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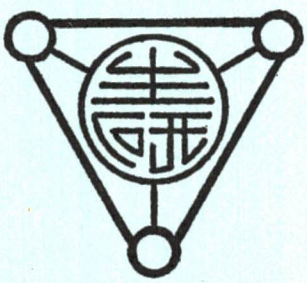

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Estimation of induced mutation rates of three genes in common wheat by the specific locus method with the aid of cytoplasmic male sterility[1)]

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Introduction

In diploid organisms, the mutation rate of an individual dominant allele to its recessive form (including deficiency) can only be efficiently estimated by treatment of their heterozygotes by a mutagen. In higher plants, however, production of heterozygotes in a large quantity is possible only in the few species, that show strict self incompatibility or give a large number of seeds from artificially cross-pollinated flowers. In small grain cereals, like barley, rice and wheat, it is almost impossible to prepare hybrid seeds in large quantities, and, therefore, mutation rates of individual alleles by the specific locus method have never been estimated on a large scale.

A number of male sterile lines with different genotypes have been produced in hybrid wheat breeding of common wheat (eg. TSUNEWAKI *et al.* 1976). This provides the unexpected opportunity of large scale production of heterozygous seeds in this otherwise completely self-fertilizing crop. During the course of producing coisogenic marker lines with the genetic background of a common wheat cultivar, *Triticum aestivum* cv. S–615 (TSUNEWAKI and KOBA 1979), a line having the three dominant genes, *B*1, *C* and *Hg* has been bred. Among the large number of male sterile lines of common wheat having the *Triticum timopheevi* cytoplasm, that of *T. aestivum* cv. Norin 29 was selected because this cultivar is homozygous recessive for all the three genes mentioned above, and has the same flowering time as cv. S–615 (FUJIGAKI and TSUNEWAKI 1976). Hybrid seeds were produced by natural out-crossing between the two lines.

The present investigation was primarily planned (1) to prove the usefulness of heterozygote production with the aid of cytoplasmic male sterility for estimation of the mutation rates of individual genes of self-pollinating cereals by the specific locus method, (2) to compare the induced mutation rates of the three dominant genes of common wheat which locate at different distances from the centromere, and (3) to determine the effects of two different mutagens, EMS (ethyl methanesulfonate) and γ-rays, on relative mutation rates of the three genes.

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The investigation failed in one respect; the presence of the *timopheevi* cytoplasm increased the sensitivity of dormant seeds to γ-rays so much that very few plants could survive after γ-ray treatment, and, consequently, the last aim of the present investigation could not be pursued. However, the results obtained demonstrated that the large quantity of heterozygotes produced with the aid of cytoplasmic male sterility is useful in making an efficient estimation of the induced mutation rate of a gene by the specific locus method, which will be reported herein.

Materials and Methods

A multiple marker line of a common wheat cultivar, *Triticum aestivum* cv. S–615 that is marked by three dominant genes, i.e., *B*1 for awn suppression, *C* for compact ear and *Hg* for hairy glumes was used as the pollen parent in the production of heterozygous seeds. Chromosomal locations of all genes are given in Fig. 1. Though the *B*1 gene is known to be located on the long arm of chromosome 5A, its distance from the centromere has not yet been reported (SEARS 1954). The *C* gene is closely linked to the centromere of chromosome 2D, though its arm location is unknown (MCINTOSH 1973). *Hg* is located on the short arm of chromosome 1A and freely recombines with the centromere (MCINTOSH 1973).

Another common wheat cultivar, *T. aestivum* cv. Norin 29 that has the genotype *b1b1 cc hghg* (phenotype=fully awned and lax ear with non-hairy glumes) was converted to a male sterile form by substituting its cytoplasm with that of *T. timopheevi* (FUJIGAKI and TSUNEWAKI 1979). This line will be referred to as (*timopheevi*)-Norin 29.

At plantation, plants of male sterile (*timopheevi*)-Norin 29 were surrounded by those of the multiple marker line of S–615. At flowering, (*timopheevi*)-Norin 29 was fertilized with the pollen of the multiple marker line because of their vicinity. However, other cultivars or lines grown adjacent to the plot of the present materials also could pollinate the male sterile Norin 29 at a lesser frequency. All seeds set on (*timopheevi*)-Norin 29 were harvested, and used for mutagen treatment.

