BEHAVIOUR OF BALANCED LETHAL GENES OCCURRING IN PROGENY OF X-RAYED RICE¹⁾

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Balanced lethals such as found in *Drosophila melanogaster* by Muller (1918) are not reported so many in animals and plants. As our related examples, three such cases are known; i. e., chlorophyll mutants in oats (Nishiyama, 1934) and barley (cf. Gardner, 1960), and an incomplete endosperm variant in maize (Bianchi and Morandi, 1962).

Some years ago, several progeny strains of X-rayed rice with anomalous leaf pigmentations were kindly supplied by Dr. Y. Nishimura. Pedigree tests were done by the present authors, and a special strain which segregates greens and yellows in a ratio of 1 : 1 was detected. The yellow seedlings soon died off but the greens persistently segregated into greens and yellows in the same ratio. A provisional assumption of two recessive lethal genes was thus made (Katayama and Shida, 1963).

The following experimental procedures have been then adopted in order to examine the validity of the assumption: 1) selfing, 2) crossing with normal green strains, and 3) irradiation with γ -rays. The results obtained from the examination are described in the present paper.

MATERIALS AND METHODS

The mutant strain studied in this paper was originated from yellow variants found in 1958 in the X₂ generation of X-rayed seeds of Norin No. 8, one of popular strains of Japanese rice. The yellow tint varied with environmental conditions at the time of sowing and sometimes showed faint yellow. The yellow individuals followed gradually to death after the three-leaf stage. For selfing, caution was taken to avoid outcrossing by cultivating them in an isolated plot and sometimes by bagging with paraffin paper when natural crossing was suspected.

In survey of segregation, seeds were sown in middle May, and the observation of the yellow leaf colour was made at one- and two- leaf stages, counting the numbers of the green and yellow seedlings. Good seed germinations were observed in general. This mutant was crossed with either of two normal green strains, Muketsu-to (a strain of paddy rice with a deep green colour and less tillers) or Dango-mochi No. 2 (a strain of upland rice).

Irradiation of dry seeds of this mutant strain with γ -rays from a 6,000 curie ¹³⁷Cs

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source was made at the National Institute of Genetics. The γ -ray intensity was 5kR/hr, and the exposures applied were 10, 20 and 30kR. The irradiated seeds were sent immediately back to our laboratory and were sown with least delay.

SEGREGATION RESULTS

(1) Selfing

Segregation in the progeny produced by selfing was investigated from 1961 to 1966, and the results are presented in Table 1. In the selfed first generation (S_1), a segregation ratio of greens : yellows = 1 : 1 was obtained. The same segregation ratio was also found in 34 lines of the second generation (S_2), and no constant green line was detected.

Table 1. Segregations in selfed progenies (1961~1966)

Generation	Phenotype of previous generation	No. of lines	Green individuals	Yellow individuals	Total number	Assumed ratio	χ^2	P
S_1	green	1	52	51	103	1 : 1	0.010	>0.9
S_2	green	34	1014	999	2013	1 : 1	0.112	0.8-0.7
S_3	green	1 15	38 313	0 309	38 622	1 : 1	0.025	0.9-0.8
S_4	green	19	433	450	883	1 : 1	0.327	0.7-0.5
		1	67	1	68			
		19	716	741	1457	1 : 1	0.429	0.7-0.5
		20	674	630	1304	1 : 1	1.485	0.3-0.2
		20	674	659	1333	1 : 1	0.169	0.7-0.5
	green*	19 11	1382 841	0 31	1382 872			
S_5	green	5	136	122	258	1 : 1	0.760	0.5-0.3
		4	115	106	221	1 : 1	0.367	0.7-0.5
		5	158	152	310	1 : 1	0.116	0.8-0.7
		4	142	155	297	1 : 1	0.490	0.5-0.3
	green*	3	204	0	204			
		1	30	1	31			
S_6	green	5	179	175	354	1 : 1	0.045	0.9-0.8
		5	281	273	554	1 : 1	0.116	0.8-0.7
		5	350	356	706	1 : 1	0.051	0.9-0.8

* Subsequent generation of constantly or predominantly green lines. Others are the progeny of the 1 : 1 segregating lines.

