Studies on Tetraploid Rice

VI. Fertility variation and segregation ratios for several characters in tetraploid hybrids of rice, *Oryza sativa*, L.¹

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Received February 21, 1955

It is a remarkable tendency of tetraploid rice that in F_1 fertility becomes higher and chromosome pairing becomes more regular than in the parental tetraploid varieties. Tetraploid hybrids between distantly related varieties of cultivated rice thus appear to have a tendency to behave like amphidiploids, which implies selective pairing of chromosomes derived from the same parent. This fact was pointed out by Cua (1951) as well as in previous papers of this series (Oka 1954 c, d).

It was also found, however, as reported in previous papers, that (1) fertility in autotetraploid varieties markedly differed according to the diploid prototype, and tended to be higher in those showing vigorous growth (at tetraploid level), (2) tetraploid F_1 hybrids showed generally a hybrid vigor to which fertility seemed to proportionate, and (3) no significant correlation was found between fertility and the number of quadrivalent chromosomes. These facts may suggest that a complementary effect of parental genes, rather than the amphidiploid-like nature of the tetraploid hybrid, was responsible for the improvement in fertility. Thus, for the study of genetical relationships between variety groups of cultivated rice, such as the "Continental" and "Insular" groups' (Oka 1953 d) it is an important problem whether or not selective pairing of chromosomes takes place in tetraploid hybrids between distantly related varieties.

In order to throw a light on this problem, the writer investigated variations in fertility and other characters in F_2 to F_7 and the F_2 segregation ratios for several characters in such tetraploid hybrids. From these studies, it was recognized that the high fertilities in F_1 could not be fixed in later generations, and that the F_2 segregation ratios agreed always with the theoretical autotetraploid ratios, so far as the present study goes.

The data presented in this paper were obtained in the Taiwan Provincial College of Agriculture, Taichung, Formosa. The writer expresses his hearty gratitude to Mr. K. H. Tsai and to Mr. T. K. Ming, who kindly helped him in the laborious investigations.

Materials and methods

Tetraploid plants were induced by Colchicine method from 71 rice varie-

¹ Contributions from National Institute of Genetics, Japan, No. 105.

ties of different Asian countries. They were inbred for several generations, and were crossed with each other (Oka 1954b). The materials used in this work, given in Table 1, are a part of those crosses; they were chosen among those between the Continental and Insular groups and they showed relatively high fertilities in F_1 .

Table 1. Parental tetraploid varieties and F_1 plants

No.	Local name	Origin	Variety group	Fertility in % Pollen	Seed
R 1	70a som cau	Indo-China	Continental	82.5	17.5
J 108	Pei-ku	Formosa	"	72.4	17.6
P 242	Malagkit Pirurutong	Philippines	Insular, Tropical	81.0	9.3
Q 325	Kaniranga	Indonesia	"	82.0	21.0
P 414	P.T.B. 10	India	Continental	76.0	4.6
J 415	A.D.T. 4	"	"	87.5	16.6
P 417	A.D.T. 14	"	"	82.5	26.2
U 421	P.T.B. 8	"	"	83.0	37.9
J 553	Kinoshita-mochi	Japan	Insular, Temperate	76.3	24.3
L 647	Padi ase banda	Celebes	Insular, Tropical	80.3	7.7

(F ₁ hybrids)					
Cross		Fertility in % Pollen	Seed	Ratio of height to parental average (%)	
L 647 × J 415		87	76	114	
J 108 × Q 325		78	47	122	
R 1 × P 242		59	33	118	
R 1 × P 417		65	25	135	
P 414 × J 553		77	38	120	
L 647 × U 421		73	40	115	

The hybrid generations after F_2 were grown in pedigrees, with two generations a year (the first and second crops). Though in the F_2 nearly all plants were taken, one plant with the highest fertility was selected from each line after F_3 . Measurements on single plant basis were recorded in regard to fertility, heading date and plant height. For observing segregation ratios, phenol reaction, endosperm property (glutinous or not), apiculus pigmentation and seed coat color were investigated by the following methods.

Phenol reaction: Unhulled rice seeds were immersed in 1.5% phenol (carbolic acid) solution and were dried gradually. The presence or absence of coloration was recorded after 24 hours. This character is determined by a pair of genes (Ph : ph) and the positive reaction is dominant over the negative reaction.

