THEORETICAL STUDIES ON THE METHODOLOGICAL PROCEDURES OF RADIATION BREEDING

III. PROCEDURES FOR OBTAINING AT LEAST TWO DESIRABLE MUTANTS IN AUTOGAMOUS PLANTS FOLLOWING SEED IRRADIATION

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1. INTRODUCTION

In the first and the second reports of this series, theoretical considerations were made on properties of the four methods for selection in irradiated populations. Each of those methods, *i.e.* the "improved ear-to-row method" (method B), the "one-plant-one-grain method" (method C), the "one-plant-two-grain method" (method D) and the "one-plant-three-grain method" (method E) was shown to be more effective for detecting at least one desirable plant in X_2 -generation as compared with the "conventional ear-to-row method". A desirable mutant is defined in this report as well as in the previous one (see Yoshida, 1962).

In identifying a desirable mutant in field experiments of selection, it is frequent to face the difficulties caused by the factors such as soil heterogeneity and other environmental variabilities. Therefore, under such difficult conditions for selecting a desirable mutant, it seems to be appropriate to consider the probability of detecting at least two desirable mutants in order to guarantee the detection of at least one desirable mutant.

In the present report, it is intended to establish methods for the discovery of two or more desirable mutants in X_2 from a minimum total of X_2 -plants, and/or X_1 - and X_2 -plants in the aggregate which will economise both labour and land.

2. MATHEMATICAL PROCEDURES AND RESULTS

(i) *Explanation of notations*

The symbols used in this report are explained below.

Let p_1 stand for a probability of occurrence of an X_2 -line containing at least one desirable mutant. From the result observed by Gaul (1958), it is possible to say that p_1 increases when X_1 -plants are grown densely. Let p_2 stand for a conditional probability of occurrence of such desirable mutant for every one of the plants in such an X_2 -line and let $q_1 = 1 - p_1$ and $q_2 = 1 - p_2$.

Let m be the number of X_2 -lines or of X_1 -plants (in case of taking seeds from one ear of one X_1 -plant) and n be the number of plants in

an X_2 -line. Therefore, the product mn is the total number of X_2 -plants.

Let P_1^{II} be the probability of detecting at least two desirable mutants among mn plants. This P_1^{II} is concerned in the following three different ways: (a) P_{11}^{II} ; the probability of detecting at least one X_2 -line containing at least two desirable mutants, (b) P_{12}^{II} ; the probability of detecting at least two X_2 -lines (which contain at least one desirable mutant per line) among m lines and (c) P_{13}^{II} ; the probability of detecting at least two desirable mutants from the whole of an X_2 -population. In this report, it is assumed that the probability of producing a desirable mutant is equal to that of detecting a desirable mutant.

(ii) *Improved ear-to-row method II (method B II)*

In the first place, the effective procedure will be established by adopting P_{11}^{II} for the aim of detecting at least one X_2 -line containing at least two desirable mutants.

The probability, P_{11}^{II} , is shown in the following equation (1):

$$P_{11}^{II} = 1 - [1 - \{p_1(1 - q_2^n - np_2q_2^{n-1})\}]^m \quad (1)$$

This P_{11}^{II} is, of course, zero when $n = 1$. Let T be

$$|\log(1 - p_1 + p_1q_2^n + p_1np_2q_2^{n-1})|.$$

Then, relative m , mn and $m+mn$ are obtained from equation (1) as $1/T$, n/T and $n+1/T$ regardless of any value of P_{11}^{II} . For calculating real m , mn and $m+mn$, $1/T$, n/T and $n+1/T$ should be multiplied by $|\log(1 - P_{11}^{II})|$ respectively.

Mutation in one locus is concerned in this report as well as in the previous ones in this series. Usually, $p_1 = 1/100$ and $p_2 = 1/5$ in rice, $p_1 = 1/740$ and $p_2 = 1/5$ in barley as described by Yoshida (1962). So that, the values of p_1 from $1/10$ to $1/10,000$ used for the calculations in table 1 may be expected with far wider ranges than those occurred actually in cereals. When $q_2 = 4/5$ and $p_1 = 1/10$ to $1/10,000$, relative mn and $m+mn$ obtained from n/T and $n+1/T$, respectively, in terms of p_1 and n are tabulated in table 1. The ratios in per cent. of mn and $m+mn$ when n varies, to mn and $m+mn$ when $n = 2$ respectively, are also shown in table 1. The values of the ratio under given n are almost constant in mn and in $m+mn$ when $p_1 = 1/10$ to $1/10,000$ and $q_2 = 4/5$.

