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The effect of wheat cytoplasm on the synthesis of wheat × rye hybrids

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Summary. Four nuclear genotypes of *Triticum aestivum* L., each substituted into the cytoplasm of *T. timococcum* Kostoff, *Aegilops ventricosa* Tausch and *T. timopheevi* Zhuk., were crossed with four homozygous *Secale cereale* L. inbred lines to produce 48 alloplasmic octoploid primary triticales. Crossability, embryo differentiation, amphihaploid plantlet recovery in vitro, and response to colchicine treatment were investigated. It was found that in general the effects of the wheat cytoplasm on the characteristics studied were of equal importance as those of the wheat and the rye nuclear genotypes. Cytoplasm can be regarded as an additional source of variation in the synthesis of primary triticales. Cross-compatibility in wheat × rye hybrids appeared to be determined by specific parental genotypes and their nucleo-cytoplasmic interactions rather than by any general compatibility of particular nuclear genotypes and/or cytoplasms.

Key words: Common wheat – Rye inbred lines – Alloplasmic triticale – Wheat cytoplasm – Wheat × rye synthesis

Introduction

Cytoplasmic influences on the manifestation of morphological and physiological traits have been reported for a great number of cultivated plants. In numerous instances male sterility has resulted from incorporating alien cytoplasms in interspecific crosses but, in addition, a wide range of traits such as vigour, maturity, yield, and seed characters were affected (Duvick 1965; Edwardson 1970; Sage 1976; Busch and Maan 1978; Maan 1979; Ward et al. 1983).

With regard to nucleo-cytoplasmic interactions in the intergeneric wheat × rye hybrid triticale (× *Triticosecale* Wittmack), various experiments were conducted. Hsam and Larter (1974) synthesized isogenic hexaploid triticales differing only in the source of their cytoplasm. Improved performance due to the female tetraploid wheat which possessed hexaploid wheat cytoplasm was noticed in terms of embryo development, plant height, and fertility.

Jouve and Soler (1978) studied the influence of *Triticum timopheevi* and *Aegilops ovata* cytoplasm on meiotic behaviour in triticale. Increased meiotic stability was detected in those forms with *Ae. ovata* cytoplasm. Sánchez-Monge (1975) reported on triticales produced with rye and wheat cytoplasm, both having the same genomes, and indicated that hexaploid triticales with rye cytoplasm appeared to be of little practical value. In a recent study Tsunewaki et al. (1984) described the effect of seven cytoplasms of alloplasmic triticale cv. 'Rosner' on various agronomic characters. None of the cytoplasms appeared to be superior compared to euplasmic 'Rosner'.

Information as to the effect of nucleo-cytoplasmic interactions on the synthesis of primary triticales is insufficient at present. Hsam and Larter (1974) presented results on crossability and embryo behaviour using 6x- and 4x-wheat cytoplasm. Stefanowska and Cauderon (1983) reported high crossability of a common wheat cultivar with *Ae. ventricosa* cytoplasm when pollinated with the cv. 'Petkus'. However, both these studies were conducted with undefined triticales produced from wheat × open-pollinated rye populations.

The present report describes for the first time the nucleo-cytoplasmic relationships in wheat affecting various characters in the production of genetically defined octoploid triticales synthesized from twelve alloplasmic wheats each crossed with four rye inbred lines.