Two kinds of mutagens, i.e., EMS and γ-rays were applied. They were applied as follows:

EMS treatment .. Dormant, dry seeds were presoaked with deionized water for 2 hr at 23°C and 1,500 seeds were placed in a beaker that contained 150 ml of 0.25 or 0.35% EMS solution, and were kept in the dark for 22 hr at 20°C. After the treatment, seeds

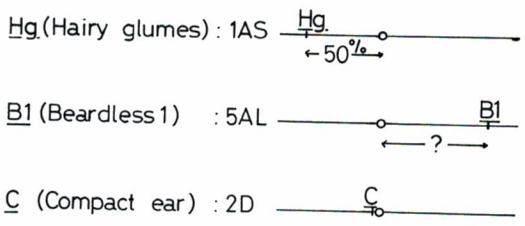

Fig. 1. Chromosomal locations of three genes, *B*1, *C* and *Hg*.

were washed with running water for about 15 min, and then sown in a seed bed. As the control, the same number of seeds were treated in the same way as the EMS-treated seeds, except that they were kept in deionized water in place of the EMS treatment.

γ-ray irradiation .. 1,500 dormant, dry seeds were irradiated with 10 or 15 kR dose of γ-rays from a ^{60}Co source at a dose rate of 450 R/min, at the Laboratory of Nuclear Radiations, Institute for Chemical Research, Kyoto University. After irradiation, seeds were soaked in running water for 20 hr at room temperature, and sown in a seed bed.