Endosperm property: Non-glutinous or glutinous was tested by iodine reaction of the endosperm. Non-glutinous (+) is a monogenic dominant against glutinous(gl).

Apiculus pigmentation: Three complementary gene pairs (C, Sp and Ap), independent of each other, are responsible (Nagao 1951); the crosses used differed in C:c.

Seed coat coloration: Red is brought about by two independent genes (Rc and Rd) and is dominant against colorless. The crosses used segregated in Rc:rc.

Results of observations

1. Fertility variation in F_2 and later generations

Though two crosses, 108×325 and 647×415 , were used, the pooled data for them will be given here, since they showed almost the same tendency. Mean fertilities and variances among and within lines in F_2 and later generations are given in Table 2.

Fig. 1. Frequency distributions for fertility in F_2 , F_3 and F_4 of $J\ 108 \times Q\ 325$Selected plants.

correlations are significant; this means that a greater part of the fertility variation is heritable.

Figure 1 shows frequency distributions for fertility in the F_2 , F_3 and F_4 of 108×325 . The above-mentioned phenomena may also be seen in Figure 1. It may then be said that the F_1 plants with high fertilities did not breed true, and the progeny segregated for fertility to let mean fertilities go down with the repetition of generations.

Variations in heading date and height are given in Table 3. In the same manner as in the case of fertility, a conspicuous segregation was found

It is shown in Table 2 that (1) mean fertility tends to go down with the repetition of generations, though plants with high fertilities have been selected in every generation, (2) differences between line means, as shown by intraline correlations, are generally large; this shows a marked segregation for fertility to occur in each generation, (3) variances within lines (among individual plants) are much larger than those in parental varieties, and do not decrease with the repetition of generations: this may mean that the fixation of homozygous lines is reached very slowly in tetraploid hybrids due to the multiplicity of chromosomes, (4) parent-progeny

Table 2. Variations of fertility in tetraploid hybrid populations

Cross	Generation	Num. of		Mean Fertility		Variance btw.		Intraline corrl.	Parent-progeny corrl.
		plants	lines	Whole plants	Selected plants	Lines	Indv.s		
L 647	L 647*	132		14.8%			156.5		
×	J 415*	8		6.3			55.5		
J 415	F ₁	1		76.0					
	F ₂	33		41.8			509.1		
	F ₃	399	32	45.5	72.4	128.7	489.6	0.154	0.286
	F ₄	505	54	29.1	51.3	173.0	189.0	0.447	0.328*
	F ₅	520	53	30.2	47.2	172.9	296.1	0.304	0.396**
	F ₆	412	44	34.0	56.1	81.3	377.0	0.168	0.142
	F ₇	427	44	26.9		142.0	268.6	0.278	0.337*
J 108	J 108*	28		10.6			104.1		
×	Q 325*	13		16.2			175.6		
Q 325	F ₁	3		47.0					
	F ₂	176		44.5			413.0		
	F ₃	444	30	31.5	66.8	173.9	359.3	0.283	0.614**
	F ₄	500	30	28.0	54.4	76.8	253.6	0.196	0.389*
	F ₅	376	29	39.9	56.3	142.5	355.8	0.251	0.559**
	F ₆	537	28	30.4	59.6	142.9	299.6	0.298	0.388*
	F ₇	482	28	37.0		130.2	379.4	0.222	0.639**

* Parental varieties grown together with hybrid populations; averages for more than three years are given.

Table 3. Variations in height and heading date and correlations between fertility and height in tetraploid hybrid populations