Materials and methods

As donors of cytoplasm three sources were used: *Triticum timococcum* Kostoff, *Aegilops ventricosa* Tausch, and *Triticum timopheevi* Zhuk. The nuclear genotypes of four winter wheat

Table 1. LS-estimates of mean values of four maternal wheat genotypes, four paternal rye inbred lines and three cytoplasms for crossability, embryo development and response to colchicine treatment. The number of cross combinations is given in parenthesis

Factor	Crossability	Embryo development		Colchicine treatment		
	% Seed set	% seeds with well-differentiated embryos	% plantlets from well-differentiated embryos	% plant survival	% amphidiploid plants	Mean no. of seeds per 2n plant
Wheat						
14	2.5 (12)	35.8 (12)	85.2 (9)	94.6 (9)	19.2 (9)	4.2 (8)
32	4.4 (12)	29.2 (12)	83.5 (8)	83.2 (9)	13.1 (9)	7.4 (6)
40	2.8 (12)	58.1 (12)	81.0 (9)	88.4 (9)	22.3 (9)	8.0 (8)
42	4.9 (12)	41.7 (12)	68.9 (9)	89.1 (9)	16.4 (9)	6.1 (6)
Rye						
L 60	3.3 (12)	44.1 (12)	83.7 (12)	90.6 (12)	13.2 (12)	2.8 (8)
L 282	8.8 (12)	43.2 (12)	75.4 (12)	87.2 (12)	25.4 (12)	7.3 (11)
L 301	1.6 (12)	44.6 (12)	79.9 (11)	88.7 (12)	14.8 (12)	9.2 (9)
L 311	0.9 (12)	33.0 (12)	–	–	–	–
Cytoplasm						
T. timococcum	2.4 (16)	30.8 (16)	84.3 (11)	83.8 (12)	14.4 (12)	7.5 (9)
Ae. ventricosa	5.2 (16)	42.9 (16)	75.3 (12)	91.1 (12)	19.4 (12)	6.0 (9)
T. timopheevi	3.4 (16)	49.9 (16)	79.3 (12)	91.7 (12)	19.6 (12)	5.7 (10)

lines *Triticum aestivum* L. (14, 32, 40, 42) were substituted into each cytoplasm by four successive backcrosses. Each of the lines carried 'Primépi' restorer genes[1]. The 12 male-fertile alloplasmic wheat parents were crosses with four winter rye inbred lines *Secale cereale* L. (L 60, L 282, L 301, L 311) each, to produce a total of 48 wheat × rye hybrid combinations.

Cultivation, crossing, and embryo culture techniques were performed as described earlier (Oettler 1983, 1984). An average of 700 florets were pollinated from each cross combination. For colchicine treatment of the hybrid plants the method of Jensen (1974) was followed by using a 0.15% colchicine solution. The treated plants were further handled as described by Oettler (1982).

For each cross combination the characters seed set, proportion of well-differentiated (with scutellum) excised embryos, and amphihaploid plantlets recovered from in vitro techniques were recorded. Response to colchicine application was assessed as percentage plant survival after treatment and proportion of amphidiploid plants or plants with diploid sectors obtained, and as the average number of seeds per amphidiploid.

Percentage data were corrected, weighted and transformed to arcsin according to Anscombe (Laubscher 1961) and Snedecor and Cochran (1967), and subjected to analyses of variance for unbalanced data (van Baaren 1973; Utz, unpublished). Mean values were obtained as least squares (LS) estimates, corrected for missing data.

Results

Wheat × rye crossability

The first column in Table 1 shows the mean seed set for the three cytoplasms, and the four wheat and rye parents. The variation among cytoplasms and wheats was small and very similar, while the rye parents differed considerably in their ability to fertilize different wheats.

Table 2 gives a more detailed account on crossability of individual parents in specific cytoplasms. Wheat parents in the *Ae. ventricosa* cytoplasm exhibited the widest range in seed set (1.8% to 9.4%). Differentiation of the rye inbreds for crossability was also best in that cytoplasm (1.6% to 13.2%).

All three sources of variation and cytoplasm × wheat interaction were significant as revealed by the analysis of variance (Table 3).

Embryo development

A large variation was observed in the proportion of seeds with well-differentiated embryos (Fig. 1) for cytoplasms and wheats (Table 1, second column). For rye inbreds the range was much narrower.

