleus in diploid species of *Oryza*. Thus, more investigations are required to solve the mechanism of recovery of DNA molecules injured by the radiation in genus *Oryza*.

Summary

In order to investigate the relationship between radiosensitivity and DNA content per nucleus of diploid species or strains in *Oryza*, this experiment was designed.

DNA content per nucleus was estimated by MSP-IV. Dormant dry seeds kept in 13% relative humidity at 20°C were exposed to 20-40 kR of ^{60}Co γ -rays at a dose rate of 19.74 kR/h.

The maximum resistance was showed in the species having A genome constitutidn, and followed by *O. brachyantha* (BB). In spite of containing about same amount of DNA with *O. sativa*, *O. officinalis* (W0002) showed no survival plant at even 20 kR irradiation.

In diploid *Oryza* species including each genome constitution, the relationship between DNA content per nucleus and 50% seedling growth (irradiation dose which is necessary to reduce seedling height to 50% of control) is summarized as follow:

$$F < B \approx A \approx C < E \cdots \cdots \begin{matrix} \text{DNA content per} \\ \text{nucleus} \end{matrix}$$

$$C < E < B < F < A \cdots \cdots \begin{matrix} 50\% \text{ seedling} \\ \text{growth} \end{matrix}$$

Based on the present result, relationship between radiosensitivity and DNA content per nucleus was not clear among the species in *Oryza*.

From these results, the author assumes that the sensitivity for radiation of the species in *Oryza* may result from the interaction both of DNA content per nucleus and ability of recovery of DNA molecules injured by the radiation.

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植物における放射線感受性

II. 二倍性イネ属植物の核当り DNA 含量と放射線感受性について

片 山 平

(九州大学農学部)

すでに, SPARROW ら (1953 ; 1961a, b ; 1962 ; 1964 ; 1965) によつて, 高等植物の放射線感受性と核容積, 中間期染色体容積 (ICV) との間には, 高い負の相関々係のあることが報告されている。

一方, イネ属植物の種, 品種, 系統についての放射線感受性は, 種間, 品種間, 系統間で, かなりの差異のあることが認められている (KAWAI 1962, FUJII and MATSUMURA 1959, NAGAMATSU and KATAYAMA 1959, FUJII 1962)。

本研究は, イネ属の二倍性植物を用いて, 種および系統間の放射線感受性と核当り DNA 含量との関係を明ら

かにすることを目的として行つた。

イネ属植物の各ゲノムに属する二倍種について, 核当り DNA 含量を測定した結果は, すでに報告したところであるが (KATAYAMA 1967), 本研究では, とくに, A, B および C ゲノムに属する種または系統に関して, 同一ゲノムに属する異なる種または系統間の核当り DNA 含量の変異をしらべた。結果は第 1 表にまとめた様に, ゲノム内では有意な差は認められなかつた。この結果は前報 (KATAYAMA 1967) のそれと同様であることを示すものである。

一方, 乾燥種子に対して, ^{60}Co の 20, 36, 45 kR を

19.74 kR/h の線量率で照射し、発芽率、幼苗長、生存率を測定し、さらに、対照区の幼苗長に対して 50% の伸長阻害を示す照射線量を推定した。

イネ属植物では、感受性には関係なく、かなりの大線量を照射した場合でも種子の発芽は完全で、その後感受性の程度に応じて幼苗は枯死する。従つて、本研究で用いた程度の照射量では、全処理区とも発芽率に差異はみられない (第 2 表)。

下種後 21 日目における生存率をまとめて第 2 表とした。同表から *O. officinalis* (W0002) は最も高い感受性を示して 20 kR で、ついで *O. punctata* (W1514) は 36 kR で、それぞれ生存個体を認めることは出来なかつた。これに対して、*O. sativa* (I-1, I-5, I-7, I-17) は強い抵抗性を示した。しかるに、これらの DNA 含量は、第 1 表にみられる様に有意な差はなく、大体、 $B \approx A \approx C$ の関係がみられている。また、DNA 含量の最も少ない *O. brachyantha* (W023) は *O. sativa* について放射線抵抗

性を示した。

幼苗長は下種後 10 日目に測定した。第 2 表から 36 kR 照射区における幼苗長は、それぞれの対照区に比較して、*O. sativa* (I-8, I-20), *O. sativa* f. *spontanea* (Y-15) および *O. officinalis* (Y-21) では約 11~24% であったのに対し、同じ *O. sativa* の I-1, I-7, I-17 では 70~80% と高い抵抗性を示し、*O. brachyantha* (W023) はこれについてだ。

核当り DNA 含量と幼苗長を 50% 阻止するために必要な線量との関係をまとめて第 1 図とした。同図からも明らかな様に、両者の間には明瞭な関係は認められない。

以上の結果から、イネ属の二倍性植物に属する種および系統間の放射線感受性は、単に、核当り DNA 含量のみによらず、照射による DNA 障害の修復現象との相互作用によると考えられる。この修復機構に関しては、さらに今後の研究にまたねばならない。