Cross	Generation	Mean (cm)	Height		Mean	Heading date		Corrl. btw. fertility & height
			I.C. Cor.*	P.P. Cor.**		I.C. Cor.*	P.P. Cor.**	
L 647	L 647	107,97#			V19, IX25#			
×	J 415	105,130#			V13, IX25#			
J 415	F ₂	123			V4			0.186
	F ₃ #	126	0.245	0.406*	IX20	0.430	0.296	0.464*
	F ₄	123	0.606	0.443**	V7	0.711	0.381	0.476**
	F ₅	116	0.291	0.461**	IV27	0.540	0.639	0.300*
	F ₆ #	108	0.367	0.469**	IX25	0.746	0.394	0.432**
	F ₇	111	0.399	0.382*	V12	0.678	0.284	0.337*
J 108	J 108	106,120#			V23, X12#			
×	Q 325	129,127#			VI2, X15#			
Q 325	F ₂	136			V24			0.549**
	F ₃ #	126	0.232	0.472*	X7	0.394	0.627	0.658**
	F ₄	134	0.410	0.645**	V28	0.691	0.749	0.156
	F ₅	126	0.396	0.790**	V14	0.464	0.813	0.763**
	F ₆ #	111	0.426	0.686**	X9	0.641	0.723	0.388*
	F ₇	119	0.418	0.403*	V26	0.582	0.644	0.497**

2nd crop

* Intraclass correlation (Intra-line)

** Parent-progeny correlation

* Between 5% and 1% levels of significance

** Beyond 1% level

for both of these characters, and it was demonstrated that the F₁ hybrids did not show a tendency to breed true. Correlations between fertility and height are also shown in Table 3. Fertility was found to be positively correlated

with height in every generation. As pointed out in previous papers (Oka 1954 a, b), the same tendency was also seen regarding variations within as well as among the varieties. It then seems that in tetraploid rice, the more vigorously the plant grows, the higher becomes the fertility.

2. Segregation ratios for several monogenic characters

Segregation ratios for phenol reaction, apiculus coloration, seed coat coloration and glutinous endosperm found in the F_2 of several crosses are given in Table 4.

Table 4. F_2 segregation ratios for several monogenic characters in tetraploid hybrids between distant varieties of rice

Crosses	Obs. Num. D.	Num. R.	Exp. Num. D.	Num. R.	(20.8:1) X^2	Exp. Num. D.	Num. R.	(35:1) X^2
(Phenol reaction)								
R 1×P 242	491	14	481.8	23.2	3.82	491.0	14.0	0.0
J 108×Q 325	163	6	151.25	7.75	0.42	164.3	4.7	0.37
L 647×U 421	247	9	244.3	11.7	0.65	248.9	7.1	0.52
(Apiculus coloration)								
R 1×P 242	494	15	485.7	23.3	3.12	494.9	14.1	0.06
R 1×P 417	247	6	241.4	11.6	2.83	246.0	7.0	0.15
J 108×Q 325	206	7	203.3	9.7	0.80	207.1	5.9	0.21
(Seed coat coloration)								
L 647×U 421	241	9	238.0	12.0	0.79	243.0	7.0	0.59
(Endosperm, glutinous or not)								
P 414×J 563	285	9	280.5	13.5	1.57	285.8	8.2	0.07

Table 4 shows that all observed ratios were between 35:1 and 20.8:1. Each of these characters is controlled by a pair of major genes and shows a 3:1 ratio in corresponding diploid hybrids. In tetraploid hybrids, a 35:1 to 20.8:1 ratio would be expected, if each of the four homologous chromosomes choosed its partner to pair at random, regardless of having been derived from the same or different parents. If the chromosomes from the same parent tended to pair with each other, however, homozygous gametes would decrease and heterozygous gametes would increase to give rise to a deficiency in plants with recessive phenotype. The above-mentioned fact that all observed ratios agree well with the expected ones in autotetraploids, may indicate that no selective pairing occurs among the four homologous chromosomes, so far as the chromosomes carrying several genes under discussion are concerned.

3. Genotypic segregation ratio for glutinous endosperm

In a tetraploid hybrid, the F_2 genotype will be of five kinds, which in the F_3 segregate as follows:

1	AAAA	$\infty:0$
8	AAAa	$\infty:0$ (or 784:1)
18	AAaa	35:1 (or 20.8:1)
8	Aaaa	3:1
1	aaaa	0: ∞

In the cross R 1(non-glutinous) $\times$ P 242(glutinous), seeds of each F_2 plant were examined as to the manner of segregation for glutinous endosperm. Since owing to sterility some plants produced less than 20 seeds, the genotype was determined by the following method. For example, when a ratio of 20 non-glutinous: 3 glutinous was found in an F_2 plant, the probability that this ratio happens will be, when the plant has +glglgl:

$$\frac{23!}{20! 3!} \left(\frac{3}{4}\right)^{20} \left(\frac{1}{4}\right)^3 = 0.0875,$$

and when the plants has ++glgl:

$$\frac{23!}{20! 3!} \left(\frac{35}{36}\right)^{20} \left(\frac{1}{36}\right)^3 = 0.0218.$$

