ダイズにおけるガンマー線による染色体異常の品種間差異

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VARIETAL DIFFERENCES IN GAMMA-RAY INDUCED CHROMOSOME ABERRATIONS IN SOYBEAN

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When the seeds of 27 soybean varieties were exposed to gamma-rays, distinct varietal differences in the frequency of chromosome aberrations were found at the first root tip mitosis. The varieties tested could be divided into resistant and sensitive groups. F_2 seeds from crosses between resistant and sensitive varieties were irradiated and from the frequency distribution of chromosome aberrations it was concluded that the intervarietal differences were controlled mainly by a single recessive gene rs_1 which had been detected by Takaki and Yamashita to control the degree of seedling growth inhibition in both seed and growing plant irradiations.

INTRODUCTION

The aim of the present study is to elucidate a genetically controlled variation in radiation induced chromosomal aberrations in soybean *Glycine max* (L.) Merrill.

Although varietal differences in radiosensitivity have been reported by many researchers with different plant species using various end-points of radiation effects, relatively few data are available at present concerning chromosomal aberrations. Gelin *et al.* (1958) reported that pea varieties differed in chromosome damages induced by X-ray irradiation of seeds. Stoilov *et al.* (1966) showed from neutron irradiation experiments of maize seeds a variation in the frequency of chromosome damages among the inbred lines used, and also between parental lines and the F_1 plants of their single or double crosses. Lawrence (1963) demonstrated that chromosome interchange frequency induced by irradiation of plants at the flowering stage differed among the inbred lines tested.

In soybean, Yamashita (1964) and Takaki and Yamashita (in press) have observed a marked intervarietal variation in growth inhibition after acute irradiation to seeds and chronic irradiation to whole growing stage. They found that the variation is controlled mostly by a recessive major gene which was denoted as rs_1 by them. In the present paper it is shown, from gamma-ray exposure of seeds, that the gene exerts its influence not only on growth depression as they have demonstrated but on chromosome damages at root tip mitosis.

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MATERIALS AND METHODS

Seeds of 27 soybean varieties were exposed to 20 kR of ^{60}Co gamma-rays at a dose rate of 1 kR per h, 20 seeds being used for each. Prior to irradiation the seeds were stored in a desiccator containing saturated solution of $NaClO_3$ for more than two weeks until they showed a constant weight. The moisture content of the seeds was then 9.1% on the average. Of the varieties used, 20 were of determinate type in which growth of the main stem terminates abruptly, and 7 were of indeterminate type in which main stem tapers gradually. Immediately after irradiation the seeds were immersed in Usuplun solution for sterilization for half an hour and placed on moist filter paper in petri dishes. They were kept in a dark temperature controlled chamber at 25°C for 48 h and root tips were fixed with glacial acetic acid. The root tips were washed, hydrolyzed at 60°C in normal HCl for 10 min, stained with Feulgen solution for 2 h and then squashed on a slide in a 10% glycerol solution. The number of chromosome fragments and bridges was counted at anaphase of first root tip mitosis. Fifty, at maximum, cells per root were observed.

In order to determine dose response curves for chromosome aberrations, seeds of two varieties with different radiosensitivity, Tachisuzunari and Senryu, were exposed to 10, 20, 30 and 40 kR of gamma-rays at a dose rate of 1 kR per h. In each treatment, about 450 cells were observed.

For a comparison of radiosensitivity measured by cytological damage and growth depression, the seeds of 26, out of the 27 varieties used for the first experiment, were irradiated with five different doses of gamma-rays ranging from 5 to 40 kR. Irradiated and control seeds were sown outside in pots, 20 seeds for each being employed. Forteen days after sowing the seedling height was measured for all of the plants. The D_{50} dose which is required to reduce the unirradiated control value by 50% was estimated for each variety.

Finally, to elucidate a genetic segregation of radiosensitivity in progenies of crosses between resistant and sensitive varieties, the following two crosses were made.

