Cytogenetics of Sterility in F_2 Hybrids of Indica $\times$ Indica and Indica $\times$ Javanica Varieties of Rice (*Oryza sativa* L.)¹

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Sterility occurs so commonly in the hybrid progenies of rice crosses that rice researchers widely recognize its importance in breeding programs and genetic studies. Most cytological and genetic studies have been concerned with crosses involving cultivars from two variety groups, indica and japonica. Engle, Chang, and Ramirez (1969) pointed out that sterility is equally important in F_1 hybrids of indica $\times$ indica and indica $\times$ javanica crosses. This paper summarizes the cytological studies on the sterile F_2 progenies of selected crosses from such combinations.

Materials and methods

Pollen and spikelet sterility of parents and of F_2 progenies were determined in six crosses: IR8 $\times$ Pankhari 203, Pankhari 203 $\times$ Rodjolele, Basmati 370 $\times$ Taichung Native 1, IR8 $\times$ Boegi Imba, Basmati 370 $\times$ Pankhari 203, and IR8 $\times$ Rodjolele. Plants grown in two replications, each consisting of 120 F_2 plants per cross and 20 plants for each parent, were studied. For pollen counts, one anther from each of 10 spikelets of the earliest emerging panicles was macerated in a drop of a solution of 1-percent iodine-potassium iodide. About 500 pollen grains per plant were counted. Three early emerging panicles from each plant were examined for spikelet sterility.

Only the most sterile F_2 plants (50 percent sterility and above) from three crosses were examined cytologically. The sporocytes were fixed in fresh Farmer's solution, transferred after 48 hours to 70 percent ethyl alcohol containing 0.05 percent ferric chloride, and examined by a modified iron-aceto-carmin technique. A minimum of 50 cells per meiotic stage per plant were observed, except at pachynema, where at least 25 cells were examined.

Results

Pollen and spikelet sterility

Table 1 shows the ranges and means of the percentages of spikelet and pollen

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sterility among the parental and hybrid materials. F_2 plants of the IR8×Boegi Imba cross produced the highest mean percentage of sterility in both spikelet and pollen counts. The same cross had the widest range of pollen sterility. The Basmati 370×Pankhari 203 cross had the widest range in percentage of spikelet sterility but the lowest mean sterility in both spikelet and pollen counts.

All F_2 distributions of pollen sterility were unimodal but generally showed negative skewness (Fig. 1). Spikelet sterility was generally higher than pollen sterility in each sterility class except at the very low range where pollen sterility was higher than spikelet sterility.

Among the parents, Basmati 370 had the highest mean in both spikelet sterility (16.4%) and pollen sterility (13.6%). Pankhari 203 had the lowest spikelet sterility (3.1%), while Rodjolele had the lowest pollen sterility (7.5%).

Table 1. Spikelet and pollen sterility percentage of F_2 populations and of their parents

Cross or parents	Sterility (%)			
	Spikelet		Pollen	
	Range	Mean	Range	Mean
Basmati 370 × Pankhari 203	0.43–98.29	5.18	4.59–53.80	14.39
Pankhari 203 × Rodjolele	0.61–64.51	11.38	3.84–86.59	15.94
IR 8 × Pankhari 203	1.08–81.29	18.99	5.06–92.31	29.02
IR 8 × Rodjolele	2.21–68.89	19.84	1.28–67.49	18.45
Basmati 370 × TN1	2.24–83.77	19.86	3.83–73.63	23.66
IR 8 × Boegi Imba	0 –74.71	21.48	6.43–99.06	33.41
Pankhari 203 (indica)	0.71–16.80	3.06	3.41–22.15	9.72
Rodjolele (javanica)	1.19–11.27	3.83	2.42–37.35	7.45
Boegi Imba (javanica)	1.98–37.07	7.79	2.77–19.52	12.83
IR 8 (indica)	3.22–27.71	8.55	2.55–15.89	8.11
Taichung Native 1 (indica)	4.15–28.02	9.94	4.41–21.13	10.68
Basmati 370 (indica)	1.61–69.11	16.37	2.48–70.50	13.59

Pachynema

Table 2 summarizes the chromosome behavior of three F_2 samples at pachytene stage. From 63 to 79 percent of the cells were normal. Loose pairing of chromosome segments, duplication-deficiency (dp-df) loops, and reciprocal translocations were observed in all the crosses examined. IR8×Boegi Imba showed the highest frequency of loose pairing (17.6%) and reciprocal translocations (6.3%), while IR8×Pankhari 203 showed the lowest values of the same aberrations. Basmati 370×TN1 produced the highest percentage of dp-df loops (3.5%) while IR8×Pankhari 203 had the lowest frequency (0.7%). Unequal bivalents ranging from 1.04 to 1.91 percent were only observed in the IR8×Pankhari 203 and Basmati 370×TN1 crosses. Inversion loops were found only in the Basmati 370×TN1 and IR8×Boegi Imba crosses at about 0.3 percent in each cross.

