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Production and characterization of alloplasmic lines of a triticale 'Rosner'*

K. Tsunewaki, M. Iwanaga**, M. Maekawa and S. Tsuji
Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606, Japan

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Summary. The transfer of cytoplasms of various *Triticum* and *Aegilops* species to a hexaploid triticale ('Rosner') has been attempted using 30 alloplasmic lines and a euplasmic line of common wheat as cytoplasmic donors. The average rate of F_1 hybrid production (seed setting rate × germination rate) following an ordinary method of crossing is only 0.09%, whereas this rate is increased to 3.1% by use of embryo culture. The first backcross of the F_1 plants with triticale pollen is again difficult, the hybrid production being 0.9%. Further backcrosses proceed smoothly in most cases. As a consequence, the following seven cytoplasms have been transferred to triticale: *T. dicoccum, T. aestivum, Ae. squarrosa, Ae. cylindrica, Ae. juvenalis, Ae. ovata* and *Ae. speltoides.* None of these alien cytoplasms causes more meiotic instability than does the triticale's own cytoplasm. Two cytoplasms of *T. dicoccum* and *T. aestivum,* both belonging to the B plasma type, have no effect upon any of triticale's characters. Two D type cytoplasms of *Ae. squarrosa* and *Ae. cylindrica* cause about 50% reduction of male fertility but exert no other remarkable effects. This fact suggests a partial functional compensation of the effect of a 1D chromosome upon interacting with D cytoplasm by a rye chromosome substituting for it in triticale. A D^2 cytoplasm of *Ae. juvenalis* causes earlier heading and complete male sterility, accompanied by some reduction of growth vigor. An M^0 type cytoplasm of *Ae. ovata* and an S type cytoplasm of *Ae. speltoides* cause a great heading delay, complete male sterility, and severe reduction of vigor. From the viewpoint of triticale breeding, none of these cytoplasms appears superior to the triticale's own cytoplasm. However, from the viewpoint of genetics, the hexaploid triticale is an effective tester for differentiating the B, S, and D plasma types.

Key words: Alloplasmic triticale – Male-sterile triticale – Wheat cytoplasm – *Aegilops* cytoplasm – Cytoplasmic relationship

Introduction

Triticale is a new cereal crop synthesized primarily from emmer wheat ($2n=28$, genome formula AABB) as female and rye ($2n=14$, RR). For continued plant improvement, it is necessary to locate new cytoplasms that collaborate better with triticale nuclei than does the emmer cytoplasm. Since rye is out-breeding, the production of hybrid varieties certainly will be among the strategies used to improve triticale, and in order to produce hybrids, it is necessary to find a male-sterile cytoplasm that does not exert any adverse effects upon other plant characters.

We have shown great genetic diversity among cytoplasms of *Triticum* (wheat) and *Aegilops* species by transferring them into 12 common wheats ($2n=42$, AABBDD) (Tsunewaki et al. 1976; Tsunewaki 1980). The limitations of this early work were set by the fact that the tester nuclei used for screening cytoplasmic differences possessed the same genomic constitution: the relatively few phenotypic differences observed no doubt resulted from interactions between different plasmons and the A, B and/or D genomes. By use of nuclei possessing different genomic constitutions, such as the AABBRR of triticale, cytoplasmic differences not revealed by our previous works may be exposed; in ad-

* Contribution No. 466 from the Laboratory of Genetics, Faculty of Agriculture, Kyoto University, Japan
** Present address: Centro Internacional de la Papa, Apartado 5969, Lima, Peru

dition, the present strategy may also shed light upon the phylogeny of *Triticum* and *Aegilops* cytoplasms.

With these goals in mind, we have attempted to transfer a large number of *Triticum* and *Aegilops* cytoplasms to the earliest registered triticale cultivar ('Rosner') by repeated backcrosses. The transfer proved to be difficult, but we have succeeded in transferring seven cytoplasms, i.e., from two *Triticum* and five *Aegilops* species. While none of the transfers appears promising for breeding purposes, three have induced complete male sterility. The results are reported below.

Materials and methods

Plant materials

Recurrent pollen parent. A triticale cultivar 'Rosner', the first licensed triticale (1969), bred by the University of Manitoba, Canada (Larter et al. 1970), was used as the recurrent pollen parent. According to J. P. Gustafson (personal communication), 'Rosner' possesses six rye chromosome pairs with rye-characteristic telomeric heterochromatin. However, 'Rosner' does not carry the rye chromosome pair 2R, with its normal banding pattern.

Cytoplasm donors. Alloplasmic lines of common wheat cultivars, 'Chinese Spring', 'Salmon' and 'Selkirk', which were bred in our laboratory (Tsunewaki 1980), were used as the cytoplasm donors to 'Rosner' triticale. Original sources of the cytoplasms are presented in Table 1. In total, 31 cytoplasms from 27 *Triticum* and *Aegilops* species, distributed among 15 plasma types, were used in the present investigation.

