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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**

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Summary. The nuclei of 12 common wheats (genome constitution *AABBDD*) were placed into the cytoplasms of *Aegilops kotschyi* and *Ae. variabilis* (both $C^uC^uS^vS^v$) by repeated backcrosses. Using these nucleus-cytoplasm hybrids, male sterility-fertility restoration relationship was investigated. Male sterility was expressed by these cytoplasms only in Slm, Splt and Mch. The other nine common wheat nuclei gave normal fertility against these cytoplasms. These cytoplasms were compared with the *Triticum timopheevi* cytoplasm that is now widely used in the hybrid wheat breeding program in order to investigate their effects on important agronomic traits of the 12 common wheats: The *kotschyi* and *variabilis* cytoplasms were as good as the *timopheevi* cytoplasm in this respect.

The F_1 hybrid between (*kotschyi*)- or (*variabilis*)-Splt and CS showed normal fertility. Segregation of the fertiles and steriles in their F_2 generations followed the simple Mendelian fashion, i.e., 3 fertile : 1 sterile. Thus, the fertility restoration in this case is mainly controlled by a single dominant gene which will be designated as *Rfv1*. To determine its location, ditelo-lBS and -lBL of CS were crossed as male parents to male sterile (*kotschyi*)- and (*variabilis*)-Splt. The F_1 hybrids between the male sterile Splt's and CS ditelo-lBS became male fertile, while those between the male sterile Splt's and CS ditelo-lBL became completely male sterile. Thus, the location of the gene *Rfv1* has been determined to be on the short arm of chromosome lB of CS. Furthermore, a close relationship between the fertility-restoring genes and the nucleolus organizer region was pointed out.

Finally, the schemes of breeding the male sterile lines of a cultivar with these cytoplasms, and its maintainer line were formulated. The following two points were considered as the advantages of the present male sterility-fertility restoration system over that using the *timopheevi* cytoplasm in breeding hybrid wheat: (1) easier fertility restoration in F_1 hybrids, and (2) no need of breeding the restorer line.

Key words: *Kotschyi-variabilis* cytoplasms – Hybrid wheat – Male sterility – Fertility restoration

Introduction

We have been carrying out a series of investigations on the genetic diversity of the cytoplasm in *Triticum* and *Aegilops* in order to clarify the phylogenetic relationships among the cytoplasms of diploid and polyploid species, and to discover the male sterility-fertility restoration system usable in hybrid wheat breeding (Endo and Tsunewaki 1975; Tsunewaki et al. 1978). In the course of these investigations, we found that the cytoplasms of *Ae. kotschyi* and *Ae. variabilis* cause male sterility in three of twelve common wheats tested (Mukai and Tsunewaki 1975; Tsunewaki et al. in press). In the present investigation, (i) genetic characteristics of these two cytoplasms were compared with those of Timopheevi wheats, and (ii) fertility restorations of these cytoplasms were screened with one of which gene analysis was carried out. These results will be described in this article and advantages of this new male sterility-fertility restoration system over the widely used *timopheevi* system will be discussed.

Materials and Methods

The following species were used in the present investigation as the nucleus and cytoplasm donors to the nucleus-cytoplasm hybrids (hereafter, called NC hybrids): twelve common wheats (2n = 42, genome constitution *AABBDD*), i.e., *Triticum aestivum* var. 'erythrospermum' (abbreviation, Tve), strain P168 (P168), cv. 'Chinese Spring' (CS), cv. 'Norin 26' (N26), strain Salmon (Slm), cv. 'Jones

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Fife' (JF), cv. 'Selkirk' (Sk), and cv. 'S-615' (S615), *T. sphaerococcum* var. 'rotundatum' (Sphr), *T. compactum* cv. 'No. 44' (Cmp), *T. spelta* var. 'duhamelianum' (Splt), and *T. macha* var. 'subletschchumicum' (Mch) as the nucleus donors, and four tetraploid species (2n = 28), i.e., *Ae. kotschyi* strain No. 2, *Ae. variabilis* strain No. 1 (both $C^uC^uS^vS^v$), *T. timopheevi* var. 'typicum', and *T. dicoccoides* var. 'nudiglumis' (both *AAGG*) as the cytoplasm donors. The NC hybrids were produced, as shown in Table 1, by repeated backcrosses of the cytoplasm donors with the nucleus donors as the recurrent pollen parent. Each NC hybrid is indicated by the name of its cytoplasm donor in parentheses hyphenated with the name of its nucleus donor. The number of backcrosses, including the initial cross, is given by a superscript to the name of the nucleus donor, when necessary.

The (*timopheevi*)-Sk was originally produced by Dr. J.W. Schmidt, University of Nebraska, USA. The initial crosses between *Ae. kotschyi* or *Ae. variabilis* and *T. aestivum* were made by Dr. S. Sakamoto, the Plant Germ-plasm Institute, Kyoto University, Japan. *T. aestivum* strain Slm is a hexaploid derivative of octoploid *Triticale*, with almost the normal chromosome complement of common wheat (Tsunewaki 1964). However, two chromosomes (1B and 2B) of Slm differ structurally from those of common wheat; the satellited arm, including the nucleolus-organizing region of the 1B chromosome, is replaced by an arm of the 1R chromosome of rye (Tsunewaki 1964; Zeller 1973), and chromosome 2B lacks the *W1* locus for wax production and the *Rfu2* locus for fertility-restoration against the *Ae. umbellulata* cytoplasm (Tsunewaki 1974). Two aneuploid derivatives of CS, ditelo-lBL and -lBS (2n = 20'' + t''), which lack the short and long arm of chromosome 1B, respectively, were supplied by Dr. E.R. Sears, University of Missouri, USA, and have been maintained in our laboratory by cytological checking.