Then, the probabilities that the genotype of the given plant was +glglgl and ++glgl will be respectively:

$$(+glglgl) \frac{0.0875 \times 8}{0.0875 \times 8 + 0.0218 \times 18} = 0.64,$$

and

$$(++glgl) \frac{0.0218 \times 18}{0.0875 \times 8 + 0.0218 \times 18} = 0.36.$$

This probability for each genotype was summed up to estimate the genotypic segregation ratio among F_2 plants. + + + +, + + + gl and + + glgl were grouped into the same class, since $\infty : 0$ and 35 : 1 were indistinguishable.

Table 5. Observed and expected segregation ratios for glutinous endosperm in the F_2 of R1 $\times$ P242

Genotype	Obs. Num. (Estimated)	Expected Chromatid segregation	Number Chromosome segregation	Considering certation
+ + + + } + + + gl } + + glgl }	231.6	193.0	203.9	231.4
+glglgl	36.4	66.5	60.4	36.2
glglglgl	4.0	12.5	7.5	4.3
Total	272.0	$X^2 = 27.12 (P < 0.01)$	14.95 ($P < 0.01$)	0.02 ($P > 0.95$)

Table 5 gives the genotypic segregation ratio thus found in the F_2 of R 1 $\times$ P 242. In Table 5, the observed ratio agrees neither with the expected ratio based on random chromatid segregation nor that based on random chromosome segregation. The gametic ratio of + + : + gl : glgl was then computed from the observed ratio in Table 5: Let the frequencies of gametes with +gl, glgl and + + be x, y and 1-x-y respectively, the frequencies of different F_2 genotypes will be as follows:

$$\begin{array}{l} \left. \begin{array}{l} + + + + \\ + + + gl \\ + + glgl \\ + glglgl \\ glglglgl \end{array} \right\} \begin{array}{l} (1-x-y)^2 + 2x(1-x-y) + 2y(1-x-y) + x^2 = 1 - 2xy - y^2 \\ 2xy \\ y^2 \end{array} \end{array}$$

By means of maximum likelihood method, the frequencies of different genotypes will be estimated as follows:

Gametes	Expected Frequency	
	Chromosome segregation	Chromatid segregation
+gl..... $x=0.551$	0.666	0.572
glgl..... $y=0.121$	0.167	0.214
++..... $1-x-y=0.328$	0.167	0.214

Comparing the estimated frequencies with expected ones, we find that gametes with ++ are much more numerous than those with glgl, while the frequencies of +gl gametes are rather less than the expected one. This may eliminate the possibility of selective pairing of chromosomes by which the heterozygous gametes should be increased. On the other hand, the deficiency in glgl gametes may suggest a certation in favor of ++ against glgl pollen, in the same manner as known in diploid hybrids. Assuming then the gametic ratio to be the expected one (average of ratios for random chromosome and chromatid segregations; $x=0.62$, $y=0.19$), the effect of certation was surveyed.

Let the fertilizing capacity of +gl and glgl pollens be $1-s_1$ and $1-s_2$ respectively, the frequencies of different F_2 genotypes will be as follows:

+++ +	}		$0.7283 - 0.5022s_1 - 0.0361s_2$	(Obs. Freq.)	$231.6/272$
+++ gl					
++ glgl					
+ glglgl			$0.2356 - 0.1178s_1 - 0.1178s_2$		$36.4/272$
glglglgl			0.0361	$-0.0361s_2$	$4.0/272$

It then follows that

$$\frac{0.7283 - 0.5022s_1 - 0.0361s_2}{0.2356 - 0.1178s_1 - 0.1178s_2} = \frac{231.6}{36.4},$$

$$\frac{0.7283 - 0.5022s_1 - 0.0361s_2}{0.0361} = \frac{231.6}{4.0},$$

and

$$\frac{0.2356 - 0.1178s_1 - 0.1178s_2}{0.0361} = \frac{36.4}{4.0}.$$

Least squares solution of these equations gave $s_1=0.730$ and $s_2=0.829$. The fertilizing capacities of +gl pollen and of glgl pollen will then be estimated to be 0.27 and 0.17 of that of ++ pollen, respectively.