Transfer of cytoplasms

Substitution backcrosses. The transfer of *Triticum* and *Aegilops* cytoplasms into 'Rosner' triticale has been achieved by successive backcrosses of the F_1's, alloplasmic common wheats × 'Rosner', with 'Rosner' as the recurrent pollen parent. Individual alloplasmic lines are indicated by the name of the cytoplasm donor in parentheses and hyphenated 'Rosner': for example, (*squarrosa*)-'Rosner' means an alloplasmic 'Rosner' line with *Ae. squarrosa* cytoplasm.

Embryo culture. As described in "Results", seeds from the crosses between all alloplasmic common wheats and 'Rosner' are mostly very shrivelled, and are rarely capable of germinating. To rescue F_1 embryos, an embryo culture technique was employed with 16 cross combinations in the following way: about two to three weeks after pollination, developing seeds were taken from the florets, sterilized 10 s in 70% alcohol and rinsed with sterilized water. Embryos were aseptically excised from the seeds under an anatomical microscope, and placed on agar slant medium in test-tubes, each containing 10 ml of the RM-64 medium (Linsmaier and Skoog 1965), supplemented with 2.0 mg/l of 2,4-D. The medium was adjusted to pH 5.6 with 1N NaOH, then sterilized by autoclaving 15 min under a pressure of 1.2 kg/cm². The cultures were incubated at 25 °C under continuous fluorescent illumination. When shoots and roots developed well, the plantlets were transplanted into pots, and allowed to grow in a temperature-controlled greenhouse.

Cytology. Somatic chromosome numbers were determined using root-tips which were pretreated 24 h in ice water (0 °C), fixed with acetic alcohol (1:3 mixture) and stained with acetocarmine. The ordinary squash method was used for cytological preparation of the somatic chromosomes. Meiotic chromosome configurations were observed using pollen mother cells (PMCs) from premature anthers which were fixed with acetic alcohol and stained with acetocarmine. In this case, the smear method was used for cytological preparation.

Table 1. Sources of cytoplasms used in the present investigation. Arranged in order of ploidy and alphabetical order of nuclear genome

Species	Nucleus		Plasma type[c]
	Ploidy[a]	Genome constitution[b] (haploid)	
Triticum boeoticum	2x	A	A
Aegilops caudata	2x	C	C
Ae. umbellulata	2x	C^u	C^u
Ae. squarrosa	2x	D	D
Ae. heldreichii	2x	M	M
Ae. uniaristata	2x	M^u	M^u
Ae. speltoides	2x	S	S
Ae. aucheri	2x	S	G
Ae. sharonensis	2x	S^1	S^1
Ae. longissima	2x	S^1	B
Ae. bicornis	2x	S^b	S^b
Ae. mutica	2x	Mt	Mt
T. dicoccoides	4x	AB	B
T. dicoccum	4x	AB	B
T. dicoccoides nudiglumis	4x	AG	G
T. araraticum	4x	AG	G
T. timopheevi	4x	AG	G
Ae. triuncialis	4x	CC^u	C^u
Ae. cylindrica	4x	CD	D
Ae. biuncialis	4x	C^uM^b	C^u
Ae. ovata	4x	C^uM^o	M^o
Ae. triaristata	4x	C^uM^t	C^u
Ae. kotschyi	4x	C^uS^v	S^v
Ae. variabilis	4x	C^uS^v	S^v
Ae. crassa	4x	DM^{cr}	D^2
Ae. ventricosa	4x	DM^v	D
T. zhukovskyi	6x	AAG	G
T. aestivum	6x	ABD	B
Ae. triaristata	6x	$C^uM^tM^{t2}$	C^u
Ae. juvenalis	6x	C^uDM^j	D^2
Ae. crassa	6x	DD^2M^{cr}	D^2

[a] $x = 7$
[b] After Lilienfeld (1951) and Kihara and Tanaka (1970)
[c] After Tsunewaki (1980) and Tsunewaki and Tsujimoto (1983)

Estimation of cytoplasmic effects

The effects of the successfully transferred *Triticum* and *Aegilops* cytoplasms on agronomic characters of 'Rosner' were investigated during two crop seasons between Fall 1979 and Summer 1982.

