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REPORT

A COMPARISON OF THE EFFECTS OF TEST ABLE ATOMIC BOMB IONIZING RADIATION AND X-RAYS ON SEEDS OF BARLEY, WHEAT AND OATS

APPENDIX NO. 25
TO THE FINAL REPORT

OPERATION CROSSROADS
JOINT TASK FORCE ONE

DIRECTOR OF SHIP MATERIAL

NAVAL MEDICAL RESEARCH SECTION

(6) OPERATION CROSSROADS.

A COMPARISON OF THE EFFECTS OF TEST ABLE ATOMIC BOMB IONIZING RADIATION AND X-RAYS ON SEEDS OF BARLEY, WHEAT AND OATS*

(21) Report of Naval Medical Research Section, Joint Task Force ONE, on Biological Aspects of Atomic Bomb Tests.

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Introduction

This study is part of the cooperative effort by the Naval Medical Research Section, Joint Task Force ONE, and the U. S. Department of Agriculture to investigate biological efects of atomic bomb ionizing radiation. A large variety of biological material including animals, seeds, grain insects, bacteria, fungi, etc., were distributed on twenty-two target ships prior to the detonation of the Test ABLE bomb July 1st 1946. For additional information regarding the biological aspects of this test, the reader is referred to papers by Draeger and Warren (3) and Randolph, et al (4).

Barley seeds were selected as one of the test objects because of their excellent fitness for determining the effects of irradiation on mutation frequency. Young and old seeds were included in order to study the effect of age on sensitivity to irradiation. Seeds of Durum (tetraploid), common wheat (hexaploid), and common oats (hexaploid) were included in the tests in order to have results from polyploid plants for comparison with information obtained from the non-polyploid barley.

Experimental Design

Samples of 2 varieties of barley, 1-year-old seeds of Trebi, and 19-year-old seeds of Moister, were exposed to radiations from the first (aerial) atomic bomb's explosion at Bikini, July 1, 1946. Samples of the seeds were kept as untreated controls and, in addition, some seeds (of Trebi, but not of Moister) were exposed to 3 dosages of X-rays (4,000r, 8,000r, and 16,000r). Seeds of one variety of durum (Carleton) and one variety of common wheat (Pilot) were handled in the same manner as the seeds of Trebi barley. That is, some seeds were kept as a control; others were exposed to the bomb radiations; and some were X-rayed (4,000 r, 8,000r, and 16,000r). Limited observations were also made on the effects of the bomb's radiations on one variety of oats (Vicland).

28 When the bomb-irradiated seeds (from several points or "stations" varying in distance from the point of explosion of the bomb) were returned to the United States, they were tested for germination in comparison with the control and X-rayed lots. It was observed that the seeds receiving the heaviest irradiation from either the bomb or X-rays were reduced only slightly in germination; therefore, only the more heavily irradiated seeds (bomb and X-rays) were continued through subsequent comparisons. Seeds given 8,000 r and 16,000 r units of X-rays were planted at Sacaton, but on the basis of preliminary observations, only plants from the 16,000r treatment were harvested.

29 The seeds (controls, bomb-irradiated, and X-rayed) were planted in early December at Sacaton, Arizona, where macroscopic and microscopic observations were made in the spring of 1947 on the plants grown from them. When mature, 4 widely separated spikes from each plant were harvested and shipped to the State College of Washington, where tests on mutation frequency were run.

30 The mutation frequencies were determined in the usual manner, Stadler (7), by planting approximately 30 seeds of each of the 4 spikes from a plant in separate progeny rows. If a clear-cut seedling mutant character appeared in 1 or 2 (rarely 2, and practically never in 3) of the head rows, it was counted as a mutant. If the character segregated in all of the 4 rows, the mutation was considered to be of spontaneous origin, prior to the start of the experiment, and was not counted as an induced mutation. Since, almost without exception, 4 spikes per plant were tested, the mutation frequencies can be expressed on a "per plant" basis by dividing the frequencies by 4. However, the 4 spikes tested would of course not reveal all the mutated sectors in a plant. So the total seeding mutation frequencies would be higher than those reported.

31 Some effort was made to find mature-plant mutants in X_2 populations; and a number were found in the barley and durum, but not in common wheat. However, the observations were on a rather limited scale, and no attempt was made to dtermine the frequency of mutation for mature-plant characters. It did seem apparent that the frequency of mature-plant mutants was considerably lower than the frequency of seeding mutants, Stadler (7).

