Basic studies on hybrid wheat breeding utilizing the *timopheevi* cytoplasm and *Rf*3 gene —— Summary of the results

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Reprinted from SEIKEN ZIHÔ, Report of the
Kihara Institute for Biological Research
No. 29, October, 1980

財団法人　木原生物学研究所　生研時報　第29号　別刷
昭和55年10月31日発行

Seiken Zihô **29**, 40～56 *(1980)*

Basic studies on hybrid wheat breeding utilizing the *timopheevi* cytoplasm and *Rf*3 gene — Summary of the results[1)]

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1. Introduction

Great success of hybrid variety in Sorghum, a self-pollinating crop, and the discovery of cytoplasmic male sterility and fertility restoration in wheat (Kihara 1951, Fukasawa 1955) stimulated the works toward production of commercial hybrid variety of common wheat (Roberts 1965). Since the discovery of Wilson and Ross (1962) on the induction of complete male sterility in common wheat with the *T. timopheevi* cytoplasm, this cytoplasm has been used almost exclusively as the source of male sterility all over the world.

Fundamental researches on hybrid wheat breeding using Japanese cultivars were initiated by the present author in 1965. At an early stage of the program, the effects of heterosis on yield and other agronomic characters were investigated using F_1 hybrids produced by hand-pollination between several Japanese and American cultivars. The results, which are shown in Table 1, indicated that 20–30% yield increase could be expected in the best hybrid combination, and encouraged us to carry out the works on hybrid wheat breeding.

Table 1. Relative yields of F_1 hybrids as compared to the best-yielding cultivars (Tsunewaki 1970a)

Relative yield (Index)	No. of F_1 combinations				No. of parents
	Jap.×Jap.	Jap.×U.S.	U.S.×U.S.	Total	
<50	0	0	1	1	4
51-70	1	2	5	8	3
71-80	0	5	6	11	3
81-90	1	5	2	8	0
91-100	4	13	0	17	2
101-110	4	5	1	10	0
111-120	4	5	0	9	0
121-130	0	1	0	1	0
131-140	1	0	0	1	0
Total	15	36	15	66	12

Note: Yields of the best-yielding cultivars (Norin 50 and Ebisu-komugi) are taken as 100.

1) Contribution from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, No. 429. The work was supported in part by a Grant-in-Aid (No. 511601) from the Ministry of Education, Science and Culture, Japan.

In the beginning, the cytoplasms of both *T. timopheevi* and *Ae. ovata* were employed. As for our works with the *timopheevi* cytoplasm, two common wheat strains, i.e., (*timopheevi*)-Bison[2] and (*timopheevi*)-Selkirk[2] were obtained in 1964 from Dr. J. W. Schmidt, the University of Nebraska, USA, as the source of this male sterile cytoplasm. Kihara and Tsunewaki (1966) found that *T. spelta* var. *duhamelianum* (hereafter, simply called Spelta) carries an effective fertility-restoring gene to this cytoplasm. This was later analyzed by Tahir and Tsunewaki (1969) by means of monosomic analysis, and a single dominant fertiliy-restoring gene was identified to be on chromosome 1B, which they designated as *Rf*3. We used this gene to produce the restorer lines to the *timopheevi* cytoplasm. Twenty-one Japanese cultivars of common wheat were obtained in 1965, and four additional ones in 1973, from the Central Agricultural Experiment Station, the Ministry of Agriculture, Forestry and Fishery, Japan. They have been used as the recipient of the *timopheevi* cytoplasm in the production of male sterile lines, and that of the *timopheevi* cytoplasm and *Rf*3 gene in the production of fertility restorer lines. The backcross method was used exclusively for their production, because of lack in experience and facilities to carry out their breeding by other means. The first F_1 hybrids were produced in 1976, and again in 1978, using the male sterile lines and fertility restorer lines mentioned above. Small scale yield trials of the hybrid wheat were carried out in several locations in two growing seasons, 1976–1977 and 1978–1979.

As for our works with the *ovata* cytoplasm, (*ovata*)-Norin 26, that was obtained from Dr. H. Fukasawa, Kobe University, Japan was used as the source of this cytoplasm. Kihara and Tsunewaki (1964) found that an alien chromosome substitution line (chromosome 1C substituted for 1D) of *T. aestivum* var. *erythrospermum* called "P168", carries an effective fertility-restoring gene to the *ovata* cytoplasm. The gene was later analyzed by means of monosomic analysis (Tahir and Tsunewaki 1971a), and a single dominant gene *Rfc*1 was identified to be on chromosome 1D. This gene was used in the production of fertility restorer lines against the *ovata* cytoplasm. In this case, too, the backcross method was employed in producing both the male sterile and fertility restorer lines. However, in 1973 the works with this male sterility-fertility restoration system were discontinued, because of great delay of heading caused by the *ovata* cytoplasm.

2. Production of male sterile lines of Japanese cultivars

In total, 25 cultivars of Japanese common wheat have been used for the production of male sterile lines. They have been backcrossed as the recurrent pollen parent to an alloplasmic common wheat with the *timopheevi* cytoplasm. Ears of the plants at the B_{10} generation are shown in Fig. 1. Selfed seed fertilities in successive backcross generations are collectively shown in Table 2. There are some problems in the reliability of the data, when partial fertility was observed; first, ears might have been bagged after some florets had flowered and had been partially outcrossed, resulting in some seed setting, even though they were completely male sterile. Second, in some years storms occurred during the flowering season, owing to which some bags were broken, resulting in seed set by

