CYTOLOGICAL AND GENETIC CHANGES INDUCED IN SORGHUM PURPUREO-SERICEUM BY THERMAL NEUTRONS

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From the study of "Reciprocal translocations induced by X-rays and thermo-neutrons in wild sorghum" (Wu and Pi, 1968), we know that thermal neutrons produce no serious effect on seed viability, but have a significant effect on inducing chromosome aberrations, and we also know that the exposure at 3.5×10^{13} n/cm^2 is the most appropriate one for thermal neutron treatment, at which most chromosome aberrations are induced and the least damage to plants occur. Based on this information, the author treated *S. purpureo-sericeum* seeds with the proper dosages of thermal neutrons 3.5×10^{13} n/cm^2 and 4.0×10^{13} n/cm^2 respectively in the present experiment, and expected to induce more types of chromosome aberrations and get more gene mutations.

No work has previously been done on the effect of thermal neutron flux on seed viability, so the author is presenting the data obtained from his investigation in this paper.

In the present study, two types of chromosome interchanges, i.e. ring-of-four chromosomes and pseudo-isochromosomes, were induced by thermal neutron irradiations and can easily be detected in the microsporocytes. Application of this data may provide a method to study the problem of whether chromosome breakage and recombination induced by irradiations occur at random. Finally, this paper deals with the relationship between the frequency of multivalents and chromosome non-disjunction in translocation heterozygotes.

MATERIALS AND METHODS

The annual wild sorghum, *Sorghum purpureo-sericeum* Aschers and Schweinf, was used in this experiment. Three lots of *S. purpureo-sericum* seeds were exposed to thermal neutron irradiations at 3.5×10^{13} n/cm^2 (flux; 3.2×10^{8} n/cm^2 sec), 3.5×10^{13} n/cm^2 (flux; 6.54×10^{8} n/cm^2 sec) and 4.0×10^{13} n/cm^2 (flux; 3.7×10^{8} n/cm^2 sec) doses separately. The thermal neutrons were generated in the thermal neutron column of the nuclear reactor at National Tsing-Hua University, for which the author is very grateful to this said institution. The irradiated and control seeds were soaked in water for 24 hours and germinated in Petri dishes. The germinated

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seeds were first planted in wooden flats in the green house, and then transplanted in the field. The germination percentage of each seed lot was recorded at the time of planting. Seedling height and number of white seedlings were determined at the time of transplanting. Suitable panicles of each plant in each experimental treatment were fixed in 1:3 ferric propionic alcohol for 24 hours. The types and frequencies of chromosome aberrations in microsporocytes, and abnormal behavior of chromosomes in meiosis were examined cytologically. The propionic carmine method was used throughout this experiment. Numbers of light green mutations and iojap mutations were taken at the heading stage. Before harvesting, data was taken on the number of tillers.

RESULTS AND DISCUSSION

1. The effect of thermal neutron flux on seed viability.

As shown in Table 1. *S. purpureo-sericeum* seeds treated with total dosage of thermal neutrons 3.5×10^{13} n/cm^2 at a constant intensity of flux 3.2×10^{8} n/cm^2 per second showed no significant reduction in germination and in seedling survival. But at the same total dosage of thermal neutron irradiation 3.5×10^{13} n/cm^2 if the flux was increased from 3.2×10^{8} n/cm^2 sec. to 6.54×10^{8} n/cm^2 sec. None of the five hundred treated seeds germinated. This result shows that the thermal neutron flux is much more effective in preventing seed viability than the total dosage of thermal neutrons. The degree of inhibition to seed viability increased with the intensity of the flux. Therefore, we should not only consider the total dosage of treatment, but also the intensity of flux, before we can expect to induce gene mutations and chromosome aberrations.

Hsieh (1961) irradiated seeds of rice with total dosage of thermal neutrons 3.6×10^{13} n/cm^2 for 15 hours and found that chromosome aberrations and gene mutations were induced, but no significant injury was observed in plant growth. The dosage that Hsieh employed was slightly stronger than the dosage of thermal neutrons which was used in this experiment, 3.5×10^{13} n/cm^2 at a constant flux intensity of 6.54×10^{8} n/cm^2 sec, but the latter caused the death of all seeds. It is suggested from these results that various species of plants have different degrees of sensitivity to irradiation intensity, and the tolerance of rice to thermal neutron treatment is greater than that of the wild sorghum. Gaustafson (1944) found that the maximum dose of X-rays a species could withstand and survive, varied widely for different species, ranging from only 5000 r of X-rays for the sunflower to 92,000 r for white mustard.