In the first experiment (1979–1980), six alloplasmic lines, all from the B_4 generation, were randomized together with a euplasmic (control) line in four replications. Three plants per

Table 2. Results of conventional crosses between alloplasmic common wheats and triticale 'Rosner'. Arranged according to the plasma type of the female parent

Cytoplasm	Plasma type	No. florets pollinated	No. seeds set	% seed set	No. seeds germinated	% germination	% cross success[a]
boeoticum	A	48	12	25	0	0.0	0.0
longissima	B	40	15	38	0	0.0	0.0
dicoccoides	B	362	298	82	0	0.0	0.0
dicoccum	B	148	103	70	0	0.0	0.0
aestivum	B	925	372	40	2	0.5	0.22
caudata	C	2,002	707	35	0	0.0	0.0
triuncialis	C	40	0	0	–	–	0.0
umbellulata	C^u	549	371	68	0	0.0	0.0
biuncialis	C^u	40	6	15	0	0.0	0.0
triaristata 4x	C^u	22	10	45	0	0.0	0.0
triaristata 6x	C^u	24	12	50	0	0.0	0.0
squarrosa	D	1,799	820	46	2	0.2	0.11
ventricosa	D	20	9	45	0	0.0	0.0
crassa 4x	D^2	24	13	54	0	0.0	0.0
juvenalis	D^2	77	36	47	1	2.8	1.30
crassa 6x	D^2	162	21	13	0	0.0	0.0
aucheri	G	26	0	0	–	–	0.0
nudiglumis	G	410	183	45	0	0.0	0.0
araraticum	G	24	5	21	0	0.0	0.0
timopheevi	G	2,343	769	33	2	0.3	0.09
zhukovskyi	G	26	13	50	0	0.0	0.0
heldreichii	M	24	18	75	0	0.0	0.0
uniaristata	M^u	40	2	5	0	0.0	0.0
ovata	M^o	1,697	571	34	3	0.5	0.18
mutica	Mt	204	126	62	1	0.8	0.49
speltoides	S	1,157	571	49	1	0.2	0.09
bicornis	S^b	22	8	36	0	0.0	0.0
sharonensis	S^l	770	324	42	0	0.0	0.0
kotschyi	S^v	343	244	71	0	0.0	0.0
variabilis	S^v	360	299	83	0	0.0	0.0
Pooled	–	13,728	5,938	43.3	12	0.20	0.09

[a] (Seed setting rate) × (germination rate)

line constituted a plot, and the following 18 characters were measured:

(1) Heading date – heading date in May (e.g., 2 means heading on May 2)
(2) Number of ears per plant
(3) Plant height (cm)
(4) Dry matter weight (g) – weight of the whole air-dried, matured plant
(5) Number of internodes – number of internodes of 3 cm or longer
(6)–(8) First to third internode length, respectively, from the top
(9) Culm diameter (mm) – diameter at the middle part of second internode
(10) and (11) Flag leaf length and width (cm), respectively
(12) Flag leaf index – flag leaf length/flag leaf width
(13) Ear length (cm)
(14) Number of spikelets per ear
(15) Spike density (cm^{-1}) – spikelet number/ear length
(16) Awn length (cm)
(17) Selfed seed fertility (%) – percent seed set of the normally developed first and second florets of bagged ears
(18) Pollen fertility (%) – percent pollen grains with one vegetative and two wedge-shaped sperm nuclei.

The characters (5) to (16) were observed using the tallest tiller of each plant.

In the second experiment (1981–1982), five alloplasmic and a euplasmic line were randomized in three replications. Five plants were grown in each plot per line, and seven characters, heading date, plant height, ear number per plant, number of spikelets per ear, selfed seed fertility, number of seeds per plant and 1,000 kernel weight, were measured from plants.

The data thus obtained were analyzed by an ordinary method of analysis of variance, and the 5% least significant difference (LSD) test was applied to classify cytoplasms into different groups.

Results

Production of F_1 hybrids

Results on the production of F_1 hybrids by ordinary crosses between alloplasmic common wheats and 'Rosner' triticale are presented in Table 2. Although relatively high seed-setting rates were obtained in most

Table 3. Results of culturing premature F_1 embryos of the crosses between alloplasmic common wheats and triticale 'Rosner'

Cytoplasm	Plasma type	No. embyros cultured	No. F_1's matured	% success
boeoticum	A	84	0	0.0
dicoccoides	B	155	5	3.2
dicoccum	B	90	2	2.2
aestivum	B	?	2[a]	–
umbellulata	C^u	143	4	2.8
squarrosa	D	143	2	1.4
cylindrica	D	?	8[a]	–
crassa 6x	D^2	?	2[a]	–
nudiglumis	G	106	7	6.6
timopheevi	G	137	7	5.1
ovata	M^o	?	12[a]	–
mutica	Mt	46	6	13.0
speltoides	S	?	1[a]	–
sharonensis	S^l	160	2	1.3
kotschyi	S^v	145	5	3.4
variabilis	S^v	146	2	1.4
Pooled		1,355	42 (+25)	3.1

?–Not recorded
[a] Total is given in parentheses, and excluded in calculation of % success

cross combinations, almost all seeds were extremely shrivelled and did not germinate. Only 12 F_1 hybrids were obtained from 13,728 florets pollinated; that is, the success rate of the crosses was only 0.09%.