To estimate the male fertility-restoring ability against the four male sterile cytoplasms, the following three characters were investigated: selfed and open-pollinated seed fertilities (per cent seed set in the first and second florets of ears bagged before flowering and of open-pollinated ears, respectively), and pollen fertility (percentage of pollen grains with one vegetative and two wedge-shaped sperm nuclei).

In order to estimate the effects of the male sterile cytoplasms on some main agronomic characters, the NC hybrids were planted in the experimental field in a split-plot design with four replications, and arranged to have twelve nuclei in the main plots, and four cytoplasms in the subplots. Data were taken from two plants in each subplot. The following seven characters were investigated: heading date (the day of emergence of the earliest ear in each plant, April 20th = 0), plant height (height of the highest stem from its base to ear tip), ear number per plant, dry matter weight (weight of air-dried plant), ear length, number of spikelets per ear, and crossed seed fertility.

Results

1 Effects of the Cytoplasms of Ae. Kotschyi and Ae. Variabilis on Fertility and Other Agronomic Characters of Common Wheat

Ears of the four cytoplasm donors, and those of NC hybrids of CS and Splt are shown in Figure 1. The selfed seed fertilities of the twelve NC hybrids of each male sterile cytoplasm and those of the normal lines of the nucleus donors are shown in Figure 2, in the form of the fertility spectrum. The spectra of two cytoplasms in each of the following two pairs, the *kotschyi* and *variabilis* cytoplasms, and the 'nudiglumis' and *timopheevi* cytoplasms, were similar to each other. Nine of the twelve NC hybrids with each of the *kotschyi* and *variabilis* cytoplasms showed normal fertility. However, these cytoplasms caused complete male sterility in Slm, Splt and Mch. On the other hand, the NC hybrids with the 'nudiglumis' and *timopheevi* cytoplasms were all male sterile except in Splt and Mch. Splt and Mch showed remarkable restoration of fertility against these two cytoplasms. Except for the Slm's nucleus, the fertility spectra of the *kotschyi* and *variabilis* cytoplasms were totally complementary with those of the 'nudiglumis' and *timopheevi* cytoplasms, i.e., the nuclei showing normal male fertility against the first two cytoplasms produced completely male sterile plants with the last two cytoplasms while the nuclei producing male sterile plants in the former produced normal male fertile plants in the latter.

Average performances on the seven agronomic characters of the normal lines of twelve common wheats and their NC hybrids are summarized in Table 2. The *kotschyi* cytoplasm did not influence any agronomic characters except dry matter weight, which was reduced about 12%. The *variabilis* cytoplasm affected three characters; plant height, ear number and dry matter weight, being reduced 4, 18 and 26%, respectively. Two Timopheevi type cytoplasms, i.e., of the *timopheevi* and 'nudiglumis' cytoplasms, also, affected a few characters; the former delayed heading 1.4 days and reduced plant height 4%, and the latter reduced plant height and crossed seed fertility 4 and 8%, respectively. Therefore, it is safely concluded that the *kotschyi* and *variabilis* cytoplasms are as good as the 'nu-

Table 1. The backcross generations of nucleus-cytoplasm hybrids used in the present investigation; the NC hybrids are indicated by the names of their nucleus and cytoplasm donors

Nucleus donor (ABD)	Cytoplasm donor *kotschyi* (C^uS^v)	*variabilis* (C^uS^v)	*'nudiglumis'* (AG)	*timopheevi* (AG)
Tve	4	4	5	10
P168	4	4	6	5
CS	8	8	5	9
N26	5	8	5	9
Slm	5	5	3	12
JF	4	4	5	7
Sk	7	5	5	14
S615	4	4	4	6
Sphr	4	4	5	9
Cmp	4	4	5	11
Splt	4	4	6	12
Mch	3	3	6	12

( ) = Genome constitution of the gamete

Table 2. Performances of the twelve common wheats and their NC hybrids on seven agronomic characters