In cells of two crosses several chromosomes completely or almost completely failed to pair. This unpaired condition occurred in 10.5 percent of the cells in

Fig. 1. Distribution of F_2 plants by pollen and spikelet sterility in six crosses. The actual number of the F_2 plants recorded in Basmati 370 $\times$ Pankhari 203 (lower left) was twice that shown on the vertical scale.

IR8 × Boegi Imba and 1.4 percent of the sporocytes in IR8 × Pankhari 203. Fragments were also observed in these two crosses. The major nucleolus was absent in some of the cells of three crosses.

Table 2. Chromosome behavior of the F₂ hybrids at pachynema

Chromosome behavior	Cells observed (%)		
	IR8 × Boegi Imba	IR8 × Pankhari 203	Basmati 370 × TN1
Normal	63.25	79.37	76.67
Loose pairing	17.58	12.23	13.73
Unequal bivalents	—	1.04	1.91
Duplication-deficiency loops	0.78	0.69	3.51
Reciprocal translocations	6.29	2.79	3.51
Inversion loops	0.26	—	0.31
Fragments	0.78	0.34	—
Absence of major nucleolus	0.52	2.09	0.31
Partial pairing	10.49	1.39	—
	Cells observed (No.)		
	381	286	313

Table 3. Meiotic behavior at diakinesis and metaphase I of the F₂ hybrids

Chromosome behavior	Cells observed (%)		
	IR8 × Boegi Imba	IR8 × Pankhari 203	Basmati 370 × TN1
12 II (normal)	74.40	78.51	84.74
2-10 I + 7-11 II	9.12	13.92	7.92
9 II + 1 VI	0.22	—	—
11-23 I + 2-26 monads	0.39	—	—
24 I	0.74	—	—
1-7 II + 10-22 I	0.51	—	—
1-7 II + 9-14 I + 2-16 monads	0.11	—	—
1-2 IV	2.28	0.71	1.62
2n-1, 2n-2, 2n-4	2.85	1.48	0.51
2n+1, 2n+2, 2n+4	2.62	1.25	0.32
Fragments	0.28	0.17	0.12
Clumped chromosomes	1.65	—	—
Absence of major nucleolus at diakinesis	1.65	—	0.12
Presence of micronucleoli at diakinesis	3.90	3.86	4.55
Precocious disjunction, forming a bridge at M I	0.11	—	—
Persistent nucleolus at M I	0.17	0.05	0.06
Mis-oriented spindles with nucleolar bodies at M I	1.65	—	—
	Cells observed (No.)		
	1754	1680	1540

Diakinesis and metaphase I

Table 3 shows that from 74 to 85 percent of the cells were normal at diakinesis and metaphase I. Cells of IR8 $\times$ Boegi Imba showed complete asynapsis of the chromosomes at a frequency of 0.7 percent (Fig. 2a). Moreover, this cross also showed many univalents, as high as 23, along with two to 26 monads. The univalents observed at diakinesis proceeded to equational division at metaphase I, result-

Fig. 2. Aberrant meiotic behavior in the F_2 hybrids. a, 24 univalents at diakinesis. b, metaphase chromosomes forming a bridge. c, persistent nucleolus at anaphase I. d, multi-spindles. e, three cells showing six groups of chromosomes. f, a group of 3 cells, one of which is devoid of chromosomes.

ing in monads which were generally located near to each other. Varying numbers of univalents, also observed in the other three crosses, ranged from 8 to 14 percent. One to two quadrivalents were found in three crosses. Hexavalent formation was noted only in the IR8 $\times$ Boegi Imba cross. Precocious disjunction followed by chromosome bridges (0.11 %) was observed at metaphase I only in the IR8 $\times$ Boegi Imba cross (Fig. 2b). Aneuploid chromosome numbers ranging from 20 to 28, were found at the frequency of one to two percent in the IR8 $\times$ Boegi Imba, Basmati

370×Taichung Native 1, and IR8×Pankhari 203 crosses.