Since ordinary crosses rarely produced functional F_1 seeds, embryo cultures were used for 16 cross combinations. The results are summarized in Table 3. The number of cultured embryos was not recorded in five of the crosses, but from 11 cross combinations, 42 F_1 hybrids were produced from 1,355 cultured embryos. The success rate of embryo cultures was 3.1%, about 30 times higher than the success rate of the ordinary crosses. Thus, we concluded that embryo culture is a useful means for obtaining F_1 hybrids between common wheat, eu- or alloplasmic, and triticale. Twenty-five additional F_1's were obtained from five other cross combinations. In total, 15 cross combinations gave F_1 hybrids.

The first backcross

The F_1 hybrids obtained from both ordinary crosses and embryo culture were backcrossed with the pollen of euplasmic 'Rosner'. The results on the first backcross are presented in Table 4.

Table 4. Results of backcrossing the F_1 hybrids, alloplasmic wheat × 'Rosner', to 'Rosner' as the pollen parent

Cytoplasm	Plasma type	No. florets pollinated	No. seeds set	% seed set	No. seeds germinated	% germination	% cross success[a]
dicoccoides	B	331	21	6.3	1	4.8	0.3
dicoccum	B	104	20	19.2	1	5.0	1.0
aestivum	B	958	28	2.9	9	32.1	0.9
Total	B	1,393	69	5.0	11	15.9	0.79
umbellulata	C^u	118	5	4.2	2	40.0	1.69
squarrosa	D	1,354	39	2.9	19	48.7	1.4
cylindrica	D	1,102	31	2.8	7	22.6	0.6
Total	D	2,456	70	2.9	26	37.1	1.06
juvenalis	D^2	700	23	3.3	9	39.1	1.3
crassa 6x	D^2	223	2	0.9	0	0.0	0.0
Total	D^2	923	25	2.7	9	36.0	0.98
nudiglumis	G	182	0	0.0	–	–	0.0
timopheevi	G	78	6	7.7	0	0.0	0.0
Total	G	260	6	2.3	0	0.0	0.00
ovata	M^o	2,384	76	3.2	23	30.3	0.96
mutica	Mt	450	13	2.9	6	46.2	1.33
speltoides	S	342	11	3.2	3	27.3	0.88
sharonensis	S^l	92	0	0.0	–	–	0.00
kotschyi	S^v	369	7	1.9	1	14.3	0.3
variabilis	S^v	142	3	2.1	0	0.0	0.0
Total	S^v	511	10	2.0	1	10.0	0.20
Pooled		8,929	285	3.2	81	28.4	0.91

[a] (Seed setting rate) × (germination rate)

Table 5. Backcrossed and selfed seed fertilities of advanced backcross generations of alloplasmic 'Rosner' lines

Cytoplasm	Generation	Backcrossed			Selfed		
		No. florets	No. seeds	% seed set	No. florets	No. seeds	% seed set
dicoccum	B_2	292	167	57.2	168	124	73.8
dicoccum	B_3	254	196	77.2	260	209	80.4
dicoccum	B_4	120	83	69.2	96	89	92.7
dicoccum	total	666	446	67.0	524	422	80.5
aestivum	B_5	294	247	84.0	344	314	91.3
aestivum	B_6	132	111	84.1	212	176	83.0
aestivum	B_7	116	94	81.0	96	88	91.7
aestivum	total	542	452	83.4	652	578	88.7
squarrosa	B_5	274	222	81.0	398	180	45.2
squarrosa	B_6	198	114	57.6	196	90	45.9
squarrosa	B_7	118	65	55.1	64	23	35.9
squarrosa	total	590	401	68.0	658	293	44.5
cylindrica	B_5	288	225	78.1	292	218	74.7
cylindrica	B_6	224	122	54.5	194	79	40.7
cylindrica	B_7	100	28	28.0	104	40	38.5
cylindrica	total	612	375	61.3	590	337	57.1
juvenalis	B_5	288	202	70.1	194	0	0.0
juvenalis	B_6	196	55	28.1	168	0	0.0
juvenalis	B_7	122	18	14.8	124	0	0.0
juvenalis	total	606	275	45.4	486	0	0.0
ovata	B_5	320	93	29.1	82	0	0.0
ovata	B_6	314	151	48.1	128	0	0.0
ovata	B_7	190	129	67.9	136	0	0.0
ovata	total	824	373	45.3	346	0	0.0
speltoides	B_5	98	18	18.4	84	0	0.0
'Rosner'	(1981)	–	–	–	358	332	92.7
'Rosner'	(1982)	–	–	–	216	184	85.2
'Rosner'	(1983)	–	–	–	128	112	87.5
'Rosner'	total	–	–	–	702	628	89.5

The over-all seed setting rate was 3.2%, which was not affected much by different plasma types. The germination rate of the B_1 seeds was about 28%, a one hundred-fold improvement over the F_1 seeds. The over-all success rate of the first backcrosses was 0.88%, about 10 times higher than that of the initial crosses. All F_1's with G, S^1 and S^v type cytoplasms were weak, and only small numbers of florets could be pollinated, the outcome being a single B_1 plant. This group of cytoplasms will not be useful in the breeding of alloplasmic triticale.