32 Cytological observations were made on microspermogenesis using the aceto-carmine smear technic, Smith (6). Observations were limited to one specimen (spike) per plant.

Results

Effects on Barley from Bomb & X-Radiations

A. Germination and Seedling Growth.

Comparisons of the germination of control, bomb-irradiated and X-rayed seeds showed little effect of the radiations on germination, even of the most heavily exposed seeds. Howver, it was noted that the mottling or "flecking" in the chlorophyll of the first seedling leaves was as heavy in the most severely bomb-irradiated as in the most heavily X-rayed material (16,000r). Moreover, it was observed that the mottling seemed more uniform among seedlings from the bomb-irradiated than from the X-rayed seeds.

B. Macroscopic Observations on Plants Grown from Irradiated Seeds.

Plants grown from control, bomb-irradiated, and X-rayed seeds were gone over carefully about a week before the heading stage in search of evidence that the radiations from the 2 sources had produced similar or dissimilar effects. During this search 3 plants from bomb-irradiated seeds were found with distinct, and 1 with a faint chimera. Only 1 distinct chimera was found in plants from X-rayed seeds, and none was found in the controls. The numbers of plants examined examined were approximately 2,500, 800, and 900 respectively.

C. Cytological Observations on Plants Grown from Irradiated Seeds.

Cytological observations were limited because the comparison of frequencies of chromosomal aberrations from the 2 sources of radiation was being carried out with more favorable material -- Zea mays, Anderson (1), Anderson et al (2), and Randolph et al (4). However, it seemed desirable to make such a comparison on a small scale with barley and wheat. The results of these observations are presented in Table 1, from which it is evident that the frequency of interchanges resulting from the bomb-irradiation was probably at least as high as the frequency resulting from the 16,000r of X-rays. Some of the different kinds of interchanges observed are illustrated in Figure 1.

D. Comparison of Mutation Frequencies in X_2.

Data on frequencies of mutation are presented in Table 2. In Trebi the bomb-irradiated material had a 15-fold increase in mutation frequency over the frequency in the control, as compared with a 10-fold increase in the X-rayed material. The difference would probably have been greater if the number of seedlings per spike (19.3) tested from the bomb-irradiated had equalled the number of (25.6) from the X-rayed lots. This difference in population size per spike is considered to have resulted from soil variability rather than to a greater reduction in vigor of plants grown from seeds given the bomb as compared with X-ray treatment. It so happened that part of the bomb-irradiated material was planted on a distinctly "poor spot" in the field. This "poor spot" was an eroded area near an irrigation ditch and a row of large trees. There was no distinct difference in the average number of seedlings in progenies of spikes with mutants as compared with progenies of spikes without mutants. The spontaneous frequency of mutations in the 19-year-old seeds of Moister barley

(0.3 ± 0.15%) was quite comparable with the frequency in the 1-year-old seeds of Trebi (0.4 - 0.13%). These values are based on (fairly) large numbers of head progenies, making the determinations reasonably accurate and reliable.

As with Trebi, the bomb radiation produced in Moister a 15-fold increase in mutation frequency. Again the number of seedlings per spike was lower from the bomb-irradiated than from the untreated lot. In this case, however, there was no distinct difference in the soil in which the plants were growing. Thus, it seemed likely that the bomb-radiation reduced the number of progeny per spike in the treated lot.

Effects on Durum and Common Wheat from Bomb and X-Radiations.

A. Germination and Seedling Growth.

As noted earlier with barley, the irradiation seemed to have little effect on germination and seedling growth of the wheats. The wheat seedlings also were similar to those of barley in the presence and distribution of "flecks".

B. Macroscopic Observations on Plants Grown from Irradiated Seeds.

A surprisingly high frequency of chimers was observed among the plants from irradiated seeds of durum wheat, but none was found in the common wheat from either control or irradiated seeds. There were 11 plants from the bomb-irradiated seeds of durum that had distinct chimeras of "off color" tissue. There were 3 plants with such chimeras in the X-rayed material. None was observed in the control populations. The numbers of plants examined were approximately 1,200, 400, and 200, respectively. From the bomb-irradiated, X-rayed, and control common wheat, there were about 1,300, 400, and 200 plants, respectively.