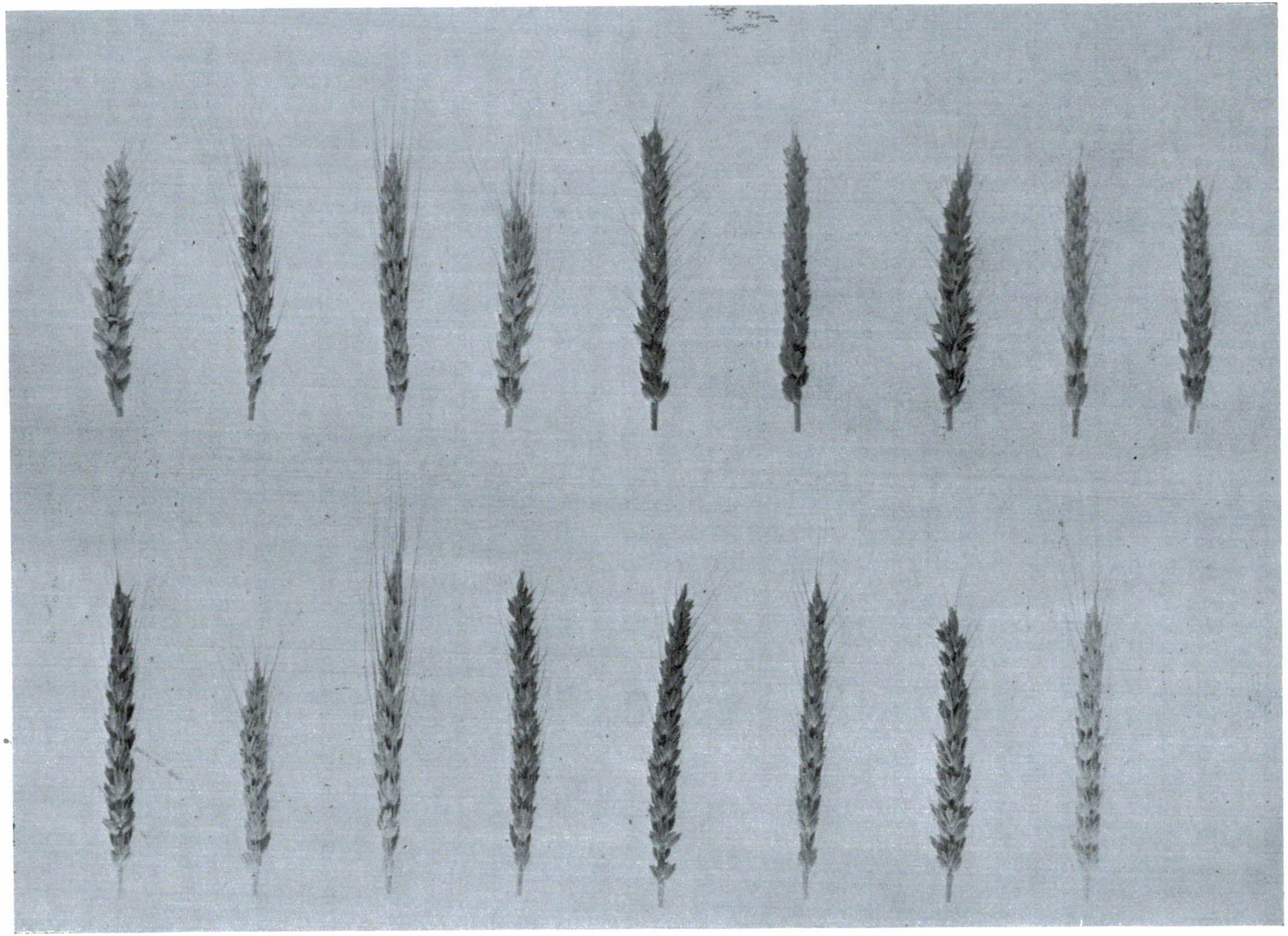

Fig. 1. Male sterile lines of Japanese common wheat cultivars, having the *timopheevi* cytoplasm.
Top row (left to right): Ebisu-, Kitakami-, Kokeshi-, Shirasagi-, Junrei-, Nanbu-, Nichirin-, Fujimi- and Mikuni-komugi.
Bottom row (left to right): Yutaka-komugi, and Norin 26, 29, 50, 53, 61, 69 and 75.

Table 2. Selfed seed fertility (%) observed in successive backcross generations of Japanese cultivars during repeated backcrosses for introducing the *timopheevi* cytoplasm into them

Cultivar	Generation											Average
	F_1	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	
Aoba-komugi	0	0	0	25	0	0	1	0	0	0	-	3
Ebisu- 〃	0	14	0	0	-	1	0	0	0	0	-	2
Fujimi- 〃	0	0	0	0	0	1	0	0	0	0	-	0
Junrei- 〃	0	14	20	5	4	0	6	18	3	2	0	7
Kitakami- 〃	0	0	0	-	-	0	0	0	0	0	-	0
Kokeshi- 〃	0	18	0	0	0	0	5	0	0	0	0	2
Mikuni- 〃	0	0	0	0	0	0	0	0	0	0	0	0
Nanbu- 〃	1	33	0	0	0	0	4	0	0	0	0	3
Nichirin- 〃	1	0	0	0	0	0	0	0	1	0	0	0
Norin 26	0	0	0	0	0	4	0	0	0	0	-	0
〃 29	0	0	0	0	0	2	0	0	0	0	0	0
〃 50	6	16	16	0	0	5	0	0	0	0	-	4
〃 52	0	0	0	8	3	11	11	0	0	0	-	3
〃 53	0	0	0	0	0	0	0	0	0	0	-	0
〃 61	0	0	0	0	0	0	1	0	0	0	0	0
〃 68	0	0	1	0	0	3	1	-	-	-	-	1
〃 69	0	2	11	7	12	20	21	6	0	11	-	9
〃 72	0	0	0	0	0	0	3	0	1	0	0	0
〃 75	0	0	0	0	0	1	0	0	0	0	0	0
Shirasagi-komugi	0	0	0	0	0	0	0	0	0	0	0	0
Yutaka- 〃	1	0	0	0	0	0	0	0	0	0	0	0
Kobushi- 〃	0	0	0	0	0	0	-	-	-	-	-	0
Omase- 〃	0	0	0	0	0	0	-	-	-	-	-	0
Sakigake- 〃	1	0	0	0	0	0	-	-	-	-	-	0
Ushio- 〃	0	0	0	0	0	0	-	-	-	-	-	0

Table 3. Performance of the male sterile and corresponding normal lines on six characters (Fujigaki and Tsunewaki 1976)

Line	Genera-tion	Heading date (day)		Plant ht. (cm)		Ear number		Flag leaf length(cm)		Seed fertility (%)			
										Selfed		Open-poll.	
		Ms	N	Ms	N	Ms	N	Ms	N	Ms	N	Ms	N
Aoba-komugi	B_7	5.2	8.8	80.8	79.5	15.4	8.7	20.2	16.9	0.1	80.0	46.4	77.9
Ebisu- 〃	B_5	4.3	4.8	76.2	76.2	15.0	14.5	20.8	18.0	1.2	95.1	44.7	91.7
Fujimi- 〃	〃	0.0	3.4	69.9	71.4	15.3	10.3	20.7	19.6	1.1	96.3	24.2	90.0
Junrei- 〃	B_7	0.5	1.9	81.8	80.2	21.0	18.1	23.4	22.0	18.2	95.9	51.7	95.4
Mikuni- 〃	〃	5.4	9.4	83.9	73.4	17.3	7.8	20.8	19.9	0.0	93.9	25.3	84.0
Nanbu- 〃	〃	6.8	7.6	84.5	83.7	8.8	7.9	22.3	19.7	0.6	94.7	27.5	91.9
Nichirin- 〃	〃	2.4	3.2	69.5	66.4	21.1	14.2	19.2	16.9	0.1	96.7	41.4	93.3
Norin 26	B_5	1.9	1.8	78.6	82.0	16.4	14.9	21.0	21.4	4.2	97.7	36.9	84.9
〃 29	B_6	7.3	8.8	118.8	112.5	20.9	15.5	26.8	24.5	0.0	94.7	45.3	97.5
〃 50	B_5	2.5	4.2	74.3	74.0	21.7	15.0	19.2	19.8	6.4	96.8	34.7	99.1
〃 53	〃	4.8	3.1	84.4	81.5	21.9	10.0	19.4	20.9	0.1	96.7	43.0	88.9
〃 61	B_6	3.3	3.2	67.9	73.2	9.8	10.2	20.4	18.1	1.6	95.9	44.7	93.1
〃 68	B_5	2.8	5.2	74.6	72.5	16.6	11.0	20.5	19.0	2.0	95.8	35.5	98.3
〃 69	〃	4.0	5.3	88.3	90.0	20.8	15.7	21.2	22.1	22.4	97.5	42.0	94.0
〃 72	B_6	1.4	2.3	76.8	74.7	11.9	8.0	18.5	15.4	5.0	97.2	49.4	94.1
〃 75	〃	16.5	17.8	113.8	118.5	15.6	14.1	23.3	24.3	0.3	89.7	23.1	92.0
Shirasagi-komugi	B_7	2.5	2.3	77.9	78.9	15.3	17.2	22.8	23.3	0.0	91.4	25.4	76.2
Yutaka- 〃	〃	2.0	4.5	82.8	76.2	23.4	10.4	23.2	20.1	0.2	96.7	44.2	83.9
Aver.		4.1	5.4	82.5	81.4	17.1	12.4	21.3	20.1	3.5	94.6	38.1	90.3

