A Note on the Cross Results of Diploid and Tetraploid Rice Plants.

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Discovering a considerable number of haploid, triploid, and tetraploid rice plants in common paddy fields, the authors made before detailed cytological studies of these spontaneous chromosomal mutations⁽³⁾⁽⁴⁾⁽⁵⁾. As to the origin of the triploid, the authors remarked as follows: Auto-triploid has usually been attributed to the union of a haploid gamete with an occasional diploid gamete. Such might also be the case in *Oryza sativa*. In the rice field, however, not only the triploid, but also the haploid and the tetraploid mutants appear together, though in less frequency. Another co-ordinate cause for all such chromosomal mutations might, therefore, be considered.

Later, E. OKURA⁽⁹⁾ tried, on a small scale, the hybridization between tetraploid and diploid races of rice. Taking tetraploid on the maternal side, he obtained 11 seeds rather easily, but 8 germinated seeds gave entirely unexpected results of 5 diploid plants and 3 tetraploid plants.

In 1943, the authors also tried hybridization between tetraploid and diploid types of rice. They then adopted the clipping method for emasculation. Sixty one flowers of tetraploids were pollinated with the pollen of diploids, and 238 flowers of diploids were pollinated with the pollen of tetraploids, but none produced the seed.

In 1948, hybridization experiments of the same kind were repeated by the authors with newly produced tetraploid types. They used at that time the hot water method (43°C for 7 minutes) for emasculation⁽²⁾ to raise the efficiency of the work. Three hundred and sixty-six emasculated tetraploid flowers were pollinated with the pollen of diploids and produced only 4 poorly developed seeds which did not germinate. Four hundred and thirty four emasculated diploid flowers pollinated with the pollen of tetraploids produced 14 perfectly developed seeds. Twelve seeds out of the 14 germinated, but they were diploid without exception, and looked entirely like the mother plant.

The results of these preliminary experiments show that the hybridization between diploid and tetraploid rice plants is not easy, if not impossible, of success, and that such results as reported by E. OKURA must be very exceptional ones.

Experiments

In 1949, the following extensive series of experiments were undertaken to determine further information on the triploid formation by the hybridization of diploid and tetraploid rice. Taking occasion, the authors also wanted to make clear, if possible, the real situation about the maternal plants occasionally produced by such rather hopeless cross-pollination, whether they are caused simply by unexpected self-pollination or by an apomictic process. For the latter purpose, heterozygous plants, especially those for the glutinous gene, were selected on the maternal side. The heterozygosity of the plant for the glutinousness was actually ascertained by the segregation of pollen-grains into two types, glutinous and non-glutinous. Throughout the experiments, diploid type was always taken on the mother side.

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Table I. Results of emasculation by the hot water method when no artificial pollination followed.

Treatment	Maternal parent (2x)	Number of flowers treated	Perfect grains obtained	Illdeveloped grains obtained	% of developed seeds	Number of germinated seeds	Number of plants grown up	Endosperm type		
								Non-gl.	Het.	Gl.
Exp. I-a	NF ₂ -hetero	1,685	19	0	1.13	13	12 (2x)	5	2	5
	F ₁ (Shinriki×Aikoku)	201	2	0	1.00	2	1 (2x)	1	0	0
	F ₁ (Aikoku×Java-28)	269	2	0	0.74	2	2 (2x)	2	0	0
	NF ₂ -glutinous	405	17	0	4.20	15	15 (2x)	0	0	15
	Total (or average)	2,560	40	0	1.56	32	30 (2x)			
Exp. I-b	NF ₂ -hetero	172	1	0	0.58	1	1 (2x)	0	0	1
	F ₁ (Shinriki×Aikoku)	74	0	0	0	0	0			
	Total (or average)	246	1	0	0.29	1	1 (2x)			
Grand total		2,806	41	0	1.46	33	31 (2x)			

Experiment I

Diploid flowers emasculated were left to the end in bags, without artificial pollination. This experiment consisted of the following 3 sub-parts (Table I).

(a). Flowers emasculated by the hot water method were left without artificial pollination. In total 2,560 flowers on 139 panicles were treated and resulted in 40 perfectly developed seeds. Of these 40 seeds, 32 seeds germinated to give 30 diploid plants.