The relative m can be obtained by subtracting mn from $m+mn$ in table 1. The value of m is largest when $n = 2$ and shows the monotone decrease with the increase of n , especially m which comes nearer to $n = 2$ decreases distinctly, that is, the ratios are 100, 37 to 38, 15, 6.9 to 7.1, 4.1 to 4.3 and 3.8 to 4.0 corresponding to $n = 2, 3, 5, 9, 20$ and 50 respectively, when $1/10 \geq p_1 \geq 1/10,000$.

In table 1 the values of mn decrease with the increase of n , are at a minimum for $n = 9$ and increase with the increase of n . The values of mn corresponding to $n = 7$ to 12 are not so much different from the minimum. The ratios are 100 for $n = 2$, 32 to 33 for $n = 7$, 31 to 32 for $n = 9$, 32 to 33 for $n = 12$ and 95 to 100 for $n = 50$.

With respect to $m+mn$ in table 1, the values of $m+mn$ decrease with the increase of n , are at a minimum when $n = 10$ and increase with the increase of n . However, the values of $m+mn$ corresponding to $n = 8, 9, 10, 12$ and 13 are not so much different from the smallest. The ratios are 100 for $n = 2, 24$ for $n = 8, 23$ to 24 for $n = 10, 23$ to 24 for $n = 13$ and 65 to 68 for $n = 50$.

The smallest mn and $m+mn$ are found for $n = 11$ and 12 respectively, when $p_1 = 1/2$ and $q_2 = 4/5$ although $p_1 = 1/2$ is omitted in table 1 and the smallest m is not found for $n = 9$ when $p_1 = 1/10$ to $1/10,000$ and $q_2 = 4/5$. Judging from the fact that $p_1 = 1/100$ in rice and $p_1 = 1/740$ in barley and that the important factor is not m but mn , the method corresponding to $n = 9$ is much better and the methods corresponding to 8 to 12 are better for the purpose of detecting at least one X_2 -line with at least two desirable mutants with the smaller number of total X_2 -plants, and/or X_1 - and X_2 -plants in the aggregate. The methods corresponding to $n = 8$ to 12 are here called the "improved ear-to-row method II" (method B II) consisting of methods B II₈, B II₉, B II₁₀, B II₁₁ and B II₁₂ corresponding to $n = 8, 9, 10, 11$ and 12 respectively.

(iii) *One-plant-one-grain method II (method C II), one-plant-two-grain method II (method D II) and one-plant-three-grain method II (method E II)*

The effective procedures will be established by considering P_{12}^{II} or P_{13}^{II} for the purpose of detecting at least two X_2 -lines (which contain at least one desirable mutant per line) among m lines or of detecting at least two desirable mutants from the whole of an X_2 -population.

P_{12}^{II} , the probability of detecting at least two X_2 -lines containing at least one desirable mutant per line among m lines, is first furnished.

$$P_{12}^{II} = 1 - \{1 - p_1(1 - q_2^n)\}^m - m[1 - \{1 - p_1(1 - q_2^n)\}]\{1 - p_1(1 - q_2^n)\}^{m-1} \quad (2)$$

When $q_2 = 4/5$, $p_1 = 1/10$ to $1/10,000$ and $P_{12}^{II} = 0.95$ and 0.99 , the values of m were calculated from equation (2). But the table is omitted here for want of space since they can be obtained by subtracting mn in table 2 from $m+mn$ in table 3. The values of m decrease with the increase of n , especially m which comes nearer to $n = 1$ decreases distinctly. Therefore, when $n = 1$, the value of m is largest.