Cross I :	Tachisuzunari (Resistant)	×	Senryu (Sensitive)
Cross II:	Sennari-komusume (Sensitive)	×	Akasaya (Resistant)

All the four varieties used in the crosses were of determinate type. F_2 seeds were irradiated with 20 kR of gamma-rays and frequencies of chromosome fragment and bridge were observed in a similar way to that described above.

RESULTS

The results obtained from cytological investigations on the root tip meristem of 27 varieties are summarized in Table 1.

A marked varietal difference in radiosensitivity can be observed in the frequencies of chromosome damages. The frequency of chromosome fragment per cell varied from

Table 1. Chromosomal aberrations at first root tip mitosis observed in 27 varieties of soybean after irradiation of seeds with 20 kR of gamma-rays

	Variety	Type of growth habit*	No. of cells observed	Proportion of cells with bridges	Fragments per cell	Aberrant cell frequency
1	Nakateppou	det	238	.055	.214	.172
2	Toshu	det	321	.069	.324	.252
3	Iwate 2	det	317	.110	.268	.268
4	Tachisuzunari	det	450	.142	.220	.271
5	Akasaya	det	364	.162	.198	.275
6	Aogin	det	368	.155	.348	.323
7	Tamatsukuri 2	det	350	.117	.466	.337
8	Karimame	det	374	.136	.476	.337
9	Mejiro 1	det	86	.186	.372	.350
10	Tojyo daizu	det	324	.176	.540	.380
11	Norin 1	det	350	.143	.437	.426
12	Shinmejiro	det	219	.224	.553	.447
13	Miyashiro jun 1	det	279	.194	.982	.552
14	Sennari komusume 1	det	325	.197	.843	.554
15	Takei 8	det	70	.271	.757	.557
16	Senryu	det	319	.273	.834	.558
17	Rikuu 17	det	241	.191	.905	.589
18	Nanbu	det	298	.218	1.030	.594
19	Karikei 1	det	361	.222	1.230	.634
20	Takiya early	det	375	.163	1.256	.646
21	Magusa kuimame 2	indet	221	.090	.317	.267
22	Kuromame	indet	328	.110	.329	.274
23	Virginia bean	indet	368	.101	.342	.280
24	Magusa kuimame 17	iedet	380	.158	.584	.395
25	Chief	indet	300	.237	.587	.503
26	Goishi shirobana	indet	165	.242	1.127	.558
27	Lexington	indet	400	.195	.970	.578

* Det and indet stands for determinate and indeterminate types of growth habit, respectively.

0.198 to 1.256 and the proportion of cells with bridges from 0.055 to 0.273. The values of these two chromosomal aberrations were highly correlated among the varieties, the correlation coefficient being 0.809 ($P<0.01$). A combination of these two values therefore might provide a better measure of radiosensitivity as regards chromosome damages than either of them. Hence, the proportion of cells with fragments and/or bridges (which will be referred to as aberrant cell frequency hereafter) was obtained for each variety. The value ranged from 0.172 to 0.646. The frequency distribution of the varieties tested for the aberrant cell frequency is given in Fig. 1.

The distribution was apparently bimodal. The varieties could be divided into two distinct groups of radiosensitivity, resistant and sensitive. However, one variety Shin-

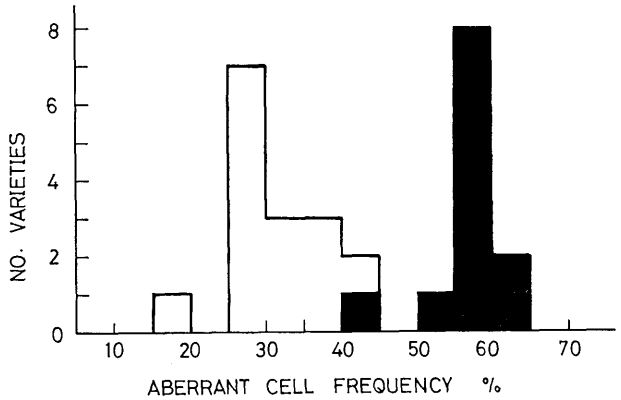

Fig. 1. Frequency distribution of 27 soybean varieties for aberrant cell frequency at first root tip mitosis after irradiation of seeds with 20 kR of gamma-rays. Open and solid column represents resistant and sensitive group, respectively.