Character Cytoplasm	Nucleus												
	Tve	P168	CS	N26	Slm	JF	Sk	S615	Sphr	Cmp	Splt	Mch	Mean
Heading date (April 20 = 0)													
aestivum	30	33	16	7	26	34	24	24	11	29	33	37	25.3
kotschyi	32	36	16	4	30	34	26	24	11	27	34	34	25.6
variabilis	31	35	17	6	29	34	24	26	9	29	31	35	25.5
'nudiglumis'	33	27	17	5	31	35	24	26	12	28	33	38	25.8
timopheevi	35	36	17	4	31	34	25	26	11	29	34	38	26.7[a]
Plant height (cm)													
aestivum	145	136	111	78	112	124	118	122	67	103	123	109	112.3
kotschyi	138	123	112	81	111	128	113	126	66	113	120	101	111.0
variabilis	137	124	110	75	103	128	115	124	64	98	112	105	107.9[b]
'nudiglumis'	137	126	102	75	103	122	114	116	63	102	124	109	107.8[b]
timopheevi	128	125	101	79	99	124	120	117	58	105	123	111	107.5[b]
Ear number													
aestivum	28	22	27	16	31	21	21	29	11	16	36	45	25.3
kotschyi	27	17	26	15	34	22	21	29	12	22	23	42	24.5
variabilis	24	16	19	13	26	22	19	22	7	17	21	42	20.7[b]
'nudiglumis'	28	28	27	17	32	22	24	28	13	16	35	41	25.9
timopheevi	19	20	23	18	36	25	29	27	8	19	38	47	25.3
Dry matter weight (g)													
aestivum	123	95	102	50	126	95	63	122	22	70	115	93	89.7
kotschyi	86	65	97	40	113	107	43	135	27	82	81	71	78.9[a]
variabilis	90	58	69	30	79	114	55	93	13	53	69	76	66.6[b]
'nudiglumis'	123	132	92	44	123	112	69	122	22	64	121	85	92.4
timopheevi	99	81	74	52	138	117	96	106	10	70	118	98	88.3
Ear length (cm)													
aestivum	16	17	9	9	11	14	12	12	5	6	–	–	11.1
kotschyi	16	16	9	9	12	13	11	13	5	6	–	–	11.0
variabilis	18	18	9	9	11	12	12	12	5	6	–	–	11.2
'nudiglumis'	18	16	9	9	12	14	13	12	6	6	–	–	11.5
timopheevi	15	15	9	9	13	15	13	13	6	6	–	–	11.4
No. of spikelets/ear													
aestivum	24	24	25	17	26	28	22	20	15	23	–	–	22.4
kotschyi	24	23	24	17	24	27	22	20	19	25	–	–	22.5
variabilis	24	24	24	17	25	26	21	22	12	24	–	–	21.9
'nudiglumis'	24	25	25	17	24	28	23	21	15	23	–	–	22.5
timopheevi	23	24	25	17	25	27	23	21	15	24	–	–	22.4
Crossed seed fertility (%)													
aestivum	91	87	92	99	77	88	85	88	83	89	90	84	87.8
kotschyi	81	82	98	86	76	92	92	88	65	90	92	81	85.3
variabilis	88	86	97	86	56	94	95	87	85	95	85	79	86.1
'nudiglumis'	87	94	68	76	57	85	88	73	74	92	88	83	80.4b
timopheevi	73	82	94	79	76	90	80	90	81	86	87	92	84.2

[a] and [b] significantly different from the normal line at the 5% and 1% level, respectively

diglumis' and *timopheevi* cytoplasms with respect to their over-all effects on main agronomic characters of common wheat.

2 Fertility Restoration against the Kotschyi and Variabilis Cytoplasms and its Gene Analysis

At the fourth backcross generation, (*kotschyi*)- and (*variabilis*)-CS, which showed the meiotic configuration of 21'' and had normal selfed seed fertilities (99% and 89%, respectively), were crossed with Splt's pollen. The F_1 plants became male fertile (selfed seed fertility was 82 and 90%, respectively), as shown in Table 3. However, the B_1 plants from the crosses of these F_1's with Splt's pollen became almost completely male sterile. In the B_3 and later backcross generations, they became completely male sterile. This result indicates that Splt carries the recessive male sterile gene(s) to these cytoplasms.

F_1 hybrids between (*kotschyi*)- or (*variabilis*)-Splt^4

and CS (pollen parent) restored normal fertility. Segregation of fertile and sterile plants in the F_2 generation was studied; the data are given in Table 4. Taking the breaking point between the fertile and sterile classes at 10%, fertiles and steriles were segregated in a 3 (fertile) : 1 (sterile) ratio. So, the fertility restoration by CS against both the *kotschyi* and *variabilis* cytoplasms is mainly controlled by a single dominant gene. However, some plants of the sterile class showed partial, though very low, fertility. In addition, B_1 and B_2 plants of (*kotschyi*)- or (*variabilis*)-CS