2. The effects of thermal neutron irradiations on several characteristics of the R_1 generation plants.

S. purpureo-sericeum seeds were exposed to thermal neutron irradiations at total dosages 3.5×10^{13} n/cm^2 and 4.0×10^{13} n/cm^2 respectively, and the flux of these two treatments was about 3.2×10^{8} n/cm^2 sec. The treated and control seed were sown and grown in the fields. The germination percentage, seedling survival, seedling height and number of tillers in both control and thermal neutron treatments are presented in Table 1.

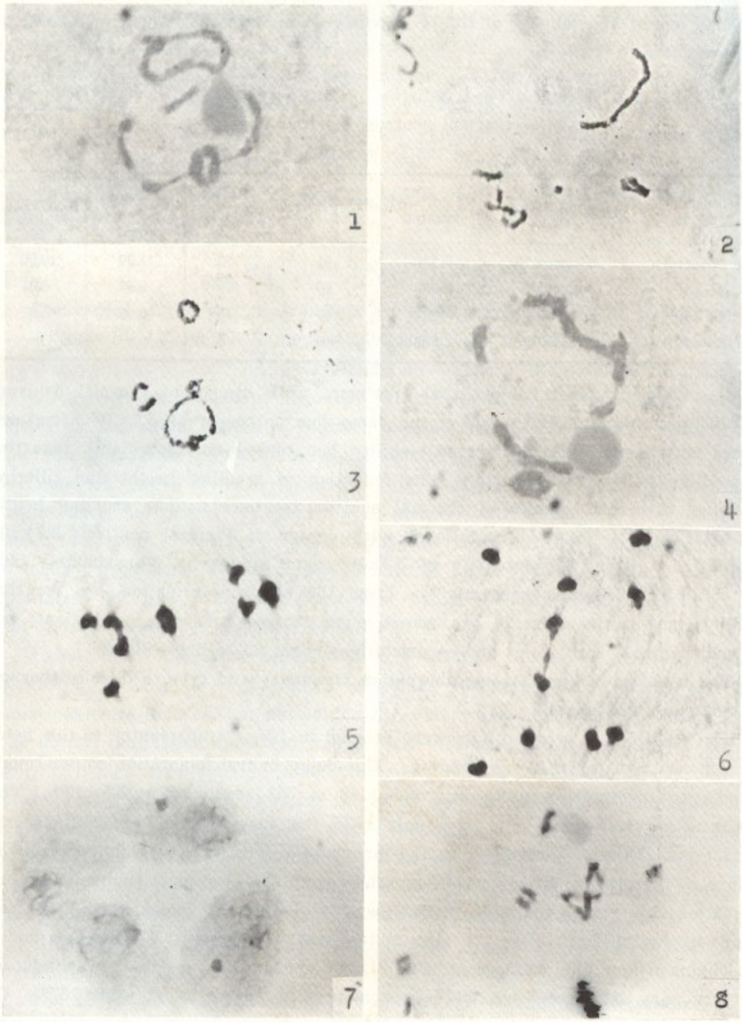
Table 1. Effects from irradiation of sorghum seeds with varying dosages of thermal neutrons on four characteristics of the R_1 generation

Total dosage (n/cm^2)	Flux (n/cm^2 sec.)	No. of seeds treated	germination (%)	Seedling survival (%)	Ave. seedling height (cm)	Ave. tiller
0 (control)	0 (control)	100	91	83	11.99	10.66
3.5×10^{13}	3.2×10^{8}	450	90	78	9.56	8.01
4.0×10^{13}	3.7×10^{8}	300	87	75	6.98	5.61
3.5×10^{15}	6.54×10^{8}	500	0	0	—	—

It is apparent from Table 1. that treatment with dosage of thermal neutrons 3.5×10^{13} n/cm^2 or 4.0×10^{13} n/cm^2 at the same flux intensity of 3.2×10^{8} n/cm^2 sec. caused no marked reduction in seed viability, but caused an appreciable reduction in seedling height and tillering. The reduction of seedling height and tillering increased with total dosage of thermal neutron treatments. The seedling height and tiller number following treatment with dosage of thermal neutrons 4.0×10^{13} n/cm^2 at a constant flux intensity of 3.2×10^{8} n/cm^2 sec. were approximately 58% and 53% of the controls respectively. From the above information, we see that seedling height and tillering are sensitive to thermal neutron irradiations, but thermal neutron irradiations has no deleterious effect on seed viability.