mejiro which had an aberrant cell frequency of 0.447 (Table 1) and seemed to fall in the resistant group in Fig. 1, has shown high aberrant cell frequency comparable to varieties in the sensitive group in later experiments. It has proven to belong to the sensitive group, and hence, the boundary between the two groups should be drawn at 0.40-0.45 instead of 0.45-0.50.

The average index of radiosensitivity and the number of varieties of the two groups are shown in Table 2.

Table 2. Average values of chromosomal aberrations of the four groups of varieties classified by growth habit and radiosensitivity

Growth habit	Radiosensitivity	No. of varieties	Proportion of cells with bridges	Fragments per cell	Aberrant cell frequency
Determinate	Resistant	11	.132	.351	.308
	Sensitive	9	.217	.932	.570
Indeterminate	Resistant	4	.115	.393	.304
	Sensitive	3	.225	.895	.578

Determinate and indeterminate types of growth habit are kept separate in the table, because they behaved differently on the degree of growth inhibition induced by seed irradiation and by chronic irradiation of growing plants. However, the mean aberrant cell frequency of resistant and sensitive groups were similar for both types of growth habit.

Analysis of variance for the aberrant cell frequency (arcsin transformed) is given in Table 3. The analysis is based on the data from roots where 50 cells at anaphase could be observed. The variation among varieties was significant in the resistant group, but not in the sensitive group.

Table 3. Analysis of variance for aberrant cell frequency in 27 soybean varieties (arcsin transformed)

Source			d. f.	S. S.	M. S.	F
Growth habit	Radiosensitivity	Variety				
Determinate	Resistant	Between	10	1102	110.2	3.7**(1)
		Within	69	2067	30.0	
	Sensitive	Between	8	417	52.1	
		Within	38	1628	42.8	
	Res.-Sens.		1	8498	8498.0	100.7**(2)
Indeterminate	Resistant	Between	3	172	57.3	
		Within	15	1391	92.7	
	Sensitive	Between	2	119	59.5	
		Within	12	469	39.1	
	Res.-Sens.		1	1539	1539.0	26.4**(3)
Det.-Indet.			1	1	1.0	
Total			160	17403		

(1) Tested by the variance within variety of determinate resistant group.
(2) Tested by the variance between varieties pooled over resistant and sensitive groups of determinate types.
(3) Tested by the variance between variety pooled over resistant and sensitive groups of indeterminate type.
** Significant at 1% level.

Figure 2 gives the dose response curves for the frequency of normal cells of resistant Tachisuzunari and sensitive Senryu. Differences in sensitivity was apparent for all the doses applied.

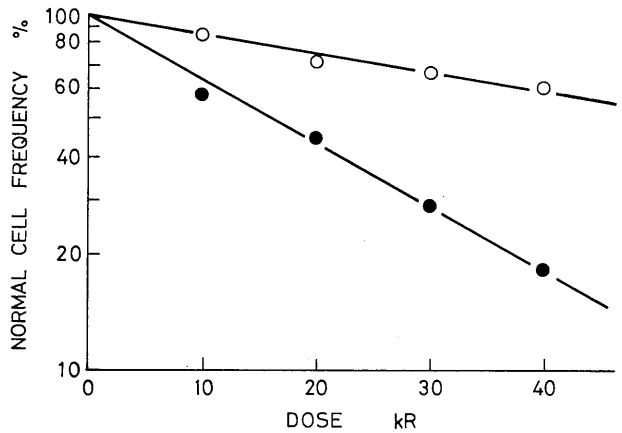

Fig. 2. Dose response curves for aberrant cell frequency of a resistant (Tachisuzunari -○-) and a sensitive (Senryu -●-) varieties.