Almost all of the S_3 lines showed the 1 : 1 segregation, but one line produced only green individuals. In the subsequent selfed generation (S_4) of this green line, 19 out of 30 lines examined bred true, while the remaining 11 lines showed segregation of yellow individuals but at a low percentage (about 3.5% on the average). Out of 79 S_4 lines of four groups derived from the 1 : 1 segregating greens, on the other hand, 78 lines were consisted of greens and yellows in the ratio of 1 : 1, and only one line was predominantly of greens except for one yellow individual. In the next generation (S_5)

of this predominantly green line, three out of four lines bred true whereas the remaining one contained one yellow seedling among the 31 descendants. The 1 : 1 segregation ratio was kept in the S_5 and S_6 when the parents were picked up from the inconstantly green (1 : 1 segregating) S_4 lines.

Moreover, the survival greens of this mutant strain underwent a normal meiosis forming 12_1 at the first metaphase, and the development of the pollen grains and their germination on the stigma were also normal. A satisfactory ripening of seeds and a high seed-setting rate were observed, being comparable with those in control rice plants.

(2) Test-crossings

Segregations in the hybrid progenies between this mutant strain and Muketsu-to or Dango-mochi are presented in Table 2.

Mutant $\times$ *Muketsu-to* : The F_1 plants had green stems and leaves. Segregations of 43 greens and 2 yellows or of 51 greens and 15 yellows were observed in the F_2 . The two yellow seedlings in the former segregation had a whitish yellow colour and

Table 2. Segregations in the hybrid progenies between the mutant line and normal green rice (1964~1966)

Generation	Phenotype of previous generation	No. of lines	Green individuals	Yellow individuals	Total number	Assumed ratio	χ^2	P
(1) Mutant $\times$ Muketsu-to								
F ₂	green	1 1	43 51	2 15	45 66	3 : 1	0.181	0.7-0.5
F ₃	green*	5 1 4 3 2	297 53 210 180 66	0 1 31 53 58	295 54 241 233 124	3 : 1 1 : 1	0.631 0.516	0.5-0.3 0.5-0.3
F ₄	green*	2 2 2	165 145 115	0 22 31	165 167 146	3 : 1	1.105	0.3-0.2
	green**	6	181	171	352	1 : 1	0.284	0.7-0.5
(2) Mutant $\times$ Dango-mochi								
F ₂	green	1 1	79 43	2 11	81 54	3 : 1	0.617	0.5-0.3
F ₃	green*	3 4 5 3	214 280 254 113	0 54 86 105	214 334 340 218	3 : 1 1 : 1	0.016 0.293	0.9-0.8 0.7-0.5
F ₄	green*	2 2 1 1	138 157 44 42	0 12 25 42	138 169 69 84	1 : 1	0	1
	green**	6	183	197	380	1 : 1	0.516	0.5-0.3

* Previous generation showed 3 : 1 segregation.

** Previous generation showed 1 : 1 segregation.

the segregation could not be regarded as a simple recessive ratio. But the yellow individuals in the latter segregation were somewhat greenish in colour and the segregation could be fitted to the 3 : 1 ratio. In the F_3 of the latter line various segregation ratios were observed. Namely, out of 15 F_3 lines examined, five bred true as greens; one contained only one yellow individual; four were consisted of more greens and less yellows than expected from the 3 : 1 ratio; three were of segregations close to a 3 : 1 ratio; and the remaining two lines approximated a 1 : 1 ratio.

When the F_4 lines were taken from the green plants of the F_3 lines which showed the 3 : 1 segregation, the following three different segregations were observed: All greens (two lines); more greens and less yellows than the 3 : 1 ratio (two lines); and greens and yellows in the 3 : 1 ratio (two lines). When the F_4 lines were derived from the 1 : 1 segregating F_3 lines, on the other hand, segregations of greens and yellows close to a 1 : 1 ratio were observed in all of the six F_4 lines.

Mutant $\times$ *Dango-mochi* : The F_1 hybrids also had a normal green colour. The segregations in the F_2 and the subsequent generations were similar to those observed in the above mentioned combination. That is, one group of the F_2 contained only two yellows among 81 plants, while the other did 43 greens and 11 yellows. The former yellows were whitish and of very low percentage. The latter yellows were somewhat greenish and fitted to a 3 : 1 ratio. This 3 : 1 segregating line showed various segregation types in the F_3 generation. Out of 15 F_3 lines investigated, three lines contained no yellow seedling; four lines with less yellows than 25%; five lines approximately 25% yellows (3 : 1); and three lines 50% (1 : 1).