C. Cytological Observations on Plants Grown from Irradiated Seeds.

Lack of time and other factors made it impossible and undesirable to examine more than a small sample of plants for interchanges and other chromosomal aberrations. The results of these cytological observations, which were limited to specimens taken from durum, are included in Table 1. From the data it is apparent that there was a high frequency of interchanges and chromosomal loss resulting from the irradiation -- regardless of source. It is obvious, even from this limited number of observations, that the rate of interchange was considerably higher in the tetraploid wheat than in the diploid barley. There was a considerably higher frequency of interchanges from the X-rayed than from the bomb-irradiated material, but the number of specimens examined was too small for the difference to be worthy of emphasis.

D. Comparison of Mutation Frequencies in X_2.

There were two remarkable results in connection with the mutation studies in the tetraploid and hexaploid wheats (Table 2). In the first place there were amazingly high (but not strikingly different) frequencies of seedling mutants in the bomb and X-irradiated durum. Relatively few observations have been made on mutation frequencies in polyploids, but the data of Stadler (8), and general experience, much of it unpublished, have indicated that the mutation rate in polyploids is very low compared with the mutation rate obtainable from irradiating similar, non-polyploid species. The reason for this, suggested by Stadler, probably lies in the duplication of genes in polyploids.

In the second place there was a considerable frequency of mutants in the common wheat -- which were, almost without exception, of a particular type, a "banded shrivel". Characteristically, "banded shrivel" seedlings develop (at the late first or second leaf stage) transverse zones of necrotic, brown tissue separated (at least in early stages) by bands of normal green tissue in the leaves. This type of mutant occurred in the barley and durum wheats, but constituted only a relatively small fraction of the total number of mutants. Except for these "banded shrivel" mutants in common whea , the mutation frequency was quite low. The frequencies from the bomb-irradiated and X-rays were about the same, 0.8 as compared with 1.0%.

Effects on Oats from Bomb Radiation

As indicated earlier, the studies on oats were limited to macroscopic observations on germination and seedling growth at Beltsville, and to a search for chimeras at Sacaton. As was noted with the barley and wheat, there was no distinct reduction in germination as a result of the bomb radiation. Likewise, the mottling resulting from the irradiation was comparable to that observed in wheat and barley. No chimeras were observed among the approximately 400 plants grown from bomb-irradiated seeds at Sacaton.

Discussion

One of the most interesting possibilities in all attempts to modify heredity is that an agent will produce new or specific types of changes. For that reason an effort was made to determine whether the types of mutations produced by radiations from the atomic bomb and X-rays were similar. From Table 3 it is apparent that there were no striking differences in the kinds of mutants obtained from the 2 sources of radiation.

It was thought to be of some interest to determine the ratio of normal to mutant seedlings in head progenies that were segregating mutants. From Table 1 it is evident that these ratios were all quite similar in barley(Ca 4:1) with the exception of hhe untreated Trebi (8.5:1). The ratios in the untreated lots were based on relatively few populations but may be suggestive in indicating the period in ontogeny when the mutations occurred. Since all of the radiation-induced mutations resulted from radiation of the dormant seeds, it is to be expected that the segregation ratios might be essentially the same, which they were, (on the average). On the other hand, spontaneous mutations can presumably occur at any time in the life cycle. Thus, it could be inferred that the 8.5:1 ratio in the untreated Trebi indicated that a considerable proportion of the 11 mutations occurred sometime after germination started. From the same sort of reasoning (again admittedly based on much too small a number of mutants for acceptance as fact), it would seem that the similarity between the ratios of normal to mutant seedlings in untreated and irradiated Moister indicates that most of the 5 spontaneous mutations may have occurred during the 19-year storage period of the dormant seeds. This problem would justify further study.

It is evident from Table 1 that the ratios of normal to mutant seedlings in durum and common wheat (4.5 to 5.6:1) were about the same as in barley -- but a little higher in every case. Why they were higher is not apparent, but the fact that they were so close indicates that the mutants in these polyploid species were monofactorial -- as presumably they were in barley. (Incompleted X_3 progeny tests indicate that the mutants in barley, durum and common wheat are in most cases at least, monofactorially determined.)