In order to examine the relationship between the radiosensitivity measured by chromosome injury and that by growth inhibition, the D_{50} for seedling growth was compared with the corresponding aberrant cell frequency (Fig. 3).

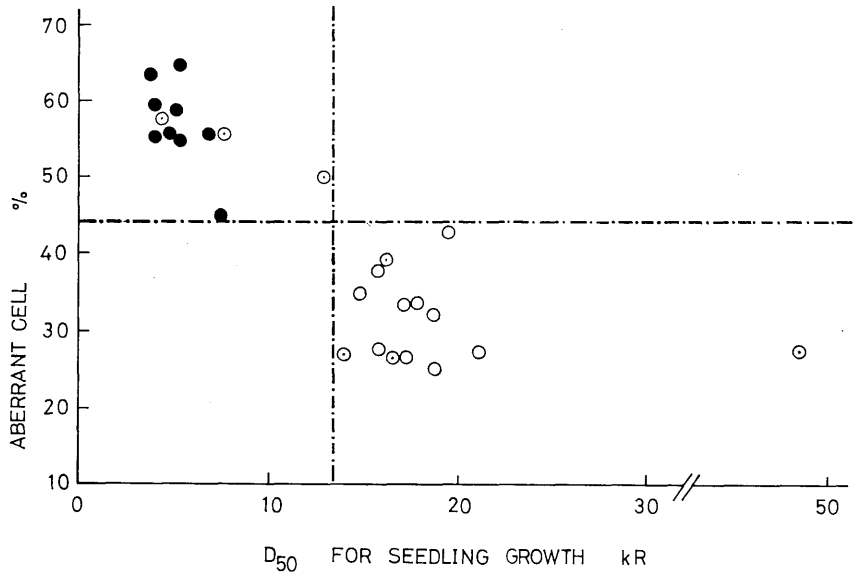

Fig. 3. Relationship between the aberrant cell frequency and the radiosensitivity as regards seedling growth inhibition. Open and solid circles stand for resistant and sensitive varieties of determinate type, respectively, and dotted circles denote varieties of indeterminate type.

The values of D_{50} for seedling growth varied from 4.1 to 21.2 kR with the exception of one indeterminate variety, Virginia bean, which proved extremely resistant, showing D_{50} of 48.5 kR. As in the case of aberrant cell frequency, the varieties used could be divided into two distinct groups according to the degree of growth inhibition. Moreover, the varieties which belonged to resistant and sensitive group as regards chromosome aberrations were found to be, without exception, in resistant and sensitive group for growth inhibition, respectively. Excluding the variety Virginia bean, the correlation between aberrant cell frequency and D_{50} for seedling growth was very high (0.902), significant at the 1% level. Within resistant and sensitive groups, however, correlation was insignificant.

The chromosomal damages found in the two F_2s and their parents are given in Table 4. The proportion of cells with bridges in the F_2 was lower than that in the resistant parents, while for the frequency of fragments per cell F_2s was intermediate between the parents. The mean aberrant cell frequency of the F_2s was also intermediate, rather near to the resitant parent, indicating that resistant is dominant over sensitive. Further to examine whether, as in the case of growth inhibition, the varietal difference in the frequency of chromosome damages is mainly controlled by the single major gene rs_1 or not, the frequency distributions of the aberrant cell frequency for the F_2 plants and their parents were compared (Fig. 4a, b).

Table 4. Chromosome aberrations of two sets of resistant and sensitive varieties and F_2 from a cross between the parents

Population	No. of roots observed	No. of cells observed	Proportion of cells with bridges	Fragments per cell	Aberrant cell frequency
Tachisuzunari (T)	17	820	.136	.228	.268
Senryu (S)	10	481	.237	.896	.563
F_2 (T×S)	62	3093	.089	.423	.298
Akasaya (A)	18	864	.131	.262	.262
Sennari komusume(S′)	15	515	.163	.913	.546
F_2 (S′×A)	127	6324	.127	.405	.314

The distribution of the F_2 plants ranged more widely than those of the parents, showing a clear heritable nature of the sensitivity in the parents. The distribution, however, did not show the bimodality to be expected from a monogenic segregation of 3 resistant to 1 sensitive. But it is possible that bimodality may be masked by a rather large variation in aberrant cell frequency. Hence, a method suggested by Allard (1956) for testing the number of genes participating the inheritance of semiqualitative characters was applied. The frequency distributions characterized by the following means and standard deviations were assumed to represent the three genotypes expected in the F_2 generation.