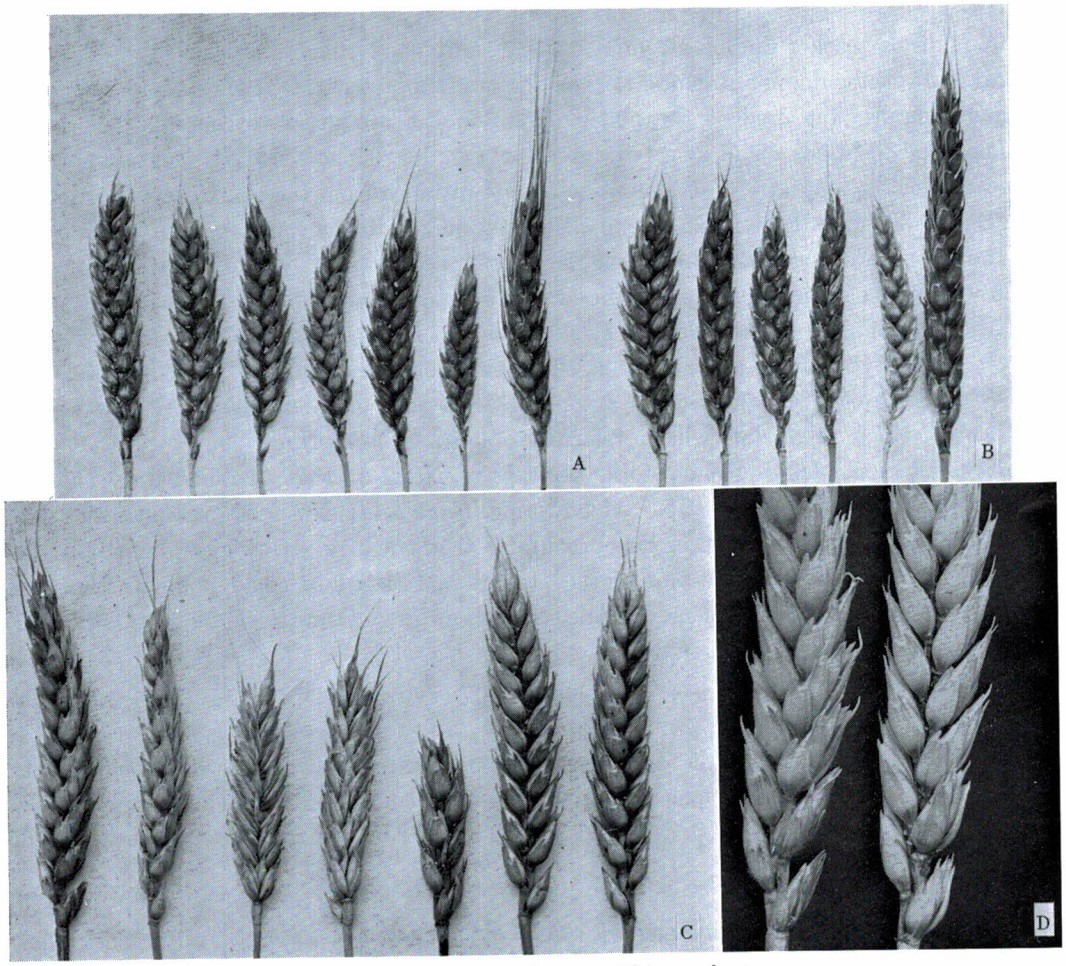

Fig. 2. Mutant ear(s) segregating among normal ears within a plant.
(A) One (last one in right) of seven ears of a 0.35% EMS-treated plant showing the mutation of *B*1 (awnless) to *b*1 (awned).
(B) One (last one in right) of six ears of a 0.35% EMS-treated plant showing the mutation of *C* (compact ear) to *c* (lax ear).
(C) Two ears (last two in right) of a 10 kR γ-rayed plant showing the mutation of *Hg* (hairy glumes) to *hg* (non-hairy).
(D) Two ears, one hairy (left) and the other non-hairy, of the above plant.

Two months after seeding, seedlings survived were transplanted into the field. At maturity, all plants were pulled out, and were classified into the real, intended hybrid type (phenotype=B1 C and Hg) and offtype (other phenotypes), based on ear characters: Plants which had some ears showing the typical F_1 characters, i.e., awnless and compact ear with hairy glumes were classified as the real hybrid, and subjected to further observation. On the contrary, plants which had no ears showing the characters expected in the real F_1, were considered as an offtype, and were discarded.

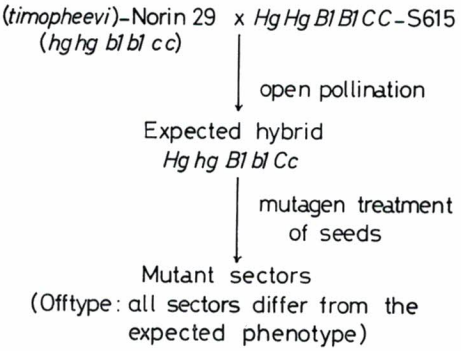

Fig. 3. The major experimental procedures.

The ears of all the plants classified as the real F_1 hybrid were counted separately, and the phenotype of each ear was examined. Ears which showed change of either three dominant characters to the recessive one were assumed to have been derived from mutation of the dominant gene responsible for that character to its recessive form. All ears of representative plants which produced mutant ear(s) are shown in Fig. 2.

Fig. 3 gives the essential part of the present experimental procedures.

Results

Table 1 summarizes the results on plant survival and frequency of real hybrids. EMS treatments lowered the survival rate by about 10% of that of the control. Both γ-ray treatments were too severe for the plants to survive; 90 to 99% plants died before or after transplantation. The frequency of offtypes among matured plants in the control plot was 41.5%, that is about 4% higher than that observed in the EMS-treated plots; their difference being significant at the 5% level. On the contrary, γ-ray irradiation increased the proportion of offtypes about 10% of that of control, the difference being significant at the 1% level. Relative sensitivities between offtypes and real hybrids against EMS differed from that against γ-rays. No reasonable explanation can be given to this phenomenon.

Frequencies of plants having mutant ears for individual characters are summarized in Table 2. Among the EMS-treated plants, the frequencies of plants with mutant ears for both *B*1 and *C* genes were about two times higher than that for the *Hg* gene. However,

Table 1. Frequencies of survived plants and real hybrids after mutagen treatment

Treatment	No. seeds sown	No. plants matured	% Matured	No. offtypes	No. real hybrids	% Offtypes[1)]
Control	1,500	1,343	89.5	557	786	41.5
0.25% EMS	1,500	1,209	80.6	464	745	38.4
0.35% 〃	1,500	1,149	76.6	423	726	36.8
Total 〃	3,000	2,358	78.6	887	1,471	37.6
10kR γ-rays	1,500	200	13.3	110	90	55
15kR 〃	1,500	11	0.7	3	8	27
Total 〃	3,000	211	7.0	113	98	54