3. The relation of chromosome aberration frequency and type to dose of thermal neutron irradiations.

The seedlings in wooden flats were delayed in being transplanted to the fields for a month because of a rainy season. This delay in transplantation caused much damage to seedlings, consequently only 168 of 352 seedlings at 3.5×10^{13} n/cm^2 treatment survived to maturity, and only 25 of the survivors showed chromosome aberrations. Most of these were a ring- or chain-of-four type, which occurred in twenty of the plants. Pseudo-isochromosomes were the next most common type of aberration observed in the microsporocytes, two plants were found which contained pseudo-isochromosomes. One plant had a two rings-of-four (Fig. 1), and one had a two chains-of-four plus a fragment (Fig. 2), and one with a ring-of-six was induced. The frequency of chromosome aberrations induced in this treatment was 16.67%. Of a total of 226 seedlings at 4.0×10^{13} n/cm^2 treatment, only 73 attained maturity, and 27 of them examined showed the presence of chromosome aberrations. A ring- or chain-of-four was the most frequent type of chromosome aberrations, in 23 plants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this type was observed. Other types of chromosome aberrations were seen, but only one of each of the following types were observed, and these were two chains-of-four plus a fragment, a ring-of-four plus two pseudo-isochromosomes (Fig. 3), a ring-of-six, and a ring-of-eight (Fig. 4). In this treatment, the frequency of chromosome aberrations rose to 43.84%. The results indicate that the exposure of 4.0×10^{13} n/cm^2 at a constant intensity of flux 3.7×10^{8} n/cm^2 per second was the better one for thermal neutron treatment, at which a greater frequency in aberrations and more types of chromosome aberrations were induced. Data for the frequencies of different types of chromosome aberrations determined from R_1 microsporocytes are presented in Table 2.

Table 2. The frequencies of different types of chromosome aberrations in R_1 generation irradiated with thermal neutrons

Total dosage (n/cm^2)	Flux (n/cm^2 sec.)	No. of plants ex-amined	No. and types of plants showing chromosome aberrations							Total no. of plants showing chromosome aberrations	*Frequency of chromo-some aber-rations (%)
			⊙1	2⊙4	2 chains-of-four + fragment	⊙6	⊙8	⊙4 + 2 pseudo-isos	2 pseudo-isos		
3.5×10^{13}	3.2×10^{8}	168	20	1	1	1			2	25	16.67
4.0×10^{13}	3.7×10^{8}	73	23		1	1	1	1		27	43.84
Total		241	43	1	2	2	1	1	2	52	24.90

* Whether homologous or non-homologous, the interchange was considered as a unit of chromosome aberration. When the data for types of chromosome aberrations were transformed into percent, ring-of-six was counted as 2 interchanges and ring-of-eight as 3 interchanges.

The author found only chromosome interchanges but no chromosome inversion in the R_1 microsporocytes in this study. Contrary results were reported by Glass (1940) who found only inversions but no interchange in the X-rayed Drospohila oöcytes. The cause of the different results is obscure. In the present experiment, whether in the controls or in irradiation treated seeds, many plants developed panicles which showed some with precociously separated bivalents at the first metaphase (Fig. 5). The cause of precocious separation of paired chromosomes was because the chromosomes of this bivalent were shorter than the others, due to the short chromosomes lacking interstitial chiasmata, therefore the bivalent separated precociously at the first metaphase. In many panicles observed there was a chromosome bridge without a fragment at the first anaphase (Fig. 6), and this may be due to

Fig. 1. Diakinesis. Two rings-of-four. Fig. 2. Diakinesis. Two chains-of-four plus a fragment. Fig. 3. Diakinesis. A ring-of-four plus two pseudo-isochromosomes. Fig. 4. Diakinesis. A ring-of-eight. Fig. 5. First metaphase. A precociously separated bivalent. Fig. 6. First anaphase. A delayed separated bivalent. Fig. 7. Tetrad stage. Micronuclei. Fig. 8. Diakinesis. Zigzag configuration.