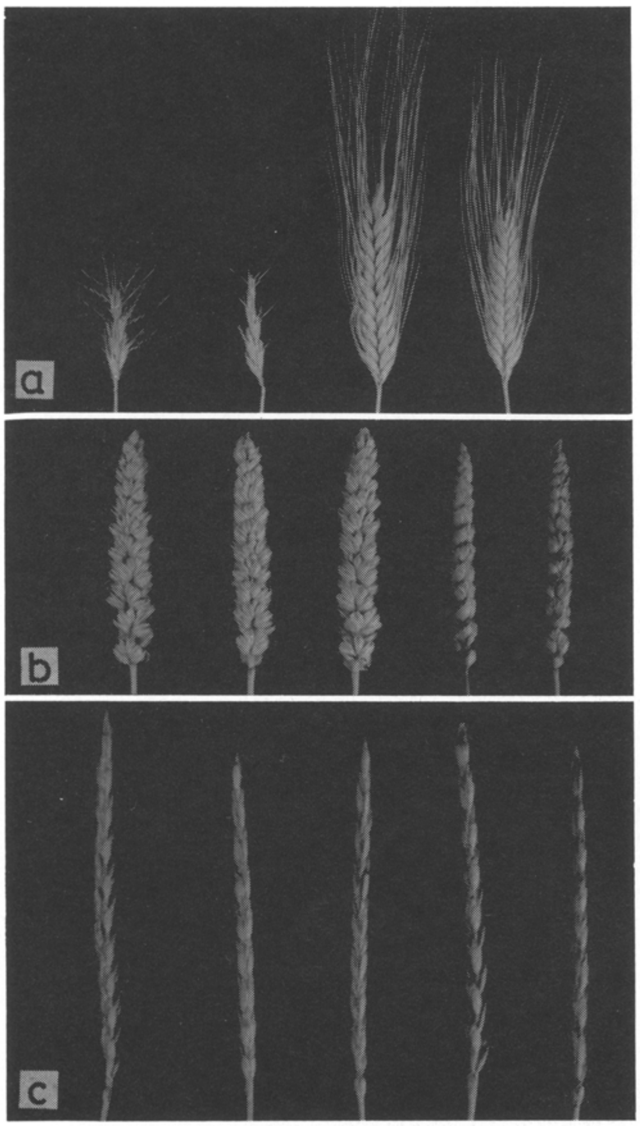

Fig. 1a-c Ears of the cytoplasm donors and their nucleus substitution lines. a Four cytoplasm donors (left to right): *kotschyi, variabilis,* 'nudiglumis' and *timopheevi,* b A nucleus donor, *T. aestivum* cv. 'Chinese Spring', and its NC hybrids with the cytoplasms of *kotschyi, variabilis,* 'nudiglumis' and *timopheevi* (left to right), c A nucleus donor, *T. spelta* and its NC hybrids with the cytoplasms of *kotschyi, variabilis,* 'nudiglumis' and *timopheevi* (left to right)

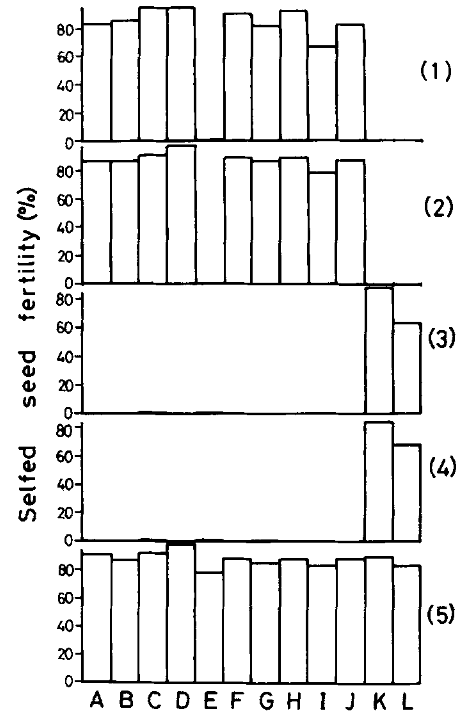

Fig. 2. Fertility spectra of five cytoplasms tested with the twelve tester nuclei. (1)-(5): The cytoplasm of *kotschyi, variabilis,* 'nudiglumis', *timopheevi* and *aestivum,* respectively, A-L: Twelve tester nuclei – Tve, P168, CS, N26, Slm, JF, Sk, S615, Sphr, Cmp, Splt and Mch (left to right)

× $Splt^2$ or × $Splt^3$ showed some selfed seed fertilities as shown in Table 3. These facts indicate that some minor genes are also involved in fertility restoration.

In order to locate the major gene for fertility restoration on a specific chromosome and its arm, male sterile Splt's with these cytoplasms were crossed with the pollen

Table 3. Changes on the selfed seed fertility of Splt's with the *kotschyi* and *variabilis* cytoplasms through successive backcrosses

Material		Fertility (%)	Place	Year
(*kotschyi*) – CS^4	× Splt	82	Field, Kyoto	1974
,,	× $Splt^2$	5	,,	1975
,,	× $Splt^3$	5	,,	1976
,,	× $Splt^4$	0	Greenhouse, Kyoto	,,
,,	× $Splt^5$	0	Field, Kyoto	1977
,,	× $Splt^6$	0	Field, Osaka	1978
,,	× $Splt^7$	0	,,	,,
(*variabilis*) – CS^4	× Splt	90	Field, Kyoto	1974
,,	× $Splt^2$	14	,,	1975
,,	× $Splt^3$	6	,,	1976
,,	× $Splt^4$	0	Greenhouse, Kyoto	,,
,,	× $Splt^5$	0	Field, Kyoto	1977
,,	× $Splt^6$	0	Field, Osaka	1978
,,	× $Splt^7$	0	,,	,,

Table 4. Segregation of the fertile and sterile plants in the F_1 and F_2 generations of the crosses, (*kotschyi*) – Splt × CS and (*variabilis*) – Splt × CS

