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Single Gene Restoration of Cytoplasmic Male Sterility in Wheat and its Implications in the Breeding of Restorer Lines

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Summary. Cultivars of *T. aestivum* crossed onto two lines with male sterility induced by the cytoplasm of *T. timopheevi* gave a high level of restoration in the F_1 generation. The ratio of fertile to sterile plants segregating in the F_2 generation was consistent with that expected for a single dominant restorer gene. The possible association between this gene and mildew resistance or some other desirable character derived from 'CI 12633', a common ancestor of the cultivars used, is discussed.

Key words: Male sterility - Wheat - Restoration - Mildew Resistance - Plasmatic Inheritance

Introduction

The commercial development of F_1 hybrid wheat depends on a satisfactory method of seed production together with a yield advantage, over the highest yielding pure line, greater than that needed to cover increased production costs.

The most promising method uses male sterility induced by the cytoplasm of *T. timopheevi* Zhuk. Male sterile lines of *T. aestivum* L. cultivars are obtained by repeated backcrossing to a source of *T. timopheevi* cytoplasm. On a large scale, male sterile seed is produced from blocks of sterile (A-line) that have been wind pollinated by alternating blocks of maintainer (B-line). F_1 seed is produced in a similar manner by replacing the B-line with a second cultivar (Restorer or R-line) capable of restoring male fertility in the F_1.

It has been shown that restoration can be complex; Wilson (1968) concluded that restoration derived from *T. timopheevi* was due to at least three dominant genes acting with cumulative effect, and Sage (1972) reported similar gene action. Mihaljev (1973) concluded that restoration in 'VK-64-28' was controlled by a polygenic system, the genes acting as partially dominant and additive.

For ease of manipulation simple inheritance of restoration is desirable, preferably a single gene which restores reliably over a range of environments. The only case reported where complete restoration in the F_1 was shown to be due to a single dominant gene was in *T. spelta* var. 'duhamelianum' where the gene was located on chromosome 1B (Tahir and Tsunewaki 1969).

Sources of genes in *T. aestivum*, capable of restoring fertility to male sterility induced by the cytoplasm of *T. timopheevi*, have been reported in European cultivars by Zeven (1968a) and Apltauerová (1967). In some of these, eg. 'Minister', 'Dominator', 'van Hoek', 'Blé de Gironde', and 'Carstens V', Zeven (1968a, 1968b) concluded that a major dominant gene provided restoration. Using monosomic analysis the major gene in 'Minister' was assigned to chromosome 1B; but chromosomes 4B and 7D were also found to contribute towards restoration (Zeven 1970, 1971). In another cultivar, 'Primepi', two genes giving restoration were located on chromosomes 1B and 5D by Bahl and Maan (1973) again using monosomic analysis.

Whenever monosomic analysis has been used to identify the chromosomes carrying restorer factors which have been derived from *T. timopheevi* or *T. zhukovskyi* two or more chromosomes have been found to be involved. A major gene on chromosomes 1A is always involved, with other genes usually on chromosomes 7D or 6B, and occasionally on chromosomes 5A, 6D and 7B (see review by Sage 1976). Thus, with the exception of that on 7D the restorer genes in Eu-

ropean wheats are carried on different chromosomes from those that carry the genes derived from *T. timopheevi*.

The *T. aestivum* pure line breeding programme at Cambridge has involved cultivars which are known to restore male sterility induced by *T. timopheevi* cytoplasm. Part of the restorer breeding programme has therefore been devoted to screening advance selections for high levels of restoring capacity. It is intended that this will provide high yielding cultivars which could be used directly as restorers, or from which restorers with improved out pollination characters could be bred. Should this restoration potential be inadequate for the range of environments met in Western Europe, this may be improved by incorporating restorer genes derived from other sources.

This paper reports on the level of restoration obtained and the segregation of fertility in the F_2 generation, and their consequences to the F_1 hybrid wheat programme.