In table 2, the values of mn and the ratio in per cent. of mn when n varies, to mn when $n = 1$ are shown when $q_2 = 4/5$, $p_1 = 1/10$ to $1/10,000$ and $P_{12}^{II} = 0.95$ and 0.99 . The values of the ratio for $P_{12}^{II} = 0.95$ are equal to those for $P_{12}^{II} = 0.99$ when given p_1 , p_2 and n are constant. The ratios generally increase with the decrease of p_1 , but they are almost constant for any given value of n when $p_1 = 1/10$ to $1/10,000$. The lowest value of mn is found under $n = 1$ since mn shows the monotone increase with the increase of n for a given value of p_1 from $1/10$ to $1/10,000$. This is applicable even when $p_1 = 1/2$. The relative values are 100 for $n = 1, 110$ to 111 for $n = 2, 121$ to 123 for $n = 3$, etc., when $p_1 = 1/10$ to $1/10,000$ as seen in table 2.

TABLE I
Values of relative mn and $m + mn$ calculated from n/T and $n + 1/T$ respectively; $q_2 = 4/5$ and $p_1 = 1/10$ to $1/10,000$ (row 1).
Ratios of $\frac{mn \text{ for a given } n}{mn \text{ when } n = 2}$ and of $\frac{m + mn \text{ for a given } n}{m + mn \text{ when } n = 2}$
in per cent. (row 2)

Relative	$\frac{n}{p_1}$	2	3	5	7	8	9	10	12	13	20	50
mn	$\frac{1}{10}$	1,149 % 100	660.6 57	432.5 38	372.7 32	361.6 31	357.1 31	357.3 31	367.0 32	375.4 33	471.4 41	1,093 95
	$\frac{1}{100}$	11,508 % 100	6,639 58	4,377 38	3,800 33	3,699 32	3,666 32	3,688 32	3,797 33	3,891 34	4,924 43	11,455 100
	$\frac{1}{1,000}$	114,942 % 100	66,372 58	43,795 38	38,064 33	37,080 32	36,747 32	36,881 32	38,076 33	39,052 34	49,452 43	115,195 100
	$\frac{1}{10,000}$	1,176,470 % 100	660,792 56	439,599 37	380,435 32	371,112 32	366,885 31	368,148 31	381,060 32	391,053 33	495,246 42	1,152,315 98
$m + mn$	$\frac{1}{10}$	1,724 % 100	880.8 51	519.0 30	425.9 25	406.8 24	396.8 23	393.0 23	397.5 23	404.3 23	495.0 29	1,115 65
	$\frac{1}{100}$	17,262 % 100	8,852 51	5,252 30	4,343 25	4,162 24	4,073 24	4,057 24	4,113 24	4,190 24	5,170 30	11,684 68
	$\frac{1}{1,000}$	172,413 % 100	88,496 51	52,554 30	43,502 25	41,715 24	40,830 24	40,568 24	41,249 24	42,056 24	51,925 30	117,397 68
	$\frac{1}{10,000}$	1,764,705 % 100	881,056 50	527,519 30	434,783 25	417,501 24	407,650 23	404,963 23	412,815 23	421,134 24	520,008 29	1,175,361 67

TABLE 2

Values of mn ; $q_1 = 4/5$, $p_1 = 1/10$ to $1/10,000$ and $P_{12}^{II} = 0.95$ and 0.99 (row 1).
 Ratios of $\frac{mn \text{ for a given } n}{mn \text{ when } n = 1}$ in per cent. (row 2)