Of the 16 cytoplasms incorporated into the F_1 hybrids, 11 were successfully transferred to the B_1 generation.

Further backcrosses and cytological studies

Further backcrosses were performed with the materials obtained in the B_1 generation. However, cytoplasmic transfers discontinued for *dicoccoides, umbellulata* and *kotschyi* cytoplasms at the B_2 generation, and *speltoides* cytoplasm at the B_6 generation. At present (Fall 1983), we are successfully maintaining the alloplasmic lines with *aestivum, squarrosa, cylindrica, juvenalis* and *ovata* cytoplasms (all in B_8), *dicoccum* cytoplasm (in B_5), and *mutica* cytoplasm (in B_2).

Backcrossed and selfed seed fertilities of alloplasmic 'Rosner' lines in three advanced backcross generations are collectively shown in Table 5.

The line with *speltoides* cytoplasm gradually intensified its growth and heading delay (about 20 days) with increased numbers of backcrosses (ref. Table 8). Consequently, the B_5 plants yielded only shrivelled seeds which failed to germinate. This line was discontinued after five backcross generations. Except for a line carrying *ovata* cytoplasm, all of the other lines (Fig. 1) produced a sufficient number of seeds, through backcrosses.

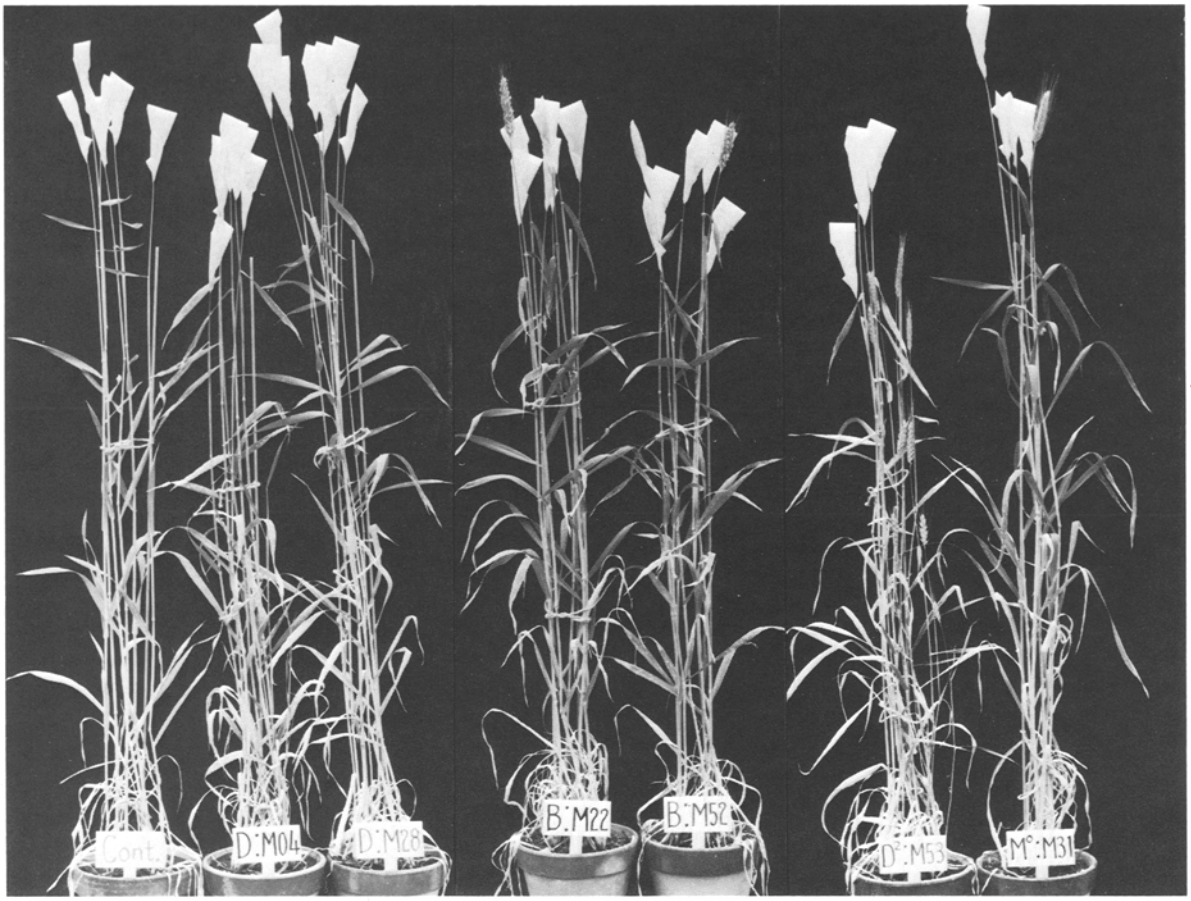

Fig. 1. Alloplasmic lines of a hexaploid triticale cultivar 'Rosner'. *From left to right:* control (euplasmic 'Rosner'), (*squarrosa*)-, (*cylindrica*)-, (*dicoccum*)-, (*aestivum*)-, (*juvenalis*)- and (*ovata*)-'Rosner'

