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MALE-FERTILITY-RESTORING MUTANTS INDUCED BY X-RAYS IN WHEAT

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SUMMARY

Some promising male-fertility-restoring mutants were obtained by X-irradiation of seeds of the male-sterile wheat, (timopheevi)Bison. One of the mutant lines thus far tested restored the male fertility of male-sterile (timopheevi)Bison and had no chromosomal change in comparison with the original Bison.

The frame for hybrid-wheat-breeding systems has been established, but many problems in this field are left to be solved by elaborate work. In order to find a more usable male-fertility-restoring gene or genes than the present ones (JOHNSON and SCHMIDT, 1968), an attempt was initiated in 1969 to artificially induce restorer mutations. This report deals with some male-fertility-restoring mutants obtained by X-irratiation of male-sterile (*timopheevi*) Bison.

MATERIALS AND METHODS

Air-dry seeds, ca. 70-80 kernels for each plot, obtained by handmade crosses of three male-sterile nucleo-cytoplasmic systems, (*ovata*)Norin 26, (*timopheevi*) Bison and (*caudata*)T.v.e., were treated with X-rays of 20 or 25 kr (105.3 r/min.) in 1969. M_1, M_2 and part of the M_3 and M_4 plants of some lines were grown in the greenhouse after vernalisation for 40 days at 50°C, and the rest of the M_3 and M_4 plants of all lines in the field at Tottori. For pollen fertility, three anthers were examined from a floret in the middle of each M_1 spike. Seed fertility was examined in bagged spikes of each plant in every generation. Meiotic behavior in microsporocytes was observed on slides made by the acetocarmine squash method.

RESULTS AND DISCUSSION

M_1

The seed germination of 91.4% or 92.5% and survivals of 90.1% or 92.5% for (*timopheevi*)Bison irradiated with X-rays of 20 or 25 kr, respectively, were higher than those for (*ovata*)Norin 26 and (*caudata*) T.v.e. It seemed, however, that these results were rather dependent on the nature of these nucleo-cytoplasmic systems. Most M_1 plants developed only one spike in the nursery box in the greenhouse.

Quite a number of spikes from X-ray-treated seeds of three cytoplasmic-male-sterile wheat lines were of restored pollen fertility, whereas no untreated plant had good pollen. As shown in Table 1, out of 156 spikes of X-ray-treated (*ovata*)Norin 26, 185 of (*timopheevi*) Bison and 52 of (*caudata*)T.v.e. 46(29.5%), 48(25.9%) and 36(69.2%) spikes, respectively, had some good pollen. One spike of (*timopheevi*) Bison was even as high as 92.4% in good pollen.

The percentage of fertility-restored spikes was 3.8 with selfed M_2 kernels, 22 being obtained only from spikes (7) of X-ray-treated (*timopheevi*)Bison (Tables 1 and 2). Such a low frequency of fertile spikes was probably caused partly by non-dehisence of anthers due to the small amount of good pollen per anther and partly by chimerical fertility restoration of spikes. Also, the non-agreement between pollen and seed fertilities of these plants may prove that the six fertility-restored plants were not hybrids by chance with some restorers.

Table 1. Percentage of good pollen in M_1 spikes of three cytoplasmic-male-sterile wheat lines treated with 20 or 25 kr of X-rays.

Male-sterile line	Dose (kr)	No. of spikes exam.	Spikes with sterile pollen only (%)	Spikes with 1-20% good pollen (%)		Spikes with >20% good pollen (%)		Spikes with viable seed(s) (%)	
	0	4	100	0		0		0	
(*o*)Norin 26	20	87	59.8	37.9	27.6	2.3	1.9	0	
	25	69	84.1	14.5		1.4		0	
	0	4	100	0		0		0	
(*t*)Bison	20	84	76.2	15.5	20.0	8.3	5.9	3.6	3.8
	25	101	72.3	23.7		4.0		4.0	
(*c*)T.v.e.	0	4	100	0		0		0	
	25	52	30.8	69.2		7.7		0	

M_2

The M_2 plants, grown in the greenhouse in 1970-1971, varied not only in seed fertility, but also in heading time, spike type, etc. Of the 22 plants, 5 were completely sterile, 5 had less than 0.10 kernel per spikelet, and 10 of the remaining 12 plants showed high seed fertility (Table 3). In general, the fertile plants were earlier in heading time than the sterile plants.