(b). After the hot water emasculation, flower glumes were clipped, but pollens were not applied. In total, 246 flowers on 13 panicles were treated and resulted in 1 well-developed seed, which gave a diploid plant.

(c). Flowers emasculated by the clipping method were left without artificial pollination. In total, 429 flowers on 20 panicles were treated and had no seed at all.

In Experiment I, about 1.5% of the flowers emasculated by the hot water method produced seeds without artificial pollination, and the 31 adult plants that grew up were diploid, without exception. When the mother plant was homogygous for the endosperm characteristic, the plants produced showed the same characteristic as the mother plant. When the mother plant was heterozygous, the plants produced were of three types, pure non-glutinous, pure glutinous and heterozygous.

Experiment II.

Emasculation was practiced by the clipping method, and the pollen was applied artificially. This experiment consisted of the following 3 sub-parts (Table II).

Table II. Results of crossing by the clipping method between 2x and 4x materials.

Treatment	Maternal parent (2x)	Number of flowers treated	Perfect grains obtained	Illdeveloped grains obtained	% of developed seeds	Number of germinated seeds	Number of plants grown up	Endosperm type		
								Non-gl.	Het.	Gl.
Exp. II-a	NF ₂ -glutinous	233	15	0	6.44	7	4 (2x)	0	0	4
Exp. II-b	NF ₂ -hetero	80	0	0	0	0	0			
	Aikoku	185	1	0	0.54	1	1 (2x)	1	0	0
	NF ₂ -glutinous	85	0	1	1.18	1	1 (2x)	0	0	1
	Total (or average)	350	1	1	0.57	2	2 (2x)			
Exp. II-c	NF ₂ -hetero	210	0	0	0	0	0			
	F ₃ -hetero	165	0	0	0	0	0			
	Aikoku	113	0	0	0	0	0			
	Total (or average)	488	0	0	0	0	0			
Grand total		1,071	16	1	1.59	9	6 (2x)			

(a). Pollination was made on the day of emasculation. In total, 233 flowers on 11 panicles were treated, and 15 well-developed seeds were obtained. Of these 15 seeds, 7 seeds germinated, but only 4 adult plants were obtained. They were all diploid plants of maternal endosperm type.

(b). Pollination was made on the next day of emasculation. In total, 350 flowers on 19 panicles were treated to result 1 well-developed seed and 1 ill-developed seed. Both of these produced diploid plants of mother's type of endosperm.

(c). Pollination was practiced 2 days after emasculation. In total, 488 flowers on 24 panicles were treated to result no seed.

Experiment III.

Emasculation was practiced by the hot water method, and pollens were applied artificially as soon as flower glumes opened after the hot water treatment (Table III).

Table III. Results of crossing by the hot water method between 2x and 4x materials.

Treatment	Maternal parent (2x)	Number of flowers treated	Perfect grains obtained	Illdeveloped grains obtained	% of developed seeds	Number of germinated seeds	Number of plants grown up	Endosperm type of 2x		
								Non-gl.	Het.	Gl.
Exp. III	NF ₂ -hetero	2,984	5	2	0.23	5	3 (2x) +2 (3x)	0	3	0
	F ₂ -hetero	13	0	0	0	0	0			
	NF ₂ -non-glutinous	168	1	0	0.60	0	0			
	Aikoku	93	0	0	0	0	0			
	F ₁ (Aikoku × Java 28)	121	1	0	0.83	0	0			
	Aikoku	52	0	0	0	0	0			
	NF ₂ -glutinous	689	7	3	1.45	8	6 (2x)	0	0	6
	Total (or average)	4,120	14	5	0.46	13	9 (2x) +2 (3x)			

In total, 4,120 flowers on 254 panicles were treated, and 14 perfect seeds and 5 poorly developed ones were obtained. Of these 19 seeds altogether, 13 seeds germinated giving only 11 adult plants, 9 diploid plants and 2 triploid plants. The percentage of the number of triploid plants against the number of the total flowers treated was 0.05%, the percentage of the diploid plants to the total flowers being 0.22%. Three diploid plants produced by the heterozygous plant for the endosperm characteristic were heterozygous, while 6 diploid plants produced by the glutinous plant were glutinous.

