

Influence of selected wheat and rye genotypes on the direct synthesis of hexaploid triticales

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Summary

In the synthesis of primary hexaploid triticales, a cross-incompatibility barrier exists when tetraploid wheat (4X) is crossed with diploid (2X) rye. Fertilization may occur, however, abnormal endosperm development usually leads to premature embryo death. Four selected tetraploid wheat lines were crossed as females with seven open-pollinated rye lines and the resulting embryos were rescued *in vitro* 13–16 days after pollination. The wheat genotypes showed a major influence on crossability (seed set), embryo development and plant recovery. The highest efficiency of amphihaploid plant recovery (18.3 plants per 100 pollinated florets) was obtained from one 4X wheat line originally selected from the cross *T. carthlicum* × *T. dicoccoides*. Some of the 3X amphihaploid plants (ABR) derived from two wheat lines showed relatively high level of partial fertility presumably as a result of meiotic restitution. Correlation analysis showed that crossability (seed set), normal hybrid embryo development *in vivo* and embryo culturability were independent of each other.

Introduction

The cross-incompatibility between wheat and rye is one of the limiting factors in the broadening of triticales germplasm base. In hexaploid triticales, the problem is compounded with premature death of 4X wheat-rye hybrid embryos between 14–20 days after fertilization. In most cases, viable plants can be obtained only after rescuing immature embryos *in vitro*.

Many studies have been conducted on the synthesis of wheat-rye hybrids but most of these studies focused on the crossability (seed set) *per se* of either hexaploid (Lein, 1943; Riley & Chapman, 1967; Lange & Wojciechowska, 1976; Wojciechowska & Lange, 1977; Jalani & Moss, 1980; Falk & Kasha, 1983) or tetraploid wheats (Jalani & Moss, 1981; Oettler, 1982; Verzea, 1990).

In the synthesis of primary hexaploid triticales, several factors other than crossability (seed set) are relevant. These include development of hybrid embryos *in vivo* and their survival when rescued *in vitro*. Also, primary triticales in the past invariably resulted from colchicine treatment, while information on the direct synthesis of hexaploid triticales via the natural fertility of their amphihaploids is lacking.

The objective of this study was to determine the genotypic influence of selected 4X wheat and rye genotypes on crossability, hybrid embryo differentiation *in vivo*, culturability of embryos *in vitro*, and natural fertility (meiotic restitution) of the synthesized amphihaploid plants. The ultimate goal is the isolation and identification of wheat and rye 'standards' that can be used as testers for routine primary triticales production (Darvey, 1990).

Materials and methods

Plant materials

Four selected tetraploid wheat lines were each crossed to seven open-pollinated spring rye lines. The wheat parents were selected on the basis of known crossability, good embryo development, and/or ability to reconstitute at meiosis in their amphihaploids. The four wheat parents were:

2 *Triticum turgidum* L. lines (4B925, 4B921)-obtained from the University of Manitoba (Canada);

2 selected lines (D and Do 1) from *T. carthlicum* (*T. persicum*) Nevski (4B280) × *T. dicoccoides* Korn. var. *spontaneonigrum* – D from Dr. Lapinski, Institute of Plant Genetics, Poznan, Poland and Do 1 from Dr. Lukaszewski, University of California, Riverside, USA.

The 7 spring rye lines used were local selections from the University of Sydney (Australia) rye breeding programme.

Plants were grown under greenhouse conditions with weekly mean minimum temperatures ranging from 11.0°C to 17.1°C and mean maximum temperatures from 26.4°C to 33.5°C.

Hybridization procedure

Wheat flowers were emasculated one to two days before anthesis and the basal, central and uppermost florets were removed prior to bagging. Receptive stigmas were dusted with rye pollen and the heads bagged again to prevent further pollination. Crossability (seed set) was assessed 10–15 days after pollination and recorded as the proportion of fertilized ovules over the total number of florets pollinated.