A ring-of-four, A ring-of-six and chromosome non-disjunction were induced in our previous work (Wu and Pi, 1968), Photomicrographs of them are omitted in this paper.

the presence of interstital chiasmata in the long chromosomes leading to the lagging at first anaphase.

4. Frequencies of interchanges between non-homologues and between homologues produced by thermal neutron irradiations.

In the present study, two of the interchange types induced in sorghum seeds by irradiations can be easily detected in microsporocytes. One of these induced most commonly is the interchange between the non-homologous chromosomes which can be recognized at meiosis as ring or chain of chromosomes. The other is the interchange between the opposite arms of the members of homologous chromosomes that results in the formation of a pair of doughnut-shaped pseudo-isochromosomes in diakinesis. At anaphase, one or both pseudo-isochromosomes lagged behind, the lagging pseudo-isochromosomes at the first and second anaphases might not be included in the daughter nuclei at first and second telophases and formed micronuclei in the tetrad stage (Fig. 7). Application of information on the interchanges between homologous and between non-homologous chromosomes. Koo (1959) provided an excellent method to study the problem of random recombination if the breakages occur at random. These two types of interchanges are highly suitable for the comparative study of their freqency of occurrence because both types are formed in the same manner, namely by a recombination involving two breaks in different chromosomes. If the breakage and recombination occur at random, then a definite proportion for each type is expected in a given organism.

On the assumption that the breakages and recombinations occur at random, a generalized expression for calculating the expected ratio of rings and pseudo-isochromosomes in any given organism was derived as follows.

For an organism with N pairs of chromosomes, the total number of possible breakage combinations is $4\cdot {}_{2N}C_2$.

Although four breakage combinations are possible in each pair of homologues, only two of them would yield pseudo-isochromosomes, therefore, the interchanges resulting in pseudo-isochromosomes, would be only one-half of the total. In an organism with N pairs of chromosomes, the expected frequency of interchanges producing pseudo-isochromosomes is 2N.

All 16 possible breakage combinations are capable of producing interchanges which would lead to the formation of rings-of-fours. For an organism having N pairs of chromosmes, the expected frequency of interchanges between non-homologues is $16\cdot {}_{N}C_2$.

Therefore, the expected ratio for the random occurrence of rings and pseudo-isochromosomes in an organism with N pairs of chromosomes is $16\cdot {}_{N}C_2/2N$, namely $4(N-1):1$. For *S. purpureo-sericeum* which has five pairs of chromosomes, the expected ratio is 16:1.

The observed values of Table 3 included the frequencies of interchanges that were induced in previous (Wu and Pi, 1968) and present experiments, so that there was a large amount of information for us to analyze accurately. The P value obtained from the X^2 test for the data of each method is larger than 0.05, suggesting non-significant deviation from the expectation. The results indicate that the breakage and recombination between homologous or nonhomologous chromosomes of *S. purpureo-sericeum* produced by various dosages of thermal neutron irradiations occurred at random. Similar results were obtained by Koo (1959) in barley and corn.

Table 3. Frequencies of interchanges between non-homologues and between homologues produced by thermal neutron irradiations in *S. purpureo-sericeum* and the chi-square tests for "goodness of fit" of observed frequencies to theoretical ratios

*Method of frequency counting	No. interchanges between non-homologues	No. interchanges between homologues	X^2	P.
1	105	7	0.02727	0.90–0.80
2	125	9	0.16841	0.20–0.15

* Method 1. Frequencies of interchanges were determined only from the cells with one ring of four or a pair of pseudo-isos, each of which was counted as one.

Method 2. Frequencies of interchanges were determined from all cells, including those with multiple interchanges. A ring of four or a pair of pseudo-isos was counted as one. A ring of six or eight was counted as two or three reciprocal interchanges, on the assumption that 4 or 6 breaks were involved in the formation of each of these larger rings.