outcrossing. Seed set due to these accidents could not be totally excluded from the data. Therefore, a line that showed complete sterility in most generations should be regarded as completely male sterile. From this criterion, we can say all cultivars except two (Junrei-komugi and Norin 69) were converted to completely male sterile lines with the *timopheevi* cytoplasm. The two cultivars, Junrei-komugi and Norin 69 seem to have very weak fertility-restoring gene(s) against this cytoplasm.

In the season of 1973–1974, all male sterile lines that were in B_5 or later backcross generations were grown together with their normal counterparts in a split plot design with four replications; the cultivars being allocated to the main plots and the normal and male sterile lines to subplots. Genetic effects of the *timopheevi* cytoplasm on heading date, plant height, ear number, flag leaf length and selfed and open-pollinated seed fertilities were investigated (Fujigaki and Tsunewaki 1976). The results are given in Table 3. The *timopheevi* cytoplasm increased ear number and reduced seed fertilities. The increase of ear number is a side effect of the sterility, that induced vigorous tillering at flowering stage. On other characters no significant effects of this cytoplasm could be detected.

3. Production of the fertility restorer lines of Japanese cultivars

Tahir (1970) and Tahir and Tsunewaki (1971b) screened the restorer to the *timopheevi*

Table 4. The results of screening the fertility restorers against the *timopheevi* cytoplasm; after Tahir (1970) and Tahir and Tsunewaki (1971b)

Species	Origin	No. strains tested	No. restorers[1]	% Restorers
T. aestivum	Japan	27	2	-
〃	Turkey	37	1	-
〃	Egypt	24	0	-
〃	N. America	5	0	-
〃	S. America	11	1[2]	-
〃	Others	7	0	-
〃	Total	111	4	3.6
T. compactum	—	3	0	0.0
T. sphaerococcum	—	4	0	0.0
T. spelta	Bulgaria	4	3	-
〃	Germany	8	5	-
〃	Hungary	2	2	-
〃	Italy	2	2	-
〃	Other	1	1	-
〃	Total	17	13[3]	76.5
T. vavilovii	—	1	0	0.0
Synthesized common wheat	—	11	0	0.0

1) Strains showing the selfed seed fertilities higher than 5% in the F_1 with a male sterile line were considered as the restorer.
2) A cultivar Gironde that gave 100% selfed seed fertility in the F_1 with a male sterile line.
3) Their fertility restoration in the F_1 with the male steriles ranged from 90 to 100%.

Table 5. Segregation of fertile and sterile plants in the di- and monosomic families of the cross, (*timopheevi*)-Bison × (Chinese Spring monosomics × *T. spelta* var. *duhamelianum*) F_1

Family	No. of plants			% Sterile	x^2-value (1:1)
	Total	Fertile	Sterile		
Disomic	201	99	102	50.7	0.0
Mono-1A	117	55	62	53.0	0.4
〃 2A	63	24	39	61.9	3.6
〃 3A	81	47	34	42.0	2.1
〃 4A	48	23	25	52.1	0.1
〃 5A	102	58	44	43.1	1.9
〃 6A	89	46	43	48.3	0.1
〃 7A	102	42	60	58.8	3.2
〃 1B	78	71	7	9.0	52.5**
〃 2B	100	55	45	45.0	1.0
〃 3B	106	48	58	54.7	0.9
〃 4B	118	57	61	51.7	0.1
〃 5B	112	53	59	52.7	0.3
〃 6B	138	74	64	46.4	0.7
〃 7B	88	48	40	45.5	0.6
〃 1D	101	54	47	46.5	0.5
〃 2D	114	54	60	52.6	0.3
〃 3D	76	38	38	50.0	0.0
〃 4D	87	48	39	44.8	0.9
〃 5D	88	52	36	40.9	2.9
〃 6D	114	63	51	44.7	1.3
〃 7D	95	37	58	61.1	4.6*

* and **: Significant at the 5% and 1% level, respectively.

cytoplasm, the results of which are summarized in Table 4. A large number of restorers were found in *T. spelta*. Beside this species, only one effective restorer was found in a collection from South America, that is a cultivar Gironde from Uruguay, showing 100% fertility restoration in the F_1 hybrid with a complete male sterile line.

Monosomic analysis was carried out on the fertility-restoring gene(s) of *T. spelta* var. *duhamelianum* (Tahir and Tsunewaki 1969) in the following way: First, Spelta was crossed to 21 monosomic lines of Chinese Spring produced by Dr. E.R. Sears (1954). In the F_1 generation, monosomics were cytologically selected, and their pollen was pollinated to a male sterile (*timopheevi*)-Bison. The seeds obtained from this test-cross were sown, and selfed seed fertility of the plants grown were examined. The results are given in Table 5. It is evident from the results that a single dominant gene of Spelta located on chromosome 1B, mainly controls the fertility restoration by this wheat, and it was named *Rf* 3. For a reference, all fertility-restoring genes, whose chromosomal locations are known, are collectively shown in Table 6.