It might also be of interest to note that the frequencies of interchanges in unselected (except for the fact that most of them came from later tillers) spikes in the barley and wheat X_1 plants were fairly comparable with the frequencies resulting from a dose of 520r units of X-rays applied to the mature pollen of diploid and tetraploid wheat, Smith (5).

However, it should be apparent that in determining the frequencies of interchanges, only one floret of one spike of a plant was examined.

This one floret did not necessarily reveal the chromosomal constitution of the entire spike nor of other spikes on the plant. The number of spikes on the plants of barley probably averaged 15 (about 10 for the durum and common wheats), so it is highly probable that the total frequency of interchanges per plant was several times that represented in Table 1.

At the present time there is no explanation for the unexpectedly high frequency of mutations in durum. It may be that the particular variety used has a very old genotype in which evolution has resulted in genic differentiation to a point where the number of duplicate genes is relatively low as compared with other tetraploid types that have been tested. Since the ratio of normal:mutant seedlings in segregating head progenies was about the same as in barley (5 to 6:1), and because of the improbability of simultaneous mutations of duplicate genes, it is likely that most (or all) of the mutants in both durum and common wheat were the result of single-factor changes.

Nor is there any ready explanation for the high proportion of mutants in common wheat that were of the "banded shrivel" type. It seems unlikely that these mutants were all the result of induced changes at a single locus. It also seems improbable that all or most of them were the result of the loss of a particular chromosome or part thereof. It is planned that an effort shall be made to determine whether these mutants seem to be genetically identical.

Summary

Comparisons were made on the biological effects on dormant seeds of barley, durum and common wheats of radiations from an atomic bomb and from known dosages of X-rays. More or less incidentally, also, the relation of age of barley seeds to susceptibility to radiation effects was studied.

Limited macroscopic observations were also made on plants grown from seeds of oats that had been exposed to atomic bomb ionizing radiations.

The results showed a marked similarity in the effects of radiation from the 2 sources. The effects studied included: 1) germincation, 2) "flecking" or mottling on the first leaves, 3) chimeras, and 4) interchanges in X_1 plants grown from the irradiated seeds; 5) seeding mutation frequencies in X_2, and 6) types of mutants obtained in X_2. Thus, it would appear that the seeds most heavily irradiated during Test ABLE received a dose of irradiation roughly equivalent to 16,000r of X-rays.

There were no clear-cut differences in the types of seedling mutants obtained from the 2 sources of irradiation nor in the relative proportions of these types.

The frequency of spontaneous mutants ($0.3 \pm 0.15\%$) from 19-year-old seeds of Moister barley was slightly lower than from 1-year-old seeds of Trebi ($0.4 \pm 0.13\%$). The bomb-induced frequency in the Moister seeds was 15 times as high as the spontaneous frequency -- the same increase as the bomb produced in seeds of Trebi.

Conclusion

Thus, the results indicate that the atomic bomb-irradiated seeds received about as much radiation as those given 16,000r units of X-radiation. It has been estimated that the mean lethal dose of total body irradiation in man is 450r and there is experimental evidence that 750r is the comparable dose for the mouse. It is evident that the atomic bomb irradiated seeds received 20-30 times the lethal dose for man or mouse.

Acknowledgments

The broad outlines for these tests were conceived by the cooperative efforts of scientific personnel of the Department of Agriculture and the Naval Medical Research Section, Joint Task Force ONE. Specific plans for

testing various kinds of biological effects of bomb-created radiations on cereals were made by L. J. Stadler, L. F. Randolph and M. T. Jenkins. Selection of the varieties of small grains to be used and collection of samples were done by G. A. Wiebe in cooperation with D. W. Robertson and Harland Stevens. X-radiation of seeds for comparison with the bomb-irradiated seeds was performed in the laboratory of L. J. Stadler. Tests on the effects of the radiations on germination and seedling growth were made by R. W. Leukel. Harland Stevens planted the material to be grown to maturity at Sacaton, Arizona. The crop was cared for by R. H. Peebles, who cooperated wholeheartedly in providing facilities for growing and studying the plants.

Macroscopic and cytologic observations on the plants grown at Sacaton were made by Luther Smith, who was also entrusted with the responsibility for determining the mutation frequencies and with the preparation of this report. Assistance in determining the mutation rates in wheat was provided by L. P. V. Johnson. Many thanks for technical help in determining the mutation frequencies are due J. G. Moseman, W. L. Nelson, and C. C. Moh, graduate students in Agronomy at the State College of Washington. As is customary in such a cooperative effort, the writer of this report must accept full responsibility for the accuracy of the observations and for the interpretation of the data presented herewith.