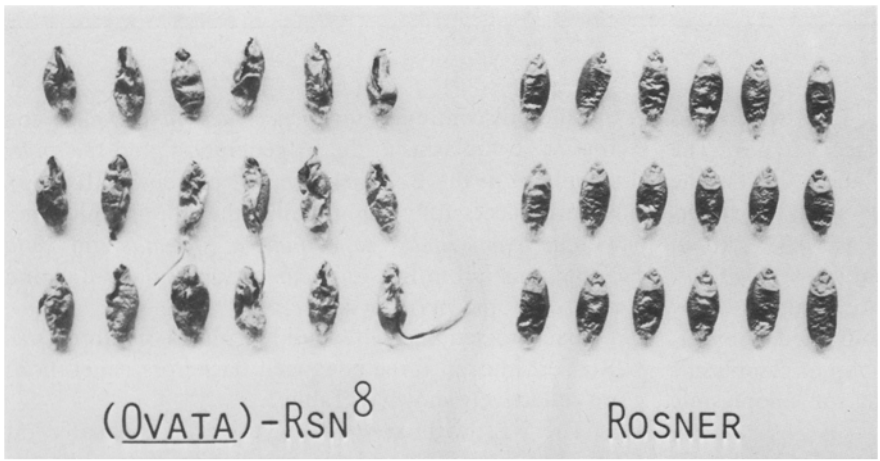

Fig. 2. Seeds of (*ovata*)-'Rosner' showing preharvest sprouting. Observed in greenhouse-grown materials. *Left:* (*ovata*)-'Rosner', *right:* control

The seed setting of (*ovata*)-'Rosner' by backcrosses is not bad, and the seeds develop well. However, most of the seeds sprout prematurely (Fig. 2), even when the plants were grown in the greenhouse and the ears were kept dry. Because of this difficulty in obtaining a sufficient number of viable seeds, (*ovata*)-'Rosner' was not included in the second experiment designed to investigate the genetic effects of alien cytoplasm upon agronomic characters of 'Rosner'.

Among seven alloplasmic 'Rosner' lines which had reached the B_4 or later backcross generations, (*juvenalis*)-, (*ovata*)- and (*speltoides*)-'Rosner' were completely

Table 6. Somatic chromosome number of B_4 plants of six alloplasmic and a euplasmic line of triticale 'Rosner'

Cytoplasm	Plasma type	Total no. plants	Chromosome no. (2 *n*)			
			41	42	43	44
'Rosner'	B	8		8		
aestivum	B	8		8		
squarrosa	D	6		6		
cylindrica	D	8		8		
juvenalis	D^2	8	1	6		1
ovata	M^o	4		4		
speltoides	S	3		2	1	

self-sterile (Table 5). The *juvenalis, ovata* and *speltoides* cytoplasms induce complete male sterility, because all three alloplasmic lines had moderate female fertility. This is the first report of male sterile cytoplasms, from *Triticum* and *Aegilops*, in triticale.

Cytological studies. In the B_4 generation of six alloplasmic lines, the somatic chromosome number was surveyed for a limited number of plants (Table 6). Of 37 B_4 plants examined, three showed aneuploid chromosome numbers, indicating that such plants resulted from some meiotic instability of chromosome pairing in the B_3 plants.

Meiotic chromosome pairing at the first metaphase was examined for the B_5 lines (Table 7). All alloplasmic lines formed some univalents but their frequencies were similar to those found in euplasmic 'Rosner'. This observation demonstrates that the nucleus of common wheat has been completely substituted for by that of 'Rosner' after five successive backcrosses. This result also indicates that at least five alien cytoplasms, i.e., those of *aestivum, squarrosa, cylindrica, juvenalis* and *ovata*, do not alter the meiotic chromosome behaviors.

Table 7. Meiotic chromosome pairing in the B_5 generation of alloplasmic 'Rosner' lines

Cytoplasm	Plasma type	No. plants obs.	No. PMCs/ plant	Univalent	Bivalent		
					Closed	Open	Total
'Rosner'	B	2	50	0.8	16.0	4.6	20.6
aestivum	B	4	48	0.7	16.2	4.5	20.7
squarrosa	D	6	41	0.9	16.2	4.3	20.5
cylindrica	D	2	45	0.8	16.0	4.6	20.6
juvenalis	D^2	4	28	0.7	16.5	4.2	20.7
ovata	M^o	2	50	0.8	15.6	5.0	20.6

Table 8. Average performances on 19 characters of the eu- and alloplasmic lines of triticale 'Rosner' (1979–1980)