In order to produce fertility restorer lines of Japanese cultivars, they have been repeatedly backcrossed as the recurrent pollen parent to (*timopheevi*)-Spelta. In each backcross generation, two to three plants showing high selfed seed fertility were selected as the female parent for further backcross. Their selfed seed fertilities are collectively shown

Table 6. Chromosomal locations of the fertility-restoring genes against various male sterile cytoplasms

Fertility-restoring gene			Male sterile cytoplasm	Reference
Chromosome	Gene	Carrier		
1A*	*Rf*1	R1, R2, R3, R4, R5	*T. timopheevi*	Bahl & Maan 1973
〃	〃	R-D, R-K	〃	Yen *et al.* 1969
1B*	*Rf*3	Splt	〃	Tahir & Tsunewaki 1969
〃	〃	Primepi	〃	Bahl & Maan 1973
〃	*Rfu*1	CS	*Ae. umbellulata*	Tsunewaki 1974
1BS*	*Rfv*1	〃	*Ae. kotschyi*	Mukai & Tsunewaki 1979
〃	〃	〃	*Ae. variabilis*	〃
1D	*Rfc*3	Cmp	*Ae. caudata*	Tsunewaki 1974
1C*	*Rfc*1	P168	〃	Tahir & Tsunewaki 1971a
〃	〃	〃	*Ae. ovata*	〃
〃	*Rfc*1 (?)	〃	*Ae. umbellulata*	Tsunewaki *et al.* 1978
〃	〃	〃	*Ae. triuncialis*	〃
〃	〃	〃	*Ae. biuncialis*	〃
〃	〃	〃	*Ae. columnaris*	〃
〃	〃	〃	*Ae. triaristata*	〃
2B	*Rfu*2	CS	*Ae. umbellulata*	Tsunewaki 1974
5D*	*Rf*6	Primepi	*T. timopheevi*	Bahl & Maan 1973
6B*	*Rf*4	R-C, R-K	〃	Yen *et al.* 1969
〃	〃	R2	〃	Bahl & Maan 1973
〃	*Rfc*2	Cmp	*Ae. caudata*	Tsunewaki 1974
6D	*Rf*5	R-C	*T. timopheevi*	Yen *et al.* 1969
7B	*Rf*7	R4	〃	Bahl & Maan 1973
7D	*Rf*2	R1, R2, R3, R4, R5	〃	〃
〃	〃	R-D	〃	Yen *et al.* 1969

*: Chromosomes carrying a nucleolar organizing region.

in Table 7. The fertile plants, that were heterozygous for the *Rf*3 gene, did not restore complete fertility, the selfed seed fertility varying from 26 to 82% depending upon the cultivars. In four cultivars, i.e., Aoba-komugi, Norin 29, Norin 53 and Norin 75, the fertility restored was 70% or higher, while the fertility did not reach 40% in Fujimi-komugi, Kitakami-komugi, Norin 61 and Yutaka-komugi. It became clear from these data that the gene *Rf*3 alone is not sufficient to restore complete male fertility in its single dose, i.e., in its heterozygous condition.

At the B_5 or B_6 generation, each rstorer line that was heterozygous for the *Rf*3 gene, was self-pollinated, and in the succeeding two to three generations selection of the true bred restorer line was carried out. Ears of some true bred restorers are shown in Fig. 2. Fertility restoration observed in such true bred restorer lines is shown in Table 8. The true bred restorer lines of Aoba-komugi, Ebisu-komugi, Junrei-komugi, Nichirin-komugi, Norin 29 and Norin 75 constantly showed about 90% fertility restoration, while those of Kitakami-komugi, Kokeshi-komugi and Shirasagi-komugi restored the fertility to lesser extent, being about 70% at best. Wilson (1968) reported that one or two fertility-restoring genes are insufficient in securing complete fertility restoration under unfavorable conditions. His view is supported in the present results which indicated that a double dose of the *Rf*3 gene is not sufficient for inducing complete fertility restoration in Japanese cultivars.

Table 7. Selfed seed fertilities (%) of male fertile plants segregated in successive backcross generations of Japanese cultivars during the transfer of the *timopheevi* cytoplasm and *Rf*3 gene to them

Cultivar	Generation							Average
	F_1	B_1	B_2	B_3	B_4	B_5	B_6	
Aoba-komugi	79	85	76	75	77	96	83	82
Ebisu- 〃	87	51	58	45	56	87	58	63
Fujimi- 〃	51	54	46	17	34	21	23	35
Junrei- 〃	67	61	79	53	56	69	70	65
Kitakami- 〃	50	27	17	21	14	29	-	26
Kokeshi- 〃	50	28	38	27	19	43	-	34
Mikuni- 〃	70	49	47	30	17	32	-	41
Nanbu- 〃	56	41	34	58	35	46	-	45
Nichirin- 〃	51	62	31	55	19	77	-	49
Norin 26	75	38	52	30	49	75	-	53
〃 29	63	62	78	58	61	98	-	70
〃 50	75	73	66	44	34	81	-	62
〃 52	55	40	75	54	-	-	-	56
〃 53	86	85	80	70	53	69	59	72
〃 61	48	21	39	47	-	-	-	39
〃 68	-	56	64	55	54	47	-	55
〃 69	76	55	63	50	69	34	-	58
〃 72	61	32	47	43	39	67	30	46
〃 75	-	75	68	85	78	71	65	74
Shirasagi-komugi	70	52	69	25	56	38	48	51
Yutaka- 〃	46	37	10	17	29	57	56	36

4. Estimation of outcrossing ability of the male sterile and the fertility restorer lines

One of the important factors for the success of hybrid wheat is whether F_1 seeds can be efficiently and economically produced by outcross between the male sterile and fertility-restoring lines. This is particularly so in Japan where weather conditions are not favorable for outcrossing.

In the fall of 1977, male sterile lines of several cultivars were planted in four replications in a plot surrounded by plants of the fertility restorer line of a cultivar that flowers on almost the same day as the male sterile lines placed in the same plot. After harvest, seed setting rate and total number of seeds set in each male sterile plant were recorded. The data obtained were subjected to an ordinary analysis of variance. The results are summarized in Table 9. From the results presented in this table, it is obvious that there are clear differences existing among the male sterile lines as for their ability of receiving pollen grains of the same restorer lines. For example, male sterile lines of Norin 61 and Nichirin-komugi showed very high outcrossing rates over 70%, followed by those of Norin 53, Ebisu-komugi and Mikuni-komugi, all of which showed seed setting rates higher than 60%. Significant differences on seed setting rate among the male sterile lines outcrossed with the same restorer line are indicated with different alphabetical letters in the last column of Table 9. Male sterile lines of Norin 61, Nichirin-komugi and Yutaka-komugi showed high seed setting rates against all restorer lines tested for them, so they can be