Embryo rescue

Embryo development was assessed 13–16 days after pollination and seeds were dissected using a stereo microscope under aseptic conditions. The dissected embryos were then classified as normal (partially or

well-differentiated with primordium and scutellum), abnormal (small globular structure) and/or seeds with no embryos. Only normal embryos were cultured *in vitro* in 9-cm disposable petri dishes since abnormal embryos generally do not develop into plants (Taira & Larter, 1978; Oettler, 1983).

A two-step embryo culture media was used. First, immature embryos were cultured in a modified Norstog medium (Taira & Larter, 1978) solidified in 0.28% agarose (Type 1A, Sigma). The cultures were then kept in the dark in an airconditioned room (22°C ± 2°C) for one to two weeks or until the embryos germinated to about 5–10 mm in length. The embryos were then transferred to MS medium (Murashige & Skoog, 1962) supplemented with IAA (1 mg/L) and BAP (1 mg/L) for further growth and differentiation. The culture bottles were placed under continuous light provided by white fluorescent lamps at a temperature of 26°C ± 2°C. After 2–3 weeks, the plants were transferred into a sand-peat mix in pots which were initially covered with, small plastic cups to minimize transpiration.

Direct synthesis of hexaploid triticale

Amphihaploid (triploid) plants obtained from cultured embryos were evaluated for fertility. These plants were not treated with any chromosome doubling agent (e.g. colchicine) and natural fertility was assessed at maturity.

Statistical analysis

Since the number of spikes pollinated was not fixed for each cross combination, a non-orthogonal analysis of deviance (ANODE) was done using generalised linear regression methods. Analysis was carried out using the GENSTAT 5 computer package (GENSTAT Reference Manual, 1987). All three variables (% seed set, % normal embryos and % plant regeneration) were analyzed as binomial-distribution variates (i.e. $0 \leq x \leq 1$) and observations from individual spikes were treated as experimental units in the analysis of deviance. ANODE is in-

Table 1. Non-orthogonal analysis of deviance for crossability (seed set), embryo differentiation *in vivo* and plantlet recovery

Source of variation	Crossability (% seed set)		Embryo differentiation (% normal embryos)		Embryo culturability (% plants recovered)	
	df	Mean deviance	df	Mean deviance	df	Mean deviance
Wheat	3	29.706**	3	37.842**	3	10.169**
Rye	6	3.650*	6	3.861*	6	3.216*
Wheat × Rye	18	1.470 ^{ns}	18	1.822 ^{ns}	18	0.916 ^{ns}
Residual	142	1.000	129	0.999	70	0.999

**0.01 > P > 0.001; *0.05 > P > 0.01; ^{ns}not significant, P > 0.05.

terpreted in the same manner as the analysis of variance (ANOVA). Linear correlation coefficients (r) were also determined for some characters.

Results

Crossability (seed set)

The analysis of deviance (Table 1) showed no significant interaction between wheat and rye parental genotypes on the proportion of fertilized ovules (P > 0.05), hence mean crossability values are pre-

sented for each parental genotype in Table 2. The wheat parents exhibited greater influence (0.01 > P > 0.001) than the rye parents (0.05 > P > 0.01) though both factors showed significant effects (Table 1).

Both *Triticum turgidum* L. lines (4B921 and 4B925) showed higher crossability with rye than the two other lines (D and Do 1). The range of crossability among the rye parents (48.7%–69.3%) was less than that obtained among the wheat parents (34.1%–74.2%) (Table 2).