Experiment IV.

Flowers once opened by the hot water treatment were artificially pollinated by the clipping method, when they closed again. This experiment consisted of the following 6 sub-parts (Table IV).

Table IV. Results of crossing by the hot water method between 2x and 4x materials.

Treatment	Maternal parent (2x)	Number of flowers treated	Perfect grains obtained	Illdeveloped grains obtained	% of developed seeds	Number of germinated seeds	Number of plants grown up	Endosperm type of 2x		
								Non-gl.	Het.	Gl.
Exp. IV-a	NF ₂ -hetero	6,789	23	4	0.40	16	11 (2x) +2 (3x)	5	4	2
	Aikoku	100	2	0	2.00	2	2 (2x)	2	0	0
	F ₁ (Shinriki × Aikoku)	1,039	12	0	1.16	7	5 (2x) +1 (3x)	5	0	0
	F ₁ (Aikoku × Java 28)	1,365	1	2	0.22	1	1 (?)			
	F ₁ (Philippine 5 × Java 28)	167	0	0	0	0	0			
	NF ₂ -glutinous	915	9	1	1.09	5	4 (2x)	0	0	4
	Total (or average)	10,375	47	7	0.52	31	22 (2x) +3 (3x)			
Exp. IV-b	NF ₂ -hetero	1,002	2	2	0.40	2	2 (3x)			
	NF ₂ -glutinous	191	1	0	0.52	1	1 (3x)			
	Total (or average)	1,193	3	2	0.42	3	3 (3x)			

(a). Artificial pollination was made 2~6 hours after emasculation. In total, 10,375 flowers on 509 panicles were treated to produce 47 perfect seeds, and 7 poorly developed ones. Of these 54 seeds altogether, 31 seeds germinated giving 26 adult plants. Twenty two plants were diploid, 3 plants were triploid, and the remaining plant undetermined. The percentage of the number of the triploid plants produced against the number of the

total flowers treated was 0.03%, and the percentage of the diploid plants produced to the total flowers was 0.21%. The endosperm of the diploid plants produced was glutinous when the mother plant was glutinous, and non-glutinous when the mother plant was pure non-glutinous. When the mother plant was heterozygous for that characteristic, three types of plants, pure non-glutinous, pure glutinous and heterozygous, were produced, the actual corresponding numbers of plants of respective types being 5, 2 and 4.

(b). Artificial pollination was carried out 10~12 hours after emasculation. In total, 1,193 flowers on 53 panicles were treated. Three well-developed seeds and 2 poorly developed ones were obtained. Three adult plants were raised from those 5 seeds, and they were triploid without exception. The percentage of the triploid against the total flowers treated was 0.25%.

(c). Artificial pollination was carried out one day after emasculation. In total, 252 flowers on 19 panicles were treated in vain.

(d). Artificial pollination was carried out 2 days after emasculation. In total, 27 flowers on 2 panicles were treated to have no seeds.

(e). Artificial pollination was carried out 5 days after emasculation. In total, 63 flowers on 4 panicles were treated without results.

(f). A hot water treatment was applied at a 2 days interval, and artificial pollination was carried out soon after the second treatment. In total, 218 flowers on 17 panicles were treated, but no seeds were obtainable from those flowers.

Experiment V.

Young flowers which did not open the glume immediately after the hot water treatment were emasculated and pollinated later by the clipping method. This experiment consisted of the following 3 sub-parts (Table V).

Table V. Results of crossing by a modified hot water method between 2x and 4x materials.

Treatment	Maternal parent (2x)	Number of flowers treated	Perfect grains obtained	Illdeveloped grains obtained	% of developed seeds	Number of germinated seeds	Number of plants grown up	Endosperm type of 2x		
								Non-gl.	Het.	Gl.
Exp. V-a	NF ₂ -hetero	237	0	1	0.42	1	0			
Exp. V-b	NF ₂ -hetero	1,692	32	4	2.13	20	20 (3x)			
	F ₁ (Shinriki × Aikoku)	131	3	0	2.29	1	1 (2x)	1	0	0
	Total (or average)	1,823	35	4	2.14	21	1 (2x) + 20 (3x)			

(a). Emasculation and pollination by the clipping method was done on the same day of the hot water treatment. In total, 237 flowers on 5 panicles were treated to result one ill-developed seed. The seed did not germinate.