The F_4 generation was taken from the 3 : 1 and 1 : 1 segregating F_3 lines. Six F_4 lines from the former segregating type showed the following four different segregations: All greens (two lines); more greens and less yellows than the 3 : 1 ratio (two lines); greens and yellows in an intermediate ratio between 3 : 1 and 1 : 1 (one line); and the both in the 1 : 1 ratio (one line). On the other hand, all the six F_4 lines from the 1 : 1 segregating type showed the same 1 : 1 segregation.

(3) γ -ray treatments

The mutant strain was irradiated with γ -rays and the progenies (R_2 - R_4) were investigated. In the R_2 segregation three new strains appeared in which found were segregations of three types, i.e., green, yellow and faint yellow. In their subsequent generations, progeny test was made and the results are summarized in Table 3 (cf. Katayama and Shida, 1967).

No. 4019 strain : In the R_2 generation from seeds exposed to 20kR of γ -rays, the segregation in three phenotypes, green, yellow and faint yellow, was 16 : 14 : 9 which is close to a 3 : 3 : 2 ratio. The most of the yellow and faint yellow individuals died after the three-leaf stage. Five R_3 lines examined contained the three phenotypes in the 3 : 3 : 2 ratio. In the R_4 , however, three lines consisting only with greens and yellows in an approximate 1 : 1 ratio but not with plants of faint yellow were found. In other three lines of the R_4 generation, the 3 : 3 : 2 segregation was observed.

No. 4023 strain : In the R_2 of seeds exposed to 30kR of γ -rays, three phenotypes were found in a ratio of 14 : 29 : 13. In the R_3 , two types of segregations were observed

Table 3. Segregations in three identical mutant strains newly obtained by γ -ray treatments

Generation	Phenotype of previous generation	No. of lines	Green individuals	Yellow individuals	Faint yellow individuals	Total number	Assumed ratio	χ^2	P
(1) No. 4019 (1964~1966)									
R ₂	green	1	16	14	9	39	3 : 3 : 2	0.129	>0.9
R ₃	green*	5	129	123	77	329	3 : 3 : 2	0.592	0.8-0.7
R ₄	green*	3 3	80 97	66 108	0 63	146 268	1 : 1 3 : 3 : 2	1.342 0.920	0.3-0.2 0.7-0.5
(2) No. 4023 (1964~1965)									
R ₂	green	1	14	29	13	56	3 : 3 : 2	5.452	0.1-0.05
R ₃	green*	3 7	50 115	51 137	0 100	101 352	1 : 1 3 : 3 : 2	0.005 4.015	>0.9 0.2-0.1
(3) No. 4025 (1964~1966)									
R ₂	green	1	14	27	4	45	3 : 3 : 2	11.237	0.01-0.001
R ₃	green*	2 4	57 65	65 71	0 44	122 180	1 : 1 3 : 3 : 2	0.525 0.296	0.5-0.3 0.9-0.8
R ₄	green*	2 4	71 104	81 112	0 63	152 279	1 : 1 3 : 3 : 2	0.658 1.177	0.5-0.3 0.7-0.5

* The next generation of the 3 : 3 : 2 segregating lines.

as in the R₄ of the strain No. 4019 (1 : 1 and roughly 3 : 3 : 2).

No. 4025 strain: In the R₂ of another 30kR-irradiated experiment, the three phenotypes also appeared but in a ratio which does not fit to the 3 : 3 : 2. However, among six R₃ lines tested, two and four lines were found to be classifiable to the 1 : 1 and 3 : 3 : 2 segregating types, respectively. Segregations observed in the R₄ lines derived from the 3 : 3 : 2 segregating R₃ were similar to those in the R₃.

These three newly obtained mutant strains may be regarded as the results of an identical mutation induced by γ -rays, because of the above almost identical genetic behaviour they showed.

DISCUSSION

In order to account for the heredity of a chlorophyll variant which occurred in the progeny of X-rayed rice and habitually segregated greens and yellows in a ratio of 1 : 1 (the yellows die soon, i.e., they are lethal), the present authors (Katayama and Shida, 1963) made a tentative assumption of balanced lethal genes as proposed by Muller (1918). Further investigations have proved the validity of the assumption, thus conclusive explanations are offered here.