Material	No. of plants			(%)	x^2–value
	Total	Fertile	Sterile	Sterile	(3 : 1)
(*kotschyi*) – $Splt^4$ × CS F_1	20	20	0	0	–
,, F_2	155	120	35	23	0.48
(*variabilis*) – $Splt^4$ × CS F_1	20	20	0	0	–
,, F_2	199	154	45	23	0.60

Table 5. Pollen and selfed seed fertilities of the F_1 hybrids between (*kotschyi*) – and (*variabilis*) – Splt (♀) and disomics or ditelo–IBS and –IBL of CS (♂)

Material		Pollen fertility (%)	Selfed seed fertility (%)
(*kotschyi*) – $Splt^4$		0.0	0.0
,,	× CS F_1	96.5	87.5
,,	× CS ditelo–1BS F_1	75.0	72.2
,,	× CS ditelo–1BL F_1	9.3	0.0
(*variabilis*) – $Splt^4$		0.0	0.0
,,	× CS F_1	91.4	86.4
,,	× CS ditelo–1BS F_1	71.4	48.5
,,	× CS ditelo–1BL F_1	11.2	0.0

Table 6. Selfed and open-pollinated seed fertilities of the F_1 hybrids between male sterile lines with the *kotschyi* or *variabilis* cytoplasm and common wheat cultivars

F_1 hybrids (♀ x ♂)			Seed fertility (%)	
			Selfed	Open-pollinated
(*kotschyi*) – Splt	× Tve	F_1	81	80
,,	× P168	,,	84	86
,,	× CS	,,	87	90
,,	× JF	,,	90	92
,,	× Sk	,,	84	93
,,	× S615	,,	82	78
,,	× Sphr	,,	76	80
,,	× Cmp	,,	85	91
611-15	× CS	,,	89	88
,,	× Kitakami-komugi[a]	,,	69	83
,,	× Kokeshi-komugi[a]	,,	70	90
,,	× Nambu-komugi[a]	,,	55	62
,,	× Ushio-komugi[a]	,,	90	92
616-15	× CS	,,	86	92
,,	× Kitakami-komugi[a]	,,	62	96
,,	× Kokeshi-komugi[a]	,,	86	88
,,	× Nambu-komugi[a]	,,	98	96
,,	× Ushio-komugi[a]	,,	100	98

611-15 = An F_2 of (*kotschyi*) – Splt × CS (selfed seed fertility = 3%)
616-15 = An F_2 of (*variabilis*) – Splt × CS (selfed seed fertility = 7%)
[a]Japanese common wheat cultivars

of two ditelosomic lines of CS. Pollen and selfed seed fertilities of the F_1 hybrids are shown in Table 5. The F_1 hybrid, (*kotschyi*)-Splt × CS ditelo-lBS was fertile, while the F_1 hybrid, (*kotschyi*)-Splt × CS ditelo-lBL was sterile (Table 5, Fig. 3). The same results were obtained with the F_1 hybrids, (*variabilis*)-Splt × CS ditelosomics. These results show that the fertility restoration by the CS nucleus is mainly due to a single dominant gene located on the short arm of its chromosome lB. This gene will be represented by the symbol, *Rfv*1, which is the first identified fertility-restoring gene to the *variabilis* cytoplasm. Splt carries its recessive allele, *rfv*1 on the same arm of the lB chromosome.

As described above, Splt with the *kotschyi* or *variabilis* cytoplasm is completely male sterile. To test the restoration ability of other common wheat nuclei against these cytoplasms, they were crossed as the pollen parent to (*kotschyi*)- and (*variabilis*)-Splt, and selfed and open-pollinated fertilities of the F_1 hybrids were investigated; the results being given in Table 6.

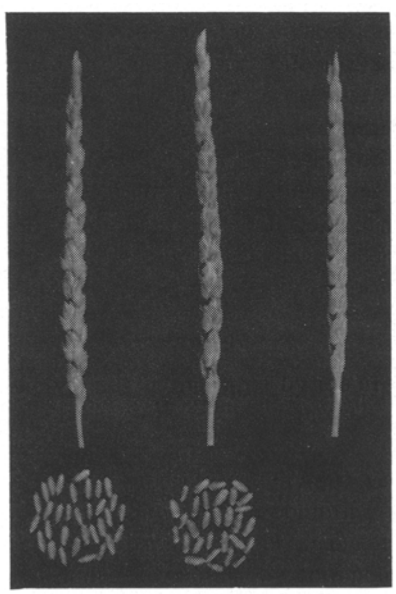

Fig. 3. Ears and seeds set on them in the F_1 hybrids between (*kotschyi*)-Splt (♀) and disomics, ditelo-1BS or-1BL of CS (♂) (left to right)

In the F_2 generation of the crosses, (*kotschyi*)-Splt × CS and (*variabilis*)-Splt × CS, one plant showing almost complete sterility was selected from each (611-15 and 616-15, respectively), and crossed with five common wheat cultivars. Selfed and open-pollinated seed fertilities of these F_1 hybrids are also given in Table 6. All these F_1 hybrids showed almost normal male fertility. Therefore, they are assumed to possess some dominant fertility-restoring gene(s) that might be the same as the *Rfv*1 of CS against the *kotschyi* and *variabilis* cytoplasms.