Materials and Methods

Five F_1 hybrids were made from crosses between male sterile (ms) lines of 'Maris Hobbit' and 'Maris Ranger' based on the cytoplasm of *T. timopheevi* and four advanced high yielding semi-dwarf winter wheat selections. These crosses were designated cms 1, 2, 3, 4 and 5 as follows:

F_1	♀ Parent	♂ Parent
cms 1	ms *(timopheevi)* - 'Hobbit[4]'	× F_6 'TJB 364/636'
cms 2	" " "	× F_6 'TJB 370/491'
cms 3	" " "	× F_6 'TL 459/3/2'
cms 4	ms *(timopheevi)* - 'Maris Ranger[5]'	× F_6 'TJB 364/636'
cms 5	" " "	× F_6 'TJB 368/268'

The F_1 seedlings, sown in small plant pots, were vernalised in a cold cabinet at 2°C day time temperature with an 8 h day for eight weeks. They were then transplanted into 23 cm diameter plant pots and grown in a glasshouse where daylight was supplemented by fluorescent lights to give a 16 h day, with night and day temperatures set at 10°C and 20°C, respectively. The number of F_1 plants grown per cross varied from four to eight, a maximum of four plants being grown in a pot.

Before flowering all ears were bagged, and at maturity the total number of spikelets, the number of spikelets with grain and the number of grain were recorded.

The F_2 progeny from each F_1 were sown in the field on 10th November 1975 as 67 spaced plants/m^2. One ear on each plant was securely bagged before flowering. At maturity the harvested ears were separated into those with and those without grain set. Where careful examination of sterile ears revealed an occasional ear with one or two grain set, these were included with the sterile ears. In the fertile ears the same ear components as on the F_1 plants were recorded.

Results

In general male fertility restoration in the F_1's was high with all four restorers tested (Table 1). Where male sterility was recorded it occurred in spikelets at the tips of the ears. The number of plants assessed per cross differed, and the number of ears and the level of grain set per ear varied between plants. This may cause some bias in comparisons of the level of restoration obtained from the different restorers. There were no differences between crosses in the number of grain set per ear and although cms 3 (ms 'Hobbit[4]' × F_5 'TL 459/3/2') and cms 4 (ms 'Maris Ranger[5]' × F_5 'TJB 364/636') had higher percentages of spikelets with grain (91 and 93 per cent, respectively), only the latter was significantly ($P < 0.05$) higher than the other crosses.

Nine F_2 families from the five crosses were classified according to fertility, none deviating significantly from a ratio of three fertile to one sterile. Where more than one family was looked at in a cross they were found to be homogenous and were thus pooled. The three crosses based on ms 'Hobbit' were homogenous and so were the two crosses based on ms 'Maris Ranger' (Table 2). The overall grain set and the percentage of fertile spikelets was high in plants to which fertility had been restored (Table 2). Variation in fertility within four respresentative F_2 families are shown in Fig. 1. In all these families the majority of the fertile progeny had over 80 per cent spikelets fertile, and an average of two or more grain per fertile spikelet.

Discussion

The degree of male fertility restoration in *T. timopheevi* cytoplasm varies according to the environment, the level of restoration in the glasshouse being in general less than that in the field (see review by Johnson and Schmidt 1968). Although our conditions for growing the F_1's cannot be regarded as being