Character[a]	Unit	Cytoplasm							5% LSD
		'Rosner'	*aestivum*	*squarrosa*	*cylindrica*	*juvenalis*	*ovata*	*speltoides*	
1	day	20.1	20.1	22.6	22.5	17.9	44.1**	39.0**	3.8
2	–	11.6	11.1	12.0	11.8	16.0	10.1	7.0	–
3	cm	102.9	105.2	102.3	101.1	89.7**	83.7**	79.0**	4.7
4	g	79.5	75.9	62.1	59.1	38.9**	37.4**	39.3**	22.0
5	–	48.1	45.8	51.5	50.6	52.0	47.5	32.3	–
6	cm	46.0	38.8	45.1	45.0	42.5	32.4*	32.6*	9.8
7	cm	22.4	22.4	21.8	20.6	17.4**	14.9**	14.8**	2.4
8	cm	14.0	13.5	12.8	12.6	11.1**	11.7*	9.8**	1.9
9	mm	6.2	6.5	5.9	5.5**	5.3**	5.4**	5.4**	0.5
10	cm	24.9	27.4	27.6	26.8	35.7**	18.1*	24.5	6.2
11	cm	1.8	2.0	1.9	1.8	2.0	1.5	1.8	–
12	–	13.6	14.0	15.0	14.9	18.3**	12.6	13.7	1.8
13	cm	9.9	10.5	10.5	10.5	9.2	11.4*	11.4*	1.1
14	–	24.8	25.0	25.1	25.6	22.4**	27.9**	26.8**	1.3
15	cm^{-1}	2.5	2.4	2.4	2.5	2.4	2.5	2.4	–
16	cm	8.4	8.5	7.9	8.1	7.3**	7.1**	6.7**	0.8
17	%	59.6	64.2	29.8**	37.7**	0.0**	0.0**	0.0**	9.4
18	%	73.6	74.4	69.0	73.4	0.0**	0.0**	0.0**	5.1

*, ** Significantly different from the 'Rosner' cytoplasm at the 5% and 1% levels, respectively

[a] See text

Table 9. Average performances on nine agronomic characters of the eu- and alloplasmic lines of triticale 'Rosner' (1981–1982)

Cytoplasm	Generation	Heading date (Apr. 20 =0)	Plant height (cm)	No. ears/plant	No. spikelets/ear	Selfed seed fert. (%)	No. seeds/plant	1,000 kernels wt (g)
'Rosner'	–	10.2	116 a	15	24.3 a	85 a	1081 a	34
aestivum	B_6	9.5	117 a	15	24.2 a	82 a	1104 a	35
dicoccum	B_3	10.9	115 a	16	24.8 a	81 a	975 a	33
cylindrica	B_6	11.3	112 a	17	22.4 b	50 b	687 b	34
squarrosa	B_6	11.6	112 a	18	22.3 b	43 b	588 b	31
juvenalis	B_6	9.2	100 b	21	20.4 c	0 c	51 c	25
5% LSD		–	4.3	–	0.8	(6.7°)	167	–

Note: The letter after each figure indicates the class to which each cytoplasm belongs
° Angle (not percent)

Agronomic performance of alloplasmic lines

The average performances of six alloplasmic lines and a euplasmic line, in the 1979–1980 field test, are summarized in Table 8. Compared with the 'Rosner' cytoplasm, *aestivum* cytoplasm exerted no significantly different effects upon the characters measured. *Squarrosa* and *cylindrica* cytoplasms reduced selfed seed fertility to about 50–60% of the control. They also reduced culm diameter to some extent. The remaining three cytoplasms exerted manifold effects on plant characters, including the induction of complete male sterility. *Juvenalis* cytoplasm produced no effect upon heading date but showed retarded growth of plants, resulting in a significant reduction of seven characters; flag leaf length and flag leaf index were increased greatly. *Ovata* and *speltoides* cytoplasms delayed heading for 19–24 days, a severe growth inhibition resulted in a reduction of most of the characters investigated.

In the 1981–1982 field test, five alloplasmic lines were investigated for seven agronomically important characters (Table 9). *Ovata* and *speltoides* cytoplasms were not included in this test for reasons stated earlier. Heading date, ear number and 1,000 kernel weight were not affected by any of the cytoplasms. Plant height was reduced by *juvenalis* cytoplasm, and spikelet number, selfed seed fertility and seed number were reduced by *juvenalis, squarrosa* and *cylindrica* cytoplasms. *Juvenalis* cytoplasm caused complete male sterility.

Discussion

On the production of alloplasmic triticale

This is the first full-scale attempt to produce alloplasmic hexaploid triticale by repeated backcrosses of the F_1 hybrids, alloplasmic common wheats × triticale, with triticale as the recurrent pollen parent. The results reveal that the cross-compatibility between alloplasmic common wheat and 6x triticale is extremely low, irrespective of the kind of cytoplasm; the percent success of ordinary crosses (number of matured hybrids per pollinated floret) was 0.09% in the data pooled for the 31 cross combinations. By means of embryo culture, however, 3.1 F_1's were obtained per 100 embryos cultured, thus greatly improving the efficiency of F_1 production. The first backcross of such F_1 plants also produces few seeds; i.e., the percent success of the first backcross was 0.88%, being about ten times higher than that of the initial crosses. Further backcrosses were easier than the first two, which is to say that the successful production of the F_1 and B_1 hybrids is the "bottle-neck" in obtaining alloplasmic triticale by this method.