Expected numbers in the last column of Table 5, which are quite consistent with the observed numbers, were computed by using these values of s_1 and s_2 . It may then be said that by considering certation, the expected ratio based on the assumption of random pairing agrees well with the observed ratio.

Discussion

The above-presented data on segregation ratios as well as on fertility variations seem to support the hypothesis that the tetraploid hybrids between

distantly related varieties of cultivated rice have no amphidiploid-like nature, though fertility as well as chromosome pairing are apparently improved in the hybrids.

It is known in diploid hybrids that no disturbance in chromosome pairing is seen even in those with the lowest fertility (Terao and Mizushima 1939, Oka 1953 b). In tetraploid hybrids, however, relatively slight structural differences in chromosomes may give rise to a differential affinity to affect the manner of pairing. The conclusion of this study that no trace of selective pairing could be demonstrated, may imply that structural differences in chromosomes between distant varieties of cultivated rice, if any, are too slight to affect the manner of pairing.

The rise of fertility due to hybridization in tetraploid rice, which can not be explained by selective pairing of chromosomes, may be explained as due to a complementary effect of genes from both parents. The genotypes in diploid varieties may generally be so constituted as to be adaptable at diploid level. At tetraploid level, they cannot be completely adaptable, while hybridization may produce gene combinations necessary for a better adaptation. The parallelism between high fertility and vigorous growth may support this view.

Similar cases of tetraploid hybrids showing an improvement in fertility have been reported in *Lycopersicon* (Lesley 1953), in *Zea* (Randolph 1941), and in *Galeopsis* (Müntzing 1943). It is possible that a fertility improvement due to hybridization is a phenomenon common to plant species in which the autotetraploids show a reduced adaptability.

The improvement in chromosomes pairing in tetraploid hybrids may also be assumed to be due to a complementary effect of genes, as it is generally accepted that the chromosome behavior is controlled by the genotype.

These considerations may lead us to the view that in the phylogenetical development of cultivated rice, structural changes in chromosomes may have played no important role, and the wonderfully rich variation among the rice varieties is mainly due to genic changes. According to the writer's experiments, hybrid sterility between rice varieties seems to be due to duplicate genes which work in the gamete as development maintainers (Oka 1953 b). A number of sets of those duplicate genes seem to work in hybrids between distant varieties. Moreover, duplicate genes have been pointed out regarding pigment formation at the stigma and other characters (the writer's unpublished data). The duplication of genes may be due either to chromosome doubling or to translocations. Though a conclusive evaluation is difficult, the present study may lower the probability of many translocations having arisen to give rise to gene duplications, since selective pairing of chromosomes has not been demonstrated in tetraploid hybrids, and may support the hypothesis that the duplication of genes in rice is due to chromosome doubling, as has been suggested by the hypothesis of secondary polyploidy (Sakai 1935).

Summary

1. In tetraploid hybrids between distantly related varieties of cultivated rice, such as between the Continental and Insular groups, the F_1 plants generally showed relatively high fertility. However, plants with different fertilities were produced after the F_2 due to segregation and the mean fertilities tended to go down with the repetition of generations. Plants showing vigorous growth tended to have high fertility.

2. In the tetraploid hybrids between distant varieties, F_2 segregation ratios so far observed for several monogenic characters (phenol reaction, apiculus pigmentation, glutinous endosperm and seed coat coloration) all agreed with the autotetraploid ratios 35 : 1 or 20.8 : 1, based on the assumption of random pairing of homologous chromosomes or chromatids.

3. The F_2 genotypic ratio for glutinous endosperm showed a significant deviation from the expected ratio: glutinous homozygotes and simplex heterozygotes were deficient. This deviation was found to be due, not to selective pairing of chromosomes, but to a certation in favor of non-glutinous pollen, in the same manner as in diploid hybrids.

4. It was concluded that tetraploid hybrids between distant varieties of cultivated rice have no amphidiploid-like nature, and that the variation in cultivated rice is mainly due to genic changes.

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