1) No. offtypes/No. matured plants

Table 2. Frequencies of real hybrids that had mutant ear(s); characters mutated are indicated by the gene symbol

Treatment	No. real hybrids	No. plants with mutant ear(s)		
		Hg	*B*1	*C*
Control	786	0 (0.00)	0 (0.00)	0 (0.00)
0.25% EMS	745	2 (0.27)	3 (0.40)	3 (0.40)
0.35% 〃	726	5 (0.69)	11 (1.52)	11 (1.52)
Total 〃	1,471	7 (0.48±0.35)	14 (0.95±0.50)	14 (0.95±0.50)
10kR γ-rays	90	6 (6.7)	5 (5.6)	6 (6.7)
15kR 〃	8	2 (25)	2 (25)	0 (0)
Total 〃	98	8 (8.2±5.4)	7 (7.1±5.1)	6 (6.1±4.7)

( ): Percent
±: 95% confidence interval of the average frequency (in percent)

their difference was not significant at the 5% level. Among the γ-rayed plants, no significant difference was observed among the three genes in the frequency of plants showing their mutations. From these results it can be said that mutation of the three genes, *B*1, *C* and *Hg* to their recessive forms occurs at almost the same frequencies.

Distribution of the plants with mutant ears among different classes of ear number was investigated; Table 3 summarizes the results. Mutagen treatment slightly decreased the number of ears per plant (0.2 to 0.7 ears reduction per plant). The average ear number of the plants which had mutant ears was slightly larger than that of all plants. However, the t-test showed no significant difference at the 5% level. This fact indicates that the occurrence of mutation in the embryo does not affect the number of tillers produced form it.

The number of ears that showed the same mutant character in individual plants is shown in Table 4. In total, 56 independent mutations were detected; among them two different mutations occurred in each of three plants (they had five, seven and 16 ears, respectively). Forty mutations (more than 70%) were expressed only in one out of 6.7 ears of the plant on the average. Only nine mutations were expressed in more than one ear of the plant (about 16%). Seven mutations were expressed only in the lateral half of each ear. These facts clearly show that each mutation is expressed mainly in one ear,

Table 3. Numbers of total and mutant ear-bearing plants in individual ear number classes

Class (No. ear/plant)	Control		0.25% EMS		0.35% EMS		γ-rayed		Total treated	
	Total	Mutant	Total	Mutant	Total	Mutant	Total	Mutant	Total	Mutant
1	5	0	10	0	12	0	10	0	32	0
2	13	0	24	0	31	1	14	0	69	1
3	75	0	68	1	84	4	13	2	165	7
4	127	0	123	0	144	2	8	1	275	3
5	185	0	176	2	150	4	8	3**	334	9
6	117	0	122	1	117	6*	12	3	251	10
7	88	0	70	1	69	3	11	4***	150	8
8	64	0	54	2	53	2	8	1	115	5
9	41	0	35	0	32	2	5	0	72	2
10	27	0	21	0	18	0	4	1	43	1
11	16	0	19	1	8	2	1	0	28	3
12	11	0	10	0	3	0	2	1	15	1
13	5	0	5	0	3	0	0	-	8	0
14	3	0	4	0	1	0	0	-	5	0
15	6	0	1	0	1	0	0	-	2	0
16	2	0	1	0	0	-	1	1***	2	1
17	0	-	0	-	0	-	0	-	0	-
18	1	0	2	0	0	-	1	0	3	0
13–16	17	0	13	0	5	0	2	1	20	1
Total	786	0	745	8	726	26	98	17	1,569	51
Ave. no. ears	6.0	-	5.8	6.6	5.4	6.0	5.3	6.9	5.6	6.4

* One plant had two mutant ears, one of which mutated from *C* to *c*, and the other from *Hg* to *hg*.
** One plant had two mutant ears, one of which mutated from *B*1 to *b*1 and the other from *C* to *c*.
*** One plant in each group had a mutant ear, in which both *B*1 and *Hg* mutated to their recessive forms.