Table 2. Data from seven fertility-restored M_1 spikes of (*timopheevi*)Bison in the greenhouse

Dose (kr)	Plant no.	% good pollen	Seed fertility: seeds / florets	Seed fertility: %
20	20-1	42.0	1/20	5.0
	20-2	6.4	1/22	4.5
	20-3	26.1	11/36	30.6
25	25-1	27.2	1/26	3.8
	25-2	92.4	3/38	7.9
	25-2	2.7	4/38	10.5
	25-3	13.2	1/32	3.1

M_3

Six M_3 families were accidentally lost. Seed fertility (Table 4) and some other characters (Table 5) were still segregating in the other 11 M_3 populations grown in the field in 1971-1972, providing ample possibility of selecting lines with heavy 1000-kernel weight and other desirable characters (Figure 1 A). The results suggest that these X-ray-induced mutants restore male fertility also under field conditions at Tottori. The parent-offspring correlation of seed fertility between the M_2 plants and M_3 populations was $r=0.705 (p<0.01)$ The seed fertility distribution of the M_3 lines suggests that at least two genes of incomplete dominance are involved in the male-fertility restoration of these induced mutants. However, this is not certain yet, because chromosomal changes induced by X-rays may have affected the fertility distribution.

Meiotic Behavior of the Mutants

Meiotic behavior was examined in some plants of the M_3 and M_4 generations grown in the greenhouse, 1972-1973. Of 40 M_3 and M_4 plants examined, 30 were disomic (Figure 1 B), 9 monosomic and 1 double monosomic. All plants observed were disomic in some M_4 lines, but in some disomics of certain M_4 lines, meiotic irregularities such as trivalents, quadrivalents and univalents at Metaphase I, laggards at Anaphase I, etc. were also observed, as a result of chromosomal changes induced by irradiation.

F_1 Hybrid between (*timopheevi*) Bison and the Mutants

In order to examine (1) whether these mutants are dominant chromosomal genes for male-fertility restoration and (2) whether they have any chromosomal changes in comparison with the original Bison, crosses were made between male-sterile (*timopheevi*)Bison (♀) and the mutants, using M_2 plant 203-11 in 1971 and M_3 plant 252(1)-1-14 in 1972. The F_1 plants grown in both the field (1971-1972) and greenhouse (1972-1973) were fertile, suggesting that the fertility restoration of the mutants was by dominant chromosomal gene or genes. An Indian restorer, NP 883, did not restore fertility under the same environmental conditions (Fig. 1 C,D).

In PMCs of the F_1 hybrids with M_3 plant 252(1)-1-14, 21_{II} at Metaphase I and regular chromosome disjunction at Anaphase I were observed.

Table 3. Seed fertility of 12 X-ray-induced, male-fertility-restoring M_2 plants of (*timopheevi*) Bison in the greenhouse

M_2 plant no.	Seed fertility %	Seed fertility Kernels/spikelet
203[1] - 1	9.5	0.30
- 2	59.6	1.70
- 6	12.2	0.38
- 8	11.2	0.27
- 9	34.4	0.85
-11	64.1	1.83
252(1)[2]-1	53.0	1.40
-3	70.3	1.61
(2)[2]-1	49.2	1.28
-2	51.7	1.37
-3	67.0	1.48
-4	90.2	2.19
Bison[3]	73.0	2.56

[1]Offspring of M_1 plant 20-3.

[2]Offspring of first and second spikes of M_1 plant 25-2.

[3]Grown in the field.

The M_3 plant 252(1)-1-14 lacked restoring genes for the male sterility of (*ovata*)Norin 26.

From these results it seems that these male-fertility-restoring mutants are quite promising in hybrid wheat breeding, though each mutant was accompanied by some undesirable characters such as weak culm. Further genetic studies are necessary.

Table 4. Seed fertility of 11 X-ray-induced male-fertility-restoring M_3 lines of (*timopheevi*)Bison in the field

M_3 line no.	No. of plants obs.	Seed-fertility classes (kernels/spikelet) 0	0.25	0.75	1.25	1.75	2.25	M ±S.D.
203 - 1	10	10						0
- 2	10	2	3	1	2	2		0.75±0.697
- 6	11	7	3	1				0.14±0.234
- 9	11	3	7	1				0.23±0.971
-11	26	3	18	3	1	1		0.38±0.389
252(1)-1	21	1	2	6	6	4	2	1.14±0.625
-3	27		4	8	9	6		1.06±0.503
(2)-1	10	8	1				1	0.25±0.707
-2	10	6		1	1	1	1	0.60±0.860
-3	17	7	1	3	6			0.59±0.572
-4	29	6	4	8	6	2	3	0.85±0.715
(*t*)Bison	7	7						0
Bison	7					2	5	2.11±0.244