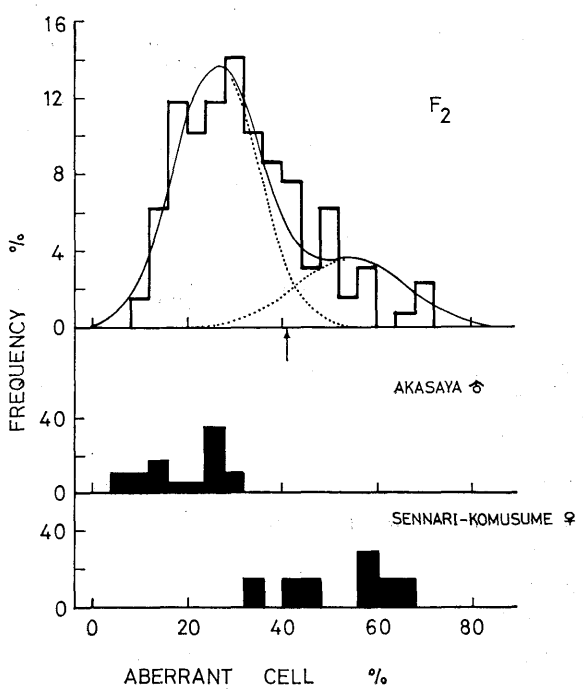

Fig. 4a.

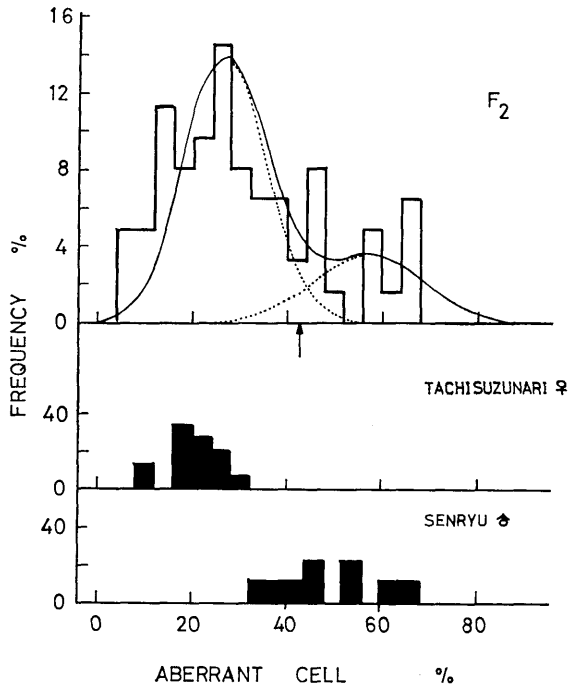

Fig. 4b.

Fig. 4a and b. Frequency distributions for aberrant cell frequency of two F_2s and their corresponding parents, following exposure of seeds to 20 kR of gamma-rays. Theoretical frequency distribution curves calculated on the basis of monogenic segregation with complete dominance are also given. Arrow indicates the point beyond which the area under the two theoretical distribution curves of the parents are equal to each other.