(b). Emasculation and pollination by the clipping method was done one day after the hot water treatment. In total, 1,823 flowers on 68 panicles were treated to have 35 perfect seeds and 4 imperfect ones. Of these 39 seeds altogether, 21 seeds germinated giving 20 triploids, and one diploid. The percentage of the triploids against the total flowers treated was 1.09%. The diploid plant was pure non-glutinous just like its mother plant.

(c). Emasculation and pollination was done after 2 days of the hot water treatment. Twenty five flowers on one panicle were treated, without seed result.

Conclusion

In 1949, the authors first succeeded in obtaining triploid rice plants by the cross of the diploid and tetraploid. In that case, emasculation was effected by hot water treatment, and the rate of success seemed to depend upon the developmental stage of flowers subjected to the treatment, and the interval between the treatment and the following artificial pollination. The following abstract of the result of the experiment will show the relationship.

- Exp. III. Emasculation was practiced by the hot water method, and the flowers were artificially pollinated as soon as flower glumes opened after the treatment0.05%
- Exp. IV-a. Flowers once opened after emasculation by the hot water method were artificially pollinated by the clipping method 2~6 hours after emasculation0.03%
- Exp. IV-b. Flowers once opened after emasculation by the hot water method were artificially pollinated by the clipping method 10~12 hours after emasculation0.25%
- Exp. V-b. Young flowers which did not open the glume soon after the hot water treatment were emasculated and pollinated by the clipping method one day after the hot water treatment1.09%

When the most common way of hybridization by the hot water method was taken, the rate of success was only 0.05%, while the percentage increased to 0.25% only by delayed pollination of about 10~12 hours. The highest percentage was, however, obtained when such a flower, which did not open its glume immediately after the hot water treatment, was pollinated one day after the treatment. The rate of success in that case was 1.09%. Hot water treatment, therefore, of flowers in the slightly premature stage followed by artificial pollination on the expected day of anthesis in normal condition seems to be most effective in producing the triploid hybrid. The triploidy of the hybrid was confirmed for each individual by direct counting of the chromosomes, though they were well furnished with known external morphological characteristics of the triploid. The possibility of artificial formation of the triploid rice by the cross of diploid and tetraploid was proved. The rate of success, however, seemed too low to attribute the origin of the triploid plant found spontaneously in farmer's fields to the cross of normal rice with an occasional tetraploid one (which is probably produced by certain highly sterile plants and grown mixed in the field).

J. KAWAKAMI^[1] examined 583,780 rice seedlings, and found 108 twins and 3 triplets. Ninety-two twins were examined by him cytologically, and 90 twins were of diploid pairs, while 1 was of diploid and triploid, and the rest was of diploid and haploid. All triplets were composed of diploids. When S. NAMIKAWA and J. KAWAKAMI⁽⁸⁾ examined 29 twins of wheat, 19 twins were of diploid pairs, 8 twins of diploid and triploid, 1 of diploid and haploid, while the rest was of diploid and tetraploid. Examining 2,201 twin plants of 16 species A. MÜNTZING⁽⁷⁾ found among those 77 triploids, 11 haploids, 2 tetraploids and 5 other deviations. He says: in contrast to other experimental methods of changing chromosome number, the twin method predominantly results in abnormality with odd chromosome multiples, triploid being most frequent. How such chromosomal abnormalities are produced in the course of twin formation is not clear, however, it may account for the spontaneous occurrence of triploid and haploid in the common field of rice.