Table 5. Some characters of 11 X-ray-induced male-fertility-restoring M_3 lines of (*timopheevi*)Bison grown in the field

M_3 line no.	No. of plants obs.	Head-ing time*	Spikes per plant M±S.D.	Culm length M±S.D.(cm)	Spike length M±S.D.(cm)	Awn length M±S.D.(cm)	Spikelets per spike M±S.D.	1000-kernel weight M±S.D.(g)
203 - 1	10	e	7.7±3.62	80.5±10.04	10.1±0.95	1.0±1.38	19.3±1.89	sterile
- 2	10	m	8.9±3.38	93.1±12.77	8.7±1.62	0.7±0.81	21.0±1.61	30.6±10.41
- 6	11	m	10.1±3.42	99.1± 9.71	8.3±1.04	1.0±0.32	23.7±1.62	34.9± 6.80
- 9	11	e	8.5±3.17	83.7±10.20	8.7±1.62	2.3±1.03	17.0±2.58	36.2± 7.81
-11	28	m	9.3±5.33	84.9±12.50	9.6±1.75	1.2±1.39	19.8±2.99	32.1± 9.00
252(1)-1	23	e	7.6±3.19	93.7± 6.28	7.8±1.05	2.7±0.91	17.1±1.57	24.8± 6.86
-3	29	e	11.2±4.48	86.1±11.00	11.2±1.30	5.0±1.04	23.8±1.99	31.2±10.94
(2)-1	10	em	6.5±3.53	83.5±18.15	9.7±2.01	4.4±2.25	17.8±2.96	30.9±16.83
-2	11	m	4.5±2.25	84.5±12.99	7.7±3.01	1.4±0.88	20.5±1.99	24.3± 6.55
-3	18	m	6.9±2.51	84.1± 7.47	8.5±1.18	5.0±1.71	17.9±1.68	27.8±11.62
-4	29	m	6.4±3.14	88.5±11.56	8.8±0.97	3.2±0.89	18.6±2.91	29.8± 8.55
(*t*)Bison	7	m	9.9±3.18	83.0± 7.23	8.0±1.46	6.3±1.14	15.7±1.65	sterile
Bison	7	m	10.6±2.07	89.3± 3.30	9.1±1.04	6.7±1.24	15.7±1.65	27.4± 6.66

* e, em, and m stand for early in, early to middle of, and middle of May, respectively.

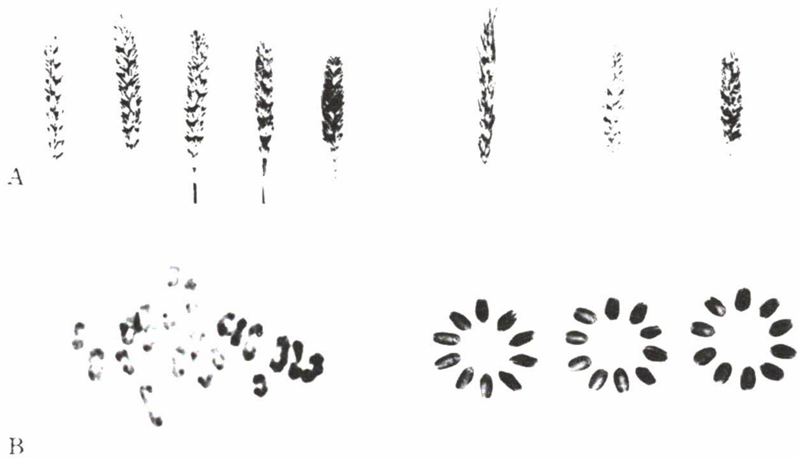

Figure 1. A: Spike of male-fertility-restoring mutants of (*timopheevi*)Bison. From left M_4 252(1)-3-16-7, M_3 203-11-15, M_3 203-11-22, M_4 252(2)-4-9-18 and M_4 252(1)-1-14-4. B: Metaphase I configuration showing 21_{II} of M_4 252(2)-4-9-18. C: Spike of (*timopheevi*)Bison (*left*), F_1 (*middle*), and M_4 252(1)-1-14-4 (*right*). D: Grain of self-fertile Bison (*left*), (*timopheevi*)Bison X M_3 252(1)-1-14 (*middle*), and M_4 252(1)-1-14-4 (*right*).

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