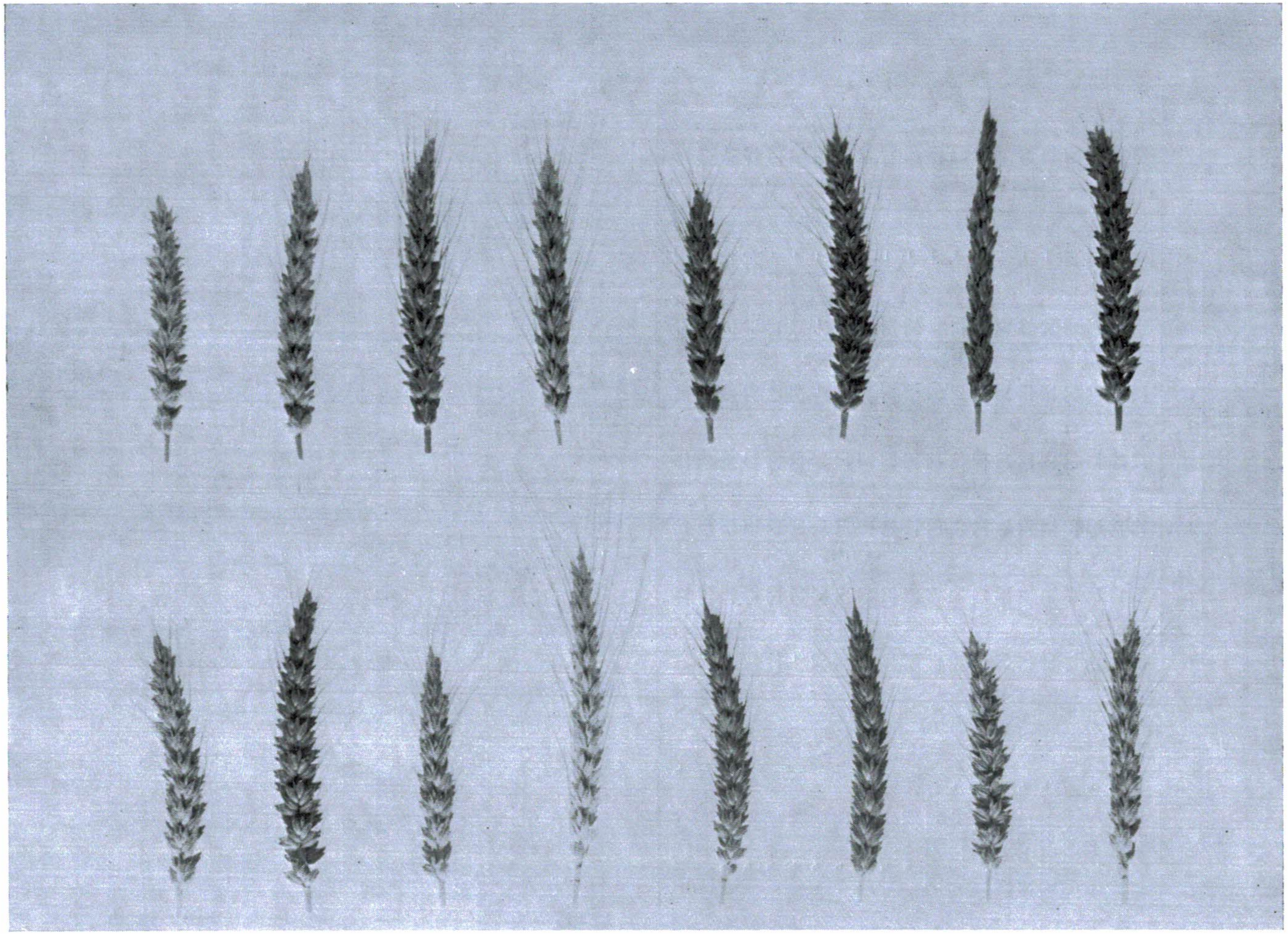

Fig. 2. Fertility restorer lines of Japanese common wheat cultivars, having the *timopheevi* cytoplasm and the *Rf* 3 gene. Top row (left to right): Aoba-, Ebisu-, Kitakami-, Kokeshi-, Shirasagi-, Junrei-, Nanbu- and Nichirin-komugi. Bottom row (left to right): Mikuni- and Yutaka-komugi, and Norin 26, 29, 50, 53, 68 and 69.

Table 8. Fertility restoration in the true bred restorer lines (homozygous for *Rf*3) of Japanese cultivars

Cultivar	1977			1978	
	Generation	Seed fertility (%)		Generation	Open-poll. seed fert. (%)
		Selfed	Open-poll.		
Aoba-komugi	B_6F_3	83	95	B_6F_4	89
Ebisu- 〃	〃	92	91	〃	82
Junrei- 〃	〃	88	82	〃	92
Kitakami- 〃	B_5F_4	50	54	B_5F_5	78
Kokeshi- 〃	〃	58	80	〃	68
Mikuni- 〃	〃	37	80	〃	83
Nanbu- 〃	B_5F_3	70	71	B_5F_4	82
Nichirin- 〃	B_5F_4	93	90	B_5F_5	98
Norin 26	〃	74	74	〃	87
〃 29	〃	88	93	〃	92
〃 50	〃	72	89	〃	83
〃 53	B_6F_3	79	94	B_6F_4	97
〃 68	B_5F_4	68	85	B_5F_5	90
〃 69	〃	72	82	〃	79
〃 72	B_6F_3	53	84	B_6F_4	85
〃 75	B_7F_3	85	94	B_7F_4	92
Shirasagi-komugi	B_5F_4	63	64	B_5F_5	68
Yutaka- 〃	B_6F_3	54	83	B_6F_4	84
Average		71	83		85

regarded as a good female parent for hybrid seed production. On the contrary, Shirasagi-komugi and Norin 50 showed generally low seed setting rates, thus they must be evaluated as a poor female parent.

A more important factor in determining the efficiency of hybrid seed production is undoubtedly the pollinator than the male sterile, female parent: A great difference was found on both the range and average of the seed setting rates of three to six male sterile lines which were outcrossed by different restorer lines, as shown in Table 10. Male sterile lines combined with each restorer line as the pollinator were not the same, so that a simple comparison can not be made between the restorer lines for their ability as the pollinator. However, it is almost certain that the restorer line of Norin 75 expressed the highest ability, while those of Norin 68 and Ebisu-komugi the lowest ability as the pollinator to male sterile lines. Number of seeds produced by a male sterile plant was almost completely proportional to its seed setting rate. Thus, it is concluded that the selection of male sterile and fertility-restoring lines, which give high outcrossing rate, is important for the efficient production of F_1 seeds. Another important conclusion we could draw from this investigation is that even under Japanese climatic conditions a seed setting rate higher than 50% can be easily obtained by selecting suitable restorer lines like those of Norin 75, Junrei-komugi, Aoba-komugi and Nichirin-komugi.

5. Yield trials of the hybrid wheat

As described in previous sections, the male sterile lines with the *timopheevi* cytoplasm

Table 9. Efficiencies of F_1 seed production in various combinations of the male sterile (with the *timopheevi* cytoplasm) and fertility restorer lines (with the *Rf*3 gene) of Japanese cultivars