5. Relationship between the multivalent percentage and chromosome non-disjunction in translocation heterozygote.

The interchange complex of the translocation heterozygote may be oriented at the first metaphase so that either alternate or adjacent chromosomes are directed toward the same pole. The alternate segregation would have the zigzag configuration (Fig. 8), while the adjacent would have the open ring. In certain species e. g. *Oenothera*, *Triticum monococcum* and *T. durum* etc. alternate chromosomes in the interchange complex pass to the same pole 70 to 90 percent of the times. Whereas in others as *Zea mays*, *Pisum sativum*, *Sorghum versicolor* and *Petunia*, alternate and adjacent segregation occur at almost equal frequencies. The orientation on the metaphase plate of the chromosomes of the interchange complex may be governed by genetic factors and mechanical conditions (Burnham, 1956). The frequencies of alternate segregation at the first metaphase of five plants heterozygous for translocation which were induced by this experiment ranged from 39.13 to 54.76 percent, and averaged 44.28 percent; that is, the two types of configurations occurred with approximately the same frequency. We observed that the interchange complex did not occur in all microsporocytes of the translocation heterozygotes, the percentages of quadrivalents which appeared at the stage of diakinesis and first metaphase of these five plants varied from 36.51 to 92.38 percent. From the information shown in Table 4. we know that the percentage of quadrivalents might be independent of the frequency of zigzag configurations in translocation heterozygotes.

Table 4. The percentages of quadrivalents and frequencies of zigzag configurations at first metaphase in each of five plants heterozygous for translocation

Plant No.	Total dosage (n/cm^2)	Chromosomal pairing type					Percentage of quadrivalents (%)
		5II	1⊙4+3II				
			Zigzag	Ring	Total	Zigzag frequency (%)	
38	3.5×10^{13}	19	21	30	51	41.18	72.85
99	3.5×10^{13}	10	23	19	42	54.76	80.77
178	4.0×10^{13}	13	16	21	37	43.24	74.00
189	4.0×10^{13}	4	20	28	48	41.67	92.38
229	4.0×10^{13}	40	9	14	23	39.13	36.51
Total	—	—	89	112	201	44.28	—

In general, the two members of a pair of homologoûs chromosomes regularly segregate to opposite poles during first anaphase in a normal diploid. Exceptions in the form of accidents occur, however, the members of a pair fail to disjoin, both passing into the same pole. This phenomenon is called unequal segregation of chromosomes or chromosome non-disjunction. In this experiment, unequal segregation of quadrivalent chromosomes at the first anaphase has been found in the translocation heterozygotes whose quadrivalent frequencies were more than 36.51 percent. As plant No. 189 with a ring-of-four, whose frequency of quadrivalent was more than 92 percent, produced chromosome non-disjunction in 7.69 percent of the quadrivalents at first anaphase. In plant No. 107 with a ring-of-six, whose frequency of the six chomosome associations jumped to 98.13 percent, about 29 percent of the six chromosome associations at first anaphase occurred as chromosome non-disjuction, these data are presented in Table 5.

From the results described above, we know that close correlation exists between the multivalent frequency and chromosome non-disjunction. that is, the frequency of multivalents is proportional to the percentage of chromosome non-disjunctions occurring in a translocation heterozygote. In interpreting this problem, the author suggests that the interchange complex with its many interstitial chiasmata have difficulty in accomplishing terminalization, and prevents regular disjunction of the chromosomes in a multiple ring. Consequently, an increase in the frequency of multivalents occur, and even chromosome non-disjunctions are induced. There is a much higher frequency of chiasmata in the six chromosome association than in the four chromosome association. Therefore, the frequencies of multivalents and chromosome non-disjunctions that occurred in six chromosome associations were more than in the four chromosome associations.