P_{12}^{II}	$p_1 \backslash n$	1	2	3	4	5	7	10	15	20	30	50
0.95	$1/10$	235.3 % 100	260.0 110	286.5 121	313.6 133	343.5 146	407.0 173	512.0 217	709.1 301	920.0 390	1,368 586	2,277 965
	$1/100$	2,370 % 100	2,632 111	2,910 123	3,208 135	3,520 149	4,186 177	5,296 223	7,350 310	9,560 403	14,190 599	23,630 997
	$1/1,000$	23,710 % 100	26,320 111	29,163 123	32,128 136	35,270 149	42,000 177	53,130 224	73,740 311	95,920 405	142,440 601	237,100 1,000
	$1/10,000$	236,750 % 100	262,200 111	291,510 123	320,480 135	352,760 149	420,430 178	530,950 224	737,480 311	960,540 406	1,424,700 602	2,373,250 1,002
0.99	$1/10$	329.1 % 100	363.2 110	399.6 121	438.4 133	479.5 146	568.4 173	715.0 217	989.1 301	1,284 390	1,908 586	3,175 965
	$1/100$	3,316 % 100	3,682 111	4,074 123	4,484 135	4,925 149	5,859 177	7,410 223	10,275 310	13,380 403	19,850 599	33,060 997
	$1/1,000$	33,180 % 100	36,860 111	40,800 123	44,960 136	49,350 149	58,770 177	74,340 224	103,160 311	134,220 405	199,260 601	331,750 1,000
	$1/10,000$	331,300 % 100	367,200 111	408,000 123	448,320 135	493,550 149	588,280 178	742,900 224	1,031,850 311	1,343,800 406	1,995,300 602	3,320,500 1,002

In table 3, the values of $m+mn$ and the ratio in per cent. between $m+mn$ when $n = 1$ and $m+mn$ corresponding to other values of n are shown for different p_1 and P_{12}^{II} respectively. The ratios increase with the decrease of p_1 , but they are almost constant under the same n when $p_1 = 1/10$ to $1/10,000$. In table 3 the ratios for given values of n for $P_{12}^{II} = 0.99$ are equal to those for $P_{12}^{II} = 0.95$ when p_1 is constant.

As shown in table 3, the value of $m+mn$ is smaller when $n = 2$ and is at a minimum when $n = 3$, and increases with the increase of n for different values of p_1 from $1/10$ to $1/10,000$ and $P_{12}^{II} = 0.95$ and 0.99 .

Then, the method corresponding to $n = 1$ needs the smallest mn and the smaller $m+mn$, the method corresponding to $n = 2$ needs the smaller mn and $m+mn$, and the method corresponding to $n = 3$ needs the smallest $m+mn$ and the smaller mn for the purpose of detecting at least two X_2 -lines (which contain at least one desirable mutant per line) among m lines. The method corresponding to $n = 1, 2$ or 3 is here designated the "one-plant-one-grain method II" (method C II), the "one-plant-two-grain method II" (method D II) and the "one-plant-three-grain method II" (method E II), respectively.

Methods C II, D II and E II can be established not only by adopting P_{12}^{II} but also by adopting P_{13}^{II} which represents the probability of detecting at least two desirable mutants among mn plants and which was suggested by Dr Kudo. This P_{13}^{II} is shown in the following equation (3):

$$P_{13}^{II} = 1 - \{1 - p_1(1 - q_2^n)\}^m - mn p_1 p_2 q_2^{n-1} \{1 - p_1(1 - q_2^n)\}^{m-1} \quad (3)$$

For the same aim of detecting at least two desirable mutants in an X_2 -population, equations (2) and (3) are concerned from the standpoint of X_2 -line with at least one desirable mutant and of desirable mutant, itself, respectively. Needless to say, P_{13}^{II} includes the case of detecting two desirable mutants not only in one X_2 -line but also two different X_2 -lines.

In table 4, the values of mn and $m+mn$, the ratios in per cent. of mn and $m+mn$ when n varies, to mn and $m+mn$ when $n = 1$ respectively, are shown when $p_1 = 1/10$ and $1/10,000$, $q_2 = 4/5$ and $P_{13}^{II} = 0.95$ and 0.99 . The results calculated from equation (3) shown in table 4 are quite similar to those from equation (2) shown in tables 2 and 3, with respect to the relation between n and mn or $m+mn$. The orders among methods C II, D II and E II obtained from equation (2) are the same with those obtained from equation (3), with regard to mn and $m+mn$. This is applicable for the order among the methods for m .

3. DISCUSSION AND CONCLUSION

(i) Comparison of methods

For the purpose of detecting at least two desirable mutants in X_2 with the smaller number of total X_2 -plants, and/or X_1 - and X_2 -plants in the aggregate, the four methods, B II, C II, D II and E II are established by considering three different probabilities, P_{11}^{II} , P_{12}^{II} and P_{13}^{II} .