Restorer line	Male sterile line	No. ears/plant	No. seeds set/plant	Seed set (%)	Fertility class[1)]
Aoba-k.	Norin 61	22.8	491	76.1	A
〃	Nichirin-k.	24.8	564	71.6	〃
〃	Norin 53	19.6	317	61.5	B
〃	Ebisu-k.	22.0	356	61.4	〃
〃	Mikuni-k.	21.1	436	60.7	〃
〃	Norin 69	19.1	309	47.5	C
〃	Average	21.6	412	63.1	
〃	5% LSD	-	-	9.8	
Ebisu-k.	Yutaka-k.	15.3	469	55.9	A
〃	Norin 50	15.1	469	46.1	〃 B
〃	〃 53	12.9	265	44.7	〃 〃
〃	Mikuni-k.	14.0	345	40.5	〃
〃	Fujimi-k.	14.4	215	39.5	〃
〃	Nanbu-k.	12.4	180	34.5	〃
〃	Average	14.0	324	43.5	
〃	5% LSD	-	-	11.6	
Junrei-k.	Norin 72	18.1	524	70.2	A
〃	Nichirin-k.	16.5	455	69.2	〃
〃	Yutaka-k.	14.6	538	67.9	〃
〃	Norin 52	16.0	549	63.0	〃 B
〃	Shirasagi-k.	15.3	376	61.9	〃 〃
〃	Norin 50	16.4	539	57.0	〃
〃	Average	16.1	497	64.9	
〃	5% LSD	-	-	8.6	
Nichirin-k.	Norin 61	22.1	396	67.0	A
〃	〃 72	22.2	544	64.1	〃
〃	Junrei-k.	21.4	502	59.8	B
〃	Fujimi-k.	18.7	265	54.7	〃 C
〃	Shirasagi-k.	18.3	300	54.3	〃 〃
〃	Norin 52	19.6	359	49.2	〃
〃	Average	20.4	394	58.2	
〃	5% LSD	-	-	7.2	
Norin 29	Nanbu-k.	12.6	349	85.2	A
〃	Kitakami-k.	12.3	203	49.1	B
〃	Norin 75	13.7	143	33.1	C
〃	Average	12.9	232	55.8	
〃	5% LSD	-	-	10.9	
Norin 68	Norin 61	17.4	238	48.0	A
〃	Yutaka-k.	18.1	279	43.7	〃 B
〃	Norin 53	15.9	190	40.6	〃 〃
〃	〃 72	22.5	445	39.6	〃 〃
〃	〃 50	21.0	346	37.7	〃 〃
〃	Shirasagi-k.	16.7	192	35.9	〃
〃	Average	18.6	282	40.9	
〃	5% LSD	-	-	11.5	
Norin 75	Norin 29	21.9	737	90.1	A
〃	Kitakami-k.	13.6	606	79.3	B
〃	Nanbu-k.	10.9	428	76.5	〃
〃	Average	15.5	590	82.0	
〃	5% LSD	-	-	4.1	

1) A; High, B; intermediate, C; low.

and the fertility restorer lines with the *Rf*3 gene in homozygous condition have been produced in Japanese cultivars of common wheat. Using them F_1 seeds were produced in 1976 and 1978 by open-pollination. The F_1 hybrids were grown in small scale field trials in two crop seasons, 1976–1977 and 1978–1979, and their agronomic characteristics were investigated. Here, the results of the 1978–1979 season will be described. In this season, the hybrids were tested in three locations, namely, Kyoto University, Kyoto (Kinki distirct), the Central Agricultural Experiment Station, Konosu (Kanto district) and Tanno Nokyo, Tanno (Hokkaido district). Their list is given in Table 11.

In Kyoto University, 30 F_1 hybrids and three check cultivars were seeded each in a single row, 3 m long, with a seeding rate of 125 kernels per row. They were arranged according to a randomized block design with four replications. The main results obtained are shown in Table 12. On the average, the F_1 hybrids became about 9 cm taller than the check cultivars, and their ear number was reduced by about 20%. The average frequency of offtypes was 34%, ranging from 7 to 66%. The seed setting rate was 9% lower on the average. The average grain yield of the F_1 hybrids was only about 58% of the check cultivars. The grain yield was highly correlated to ear number per unit length of row ($r=0.74$, $df=28$), but negatively correlated with the frequency of offtypes ($r=-0.81$, $df=28$). Most offtypes were hybrids between the male sterile parents and nonrestorer type plants grown in other parts of the field, and, consequently, became male sterile. This fact explains why such high negative correlation was observed between the two characters.

The F_1 hybrid, Norin 72 (male sterile) × Nichirin-komugi (restorer), outyielded about 14 % the average of three check cultivars, and about 4% the best check cultivar, though their difference was not statistically significant. From the results of analysis of variance and the 5% LSD test, three entries 824 (Norin 72 × Nichirin-komugi F_1), 842 (Nichirin-komugi) and 841 (Junrei-komugi) were rated to the best yielder class. Four other F_1 hybrids, namely, Entry 820 (Junrei-komugi × Nichirin-komugi), 802 (Mikuni-komugi × Aoba-komugi), 833 (Norin 72 × Norin 68) and 801 (Ebisu-komugi × Aoba-komugi) were rated to the second best yielder class, to which a check cultivar Norin 61 belonged.

The main part of the results obtained in the Central Agricultural Experiment Station with a collaboration of Dr. H. Maeda is given in Table 13. In this case, a single cultivar

Table 10. Differences due to the restorer lines (=pollinator) on the number of seeds set per plant and the seed setting rate of male sterile (ms) lines

Restorer (pollinator)	No. ms lines tested	No. seeds/plant		Seed set (%)	
		Average	Range	Average	Range
Norin 75	3	5.9×10^2	$4.3-7.4\times10^2$	82	77–90
Junrei-komugi	6	5.0× 〃	3.8–5.5× 〃	65	57–70
Aoba-komugi	6	4.1× 〃	3.1–5.6× 〃	63	48–76
Nichirin-komugi	6	3.9× 〃	2.7–5.4× 〃	58	49–67
Norin 29	3	2.3× 〃	1.4–3.5× 〃	56	33–85
Ebisu-komugi	6	3.2× 〃	1.8–4.7× 〃	44	35–56
Norin 68	6	2.8× 〃	1.9–4.5× 〃	41	36–48

Table 11. F_1 hybrids tested in the crop season 1978–1979

Entry	Parent				Seed set of male steriles (%)
	Male sterile (♀)		Restorer (♂)		
	Cultivar	Generation	Cultivar	Generation	
F_1 hybrid					
801	Ebisu-k.	B_9	Aoba-k.	B_6F_4	61
802	Mikuni-k.	B_{11}	〃	〃	61
803	Nichirin-k.	〃	〃	〃	72
804	Norin 53	B_9	〃	〃	62
805	〃 61	B_{10}	〃	〃	76
806	〃 69	B_9	〃	〃	48
807	Fujimi-k.	〃	Ebisu-k.	〃	40
808	Mikuni-k.	B_{11}	〃	〃	41
809	Nanbu-k.	〃	〃	〃	35
810	Yutaka-k.	B_{10}	〃	〃	56
811	Norin 50	B_9	〃	〃	46
812	〃 53	〃	〃	〃	45
813	Nichirin-k.	B_{11}	Junrei-k.	〃	69
814	Shirasagi-k.	〃	〃	〃	62
815	Yutaka-k.	B_{10}	〃	〃	68
816	Norin 50	B_9	〃	〃	57
817	〃 52	〃	〃	〃	63
818	〃 72	B_{10}	〃	〃	70
819	Fujimi-k.	B_9	Nichirin-k.	B_5F_5	55
820	Junrei-k.	B_{11}	〃	〃	60
821	Shirasagi-k.	〃	〃	〃	54
822	Norin 52	B_9	〃	〃	49
823	〃 61	B_{10}	〃	〃	67
824	〃 72	〃	〃	〃	64
825	Kitakami-k.	B_8	Norin 29	〃	49
826	Nanbu-k.	B_{11}	〃	〃	85
827	Norin 75	B_{10}	〃	〃	33
828	Shirasagi-k.	B_{11}	Norin 68	〃	36
829	Yutaka-k.	B_{10}	〃	〃	44
830	Norin 50	B_9	〃	〃	38
831	〃 53	〃	〃	〃	41
832	〃 61	B_{10}	〃	〃	48
833	〃 72	〃	〃	〃	40
834	Kitakami-k.	B_8	Norin 75	B_7F_4	79
835	Nanbu-k.	B_{11}	〃	〃	77
836	Norin 29	B_{10}	〃	〃	90
837	Kokeshi-k.	〃	Norin 29	B_5F_5	12
838	Idaed	B_{11}	〃	〃	60
839	Kokeshi-k.	B_{10}	Norin 75	B_7F_4	30
840	Idaed	B_{11}	〃	〃	71
Check cultivar					
841	Junrei-k.				
842	Nichirin-k.				
843	Norin 61				
844	Haruhikari				