Table 5. Unequal segregation of chromosomes at first anaphase in interchange complex

Plant No.	Chromosomal pairing type	Frequency of multivalents	5:5.	6:4.	Total	% of non-disjunction.
Control	5II	0	112	0	112	0
229	1⊙4+3II	36.51	75	0	75	0
189	1⊙4+3II	92.38	96	8	104	7.69
107	1⊙6+2II	98.13	63	26	89	29.21

6. Chlorophyll deficient mutations induced by thermal neutrons.

In this exepriment, three kinds of chlorophyll mutations, i. e. white, iojap and light green, were found among the plants from seeds irradiated with thermal neutrons. Of a total of 18 mutants, 50% were white, 38.89% were light green and 11% were iojap. Data for chlorophyll mutations are given in Table 6.

Table 6. Kinds and frequencies of chlorophyll deficient mutants induced by thermal neutrons

Kind of chlorophyll mutation	Dosage (n/cm^2)	No. of plants showing mutation in each treatment	Total no. of plants showing each kind of mutation	No. of plants tested	Percent of each kind of mutation
White	3.5×10^{13}	5	9	578	1.56
	4.0×10^{13}	4			
iojap	3.5×10^{13}	2	2	241	0.83
	4.0×10^{13}	0			
light gree	3.5×10^{13}	5	7	241	2.92
	4.0×10^{13}	2			

Owing to the lack of chlorophyll for the carrying out of photosynthesis, all the white seedlings died after the food reserve from their endosperm was exhausted. The similar mutant character "white seedling" was also reported in corn by McClintock (1941), he suggested that this kind of mutation was really derived from very small, nonlethal particular deficiencies. These deficiencies, when homozygous, produced phenotypic effects similar to those obtained after gene mutation that presumably did not involve chromosome loss.

Seven plants with light-green mutation had vigorous growth, broader leaves, more tillers and later heading habits than the others, but only empty glumes were produced, this may be attributed to mutation.

Two iojap plants were found in this experiment. They grew as good as the others in the experimental fields, but their panicles did not develop very well so their chromosome behavior in meiosis could not be studied. Rhoades (1946) reported a nuclear gene iojap in corn when homozygousity greatly increases the mutation rate of the plastid primordia from normal to defective and caused the appearance of green and white striping on leaves. The iojap mutants are usually induced by irradiations. Once formed by this nuclear process, such striping characteristics are transmitted as though they were completely determined by a cytoplasmic particle transmitted only through the maternal cytoplasm.

As regards to the effect of inducing mutations, it makes no significant difference in the results, whether the dose of the thermal neutrons is 3.5×10^{13} n/cm^2 or where it is 4.0×10^{13} n/cm^2 if the flux intensity of 3.2×10^8 n/cm^2 per second remains the same. The results are expressed in Table 6.

SUMMARY

Seeds of *S. purpureo-sericeum* irradiated with dosages of thermal neutrons 3.5×10^{13} n/cm^2 or 4.0×10^{13} n/cm^2 at the flux intensity of 3.2×10^8 n/cm^2 sec. showed no marked decrease in seed viability, but these treatments caused an appreciable reduction in seedling height and plant tillering. At the same total dosage of thermal neutrons 3.5×10^{13} n/cm^2, if the flux was increased from 3.2×10^8 n/cm^2 sec. to 6.54×10^8 n/cm^2 sec., caused all the treated seeds to die. It showed that the flux was more effective in damaging seed viability than the total dosage of thermal neutrons.

In the present study, we learn that the exposure of 4.0×10^{13} n/cm^2 at a constant intensity of flux 3.7×10^8 n/cm^2 sec. is the better one for thermal neutron treatment, as this yields the most aberrations and at which the largest numbers of types of chromosome aberrations were induced. Most of them were a ring-, or chain-of-four, and the next most common aberration was pseudo-isochromosomes.

The application of Koo's expression, the theoretical ratio of rings and pseudo-isochromosomes in *S. purpureo-sericeum* was obtained. Results from the comparisons of observed data for *S. purpureo-sericeum* with the theoretical ratio indicates that the interchanges between chromosomes homologous or nonhomologous were random.

From the information shown in this experiment, we know that the frequency of multivalents may be independent of the zigzag frequency in a translocation heterozygote, while the frequency of multivalents is proportional to the percentage of chromosome non-disjunction in a translocation heterozygote.

Three kinds of chlorophyll mutations were found among the plants grown from seeds irradiated with thermal neutrons. Of a total of 18 mutants, half of them were white, 38.89% were light green and 11% were iojap.

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