Discussion

1 Chromosomal Location of the Fertility-Restoring Genes against Various Male Sterile Cytoplasms

From monosomic analysis, Tahir and Tsunewaki (1969) found that a single dominant fertility-restoring gene (*Rf3*) against the *timopheevi* cytoplasm is located on chromosome lB of Splt. The present investigation revealed that the short arm of the same chromosome of CS carries a dominant fertility-restoring gene (*Rfv*1) against the *kotschyi* and *variabilis* cytoplasms. Two genes, *Rf3* and *Rfv*1 located on the same chromosome, lB, act in a completely opposite way, except for Slm, to two kinds of cytoplasms, as shown in Fig. 2. To know whether the two genes are allelic with each other, a further study is in progress.

Previous workers have determined chromosomal locations of fertility-restoring genes for various male sterile cytoplasms, i.e., Tahir and Tsunewaki (1969), Yen et al. (1969) and Bahl and Maan (1973) for *T. timopheevi* cytoplasm; Tahir and Tsunewaki (1971) for *Ae. ovata* cytoplasm, and Tsunewaki (1974) for *Ae. caudata* and *Ae. umbellulata* cytoplasms. According to the results of Tsunewaki et al. (1978), Tve's with C^u type plasmas, i.e., the *umbellulata, triuncialis, biuncialis, columnaris* and *triaristata* cytoplasms, were completely male sterile, while Pl68's with these cytoplasms were fertile. Pl68 is a hexaploid offspring of the F_1 hybrid, Tve × *Ae. caudata* (Kihara 1959), in which chromosome lD of Tve was replaced by *Ae. caudata* chromosome lC (Muramatsu 1959). Thus, we can conclude that chromosome lC carries a fertility-restoring gene(s) for the C^utype plasmas. All results reported till now are summarized in Table 7. So far 13 genes are located on ten chromosomes. Of those genes, six are carried by the chromosomes of homoeologous group 1, three by those of homoeologous group 6 and two by those

Table 7. Chromosomal locations of the fertility-restoring genes against various male sterile cytoplasms

Fertility-restoring gene			Male sterile cytoplasm	Reference
Chromosome	Gene	Carrier		
1A[a]	*Rf1*	R1, R2, R3, R4, R5	*T.timopheevi*	Bahl & Maan 1973
,,	,,	R–D, R–K	,,	Yen et al. 1969
1B[a]	*Rf3*	Splt	,,	Tahir & Tsunewaki 1969
,,	,,	Primepi	,,	Bahl & Maan 1973
,,	*Rful*	CS	*Ae.umbellulata*	Tsunewaki 1974
1BS[a]	*Rfvl*	,,	*Ae.kotschyi*	Present result
,,	,,	,,	*Ae.variabilis*	,,
1D	*Rfc3*	Cmp	*Ae.caudata*	Tsunewaki 1974
1C[a]	*Rfcl*	P168	,,	Tahir & Tsunewaki 1971
,,	,,	,,	*Ae.ovata*	,,
,,	*Rfcl (?)*	,,	*Ae.umbellulata*	Tsunewaki et al. 1978
,,	,,	,,	*Ae.triuncialis*	,,
,,	,,	,,	*Ae.biuncialis*	,,
,,	,,	,,	*Ae.columnaris*	,,
,,	,,	,,	*Ae.triaristata*	,,
2B	*Rfu2*	CS	*Ae.umbellulata*	Tsunewaki 1974
5D[a]	*Rf6*	Primepi	*T.timopheevi*	Bahl & Maan 1973
6B[a]	*Rf4*	R–C, R–K	,,	Yen et al. 1969
,,	,,	R2	,,	Bahl & Maan 1973
,,	*Rfc2*	Cmp	*Ae.caudata*	Tsunewaki 1974
6D	*Rf5*	R–C	*T.timopheevi*	Yen et al. 1969
7B	*Rf7*	R4	,,	Bahl & Maan 1973
7D	*Rf2*	R1, R2, R3, R4, R5	,,	,,
,,	,,	R–D	,,	Yen et al. 1969

[a] chromosomes carrying a nucleolar organizing region

Tabel 8. Fertility restoration ability of different types of restorer lines against three male sterile cytoplasms

Male sterile cytoplasm	Fertility-restoring gene	No. cultivars tested	Average seed fertility (%)	
			Selfed	Open-pollinated
timopheevi	*Rf3* heterozygous	21	55	80
,,	,, homozygous	18	71	83
,,	*Rf1*, *Rf2* and *Rf3* homozygous	24	91	93
kotschyi	*Rfv1* heterozygous	13	80	85
variabilis	,, ,,	5	86	94

of homoeologous group 7. These facts seem to indicate that at least some of them have a common origin, as already suggested by Tsunewaki (1970).