TABLE 3

Values of $m + mn$; $q_2 = 4/5$, $P_1 = 1/10$ to $1/10,000$ and $P_{12}^{II} = 0.95$ and 0.99 (row 1).
 Ratios of $\frac{m + mn}{m + mn}$ for a given n in per cent. (row 2)

P_{12}^{II}	P_1	n	1	2	3	4	5	7	10	15	20	30	50
0.95		1/10	470.6 % 100	390.0 83	382.0 81	392.0 83	412.2 87	465.1 99	563.2 119	756.4 160	966.0 205	1,414 300	2,323 492
		1/100	4,740 % 100	3,948 83	3,880 82	4,010 85	4,224 89	4,784 101	5,826 123	7,840 165	10,038 212	14,663 309	24,103 509
		1/1,000	47,420 % 100	39,480 83	38,884 82	40,160 85	42,324 89	48,000 101	58,443 123	78,656 166	100,716 212	147,188 310	241,842 510
		1/10,000	473,500 % 100	393,300 83	388,680 82	400,600 85	423,310 89	480,490 101	584,050 123	786,650 166	1,008,570 213	1,472,190 311	2,420,720 511
0.99		1/10	658.2 % 100	544.8 83	532.8 81	548.0 83	575.4 87	649.6 99	786.5 119	1,055 160	1,348 205	1,972 300	3,239 492
		1/100	6,632 % 100	5,523 83	5,432 82	5,605 85	5,910 89	6,696 101	8,151 123	10,960 165	14,049 212	20,512 309	33,721 509
		1/1,000	66,360 % 100	55,290 83	54,400 82	56,200 85	59,220 89	57,165 101	81,774 123	110,037 166	140,931 212	205,902 310	338,385 510
		1/10,000	662,600 % 100	550,800 83	544,000 82	560,650 85	592,260 89	672,320 101	817,190 123	1,100,640 166	1,410,990 213	2,061,810 311	3,386,910 511

TABLE 4

Values of mn and $m + mn$; $q_2 = 4/5$, $p_1 = 1/10$ and $1/10,000$ and $P_{13}^{II} = 0.95$ and 0.99 (row 1).

Ratios of $\frac{mn \text{ for a given } n}{mn \text{ when } n = 1}$ and of $\frac{m + mn \text{ for a given } n}{m + mn \text{ when } n = 1}$ in per cent. (row 2)

Light numbers = mn ; bold numbers = $m + mn$

P_{13}^{II}	n p_1	1	2	3	4	5	7	10	15	20	30	50
0.95	$1/10$	235.3 % 100	253.8 108	271.5 115	290.8 124	312.5 133	350.0 149	405.0 172	502.5 214	610.0 259	870.0 370	1,425 606
		470.6 % 100	380.7 81	362.0 77	363.5 77	375.0 80	400.0 85	445.5 95	536.0 114	640.5 136	899.0 191	1,454 309
	$1/10,000$	236,750 % 100	260,040 110	277,500 117	300,000 127	322,500 136	360,500 152	420,000 177	534,450 226	642,600 271	909,000 384	1,499,500 633
		473,500 % 100	390,060 82	370,000 78	375,000 79	387,000 82	412,000 87	462,000 98	570,080 120	674,730 143	939,300 198	1,529,490 323
		329.1 % 100	357.0 108	388.5 118	416.0 126	445.0 135	507.5 154	610.0 185	765.0 232	980.0 298	1,365 415	2,340 681
		658.2 % 100	535.5 81	518.0 79	520.0 79	534.0 81	580.0 88	671.0 102	816.0 124	1,029 156	1,411 214	2,285 347
0.99	$1/10,000$	331,300 % 100	360,500 109	393,390 119	430,000 130	461,250 139	525,910 159	627,500 189	813,750 246	983,000 297	1,405,500 424	2,304,500 696
		662,600 % 100	540,750 82	524,520 79	537,500 81	553,500 84	601,040 91	690,250 104	868,000 131	1,032,150 156	1,452,350 219	2,350,590 355

Method B II consists of method B II₈, B II₉, B II₁₀, B II₁₁ and B II₁₂ which are established by considering P_{11}^{II} and which are used as the ear-to-row methods. Method B II₉ is the most effective in method B II. In method B II₉, nine grains yielded from every one ear of the X_1 -plants of the number required for detecting at least one X_2 -line with at least two desirable mutants are sown to obtain corresponding X_2 -plants.