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Fig. 1.

One of the effects of irradiation from X-rays and from the atomic bomb was chromosome breakage which permitted exchanges between non-homologous chromosomes. Such interchanges resulted in altered configurations at the first meiotic division.

A. A normal metaphase, showing 7 pairs of chromosomes.

B. Diakinesis, showing 5 pairs plus a ring of 4 chromosomes resulting from and interchange between 2 of the 7 pairs of chromosomes.

C. First metaphase, showing 4 pairs plus a ring of 6 chromosomes resulting from interchanges involving 3 pairs of chromosomes.

D. First metaphase, showing 3 pairs of chromosomes plus 2 separate rings of 4 chromosomes.

(NOTE: This page should face Fig. 1, in the final preparation of report.)

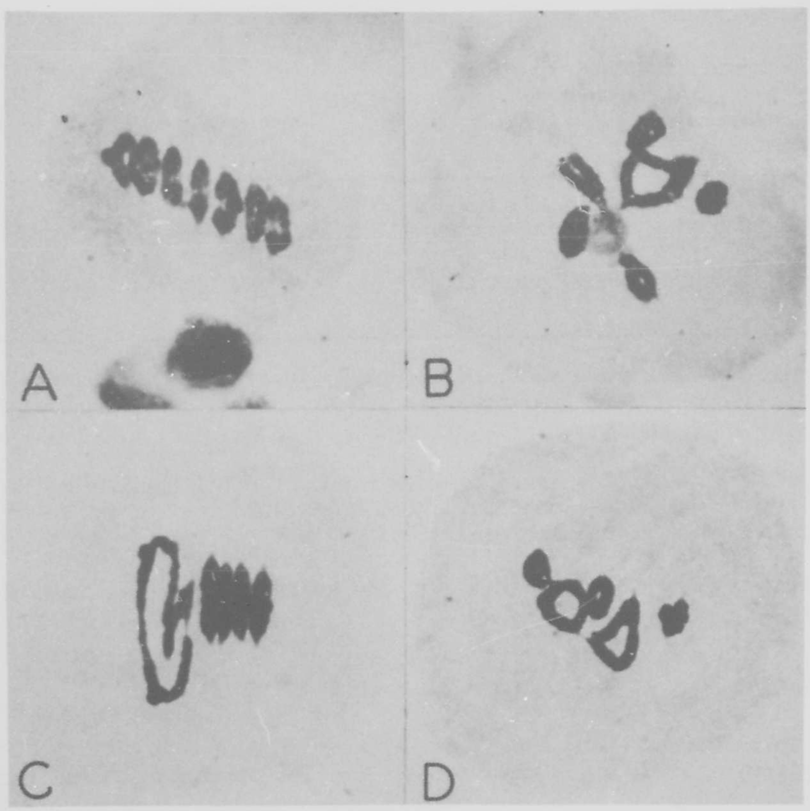

Fig.- 1

Table 1.

Comparison of Frequencies of Interchanges in Plants Grown from Bomb-Irradiated and X-Rayed (16,000r) Dormant Seeds of Barley and Durum Wheat.

Stock	Treatment	Spikes with indicated meiotic configurations Normal 1/ No.	R_4 No.	R_6 No.	Other No.	Total No.	Ring of 4 per spike 2/
Barley (Trebi)	Bomb-irradiated	77	18	3	2 3/	100	0.24
	X-rayed	64	12 4/	0	0	76	0.16
Durum (Carleton)	Bomb-irradiated	16	11	1	2 5/	30	0.57
	X-rayed	12	10	2	7 6/	30	0.83

1/ "Normal" in the sense that there were 7 (14 of durum) bivalents in the particular sector of the spike sampled by the pollen mother cells under examination.

2/ Calculated by considering a R_6 as 2 rings of 4, although a R_6 can be derived from 3 breaks (one per chromosome pair) instead of 4--the minimum number of breaks required to account for 2 rings of 4.

3/ These two plants has 2 univalents, one apparently smaller than the other in each case.

4/ One of these spikes had a R_4, 4 bivalents, and 2 univalents, one of which was smaller than the other.