All the three crosses showed several micronucleoli at diakinesis, and fragments and persistent nucleolus at metaphase I. Clumped chromosomes and mis-oriented spindles with nucleolar bodies at metaphase I were found only in the IR8×Boegi Imba cross with a frequency of 1.7 percent in both aberrations.

Anaphase I and telophase I

Table 4 shows that from 67 to 94 percent of the cells were normal in the three crosses. Chromosomal abnormalities, such as unsplit bivalents, unequal segrega-

Table 4. Meiotic behavior of the F₂ hybrids at anaphase I and telophase I

Chromosome behavior	Cells observed (%)		
	IR8 × Boegi Imba	IR8 × Pankhari 203	Basmati 370 × TN1
Normal	67.25	89.73	94.02
Unsplit bivalents	2.44	3.83	1.51
Unsplit II + fragments	0.08	—	—
Unequal disjunction	0.59	0.86	0.09
Laggard	7.76	0.95	3.03
Bridge (1-4)	3.79	1.34	0.37
Bridge + laggard	0.33	—	—
Bridge + fragments	0.75	—	0.09
Bridge + laggard + fragments	0.16	—	—
Fragments	0.25	—	—
Late disjunction	1.51	0.47	0.47
Persistent nucleolus at A I	0.25	0.28	—
Monads (2-8)	0.25	0.47	0.18
Trinucleate cell at T I	0.84	—	0.09
Mis-oriented spindles with nucleolar bodies	11.30	—	0.09
Mis-oriented spindles without nucleolar bodies	2.36	—	—
Normal spindles with nucleolar bodies	—	2.01	—
Cells observed (No.)			
	1185	1042	1054

tion of chromosomes, laggards, and anaphase bridges were also observed. IR8×Pankhari 203 had the highest percentage of unsplit bivalents (3.8%) and unequal segregation of the chromosomes (0.9%), while IR8×Boegi Imba produced the highest frequency of laggards (7.8%) and bridges (3.8%). Late disjunction occurred most frequently in IR8×Boegi Imba and monads appeared most frequently in IR8×Pankhari 203. Three-nucleated cells and mis-oriented spindle fibers with nucleolar bodies at telophase I were observed in the IR8×Boegi Imba and Basmati 370×TN1 crosses. Both aberrations were observed at the highest frequency in IR8×Boegi Imba. A bridge and a fragment were observed in the IR8×Boegi Imba and Basmati 370×TN1 crosses. The nucleolus persisted (Fig. 2c) at ana-

phase I in IR8 $\times$ Boegi Imba (0.3%) and in IR8 $\times$ Pankhari 203 (0.3%). Unsplit bivalents with fragments, bridge and a laggard, bridge plus laggards and fragments, and fragments alone occurred only in the IR8 $\times$ Boegi Imba cross. Several cells of IR8 $\times$ Boegi Imba showed multispindles (Fig. 2d). One such cell resulted in a hexad at the end of meiosis (Fig. 2e). One of the cells of IR8 $\times$ Boegi Imba was found devoid of chromosomes at meiosis II (Fig. 2f).

Discussion

Although the six crosses did not differ markedly in the mean pollen sterility of F_2 plants, only three F_2 populations produced highly sterile plants of more than 85 percent sterility. But plants which were highly pollen-fertile were found in each of the six F_2 populations. The population ranges and means are similar to those reported by Raj (1968) from two indica $\times$ indica and two indica $\times$ javanica crosses.

The association between F_1 plants and F_2 populations in pollen sterility was not consistent in different crosses. Among the six crosses, IR8 $\times$ Pankhari 203 produced the most sterile F_1 hybrids and Pankhari 203 $\times$ Rodjolele ranked second in pollen sterility (Engle, Chang, and Ramirez 1969). In the F_2 generation IR8 $\times$ Boegi Imba gave the highest mean sterility (33%) and the most sterile plants. IR8 $\times$ Pankhari 203 ranked second in pollen sterility and Basmati 370 $\times$ TN1, third. Moreover, the differences between F_2 means were generally smaller than those between F_1 means. Raj (1968) also found a higher pollen sterility in the F_1 hybrids than the mean of F_2 plants.