Table 1. Fertility of ears in plants from five restored F_1 hybrids

Male sterile parent	F_1	plant	No. ears	No. grain per ear	s.d. within palnts	% spikelets with grain	s.d. within plants
'Hobbit'	cms 1	1*	11	48.8	11.99	82	13.0
		2	4	32.5		63	
		3	2	59.0		86	
		4	2	31.5		57	
		wtd mean		44.6		76	
	cms 2	1*	9	46.1	17.52	70	15.8
		2	3	50.3		79	
		3	5	46.2		85	
		4	6	37.8		85	
		5	2	26.0		61	
		wtd mean		43.0		77	
	cms 3	1*	4	51.3	11.15	98	7.9
		2	4	52.3		95	
		3	5	41.6		85	
		4*	4	41.8		91	
		5	1	12.0		91	
		wtd mean		44.5		91	
'Maris Ranger'	cms 4	1	4	48.5	7.08	96	7.5
		2	5	28.2		79	
		3	6	43.8		91	
		4*	6	35.8		94	
		5*	5	44.6		95	
		6*	4	51.3		95	
		7*	6	52.0		98	
		wtd mean		43.1		93	
	cms 5	1	4	46.8	7.44	99	12.5
		2	5	35.4		83	
		3	5	37.2		87	
		4	5	32.8		76	
		5	3	23.3		71	
		6*	9	37.2		75	
		7	4	37.0		62	
		8	4	28.8		80	
		wtd mean		35.4		79	

* Denotes plants subsequently studied as F_2 families

Table 2. Segregation for fertility restoration and mean fertility of ears in fertile progeny from five F_2 populations

Male sterile parent	'Hobbit'				'Maris Ranger'		
F_2	cms 1	cms 2	cms 3	Heterogeneity	cms 4	cms 5	Heterogeneity
No. of families	1	1	2		4	1	
No. of fertile plants	94	92	109		186	65	
No. of sterile plants	36	29	35		55	15	
χ^2 (3-1)	0.50	0.07	0.04	0.59	0.61	1.67	0.53
$P > 0.2$	NS	NS	NS	NS	NS	NS	NS
No. of grain per ear and s.d. (within plants)	50.7 (8.95)	52.3 (10.51)	45.9 (8.79)		45.1 (12.55)	45.2 (10.20)	
% spikelets with grain and s.d. (within plants)	98.4 (2.97)	96.9 (4.56)	95.7 (9.38)		90.5 (13.01)	94.8 (8.37)	