The detailed representation of the means (Table 2) revealed that the wide range in variability of wheat parents was particularly pronounced in *T. timococcum* cytoplasm (6.7% to 56.3%). The rye parents also exhibited the widest range (11.5% to 38.2%) in that cytoplasm. The *Ae. ventricosa* cytoplasm, however, induced little differentiation.

The analysis of variance (Table 3) displayed significant differences due to the rye parents and the cytoplasms.

Since rye inbred line L 311 in most cross combinations failed to give viable plantlets in vitro, it was excluded from further examination. The proportion of

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[1] The wheat material was kindly made available by Dr. F. Keydel, Freising-Weihenstephan

Table 2. LS-estimates of mean values of four maternal wheat genotypes in three cytoplasms each and crossed with four rye inbred lines for crossability, embryo development and response to colchicine treatment. The number of cross combinations is given in parenthesis

Factor		Crossability	Embryo development		Colchicine treatment		
		% Seed set	% seeds with well-differentiated embryos	% plantlets from well-differentiated embryos	% plant survival	% amphidiploid plants	Mean no. of seeds per 2n plant
		T. timococcum					
Wheat	14	1.8 (4)	31.0 (4)	91.9 (3)	93.7 (3)	12.7 (3)	1.9 (3)
parent	32	3.1 (4)	6.7 (4)	96.3 (2)	68.8 (3)	6.3 (3)	10.8 (1)
(female)	40	2.4 (4)	56.3 (4)	75.4 (3)	82.9 (3)	23.8 (3)	10.2 (3)
	42	2.2 (4)	29.3 (4)	73.8 (3)	89.9 (3)	14.8 (3)	7.2 (2)
Rye	L 60	2.1 (4)	38.2 (4)	93.9 (4)	86.3 (4)	10.1 (4)	4.0 (3)
parent	L 282	6.2 (4)	36.1 (4)	79.4 (4)	78.2 (4)	17.3 (4)	7.2 (3)
(male)	L 301	0.9 (4)	37.6 (4)	79.7 (3)	86.9 (4)	15.8 (4)	11.3 (3)
	L 311	0.5 (4)	11.5 (4)	–	–	–	–
		Ae. ventricosa					
Wheat	14	3.1 (4)	45.3 (4)	78.6 (3)	93.5 (3)	18.3 (3)	4.4 (2)
parent	32	6.4 (4)	35.2 (4)	68.1 (3)	84.2 (3)	24.7 (3)	10.9 (3)
(female)	40	1.8 (4)	55.6 (4)	87.9 (3)	96.9 (3)	22.3 (3)	6.7 (2)
	42	9.4 (4)	35.6 (4)	66.6 (3)	89.8 (3)	12.2 (3)	1.9 (2)
Rye	L 60	3.7 (4)	33.9 (4)	79.6 (4)	90.2 (4)	10.7 (4)	2.6 (2)
parent	L 282	13.2 (4)	48.4 (4)	65.3 (4)	91.2 (4)	31.1 (4)	7.6 (4)
(male)	L 301	2.1 (4)	40.8 (4)	81.0 (4)	91.8 (4)	16.3 (4)	7.7 (3)
	L 311	1.6 (4)	48.6 (4)	–	–	–	–
		T. timopheevi					
Wheat	14	2.6 (4)	31.0 (4)	85.3 (3)	96.8 (3)	26.7 (3)	6.4 (3)
parent	32	3.8 (4)	45.6 (4)	86.0 (3)	96.6 (3)	8.4 (3)	0.4 (2)
(female)	40	4.2 (4)	62.5 (4)	79.6 (3)	85.5 (3)	20.9 (3)	7.1 (3)
	42	3.2 (4)	60.3 (4)	66.4 (3)	87.7 (3)	22.2 (3)	9.1 (2)
Rye	L 60	4.2 (4)	60.2 (4)	78.2 (4)	95.4 (4)	18.7 (4)	1.7 (3)
parent	L 282	7.1 (4)	45.2 (4)	81.4 (4)	92.3 (4)	27.7 (4)	7.0 (4)
(male)	L 301	1.9 (4)	55.2 (4)	79.0 (4)	87.3 (4)	12.3 (4)	8.5 (3)
	L 311	0.6 (4)	38.9 (4)	–	–	–	–