We have attempted the transfer of 31 *Triticum* and *Aegilops* cytoplasms to 'Rosner' triticale, but only seven cytoplasms have been successfully transferred. More than 500 florets were crossed with alloplasmic common wheats having *caudata, umbellulata, timopheevi, sharonensis* or *kotschyi* (*variabilis* cytoplasm, inclusively) cytoplasm, but with no success. It is evident from these results that *Triticum* and *Aegilops* cytoplasms of C, C^u, G, S^l and S^v plasma types are difficult to transfer to triticale.

On the value of Triticum and Aegilops cytoplasms for triticale breeding

Alloplasmic lines of 'Rosner' with *aestivum* and *dicoccum* cytoplasms performed as well as the normal line in the two tests (1979–1980 and 1981–1982). These results are not unexpected since the cultivar 'Rosner' received its cytoplasm from *T. durum* (Larter et al. 1970), and since we cannot find any cytoplasmic differences among the common and emmer wheats, including *T. aestivum, T. dicoccum* and *T. durum* (Maan 1973; Tsunewaki et al. 1976; Tsunewaki 1980; Tsunewaki and Tsujimoto 1983).

Larter and Hsam (1973) and Hsam and Larter (1974a, b, c) have reported different effects of common and emmer wheat

cytoplasms on such various characters of triticale as seed density (g/cc), seed fertility, univalent frequency, plant height, number of ears per plant, ear length, number of florets per ear, RNA, cellular protein and histone contents, and high molecular weight seed proteins. A possible cause for the discrepancies between their results and ours could be the differences in experimental conditions; their materials were grown in a greenhouse under almost constant light period and temperature, whereas our experiments were conducted under field conditions.

Five other cytoplasms incorporated into triticale show more or less adverse effects upon various agronomic characters: *ovata* and *speltoides* cytoplasms induce a great delay in heading accompanied by growth inhibition and complete male sterility; *juvenalis* cytoplasm causes reduced vigor and complete male sterility; *squarrosa* and *cylindrica* cytoplasms result in some reduction in spikelet number, selfed seed fertility and total seed production. Regrettably, we find no cytoplasms superior to the cytoplasm of 'Rosner' among the seven *Triticum* and *Aegilops* cytoplasms studied.

Three *Aegilops* cytoplasms, i.e., *juvenalis, ovata* and *speltoides* are found to cause complete male sterility. In addition, *ovata* and *speltoides* cytoplasms induce a delay of heading and reduced vigor, rendering them useless for hybrid triticale breeding. On the contrary, *juvenalis* cytoplasm induces a one to two day earlier heading, and about 15% reduction of plant height. These characteristics may possess practical usefulness. At the same time, however, *juvenalis* cytoplasm causes some reduction of vigor, e.g., reduced spikelet number. In addition, *juvenalis* cytoplasm causes partial pistillody, resulting in partial female sterility (ref. Table 5). These are unfavorable characteristics.

Genetic differentiation among Triticum and Aegilops cytoplasms

In our studies of the genetic diversity of *Triticum* and *Aegilops* cytoplasms, we used 12 common wheats belonging to five subspecies of *Triticum aestivum* as screens for different cytoplasmic effects (Tsunewaki et al. 1976; Tsunewaki 1980; Tsunewaki and Tsujimoto 1983). In these studies, the cytoplasms belonging to S (*speltoides*), D (*squarrosa, cylindrica,* and others) and D^2 (*juvenalis* and others) plasma types could not be distinguished from B plasma type (emmer and common wheat). In the present investigation, four plasma types, B, D, D^2 and S exerted different effects upon 'Rosner' triticale: B type cytoplasms exert no specific effects; D type cytoplasm induces about 50% reduction of male fertility; D^2 type cytoplasm induces complete male sterility and some reduction of vigor; and S type cytoplasm induces complete male sterility, heading delay and reduction of vigor. Triticale 'Rosner', then, is useful as a tester for classifying plasma types that can not be distinguished by use of common wheat testers.

It is a well established fact that the 1D chromosome of common wheat is indispensable for wheat plants to survive with a D type cytoplasm (Tsuji and Murata 1976; Maan 1978; Ohtsuka 1980). In the present investigation, alloplasmic 'Rosner' lines with *squarrosa* and *cylindrica* cytoplasms grow well and are fertile, but inferior to the euplasmic line. Because this triticale lacks the 1D chromosome, the present results indicate that a certain rye chromosome(s), most likely, 1R, carries homoeoallele(s) of the genes on the 1D chromosome, which recover to a great extent the vigor and fertility impaired by the D type cytoplasm.

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