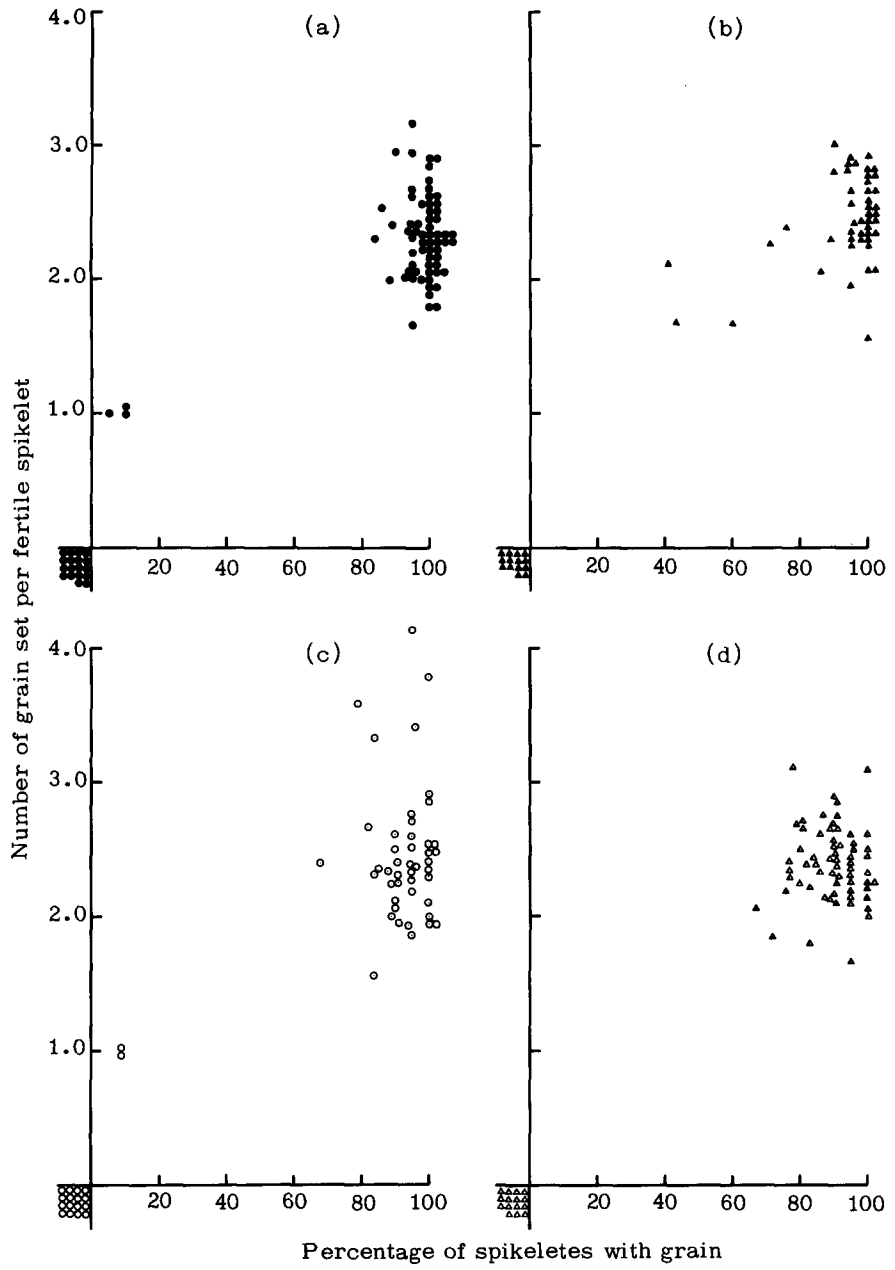
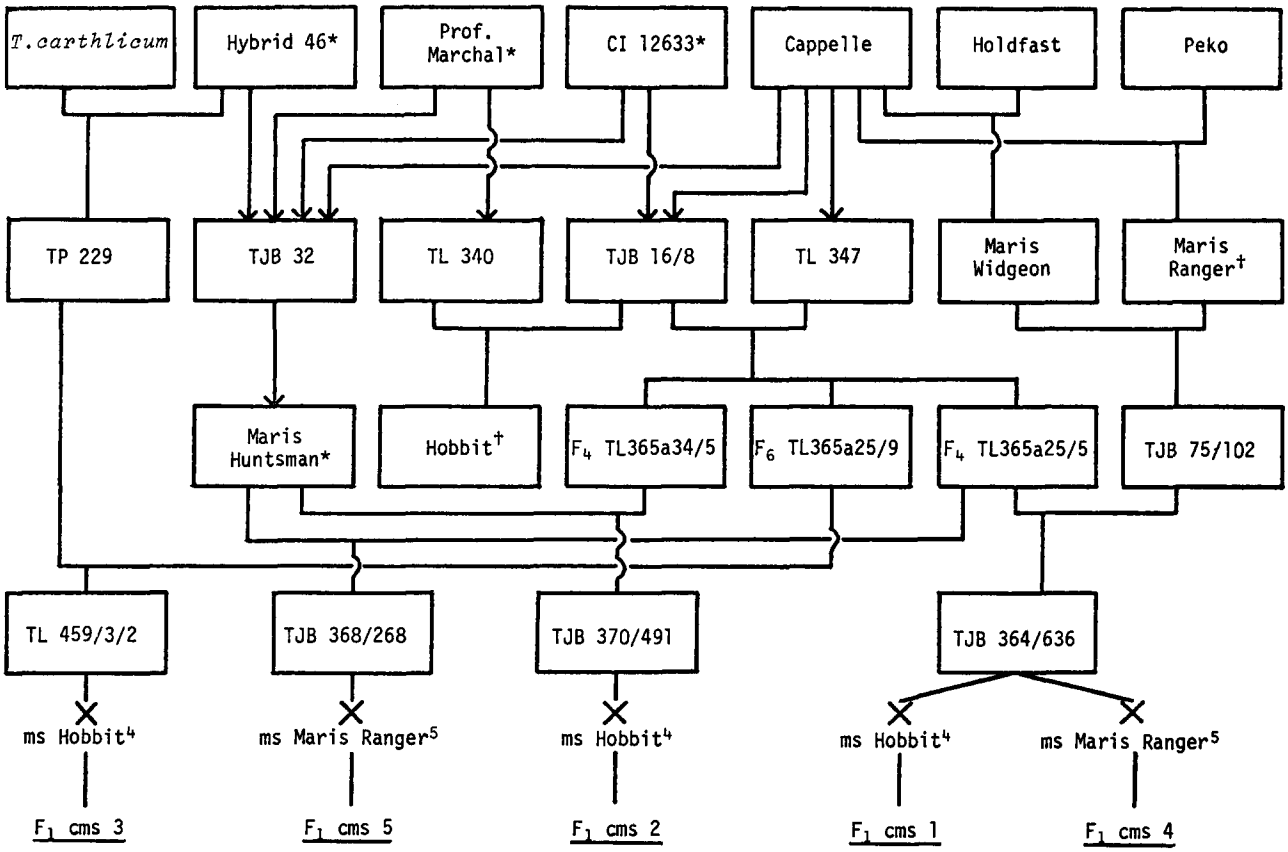