Table 2. Mean crossability, hybrid embryo differentiation, embryo culturability and efficiency of amphihaploid plant recovery of four tetraploid wheats each crossed to seven open-pollinated spring rye lines

Factor (Treatment)	Crossability (% seed set ± s.e.) ^a	Embryo differentiation (normal embryos/ 100 seeds ± s.e.) ^b	Embryo culturability (plants/100 embryos ± s.e.) ^c	Efficiency (plants/100 pollinated florets)
A. Wheat				
4B921	74.2 ± 2.6 (1394)	33.4 ± 4.7 (576)	14.1 ± 4.2 (213)	4.6
4B925	63.3 ± 3.1 (1553)	56.6 ± 4.5 (295)	18.5 ± 4.8 (191)	5.1
D	52.0 ± 2.4 (1438)	78.9 ± 2.8 (419)	44.5 ± 5.1 (287)	18.3
Do 1	34.1 ± 3.6 (951)	82.9 ± 3.4 (271)	25.1 ± 5.3 (186)	7.1
B. Rye				
CP rye	69.3 ± 4.4 (714)	46.3 ± 7.2 (230)	37.6 ± 6.1 (139)	12.0
Rye 75	64.0 ± 4.8 (760)	76.0 ± 4.8 (258)	17.7 ± 5.3 (132)	8.6
31B-767	61.2 ± 4.3 (804)	62.5 ± 6.5 (287)	22.6 ± 6.1 (159)	8.7
C rye	57.8 ± 4.9 (839)	62.7 ± 4.4 (283)	15.0 ± 5.4 (156)	5.4
R 226	53.3 ± 4.6 (814)	71.0 ± 8.8 (213)	36.6 ± 7.4 (148)	13.8
K 57	51.4 ± 4.8 (738)	51.8 ± 8.0 (172)	42.4 ± 12.8 (79)	11.3
R 258	48.7 ± 4.3 (667)	58.1 ± 9.9 (118)	33.1 ± 9.7 (64)	9.4

^{a,b,c} Figures in parenthesis refer to number of florets pollinated, number of embryos excised and number of embryos cultured *in vitro*, respectively.

Embryo development

No significant interaction was detected between wheat and rye parents on the proportion of normal hybrid embryos ($P > 0.05$) (Table 1). Both wheat and rye parents showed significant effects with the wheat parents showing a greater influence ($0.01 > P > 0.001$) than the corresponding rye lines ($0.05 > P > 0.01$).

The mean values for normal hybrid embryo differentiation (% normal embryos) of each parental wheat and rye genotype are summarized in Table 2. In contrast to crossability (% seed set), wheat lines 'D' and 'Do 1' showed superior normal hybrid embryo differentiation *in vivo* (78.9% and 82.9%, respectively). The rye parents did not differ markedly with 'Rye 75' giving the optimum response (76.0%) (Table 2).

Embryo culturability and plant recovery

The mean effects of the wheat and rye genotypes on the survival and development of cultured embryos *in vitro* is presented in Table 2. The ANODE indicated a greater influence of wheat genotypes ($0.01 > P > 0.001$) than the rye parents ($0.05 > P > 0.01$)

though both factors showed significant effects (Table 1). Among the wheat parents, the 'D' line gave the highest proportion of regenerated plants (44.5%) while the two *T. turgidum* lines (4B921 and 4B925) gave significantly lower plant recovery (14.1% and 18.5%, respectively) (Table 2). It is noted that '4B921' which gave the highest crossability produced the lowest proportion of regenerated plants.

Considerable variation was also observed among the rye parents with 'K 57' giving 42.4% regenerated plants in contrast to only 15.0% plants regenerated in 'C rye'.

Fertility of 3x amphihaploids

Due to space limitations, the amphihaploids were sown in two greenhouses. 77 of the 207 amphihaploid plants recovered were grown at 21°C/16°C (day/night) and the rest were grown in a greenhouse with a temperature from 12°C-18°C. The two *T. turgidum* lines (4B921 and 4B925) did not produce any selfed seeds in either greenhouse (Table 3). In contrast, 'D' and 'Do 1' gave partially fertile plants (13.5% and 9.8%, respectively). However, the partially fertile plants were obtained only in the

Table 3. Mean effects of wheat and rye genotypes on the fertility of triploid (ABR) wheat-rye amphihaploids