In methods C II, D II and E II which are established by considering P_{12}^{II} or P_{13}^{II} and which are used as the bulk-methods, one, two or three grains respectively, taken from every one ear of the X_1 -plants of the number required to detect at least two X_2 -lines (which contain at least one desirable mutant per line) among m lines or to detect at least two desirable mutants among mn plants. The orders among methods C II, D II and E II obtained by considering P_{12}^{II} are the same with those obtained by considering P_{13}^{II} with respect to m , mn and $m+mn$. However, the sizes of m , mn and $m+mn$ calculated from equation (2) are larger than those calculated from equation (3) except those for $n = 1$, when constant p_1 , p_2 and n are given and $P_{12}^{II} = P_{13}^{II}$.

With regard to the number of grains taken from every one ear of the X_1 -plants, methods C II, D II and E II are identical with methods C, D and E which are detected at least one desirable mutant in an X_2 -population as reported by Yoshida (1962). But the sizes of m , mn and $m+mn$ in methods C II, D II and E II are larger than those in methods C, D and E, respectively.

Although the tables are omitted for want of space, the values of m in the different methods will fall in the following order:

$$C II > D II > E II > B II_9$$

where $>$ means "larger". Although m is not so important in the practice of radiation breeding, it is necessary to prepare more X_1 -seeds in method C II than in other methods.

With respect to the values of mn , we have the following order from the values given in table 1 to 4: $B II_9 > E II > D II > C II$. The value of mn is very important in order to economise both labour and land in the practice of radiation breeding. The same order with mn will be obtained for $m+mn$ when X_1 is planted densely and X_2 is planted ordinarily. With respect to the values of $m+mn$, it is possible to have the following order from the values given in table 1 to 4:

$$B II_9 > C II > D II \gtrsim E II$$

where $\sim$ means "nearly equal".

(ii) Application fields of methods B II, C II, D II and E II

Yoshida (1962) described that when the discrimination of a mutant is difficult method B will be useful and that when a mutant is easily distinguishable, method C, with dense planting in X_1 , will frequently

be much better and in some cases methods D and E, especially D, will be more useful in the practice of radiation breeding than method C.

Each of methods B II₉, C II, D II and E II will be applicable in radiation breeding. However, no single method is known to cover all the advantages in the methods, each being superior to the "conventional ear-to-row method".

The following application fields of the methods to the practice of radiation breeding will be concerned. When the discrimination of a desirable mutant is very difficult, method B II₉ will be useful because at least two homozygous *aa* plants in an X_2 -line can be detected under given probability. Methods B II₈, B II₁₀, B II₁₁ and B II₁₂ are also useful because *mn* and *m+mn* are not so much different from method B II₉. When the discrimination of a desirable mutant is not so easy as methods C, D and E can be used or when it is desired to increase an expectation of the number of desirable mutants to be found in methods C, D and E, methods C II, D II and E II, with dense planting in X_1 , will be applicable instead of methods C, D and E respectively, for the purpose of decreasing chance of losing desirable mutants to ensure the object of radiation breeding.

4. SUMMARY

1. The aim is to establish effective selection procedures by which two or more desirable mutants in the X_2 -generation following seed irradiation can be detected in a minimum total of X_2 -plants or of combined X_1 - and X_2 -plants.

2. Four methods are considered which achieve this aim, they are:

- (i) improved ear-to-row method (B II),
- (ii) one-plant-one-grain method (C II),
- (iii) one-plant-two-grain method (D II) and
- (iv) one-plant-three-grain method (E II).

These methods have been evaluated by reference to the probability of

- (i) detecting at least one X_2 -line containing two or more mutants,
- (ii) detecting at least two X_2 -lines each containing one or more mutants and
- (iii) detecting at least two mutants in the whole X_2 population.

3. No single method is clearly superior to the other three in all respects but all four methods are superior to the conventional ear-to-row method in overall efficiency.

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