Now suppose l_1 and l_2 as two recessive lethal genes induced by the earlier X-ray treatment, which are unable to undergo normal chlorophyll formation, and L_1 and L_2 as the respective normal dominant alleles. Existence of both the L_1 and L_2 results in

normal green, but when either of the loci is homozygous for the recessive genes (i.e., l_1l_1 or l_2l_2) the yellow colour appears and plants become lethal. Further suppose that L_1 gene is located very close to l_2 gene, and the same situation for l_1 and L_2 genes. That is, two sets of two complementary genes (L_1 plus l_2 and l_1 plus L_2) located very close to each other are carried by a set of homologous chromosomes. A green plant of this mutant strain has L_1 and l_2 on one of the homologous chromosomes (designated here as $\overline{L_1l_2}$) and l_1 and L_2 on the other ($\overline{l_1L_2}$). Recombination between L and l genes is supposed to occur scarcely. Selfing of the greens brings about $\overline{L_1l_2} \overline{L_1l_2}$, $\overline{L_1l_2} \overline{l_1L_2}$ and $\overline{l_1L_2} \overline{l_1L_2}$ in a ratio of 1 : 2 : 1, and the two homozygous types, either for l_1 or l_2 gene, are yellow and lethal, and the heterozygous type green. Consequently this line segregates habitually greens and yellows in the 1 : 1 ratio as described above. Rare occurrence of constant green lines may be explained by mutable recurrence from l_1 and l_2 to L_1 and L_2 respectively. Stress should be laid on significance of the heredity due to balanced lethal genes as a mechanism which protects permanent heterozygosity (cf. Sinnot *et al.* 1950). This also relates to the problem of heterosis breeding.

The F_1 hybrids between this mutant strain and normal green rice were green, and the F_2 showed two different segregations of greens and yellows, i.e., a 3 : 1 and a predominantly green-producing segregations. The yellows in the former segregation were somewhat greenish and those in the latter were whitish in colour¹⁾. This may be explained at least partly as follows. Since the gene component of the original mutant is $\overline{L_1l_2} \overline{l_1L_2}$, the F_1 hybrids must have either of the components, $\overline{L_1l_2} \overline{L_1L_2}$ or $\overline{l_1L_2} \overline{L_1L_2}$. Thus, if there is no competitive fertilization between pollens with different genotypes, only the 3 : 1 segregation is expected in the F_2 . However, if a competition between the double dominant pollen and the lethal-gene-carrying pollen occurs, being the former pollen surpassing over the latter in fertilization, green F_2 individuals must be increased. As the segregation of the yellows at a low percentage was observed in the present study, it may be assumed that such a competitive fertilization between pollen grains with different genotypes had occurred (cf. Nishiyama, 1936). Although it is uncertain which of the $\overline{L_1l_2}$ and $\overline{l_1L_2}$ pollens is inferior to $\overline{L_1L_2}$ pollen, it may be assumed provisionally that the $\overline{l_1L_2}$ pollen (brings whitish seedling) is inferior to the $\overline{L_1L_2}$ pollen but the $\overline{L_1l_2}$ pollen (brings greenish seedling) is not. With this assumption, the $\overline{l_1L_2} \overline{L_1L_2}$ F_1 produces more greens and less yellows than the 3 : 1 ratio in the F_2 , while the $\overline{L_1l_2} \overline{L_1L_2}$ F_1 gives the both in the 3 : 1 ratio in the next generation. However, some types of anomalous segregations observed in the F_3 or F_4 derived from the 3 : 1 segregating F_2 or F_3 can not be explained only by this assumption (see Table 2). Namely, a mutational change of L_1 to l_1 is necessary to produce the 1 : 1 segregating lines, and changes of L_1 to l_1 and of l_2 to L_2 are necessary to result in the green-predominant segregation. Also, it is necessary to consider that these changes must have occurred at high frequencies. At any rate, the research conducted so far does not supply sufficient data to discuss more on these points.

In the progeny of γ -rayed original mutant strain, a new mutant strain which showed

1) This relation in yellows of which show whitish or greenish perhaps will be seen in the progeny of selfing, but the present investigations for distinction were not enough.

a segregation ratio of greens : yellows : faint yellows = 3 : 3 : 2 was found. The occurrence of this new mutant may be explicable by considering a γ -ray-induced recessive mutation at a locus (A to a) which is independent of above L_1 and L_2 loci but is concerned in chlorophyll formation, and by assuming that this gene behaves hypostatistically to the balanced lethal genes.