Fig. 1. Fertility of ears in F_2 populations, cms 3/1 (a) and cms 3/4 (b) derived from the cross ms 'Hobbit[4]' × F_5 'TL 459/3/2' and cms 4/5 (c) and cms 4/7 (d) derived from the cross ms 'Maris Ranger[5]' × F_6 'TJB 364/636'

particularly adverse for restoration, the level of fertility in all crosses was good for glasshouse grown plants (Table 1).

According to Wilson (1968), the most sterile environments, which are consequently the most difficult in which to restore male fertility, occur in northern latitudes where the growing seasons are short and temperatures cool. As the British Isles fall into this category, powerful restoration may be necessary and as Wilson suggests, consideration may have to be given to the development of female lines that are easy to restore. We have no previous knowledge as to the ease of restoration of the two male sterile lines used in this work, but where 'TJB 364/636' was crossed onto both, the F_1 with 'Hobbit' (cms 1) had 76 per cent of the spikelets with grain, and that with 'Maris Ranger' (cms 4) 93 per cent (Table 1).

The variation in fertility of ears within F_1 plants (Table 1, Fig. 1) demonstrates the difficulty in comparing the level of restoration achieved by the differ-



* Varieties known to give restoration
† B-lines of male steriles used

Fig.2. Pedigree of F_1 Hybrids cms 1-5 showing possible sources of restoration

ent restorers. The chance bagging of a less fertile ear on a few plants in some F_2 families, eg. cms 3/5 (Fig.1), may account for the few plants which are less fertile than the majority.

In five families there were between one and three F_2 plants having one or two fertile spikelets, each of which set one grain, eg. cms 3/1 and cms 4/5 (Fig. 1). Because they had so few grain and were so different from the majority of the fertile plants they were counted as being sterile. When such plants were counted as being fertile rather than sterile, only in one family, F_2 cms 4/4 did the data fail to fit a 3:1 ratio ($P = 0.03$).

The ratio most likely to be confused with a 3:1 is that due to the presence of both a recessive and a dominant gene for restoration, where the expected F_2 ratio would be 13 fertile to 3 sterile plants. When this possibility was tested, the fit to a 3:1 ratio was in general closer than to a 13:3 ratio; the summed data for the five populations (546 fertiles to 170 steriles) differed significantly from a 13:3 ratio ($P < 0.001$). If a recessive restorer gene was involved it would not be expressed in the F_1 and would only be manifest where it was homozygous in F_2 progeny. The evidence presented is thus consistent with that expected for a single dominant gene.