Table 3. Mean squares of wheat×rye hybrids in three cytoplasms for crossability, embryo development and response to colchicine treatment. Percentages were transformed to arcsin

Source	Crossability		Embryo development				Colchicine treatment					
	% Seed set		% seeds with well-differentiated embryos		% plantlets from well-differentiated embryos		% plant survival		% amphidiploid plants		Mean no. of seeds per 2n plant	
	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
Wheat (W)	3	23.4*	3	10.2	3	1.7	3	3.9	3	2.6	3	19.8
Rye (R)	3	245.6**	3	121.5**	2	76.4**	2	11.4*	2	10.7*	2	32.2
W×R	9	2.6	9	2.6	6	2.2	6	2.9	6	1.5	6	19.5
Cytoplasm (C)	2	63.3**	2	49.2**	2	36.7*	2	24.1**	2	4.9	2	1.6
C×W	6	23.9*	6	13.7	6	14.7	6	2.9	6	1.4	6	33.3
C×R	6	9.5	6	14.6	4	8.2	4	5.6	4	3.1	4	2.7
Residual	18	6.7	18	5.5	11	6.5	12	2.8	12	2.4	4	8.6

*,** Significant at the 5% and 1% levels of probability, respectively

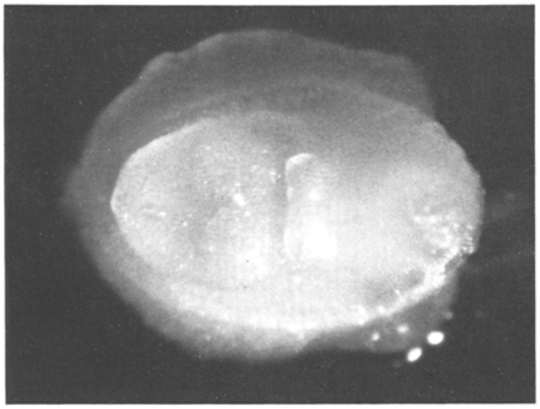

Fig. 1. Differentiated 17- to 19-day-old embryo with scutellum

plantlets regenerated in vitro from well-differentiated embryos (Table 1) was, in general, high (overall mean 79.9%), with a narrow range for cytoplasms and ryes. The low mean value of the wheat line 42 resulted from a low recovery rate in cross combinations with all three cytoplasms (Table 2). The percentages of wheat genotype 32 differed considerably, with a high 96.3% recovery rate in *T. timococcum* cytoplasm and a low 68.1% in *Ae. ventricosa* cytoplasm.

Significant effects due to rye lines and cytoplasms were detected by the analysis of variance (Table 3).

Colchicine treatment

Mean values of response to colchicine treatment are presented in the last three columns of Table 1. The proportion of plant survival was high throughout and varied little among cytoplasms, wheats and ryes. The mean proportions of fertile amphidiploid plants or plants with diploid sectors were generally low, with the widest range among rye parents (13.2% to 25.4%). Average number of seeds per diploid plant was low also and varied little among cytoplasms and wheats, but exhibited a wide range for rye parents (2.8 to 9.2 seeds per plant).

Among individual parents in specific cytoplasms (Table 2) plant survival after colchicine treatment varied little. Greater variability in percentage values was exhibited by amphidiploid plants or plant sectors obtained among the wheat parents for cytoplasm *T. timococcum* and *T. timopheevi*, with the lowest values for line 32 (6.3% and 8.4%, respectively). Paternal genotypes differed most in *Ae. ventricosa* cytoplasm for proportion of doubled plants.