Genotype	Total plants evaluated ^a	Percent partially fertile plants	Mean no of seeds		Total selfed seeds produced
			per plant	per fertile plant	
Wheat					
4B921	27 (7)	0	—	—	—
4B925	35 (3)	0	—	—	—
D	104 (45)	13.5	1.1	8.4	117
Do 1	41 (22)	9.8	0.8	8.0	32
Rye					
CP rye	40 (7)	0	—	—	—
Rye 75	15 (6)	20.0	0.9	4.7	14
31B-767	33 (17)	9.1	0.4	4.3	13
C rye	22 (7)	13.6	1.0	7.3	22
R 226	50 (22)	2.0	0.02	1.0	1
K 57	35 (13)	22.9	2.8	12.4	99
R 258	12 (5)	0	—	—	—

^aFigure in parenthesis indicates the number of plants evaluated at 21°C/16°C (day/night) greenhouse; the rest were grown in a different greenhouse with mean minimum and maximum temperatures of 12 and 18°C, respectively.

21°C/16°C glasshouse suggesting that growing conditions influence the degree of fertility. More selfed seeds were produced by 'D' line (117 seeds) compared to 'Do 1' (32 seeds); however, the mean number of seeds per plant was similar in both parents.

Two of the rye lines (CP rye and 31B-767) did not produce partially fertile amphihaploids. The most responsive rye line (K 57) gave 22.9% partially fertile amphihaploids (Table 3).

Correlation among characters

The degree of association (correlation) of the three characters relevant to amphihaploid plant production, namely, crossability or seed set (SS), normal hybrid embryo development *in vivo* (NE) and embryo culturability (EC) was determined. Correlation coefficients (r) are summarized in Table 4. The three characters were not highly associated and appeared to be independent of each other. Among the rye parents, there seemed to be a consistent but non-significant negative correlation between SS and NE and between SS and EC.

In order to determine which of the three characters had the greatest influence on the final efficiency of amphihaploid plant recovery (PR), each char-

acter was checked for its correlation to PR. It was noted that EC had the greatest influence on PR.

Discussion

The effectiveness of routinely producing primary triticales from 4X wheat × rye crosses depends on many factors including time and cost constraints, the efficiency of crossability (seed set), development and differentiation of hybrid embryos *in vivo* and culturability of embryos when rescued *in vitro*. In previous reports, primary triticales invariably resulted from colchicine treatment of amphihaploid plants. In the present study, we have shown an efficient direct synthesis of triticales via meiotic restitution in amphihaploids which can overcome one of the key limitations to the efficiency of primary triticales production.

The level of crossability obtained in three of the four tetraploid wheat parents (52%–74%) showed a marked improvement over maximum crossability (seed set) values reported earlier in the literature (Kaltsikes, 1974; Oettler, 1982, 1983, 1984; Verzea, 1990; Immonen et al., 1990) but was of the same range as that reported by Taira et al. (1978). The significant effects of both the wheat and rye genotypes

Table 4. Linear correlation coefficients (r) of four characters (crossability, normal hybrid embryo development *in vivo*, embryo culturability and plant recovery per 100 florets pollinated) among wheat and rye genotypes

Parent	Character associated ^a					
	SS/NE	SS/EC	NE/EC	SS/PR	NE/PR	EC/PR
Wheat (n = 4)						
4B921	0.108	-0.409	-0.834*	-0.466	0.219	0.193
4B925	0.212	-0.076	-0.183	0.366	0.087	0.870**
D	0.372	-0.759	-0.397	-0.286	0.323	0.686
Do 1	0.660	-0.417	-0.224	0.135	0.196	0.834*
Rye (n = 7)						
CP rye	-0.793	-0.897	0.530	-0.564	0.915	0.376
Rye 75	-0.661	-0.450	0.397	-0.187	0.534	0.877
31B-767	-0.804	-0.067	0.482	-0.252	0.607	0.982*
C rye	-0.696	-0.313	0.395	-0.210	0.358	0.993**
R 226	-0.618	-0.178	-0.176	-0.344	0.420	0.789
K 57	-0.721	-0.780	0.617	-0.534	0.754	0.866
R 258	-0.505	-0.367	0.555	0.253	0.645	0.594

^aSS seed set; NE normal embryos; EC embryo culturability; PR plantlet recovery per 100 florets. *0.05 > P > 0.01; **0.01 > P > 0.001.

on crossability is in agreement with the results obtained by other workers (Taira et al., 1978; Oettler, 1982, 1983, 1984). Furthermore, the greater influence of the wheat genotypes on crossability than their corresponding rye parents confirms the observations made by Jalani & Moss (1981) and Oettler (1984).