In the above described experiments of $2x \times 4x$, about 0.3% of the flowers produced maternals (plants in all respects like the mother plant). These maternals may have arisen through one of those processes, parthenogenesis of real germcell accompanied by a doubling of the maternal chromosomes, vegetative apogamy, and accidental selfing or contamination of pollens. If they are produced by vegetative apogamy, they will have exactly the same genetic constitution as the mother plant, no matter with its homo- or heterozygosity. In the case of parthenogenesis accompanied by a doubling of the chromosomes, if the mother plant is homozygous the maternals will show exactly the same homozygous genetic constitution as the mother plant. If the mother plant is heterozygous, however, the maternals will show homozygous genetic constitutions of two or more types, but no heterozygous one. In the case of pollen contamination, no such a general rule is conceiv-

able. To investigate the process of maternal formation on such a basis, heterozygous plants especially those for glutinous gene were often selected on the mother side in the experiments.

In the experiment of 1949, 69 maternals were obtained altogether. Of those, 12 maternals produced by pure non-glutinous mother plants were exclusively pure non-glutinous, and 30 maternals produced by pure glutinous mother plants were exclusively pure glutinous. Twenty seven maternals produced by heterozygous mother plants for glutinous characteristic were of three types, pure non-glutinous, heterozygous, and pure glutinous, the actual number of plants for each type being 10, 9 and 8 in the same order. At least, therefore, the majority of the last mentioned 27 maternals can hardly be attributed to apogamy or parthenogenesis provided above, leaving pollen contamination as the only process accountable for. It suggests, in turn, the same process for other 42 maternals just referred to. This statement does not deny the possibility of parthenogenesis of that type, but it is suggested that the chance of occurrence of that process will be far less than it might be expected by some workers. No haploid parthenogenesis occurred in the course of the experiments.

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稲の diploid と tetraploid 系統間の交配結果について

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1. 1943年と1948年とに著者等は稲の triploid を作る目的で diploid と tetraploid との間に相反交配を試みた。兩年を通じて 427 個の tetraploid の花と、672 個の diploid の花とに行った交配からは全く triploid が得られなかった。

2. 1949年には常に diploid を母方にとり、交配方法を多少ずつ変えて、更に大規模の実験を試みた。実験につかった総花数は 22,639 に及んだ。その結果、28 個の triploid を得た。交配の仕方と triploid の出来た率との関係は次のようである。

本文実験 III. 温湯除雄後開花したものに直ちに授粉した場合……………0.05%

本文実験 IV-a. 温湯除雄によって一度開花した花を 2～6 時間後に切額法によって授粉した場合……………0.03%

本文実験 IV-b. 温湯除雄によって一度開花した花を 10～12 時間後に切額法によって授粉した場合……………0.25%

本文実験 V-b. 温湯除雄によって開花しなかった花を一日後に切額法によって授粉した場合……………1.09%

3. 上述の如く、 $2x \times 4x$ によって極めて低率ながら $3x$ の作出に成功した。その場合の実験成績に照して、開花 1 日前位の花を温湯処理して「処理の 1 日後に授粉する方が最も効果的」のようである。その場合でも成功率は約 1% であった。

4. 農家の圃場に偶然発見される triploid の成因については、これを栽培の diploid 稲と、その圃場に別の事

情からすでに混在する tetraploid との交配結果と見ることは出来ないようである。

5. 稲に於ても他の植物と同様に双子が生ずることがある。一般に、双子の片方が haploid か triploid 又は tetraploid となる場合のあることが知られている。それらの中、最も起り易いのは triploid で最も起り難いのは tetraploid である。農家の水田では、最も遭遇し易いのは triploid、次は haploid と tetraploid のようである。これらの triploid と haploid は、少なくともその大部分は双子の片方として生じたもの、tetraploid の大部分は或種類の高度不稔個体の子孫として生じたものと考えられる。

6. 1949 年の交配実験を通じて約 0.3%, 69 個体、の母本に似た次代植物が得られた。これらは母体が、純糯性の場合には純糯性を、純粳性の場合に純粳性を示した。母本がそれらの性質について雑種性の場合には純粳性のもの、雑種性のもの、純糯性のものの三種類を生じた。

7. 1949 年の実験では特に花粉の混淆のないように注意したつもりであったが、上記の母本に似た個体(少なくともその大部分)は、染色体の倍加を伴ったパーセノゼネシスの結果ではなく、矢張り思わざる自花授粉の結果であったと見ざるを得ない。

8. この実験に於ては triploid 以外の haploid 或はその他の異常染色体数の個体は発見されなかった。(摘要)