Seed set of amphidiploid plants was variable, but generally low, ranging from 0.4 seeds per plant of wheat line 32 in *T. timopheevi* cytoplasm to 11.3 seeds per plant of rye line L 301 in cytoplasm *T. timococcum*.

With regard to the characteristic plant survival, rye and cytoplasm were found to be statistically significant sources of variation, while for the trait amphidiploid plants obtained, it was the former source only (Table 3).

Discussion

Wheat × rye crossability

The mean seed set in general (Table 1) was of the order to be expected for European common wheats, which predominantly are of low crossability (Krolow 1970; Tanner and Falk 1981; Oettler 1982, 1984). The results clearly demonstrate that wheat cytoplasm, in addition to wheat and rye nuclear genotypes, appears to be of equal importance in wheat×rye crossability (Table 3). In a recent study, Stefanowska and Cauderon (1983) also found such a similar effect on crossability, which resulted in higher seed set, when utilizing the genome of a French common wheat in *Ae. ventricosa* cytoplasm. In the present study, however, none of the three cytoplasms enhanced mean seed set above the known level for adapted European common wheats, which normally ranges between 0% and 10%, although wheats with *Ae. ventricosa* cytoplasm tended to have higher crossabilities (Table 2).

Embryo development

With regard to embryo differentiation, the mean proportions of well-differentiated embryos (Table 1) were consistent with another recent study (Oettler 1984). Nucleo-cytoplasmic interactions were revealed in specific cross combinations (Table 2), as for example for wheat line 32 in *T. timococcum* cytoplasm with a low 6.7% as compared to the mean of the wheat parent and the cytoplasm.

The lack of significant effects due to maternal nuclear genotypes – all belonging to the hexaploid wheats – confirms earlier findings (Moss 1970; Oettler 1984) that the degree of embryo differentiation is primarily determined by the wheat species rather than by genotypes within the species.

The mean proportion of plantlets recovered in vitro is of the order found previously in the common wheat cultivar 'Okapi'. The almost complete failure of the well-differentiated embryos of rye genotype L 311 to survive in vitro clearly indicates the existence of strong and unpredictable parental nuclear genotype and/or nucleo-cytoplasmic interactions, since this line was not known to possess any severe cross-incompatibility with common wheat.

Colchicine treatment

The mean proportion of plant survival after colchicine treatment in *Ae. ventricosa* and *T. timopheevi* cytoplasm (Table 1) was higher as compared to other studies with hexaploid wheat cultivars (Oettler 1982, unpublished).

The lower value obtained for *T. timococcum* cytoplasm corresponds to previous results. It was mainly due to the poor performance of wheat line 32, which indicates a specific nucleo-cytoplasmic interaction.

The mean rate of amphidiploid plants or plants with doubled fertile sectors was of the order to be expected for hexaploid wheat×rye crosses (Tanner 1981; Oettler 1982; Cooper and Driscoll 1985). The only statistically significant source of variation for this trait in the present material were the paternal rye lines. But Table 2 indicates that nucleo-cytoplasmic interactions do exist, which are particularly obvious for wheat genotype 32.

The generally low fertility of the amphidiploid plants and the range of seed set of the cross combinations is in good agreement with earlier investigations (Oettler 1982, unpublished). Cytoplasm did not contribute to improve fertility of the hybrids, but nucleo-cytoplasmic interactions appear to be present, leaving scope for selection.

The data indicate that, in addition to wheat and rye nuclear genotypes, cytoplasm also plays a decisive role in the synthesis of primary triticales and that strong nucleo-cytoplasmic interactions exist for the traits investigated. However, the effect of the cytoplasm did not appear to be more important than the effects of the existing nuclear genetic variability for wheat and rye parents. None of the three cytoplasms was superior with respect to wheat×rye production. Thus, the suitability of parental genotypes and cytoplasms to establish primary triticales is primarily determined by the specific combinations used and/or their nucleo-cytoplasmic interactions.

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