Table 4. Number of ears showing the same kind of mutation in individual plants

No. ears/plant	No. mutant ears			
	1/2*	1	2	3
2		1		
3		6	1	
4	1	1	1	
5	2	6	2	
6	3	7	1	
7		9		
8	1	3	1	
9		2		
10			1	
11		3		
12				2
16		2		
Total	7	40	7	2
(%)	(12.5)	(71.4)	(12.5)	(3.6)

* A lateral half of an ear showed mutant character.
Note) In one plant in each ear number class 5, 6, 7 and 16, two independent mutations occurred, which are scored separately in this table.

and very rarely in more ears, and rarely in the lateral half of the single ear, when ear number per plant is about five to six on the average.

Discussion

One of the main concerns of the present investigation was to prove that the mutation rate can be estimated efficiently by the specific locus method in self-fertilizing small grain cereals with the aid of cytoplasmic male sterility, which makes feasible the production of hybrid seeds in large quantity. In the present investigation, 11 male sterile plants of (*timopheevi*)-Norin 29, which were surrounded by the pollinator plants homozygous for *B*1, *C* and *Hg* genes, produced more than 8,000 seeds, namely, about 700 seeds/plant by natural out-crossing. About 90% of them were viable. Almost 40% of the plants raised were the offtype, i.e., they derived from fertilization of the male sterile plant by a pollen source other than the *B*1 *C* *Hg*-carrier. This percentage can be reduced by growing the parental lines in an isolated field.

In the case of EMS treatment, which only slightly reduced the survival rate, almost 50% of the seeds sown produced real hybrids, and 0.5 to 1.0% of matured hybrids showed mutation of each gene. These results indicate that the present method of producing a large number of hybrids for specific genes with the aid of cytoplasmic male sterility is useful not only in estimating the mutation rate but for all studies on mutation of a specific gene.

Other concerns of the present investigations are to obtain preliminary information on the following three points; (1) relative mutation rates of the three genes, *B*1, *C* and *Hg*, (2) their relation to the distances between the loci and centromeres, and (3) change in the relative mutation rates of the three genes caused by the use of different mutagens.

As to the relative mutation rates of the three genes, no significant difference was detected between them either by EMS treatment or by γ-ray irradiation; frequencies of plants having mutant ears were 0.48 to 0.95% by EMS treatment, and 6 to 8% by γ-ray irradiation, though the data of the latter treatment is not reliable because very few plants survived.

As shown in Fig. 1, the *C* gene is closely linked to the centromere of chromosome 2D, while the *Hg* gene recombines freely with the centromere of chromosome 1A. Common wheat is a hexaploid species, and due to this high ploidy level, deficiency of even a whole chromosome does not impair plant vigor at all. Thus, a cell deficient of a chromosome segment is not subjected to strong selection against it during the development of the plant. If a mutagen causes chromosome breakage more frequently than the so-called point mutation, we may expect higher 'apparent' mutation rate for the *Hg* gene than for the *C* gene. The results of both mutagen treatments did not indicate any higher incidence of mutation in the *Hg* locus than in the *C* locus. Thus, it can be concluded that a large portion of the mutations detected as mutant ears in the treated (M_0) generation is of the point mutation type, and not due to chromosome breakage.

Differential effects of the two mutagens, EMS and γ-rays could not be tested because the present γ-ray irradiation damaged the plants too severely. However, the data so far

obtained showed no sign of difference in their mutagenic effects on the three genes.

Summary

Mutation rates of three dominant genes in common wheat, i.e., *B*1 for awn suppression, *C* for compact ear, and *Hg* for hairy glumes (their distances from the respective centromere are unknown, almost zero and over 50%, respectively) have been estimated by the specific locus method, where EMS and γ-ray treatments were given to the heterozygous seeds produced by the use of cytoplasmic male sterility. The results have demonstrated the following points;

(1) Hybrid seed production by the use of cytoplasmic male sterility provides a good means of estimating the mutation rate of a gene by the specific locus method in self-pollinating cereals.

(2) The mutation rate expressed by the frequency of M_0 plants carrying the mutant ear among all matured plants is not significantly different among the three genes at the 5% level.

(3) The distance between the locus and the centromere is not closely related to the observed mutation rate, indicating that the majority of mutations produced are of the point mutation type.

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