	Cross I		Cross II	
	Mean	Standard deviation	Mean	Standard deviation
	%	%	%	%
Dominant homozygote and heterozygote	26.8	8.05	26.2	8.05
Recessive homozygote	56.4	9.75	54.6	9.75

The means of the dominant homozygote and the heterozygote were very similar to those of the resistant parent, while the means of the recessive homozygote are those of the sensitive parent. The standard deviation was estimated from the variance (arcsin transformed) within variety of the resistant and sensitive groups of the determinate type, this being considered to be the best estimate available of the environmental variance of each genotype. By reference to tables of the normal probability integral, theoretical frequency distributions representing the three genotypes were constructed. From these expected frequency distribution representing each F_2 was obtained

by combining the theoretical distributions of dominant and recessive phenotype in a ratio of 3 :1. For the cross II the expected frequency distribution thus obtained was similar to the observed one and a χ^2-test showed the fit to expectation to be satisfactory. For the cross I, however, there is a minor discrepancy between the expected and observed frequency distributions. Namely, the observed distribution corresponding to the resistant parent deviated slightly toward low aberrant cell frequency from the expected one. For both crosses any hypothesis in which more than one gene with equal effect were assumed could not result in better fit for the actual distributions than the one gene hypothesis. When the distributions of the F_2s were divided into two parts at a point beyond which the area under the two expected parental distribution curves are equal, the frequencies of the resulting two parts were in a ratio of 47 :15 for the cross I and 97 :30 for the cross II. The ratios differed insignificantly from an expected ratio of 3 :1 for monogenic segregation. Therefore, it can be concluded that a single gene pair could account for the predominant part of the genetic variability of the F_2s, though it does not necessarily exclude further influence of some minor genes on the segregations.

DISCUSSION

According to Takaki and Yamashita (in press), the major part of the intervarietal differences in radiosensitivity for growth inhibition induced by seed irradiation in soybean is due to a single gene pair Rs_1-rs_1, with the resistant type being dominant. They showed that an Rs_1Rs_1 variety has D_{50} for seedling growth about four times as high as that for the genotype rs_1rs_1. This distinct difference among varieties in the degree of growth depression could be confirmed in the present experiment. It was found that all resistant varieties as regards growth depression were resistant with respect to chromosomal aberrations and all of the sensitive varieties were sensitive, and that the variation in chromosomal aberrations between resistant and sensitive groups occupied the predominant part of the total variation. Hence, it may be concluded that the chromosomal radiation injuries are also controlled mainly by the Rs_1-rs_1. This conclusion can be supported by the result that the theoretical frequency distributions computed on the basis of a monogenic segregation with complete dominance were in fairly good agreement with the actual ones in two F_2 populations derived from crosses between resistant and sensitive varieties.

Other minor heritable factors, however, may not be indispensable in the control of radiosensitivity as regards chromosome damages, especially among the resistant varieties of the determinate type, since a significant variation in the mean aberrant cell frequency was detected among these varieties.

Takaki and Yamashita (in press) have reported heterosis for resistance to gamma-rays measured by seedling height in a cross between resistant and sensitive soybean varieties. Investigating chromosomal aberrations after neutron irradiation of maize seeds, Stoilov *et al.* (1966) also have shown that inbred lines were more strongly affected than their hybrids. It is not unlikely, therefore, that the minor discrepancy between the expected and observed frequency distributions of F_2 in the cross I reflects some

heterosis effect of the major or minor gene(s).

Although there existed a close correlation between the varietal sensitivity in terms of chromosome aberrations and growth inhibition, it does not necessarily follow that the former radiosensitivity is the direct cause of the latter. It has long been accepted by many radiobiologists that growth depression of roots and shoots due to irradiation can be ascribed to chromosomal aberrations induced in the meristems (Van't Hof and Sparrow 1963; Read 1959). Nevertheless, data against this general thought were also presented by some researchers (Davies 1963; Evans 1965). Ukai (1968) in his studies on differential radiosensitivity of rice varieties has shown that proportion of cells with bridges at first root tip mitosis was practically similar in varieties differing as much as three times in D_{50} for root and seedling growth. Therefore, the present authors are in favour of interpreting the result reported here as showing that in soybean the gene pair Rs_1-rs_1 controls both chromosome aberration and growth inhibition, rather than that the difference in the frequency of chromosome aberrations controlled by the major gene leads to the difference in growth inhibition as an end effect of radiations.

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