SUMMARY

1. In this paper the authors dealt with the heredity of a chlorophyll variant found in the progeny of X-rayed rice, which segregates habitually green and yellow seedlings at a ratio of 1 : 1, the yellows of which die soon. The genetic behaviour had already been explained by assuming two recessive lethal genes. Further survey of the selfed progeny was carried out as well as the examination of the mutant strain by testcrossing with other normal green rice and by γ -ray treatments.

2. Assumed were two recessive lethal genes, l_1 and l_2 , which are unable to undergo normal chlorophyll formation, and the respective normal genes, L_1 and L_2 . Coexistence of L_1 and L_2 produces normal greens, and loss of either of the dominant genes results in yellows. Also, both the loci are so closely linked to each other that recombination occurs scarcely between the loci, and L_1 and l_2 or l_1 and L_2 are located on the same chromosome. That is, the green plants of the mutant strain has $\overline{L_1 l_2} \overline{l_1 L_2}$ gene component, and the selfing gives $\overline{L_1 l_2} \overline{L_1 l_2} : \overline{L_1 l_2} \overline{l_1 L_2} : \overline{l_1 L_2} \overline{l_1 L_2}$ in a ratio of 1 : 2 : 1, the two homozygous types either for l_1 or l_2 gene being yellow and the heterozygote green.

3. Rare occurrence of constant green line in this mutant strain seems to have been caused by mutable recurrence from l_1 and l_2 to L_1 and L_2 respectively. And it is proposed that the heredity due to such balanced lethal genes has a significance as a mechanism which protects permanent heterozygosity, having a relation to heterosis breeding.

4. The F_1 hybrids between this mutant strain and normal green rice were green, and the F_2 showed two different segregations; i.e., the one with 3 greens : 1 somewhat greenish yellows, and the other with predominant greens : a few whitish yellows.

5. The latter segregation may be explicable by a competitive fertilization. Since the gene components of the F_1 plants are $\overline{L_1 l_2} \overline{L_1 L_2}$ or $\overline{l_1 L_2} \overline{L_1 L_2}$, if no competition occurred between the pollens with different components, a 3 : 1 segregation would be expected, while if the pollens underwent a competition, a distorted ratio would result. However, some inexplicable problems still remain to be solved.

6. Gamma-ray treatments of this mutant strain induced a new mutant strain which showed a segregation of greens, yellows and faint yellows in a ratio of 3 : 3 : 2. An explanation that a recessive mutation (A to a) was newly induced may be given. This locus is considered to be independent of above L_1 and L_2 loci but to be concerned in chlorophyll formation, and this gene seems to behave hypostatically to the balanced lethal genes.

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LITERATURE CITED

- Bianchi, A., and A. Morandi, 1962 A case of balanced lethal factors in maize. *Heredity* **17**: 409-414.
- Gardner, E. J., 1960 Principle of genetics. Jhon Wiley & Sons, Inc. New York. pp. 231-233.
- Katayama, Y., and S. Shida, 1963 Studies on the contamination and the variation of crop plants caused by radioactivity III. Anomalous lines of leaf pigment occurring in X-rayed rice progeny. *Bull. Fac. Agr., Miyazaki Univ.* **8**: 332-339.
- Katayama, Y., and S. Shida, 1967 Studies on the contamination and the variation of crop plants caused by radioactivity IV. Leaf pigment variants found in γ -rayed rice and barley progenies. *Bull. Fac. Agr., Miyazaki Univ.* **14**: 369-376.
- Muller, H. J., 1918 Genetic variability, twin hybrids, and constant hybrids in a case of balanced lethal factors. *Genetics* **3**: 422-499.
- Nishiyama, I., 1934 The genetics and cytology of certain cereals VI. Chromosome behavior and its bearing on inheritance in triploid *Avena* hybrids. *Mem. Coll. Agr., Kyoto Imp. Univ.*, No. 32: 1-157.
- Nishiyama, I., 1936 Balanced lethals in *Avena* (A preliminary note). (In Japanese). *Japan. J. Genetics* **12**: 41-42.
- Sinnott, E. W., L. C. Dunn, and Th. Dobzhansky, 1950 Principles of genetics. McGraw-Hill Book Company, Inc. New York. 4th Ed. pp. 271-332.