Tsunewaki (1974), also proposed that the nucleolus-organizing region, or the nucleolus itself, might play an important role in fertility restoration. Common wheat has four pairs of satellited chromosomes, two prominent (chromosome 1B and 6B) and two faint (1A and 5D), among the 21 pairs of chromosomes. Eight of the 13 known restoring genes are located on the chromosomes having the nucleolar organizer. The incidence of those genes to the satellited chromosomes is significantly higher than that expected by random distribution. The present result that the *Rfv*1 gene is located on the short arm having a nucleolar organizer of chromosome 1B further supports the postulated relationship between the fertility-restoring gene and the nucleolus organizing region. In *Nicotiana*, the same relationship was pointed out by Gerstel et al. (1978). *Nicotiana tabacum* with the cytoplasm of *N. repanda* showed male sterility. Introduction of a satellited fragment chromosome from *N. repanda* restored pollen fertility of the male sterile *N. tabacum*. Thus, formation of nucleoli by the organizers of *N. repanda* in *N. repanda* cytoplasm has been suspected to be a necessary condition for fertility restoration: both their and our results point to a close relationship between the nucleolar organizer and fertility restoration.

2 *Utilization of the New Male Sterility-Fertility Restoration System for Hybrid Wheat Breeding*

The male sterility-fertility restoration system widely used at present in the hybrid wheat breeding consists of the *timopheevi* cytoplasm and fertility-restoring genes, *Rf*1 to *Rf*7 against this cytoplasm. However, complete and stable fertility restoration is not always assured by this system, which is the critical junction for the success of hybrid wheat.

As shown in Table 2, the *kotschyi* and *variabilis* cytoplasms gave almost no deleterious effects on important agronomic characters of wheat. In this respect, they are completely equivalent to the *timopheevi* cytoplasm, though the *kotschyi* cytoplasm appears slightly better than the *variabilis* cytoplasm. As described in the previous section, the F_1 hybrids between male sterile lines of common wheat with the *kotschyi* or *variabilis* cytoplasm and various cultivars showed almost normal fertility. Average selfed seed fertilities of these F_1 hybrids were compared with those of three kinds of fertility restorer lines with the *timopheevi* cytoplasm, as shown in Table 8. The seed fertilities of the F_1 hybrids having the *Rfv*1 gene in the heterozygous condition were almost the same as those of the restorer lines homozygous for the three *Rf* genes against the *timopheevi* cytoplasm. From these results, we may conclude that the restoration ability of a single *Rfv*1 gene dose against the *kotschyi* or *variabilis* cytoplasm is higher than that of two *Rf*3 gene doses against the *timopheevi* cytoplasm, being almost the same as that of three *Rf* genes in the homozygous condition in the latter cytoplasm. The occurrence of these easier fertility restorations in the *kotschyi* and *variabilis* cytoplasms rather than in the *timopheevi* cytoplasm is seen in the fact that nine of the twelve tester nuclei became fully fertile with both the *kotschyi* and *variabilis* cytoplasms, while only two of them became fertile with the *timopheevi* cytoplasm; namely, a weaker sterilization effect of the former than that of the latter. Since selection of plants having the multiple fertility-restoring genes is difficult, this new system consisting of the *Rfv*1 gene and the *kotschyi* or *variabilis* cytoplasm seems to have a great advantage in hybrid wheat breeding over the system utilizing the *timopheevi* cytoplasm.

As described above, most common wheat cultivars so far tested serve as the restorer line. Therefore, in the new system, male sterile and maintainer lines must be bred, instead of the male sterile and restorer lines necessary in the present system utilizing the *timopheevi* cytoplasm.

Methods for breeding the male sterile line with, for example, the *kotschyi* cytoplasm and its maintainer line are proposed as shown in Figure 4. First, male sterile (*kotschyi*)-Splt is crossed to a cultivar 'A'. The F_1 hybrid is successively backcrossed several times with pollen of the

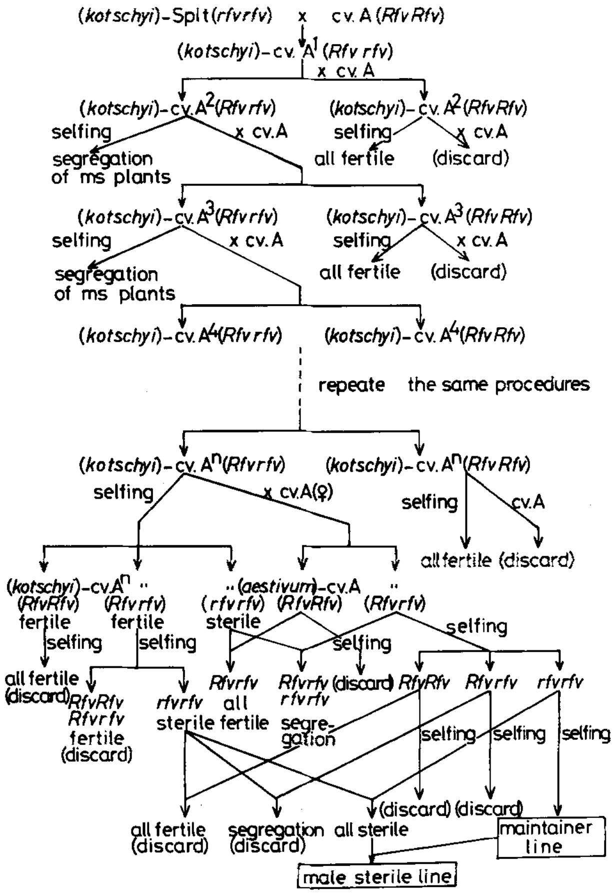

Fig. 4. The schemes of breeding a male sterile line with the *kotschyi* cytoplasm, and its maintainer line of a cultivar 'A'

same cultivar. In each backcross generation, selfing of the backcrossed female parent should be made to test their genotype; only the progenies of *Rfv*1*rfv*1 type parents are used for further backcrossing. After a sufficient number of backcrosses have been made, the male sterile line will be established by selfing the (*kotschyi*)-*Rfv*1*rfv*1 type plants.