The difference between the pollen and spikelet sterility in the low sterility classes could be due to environmental effects. It is possible that the strong rains and winds which frequently occurred during the wet season affected seed setting more than they did to the development of pollen.

The negatively skewed F_2 distributions in our crosses were also found in the F_2 populations of three partially sterile indica $\times$ japonica crosses (Jennings 1966) and in two F_2 populations each of indica $\times$ indica and indica $\times$ javanica crosses (Raj 1968). The lack of discontinuous F_2 distribution in our crosses as well as in many indica $\times$ japonica crosses (Oka 1953, Henderson 1964, Sampath 1964, Jennings 1966, Engle, Ramirez and Chang 1969) fails to support the postulate of Ratho and Balakrishna Rao (1970) that intervarietal hybrid sterility is controlled by a single pair of alleles.

A comparison of the chromosomal and other cytological aberrations of the F_1 and F_2 hybrids showed that the F_2 plants showed a greater variety of meiotic aberrations and, in some cases, a higher frequency of certain aberrations. At pachynema, the sterile F_2 plants had a higher frequency of normal cells and a lower frequency of loose pairing than did the F_1 plants. On the other hand, the F_2 plants showed aberrations such as duplication-deficiency loops, reciprocal translocations, inversion loops, fragments, absence of major nucleolus, and partial pairing, which were not observed in the F_1 hybrids.

At diakinesis and metaphase I, the F_2 plants showed a lower proportion of normal cells than did the F_1 plants. In IR8 $\times$ Boegi Imba, univalents and monads

appeared at higher frequencies in the F_2 than in their parents. Quadrivalents were also more frequent in the F_2 plants of IR8 $\times$ Boegi Imba and Basmati 370 $\times$ TN1. Aneuploid chromosome numbers were found in three F_2 populations but not in the F_1 plants. Similarly, fragments and micronucleoli were new aberrations found in the F_2 plants only. The F_2 plants of IR8 $\times$ Boegi Imba showed additional aberrations such as precocious disjunction, clumped chromosomes, and mis-oriented spindles which were not found in the F_1 hybrids.

At anaphase I and telophase I, the F_2 plants again produced a lower proportion of normal cells than did the F_1 plants. New aberrations appeared in the F_2 plants as unsplit bivalents, unequal and late disjunction, half-univalents, mis-oriented spindles, nucleolar bodies, and trinucleate cells.

In the previous study of F_2 plants of indica $\times$ japonica crosses (Engle, Ramirez, and Chang 1969), complexes of meiotic aberrations also appeared in the F_2 plants of distant crosses. The wide range of aberrations found in our material surpasses those reported in any previous study on rice. Moreover, the geographic origin of the parents concerned is not necessarily related to the extent of sterility or to the frequency of aberrant sporocytes. While some of the aberrations such as loose pairing at pachynema, univalents and quadrivalents at metaphase, and bridge formation at anaphase indicate structural differences in chromosome homology, other types of aberrations such as micronucleoli, half-univalents, mis-oriented spindles, and trinucleate cells could be affected by complex gene interactions. Aberrant chromosome disjunction at anaphase I in all four crosses suggests a genic cause. The frequency of univalents or of half-univalents is not directly related to the frequency of loose pairing at pachynema. Moreover, the frequency of any type of chromosomal aberration does not appear to be associated with the pollen sterility of the concerned F_2 plants.

In our earlier papers we postulated the probable role of genic imbalance in hybrid sterility (Demeterio *et al.* 1965, Engle, Ramirez, and Chang 1969). Such a combination of chromosomal and genic differentiation between parents also appears to operate in the F_2 plants of indica $\times$ indica and indica $\times$ javanica crosses. Such complex gene interactions in rice crosses now appear to be found quite often.

Summary

Pollen and spikelet sterility in F_2 populations of three indica $\times$ indica crosses and three indica $\times$ javanica crosses were studied. The most sterile of the F_2 plants from three crosses were examined cytologically. The most common indications of structural hybridity at pachynema were loose pairing, partial pairing, and reciprocal translocations. Univalents, quadrivalents, aneuploid chromosome numbers, and fragments were observed at diakinesis and metaphase I in all three crosses. Unsplit bivalents, unequal or late disjunctions of chromosomes, laggards, and anaphase bridges were found at anaphase I and telophase I. Moreover, a variety of non-chromosomal aberrations was found at each meiotic stage. The wide array of meiotic aberrations suggests both structural hybridity and complex gene interactions.

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