5/ One spike had a R_6 and a R_4; another had a R_4 and 3 univalents plus 10 pairs, a total of 27 chromosomes, one less than the normal number (28).

6/ Four of the 7 spikes had 2 R_4, one of them having also a part of a chromosome missing. The other three had only 27 chromosomes, but each of the three had an interchange in addition to the loss of a chromosome.

Table 2.

Comparison of Mutation Frequencies from Untreated, Bomb-Irradiated, and X-rayed (16,000r) Seeds of Barley and Wheat.

Stock	Treatment	Spikes Tested Total	With Mutants			Progeny per Spike Without Mutants	With Mutants	Ratio of Normal to Mutant Seedlings in Progenies Segregating Mutants
		No.	No.	%	σ	Ave.No.	Ave.No.	Average
Barley (Trebi, seeds 1 year old)	Control	2479	11	0.44	.13	24.8	26.0	8.46:1
	Bomb-Irradiated	6783	503	7.4	.32	19.3	19.9	3.97:1
	X-rayed	2550	138	5.4	.45	25.6	24.2	4.25:1
Barley (Moister, seeds 19 years old)	Control	1536	5	0.33	.15	28.5	28.2	3.55:1
	Bomb-Irradiated	3143	173	5.5	.41	22.9	23.4	4.02:1
Durum (Carleton)	Control	912	0	0.0	0	--	--	---
	Bomb-Irradiated	4763	190	4.0	.28	26.8	25.3	4.74:1
	X-rayed	1692	46	2.7	.39	28.7	26.9	5.18:1
Common Wheat (Pilot)	Control	1149	0	0	0	--	--	---
	Bomb-Irradiated	7324	58	0.8	.10	24.2	24.1	5.62:1
	X-rayed	2400	23	1.0	.20	24.7	25.3	4.52:1

Table 3.

Comparison of Mutants Obtained From Bomb-Irradiated and X-rayed

(16,000r) Dormant Seeds of Barley, Durum, and Common Wheat

Mutant Type	Trebi Barley						Moister Barley				Durum Wheat 1/				Common Wheat 1/			
	Control		Bomb Irradiated		X-rayed		Control		Bomb Irradiated		Bomb Irradiated		X-rayed		Bomb Irradiated		X-rayed	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
White 2/	2	17	220	44	70	51	1	14	93	57	76	40	16	35	1	2	0	0
Yellow-green	4	42	87	17	17	12	0	0	30	17	24	13	8	17	0	0	0	0
Yellow	1	8	48	9	16	12	2	29	11	6	32	17	9	20	2	3	2	9
Virescent	2	17	41	8	10	7	0	0	9	5	2	1	1	2	1	2	0	0
Shrivels	0	0	33	7	14	9	0	0	5	3	20	10	6	13	44	76	21	91
Others	2	15	74	15	12	9	2	29	20	12	36	19	6	13	10	17	0	0
Total	11		503 3/		138 4/		5 5/		173 6/		190 7/		46 8/		58 9/-		23 10/	

1/ No mutants were found in the untreated control.

2/ This class, like all the others, includes a wide range of types (though in some cases clearly distinguishable). In other words, there were dozens of kinds of mutants with almost a continuous gradation between types, but they were combined into more or less homogeneous groups for this table.

3/ 19 head progenies segregated 2 distinguishable mutant characters, and 4 plants segregated the same or 2 indistinguishable mutants in 2 (in 1 case 3) head progenies.

4/ 12 head progenies segregated segregated 2 distinguishable mutant characters, and 1 plant segregated the same or 2 indistinguishable mutants in 2 head progenies.

5/ 1 head progeny segregated 2 distinguishable mutant characters.

6/ 4 head progenies segregated 2 distinguishable mutant characters, and 8 plants segregated the same or 2 indistinguishable mutants in 2 head progenies.

7/ 4 head progenies segregated 2 distinguishable mutant characters, and 4 plants segregated the same or 2 indistinguishable mutants in 2 head progenies.

8/ 3 head progenies segregated 2 distinguishable mutant characters, and 4 plants segregated the same or 2 indistinguishable mutants in 2 head progenies.

9/ 6 plants segregated the same or 2 indistinguishable mutants in 2 head progenies.

10/ 4 plants segregated the same or 2 indistinguishable mutants in 2 head progenies.