To breed its maintainer line, the cultivar 'A' is crossed as female parent to (*kotschyi*)-cv. 'Aⁿ', which has the nuclear genotype *Rfv*1*rfv*1. In the next generation, two types of plants, i.e., (*eastivum*)-*Rfv*1*Rfv*1 and -*Rfv*1*rfv*1, segregate. By testcrossing them to (*kotschyi*)-*rfv*1*rfv*1, the latter can be selected. Its selfed offspring give only fertile plants, though three genotypes, (*aestivum*)-*Rfv*1*Rfv*1, -*Rfv*1*rfv*1 and -*rfv*1*rfv*1, segregate. Again, they must be testcrossed to (*kotschyi*)-*rfv*1*rfv*1 after which the plants of the genotype (*aestivum*)-*rfv*1*rfv*1 can be selected; its selfed offspring is used as the maintainer line. Breeding procedures for the male sterile and maintainer lines are common and almost the same. Only in the last part where the *rfv*1 gene is transferred from the *kotschyi* to *aestivum* cytoplasm for the production of the maintainer line is the breeding procedure different; this is another advantage of the present male sterility-fertility restoration system utilizing the *kotschyi* or *variabilis* cytoplasm. This system is similar in its essential features to that proposed by Franckowiak et al. (1976), who tried to utilize the cytoplasm of *Ae. squarrosa*. However, no male sterile genes which function specifically in the *squarrosa* cytoplasm, have yet been found nor induced artificially. On the contrary, we already have a male sterile gene, *rfv*1 that specifically interacts with the *kotschyi* and *variabilis* cytoplasms.

Literature

Bahl, P.N.; Maan, S.S.: Chromosomal location of male-fertility restoring genes in six lines of common wheat. Crop Sci. **13**, 317-320 (1973)

Endo, T.R.; Tsunewaki, K.: Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. On the origin of the cytoplasm of *Aegilops triuncialis* L. Seiken Ziho **25-26**, 55-66 (1975)

Franckowiak, J.D.; Maan, S.S.; Williams, N.D.: A proposal for hybrid wheat utilizing *Aegilops squarrosa* L. cytoplasm. Crop Sci. **16**, 725-728 (1976)

Gerstel, D.U.; Burns, J.A.; Burk, L.G.: Cytoplasmic male sterility in *Nicotiana*, restoration of fertility and the nucleolus. Genetics 89, 157-169 (1978)

Kihara, H.: Fertility and morphological variation in the substitution backcrosses of the hybrid *Triticum vulgare* × *Aegilops caudata*. Proc. X. Int. Congr. Genet. **1**, 142-171 (1959)

Mukai, Y.; Tsunewaki, K.: Genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. II. Comparison of the cytoplasms between four 4x *Aegilops* polyeides species and their 2x relatives. Seiken Ziho **25-26** 67-78 (1975)

Muramatsu, M.: Homology of chromosomes of *Aegilops caudata* with common wheat. Wheat Inf. Serv. **9-10**, 32-33 (1959)

Tahir, Ch.M.; Tsunewaki, K.: Monosomic analysis of *Triticum spelta* var. *duhamelianum*, a fertility restorer for *T. timopheevi* cytoplasm. Jap. J. Genet. **44**, 1-9 (1969)

Tahir, Ch.M.; Tsunewaki, K.: Monosomic analysis of fertility restoring genes in *Triticum aestivum* strain P168. Can. J. Genet. Cytol. **13**, 14-19 (1971)

Tsunewaki, K.: Genetic studies of a 6x-derivative from an 8x *Triticale*. Can. J. Genet. Cytol. **6**, 1-11 (1964)

Tsunewaki, K.: Homoancestral genes in relation to parallel variations in wheat and *Aegilops*. Seiken Ziho **22**, 77-81 (1970)

Tsunewaki, K.: Monosomic analysis of two restorers to *Ae. caudata* and *Ae. umbellulata* cytoplasms. Jap. J. Genet. **49**, 425-433 (1974)

Tsunewaki, K.; Mukai, Y.; Endo, T.R.: On the descent of the cytoplasms of polyploid species in *Triticum* and *Aegilops*. Proc. V. Int. Wheat Genet. Symp. (in press)

Yen, F.S.; Evans, L.E.; Larter, E.N.: Monosomic analysis of fertility restoration in three restorer lines of wheat. Can. J. Genet. Cytol. **11**, 531-546 (1969)

Zeller, F.J.: 1B/1R wheat-rye chromosome substitutions and translocations. Proc. IV. Int. Wheat Genet. Symp. 209-221 (1973)

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