The three possible sources of restoration found in 'TJB 364', 'TJB 368', 'TJB 370' and 'TL 459' are 'Hybrid 46', 'Professeur Marchal' and 'C.I. 12633' (Fig.2). Of these 'Professeur Marchal' gave the highest level of restoration when crossed onto male sterile *(T.timopheevi)* - 'Bison' (Zeven 1968a). Both 'TJB 368' and 'TJB 370' could have derived restoring genes from all three sources, the restoration from 'C.I. 12633' possibly coming through both parents, 'Maris Huntsman' and 'TL 365a 25/5' for the

former, and 'Maris Huntsman' and 'TL 365a 34/5 for the latter. Two sources of restorer genes, 'Hybrid 46' and 'C.I. 12633', are possible for 'TL 459' whilst the only source for 'TJB 364' is from 'C.I. 12633' which is the only source of restoration common to all four restorers reported and was used extensively at Cambridge as a source of mildew *(Erysiphe graminis* f. sp. *tritici)* resistance.

The mildew resistance in 'C.I. 12633', (*T. timopheevi*, 'P.I. 94761' × [('Illinois No. 1', 'C.I. 11933' × 'Chinese Spring', 'C.I. 6223') selection 2666A2-2-15-6-3]^3), is due to a single dominant gene Pm2 (Mlx) conferring resistance at the 1-leaf stage and in the adult plant by this and a second dominant gene Pm6 (Mlf) (Allard and Shands 1954; Nyquist 1963; Briggle 1966; Jørgensen and Jensen 1972, 1973). Allard and Shands concluded that the genes were derived from *T. timopheevi* Zhuk, and suggested that several mildew resistance genes may be on a chromosome segment which was transferred.

If restoring gene(s) linked with the mildew resistance genes were transferred from *T. timopheevi* to 'C.I. 12633', selection for mildew resistance in breeding material derived from crosses involving this cultivar might, if there was close linkage, also retain the restorer gene(s). This would explain the relatively high frequency of restorers of *T. timopheevi* cytoplasm in the *T. aestivum* pure line breeding programme. Nyquist (1963) however, suggested that although Pm2 (Mlx) may have been transferred from *T. timopheevi*, it was possible that the gene came from 'Illinois No. 1'. Support for the gene being derived from a source other than *T. timopheevi* came from McIntosh and Baker (1970) who located Pm2 on chromosome 5D (one of the two 'Primepi' chromosomes carrying a restorer gene).

Jørgensen and Jensen (1973) suggested that the second mildew resistance gene Pm6 (Mlf) in 'C.I. 12633' is on chromosme 2B because it was linked to stem rust resistance, probably gene Sr9c (SrTt) (Jørgensen and Jensen 1972), which was located on this chromosome by Sears and Loegering (1968). Thus, this second mildew resistance gene is on a chromosome which has not been reported as having a major restoring gene derived from *T. timopheevi*.

An association between selection for mildew resistance and the presence of the restoring gene in the lines reported on here can only exist if the restoring gene is found to be on chromosome 5D or 2B. For the gene to be on 5D a translocation from one of the *T. timopheevi* genomes would have to be involved. If there is no association between these two characters, it may be that restorer gene(s) are associated with some other desirable attribute such as vigour or yield which would also result in the relatively high frequency of restoration in wheats bred at Cambridge. Efforts will be made to investigate these possibilities using cytogenetic and conventional analytical procedures.

A single gene, with the high level of male fertility restoration shown by the four cultivars tested, would greatly simplify the breeding of restorer lines. Restorer sources identified in this way could be used directly in combination with male sterile lines for making F_1's, but such cultivars often have poor anther extrusion and do not make good male parents. If, as in the case of the cultivars reported here, they are also semi-dwarfs, their cross pollinating capacity is further reduced in crosses with taller male sterile lines. Cultivars to be used as restorer lines must have adequate anther extrusion for cross pollination. The good anther extrusion which was observed amongst the F_2 progeny from these crosses should produce restorers with greater cross pollinating potential than their parents.

Initial selection from these crosses for restoration, anther extrusion and agronomic characters, will be followed as soon as possible by test crosses, perhaps in the F_4 generation, to a range of three or more male sterile lines. This will serve to assess their cross pollinating potential and capacity for restoration of fertility when in the heterozygous condition. They can then be assessed for their combining ability, as measured by performance of the F_1's in replicated micro plot yield trials.

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