Norin 61 was used as the check. On the average, 15 F_1 hybrids showed one day delay of heading, 12 cm taller height, 15% increase of ear length, no difference on ear number/m², 35% yield reduction, 2% decrease of liter weight and 7% increase of 1,000 kernel weight.

Table 12. Agronomic characteristics of the F_1 hybrids tested in Kyoto University, 1978–1979

Entry	Plant height (cm)	Ear no./ 1m row	Freq. of offtypes (%)	Seed-set (%)	Yield (kg/10a)
F_1 hybrid					
824	104.8	149.5	7.3	95	470
820	102.0	149.5	14.8	93	374
802	103.0	129.5	14.3	94	362
833	104.0	124.0	39.8	89	362
801	105.8	133.0	22.8	93	353
808	109.3	110.5	23.5	91	322
818	113.3	133.5	17.0	90	300
821	97.8	111.5	21.0	90	300
816	112.5	135.0	33.8	92	274
803	102.0	101.5	7.0	93	256
819	91.0	105.5	14.8	87	240
804	107.8	105.5	25.0	92	238
811	108.0	108.0	33.3	85	230
831	105.8	128.5	49.0	95	227
822	112.5	118.0	40.0	89	222
823	94.5	103.5	17.0	82	216
830	103.0	115.5	41.8	91	216
829	96.8	125.0	53.0	81	211
812	108.8	114.0	46.0	86	204
828	98.8	92.5	34.8	92	203
817	121.5	124.5	54.3	79	196
815	114.8	117.0	53.8	81	195
805	102.3	90.5	15.3	89	186
813	107.3	88.0	42.3	91	173
806	107.0	93.0	59.5	95	169
814	105.8	95.5	56.0	85	162
809	110.3	91.0	49.8	81	152
810	109.8	119.0	66.0	79	141
832	109.0	98.0	45.5	80	140
807	94.5	66.0	23.8	88	138
Average	105.5	112.5	34.1	88.2	241
Check cultivar					
842	92.8	143.0	0.0	97	452
841	96.0	132.0	0.0	98	419
843	101.3	135.5	0.0	96	367
Average	96.7	136.8	0.0	97.1	413

The yield reduction must have been resulted from partial sterility, because other yield components did not differ from or were even better than those of the check cultivar. The yield of the F_1 hybrids observed in the Central Agricultural Experiment Station showed a high positive correlation to that observed in Kyoto University ($r=0.79$, $df=14$). Here, again, Entry 824 gave the best yield (411 kg/10 a) among all the F_1 hybrids tested, which was almost comparable to the yield of Norin 61 (420 kg/10a). In addition, this hybrid headed four days earlier than Norin 61. Though it showed a higher grade of lodging than the check due to taller height, the hybrid Norin 72 × Nichirin-komugi appeared to be interesting from the practical point of view (Maeda personal communication).

Ten F_1 hybrids of spring type were tested by spring sowing in an experimental field of

Table 13. Agronomic characteristics of the F_1 hybrids tested in Central Agricultural Experiment Station, 1978–1979 with collaboration of Dr. H. Maeda

Entry	Heading date	Culm length (cm)	Ear length (cm)	Ear no./m²	Yield (kg/10a)	Liter weight (g)	1000 kernel weight (g)
F_1 hybrid							
801	4.23	97	11.3	321	349	802	38.8
802	4.23	93	10.8	314	308	812	36.7
803	4.23	93	11.7	343	309	805	36.6
804	4.23	101	11.7	362	267	804	39.0
805	4.22	92	12.2	350	325	817	41.1
806	4.24	99	11.6	293	224	790	46.4
810	4.23	100	11.1	300	139	795	39.9
813	4.23	96	11.2	340	217	804	36.5
815	4.24	106	10.8	343	158	805	39.3
818	4.18	103	10.7	384	308	787	38.7
823	4.22	91	10.8	352	334	808	40.4
824	4.17	99	10.7	372	411	782	33.1
829	4.24	101	10.8	394	210	804	35.7
832	4.23	93	11.3	330	218	803	39.6
833	4.19	91	11.1	341	298	798	38.4
Average	4.22	97	11.2	343	272	801	38.7
Check cultivar							
843	4.21	85	9.7	345	420	819	36.2

Table 14. Agronomic characteristics of the F_1 hybrids tested at Tanno Nokyo, Hokkaido, in 1978 with collaboration of Mr. Y. Sakai and Mr. Y. Nishimura

Entry	Heading date (July)	Maturity (August)	Plant height (cm)	Ear length (cm)	Seed-set (%)	Yield (kg/10a)	Relative yield	1000 kernel wt. (g)
F_1 hybrid								
825	8	14	97.8	8.0	32.3	39	19	36.8
826	8	15	84.7	8.0	13.2	35	17	40.5
827	8	12	96.6	7.1	19.9	46	22	41.2
828	6	11	93.6	7.2	45.2	139	67	38.8
829	5	11	83.1	7.4	34.8	83	40	43.9
837	5	11	89.1	7.9	27.6	89	43	40.5
838	3	12	72.8	6.8	26.4	69	33	42.0
839	2	10	85.1	7.3	32.2	66	32	40.0
840	1	7	89.5	6.7	24.5	156	75	39.7
841	5	11	95.9	7.5	49.4	272	131	36.8
Check cultivar								
844	10	12	94.9	8.2	83.5	207	100	34.0

the Tanno Nokyo, Tanno Town, Hokkaido with collaboration of Mr. Y. Sakai and Mr. Y. Nishimura. In this case, each F_1 hybrid was grown in a single row with no replication. A spring type cultivar Haruhikari was used for check. The results obtained are shown in Table 14. Most of the F_1 hybrids showed better grass type, including uniform and early heading and increased ear number. However, all of them showed very poor anther development, and no pollen was shed in the field. The *Rf* 3 gene did not function at all as the fertility-restoring gene under spring sowing condition in Hokkaido. All grains set on the hybrids were produced by outcrossing with the check cultivar. Entry 840, i.e., Idaed (male sterile) × Norin 75 (restorer) F_1, of which row was located next to Haruhikari's row, outyielded 31% over Haruhikari. This fact indicates that the F_1 hybrid has great yield potential in the spring sowing area of Hokkaido. If some effective fertility-restoring gene can be found, the hybrid wheat will become most promising in this area among all other parts of Japan.

6. Summary

(i) Male sterile lines of 25 Japanese cultivars of common wheat were produced by transferring the *timopheevi* cytoplasm into them by repeated backcrosses (mostly ten times). All but two cultivars were converted to the completely male sterile by this cytoplasm.

(ii) Several sources of the fertility-restoring gene(s) to the *timopheevi* cytoplasm were discovered, of which the *Rf3* gene locating on the 1B chromosome of *T. spelta* was introduced into 21 Japanese cultivars by repeated backcrosses (mostly five times). Fertility restoration by this gene was incomplete even in its double dose.

(iii) More than 50% of florests of male sterile lines could be fertilized by outcrossing under a suitable arrangement of the male sterile and restorer lines. Among both of them, clear differences were observed on their outcrossing ability.

(iv) F_1 hybrids produced by open-pollination between the male sterile and restorer lines were tested on their agronomic characteristics in small scales in several locations in Japan. The best F_1 hybrid, Norin 72 (male sterile) × Nichirin-komugi (restorer) consistently showed high yield, which was totally comparable or even higher than that of the leading cultivar, Norin 61, with a few days earlier heading.

(v) The hybrid wheats having the *Rf3* gene for fertility restoration have no practical value in the spring-sowing area in Hokkaido due to inability of this gene to function properly under such condition. However, their yield potential seemed to be much higher in this area than in any other parts of Japan.

Acknowledgements

The present investigations were carried out with the collaboration of Dr. T. Hori, Dr. C.M. Tahir, Dr. Y. Nakai, and Mr. J. Fujigaki from time to time. Also, technical assistances of Mr. R. Matsuo, Mrs. M. Kawamura, Miss S. Yoshii, Miss M. Nagaoka and Mrs. N. Fujigaki were indispensable. Some parts of the investigations were carried out at the Central Agricultural Experiment Station, Konosu, and the Tanno Nokyo, Tanno Town, Hokkaido, with collaboration of Dr. H. Maeda, Mr. Y. Sakai and Mr. Y. Nishimura. The materials obtained from Dr. J.W. Schmidt, Agronomy Department, University of Nebraska, Dr. E.R. Sears, Agronomy Department, University of Missouri, and Dr. H. Fukasawa, Faculty of Science, Kobe University, were indispensable for the present investigations. The author wishes to express his sincerest gratitude to all of them.

The works were finanically supported by research grants from the Ministry of Agriculture, Forestry and Fishery to "Researches on breeding hybrid varieties of cereals utilizing cytoplasmic male sterility" from 1966 to 1972, and to "Researches on breeding hybrid wheat utilizing cytoplasmic male sterility and on its agronomic characteristics" from 1973 to 1977, by those from the Ministry of Education, Science and Culture to "Basic researches on hybrid wheat breeding" (Grant-in-Aid No. 60002 in 1971, and No. 660002 in 1972), and by those from the Nisshin Seifun Foundation to "Researches on breeding hybrid wheat" from 1977 to 1979. To all of them the author wishes to express his cordial thanks.

References

BAHL, P.N. and S.S. MAAN 1973. Chromosomal location of male fertility restoring genes in six lines of common wheat. Crop Sci. 13: 317–320.

FUJIGAKI, J. and K. TSUNEWAKI 1976. Basic studies on hybrid wheat breeding. VII. Characteristics of the male sterile lines of common wheat cultivars. Japan. J. Breed. 26: 179–186.

FUKASAWA, H. 1955. Studies on restoration and substitution of nucleus of *Aegilotricum*. II. The interrelationships between *ovata* cytoplasm and fertility restoring factors. Cytologia 20: 211–217.

KIHARA, H. 1951. Substitution of nucleus and its effects on genome manifestations. Cytologia 16: 177–193.

———, and K. TSUNEWAKI 1964. Some fundamental problems underlying the program for hybrid wheat breeding. Seiken Ziho 16: 1–14.

———, and ——— 1966. Basic studies on hybrid wheat breeding, carried out at the National Institute of Genetics. Seiken Ziho 18: 55–63.

MUKAI, Y. and K. TSUNEWAKI 1979. Basic studies on hybrid wheat breeding. VIII. A new male sterility-fertility restoration system in common wheat utilizing the cytoplasms of *Aegilops kotschyi* and *Ae. variabilis*. Theoret. Appl. Genet. 54: 153–160.

ROBERTS, T.H. JR. 1965. DeKalb's commitment to hybrid wheat. Rep. Wheat Quality Conf., Crop Quality Council, Minneapolis: 40–46.

SEARS, E.R. 1954. The aneuploids of common wheat. Res. Bull. Mo. Agr. Exp. Stat. No. 572, pp. 59.

TAHIR, C.M. 1970. Distribution of fertility-restoring genes in hexaploid wheats for *Ae. ovata* and *T. timopheevi* cytoplasms. I. Japan. J. Breed. 20: 1–6.

———, and K. TSUNEWAKI 1969. Monosomic analysis of *Triticum spelta* var. *duhamelianum*, a fertility restorer for *T. timopheevi* cytoplasm. Japan. J. Genet. 44: 1–9.

———, and ——— 1971a. Monosomic analysis of fertility restoring genes in *Triticum aestivum* strain P168. Can. J. Genet. Cytol. 13: 14–19.

———, and ——— 1971b. Distribution of fertility-restoring genes in hexaploid wheats for *Ae. ovata* and *T. timopheevi* cytoplasms. II. Japan. J. Breed. 21: 49–51.

TSUNEWAKI, K. 1970. Basic studies on hybrid wheat breeding. III. Heterosis in F_1 hybrid between Japanese and U.S.A. varieties. Japan. J. Breed. 20: 69–74.

——— 1974. Monosomic analysis of two restorers to *Ae. caudata* and *Ae. umbellulata* cytoplasms. Japan. J. Genet. 49: 425–433.

———, Y. MUKAI and T.R. ENDO 1978. On the descent of the cytoplasms of polyploid species in *Triticum* and *Aegilops*. Proc. V Int. Wheat Genet. Symp. 1: 261–272.

WILSON, J.A. 1968. Hybrid wheat developments with *Triticum timopheevi* Zhuk. derivatives. Proc. III Int. Wheat Genet. Symp.: 423–430.

———, and W.M. ROSS 1962. Male-sterility interaction of the *Triticum aestivum* nucleus and *Triticum timopheevi* cytoplasm. Wheat Inf. Serv. 14: 29–30.

YEN, F.S., L.E. EVANS and E.N. LARTER 1969. Monosomic analysis of fertility restoration in three restorer lines of wheat. Can. J. Genet. Cytol. 11: 531–546.