It is generally believed that the same genes (Kr_1 and Kr_2) controlling the crossability in 6X wheat \times rye crosses (Lein, 1943) also operate in 4X wheat \times rye crosses. However, Jalani & Moss (1981) observed that the incompatibility system operating in 4X wheat \times rye crosses involves factors other than the action of crossability (Kr) genes, especially since the resulting hybrid seed from such crosses is generally devoid of endosperm and non-viable. According to Jalani and Moss (1981), the cross-incompatibility of 6X wheat \times rye crosses is a result of the interaction between the pollen and the ovary, whereas the cross-incompatibility in 4X wheat \times rye crosses is manifested both prior to fertilization (due to action of crossability genes) and during embryo and endosperm development. This suggests that the cross compatibility between 4X wheat and rye depends not only on their crossability alleles *per se* but also on their ability to produce either viable F_1 seeds *in vivo* or normally differentiated embryos which can develop into plants when rescued *in vitro*.

We have shown in the present study that crossability (seed set) is not the major determinant for an efficient amphihaploid plant recovery. For example, the highly crossable wheat genotypes (4B925 and 4B921) did not result in optimal plant recovery whereas other wheat lines with intermediate crossability (D and Do 1) gave a higher proportion of well-differentiated embryos and amphihaploid plant regeneration.

Although embryo size and degree of embryo differentiation were not recorded, it was apparent that the larger size and better differentiation of the hybrid embryos obtained from 'D' and 'Do 1' were responsible for their high proportion of surviving embryos and high plant recovery *in vitro*. This suggests that *T. carthlicum* and/or *T. dicoccoides* carry gene (s) that enhance hybrid embryo development *in vivo*.

From correlation analyses, it was also evident

that efficiency of plant recovery was highly associated with embryo culturability rather than with crossability. This clearly indicates that other factors (e.g. embryo quality) are equally if not more important than crossability for amphihaploid plant production. These factors therefore need to be taken into consideration in selecting desirable wheat and/or rye parents for routine primary triticale production.

One of the outstanding features in two of the wheat parents (D and Do 1) was their ability to produce some functional gametes in their amphihaploids. Generally, amphihaploid plants obtained from 4X wheat \times 2X rye crosses are completely sterile. These two wheat lines, however, have shown partial fertility in their amphihaploids presumably as a result of meiotic restitution. Meiotic restitution in amphihaploids leads to the production of functional unreduced ($2n = 21$) gametes (via sister-chromatid separation at the first meiotic division) as previously observed and reported in other intergeneric or interspecific hybrids (Maan & Sasakuma, 1977; Xu & Dong, personal communication).

In wheat \times rye, preliminary results have shown that meiotic restitution also occurs, however, the level of fertility is generally low (Chauhan & Patel, 1978; Darvey & Durvasula, 1980). Since both sources of *T. turgidum* used in this study failed to produce seed in any of their resulting amphihaploids, it is likely that the other *Triticum* species (*T. carthlicum* and/or *T. dicoccoides*) contributed to the partial fertility of the amphihaploids. Recent observations in our laboratory on a *T. carthlicum*-*S. cereale* amphihaploid which likewise produced unreduced gametes and selfed-seed confirm the above implication and further suggest that the same factor(s) may likewise contribute to the meiotic restitution which occurred in amphihaploids involving the same source of *T. carthlicum* crossed with *T. tauschii* (Xu & Dong, personal communication).

It is important to note that the level of partial fertility obtained in the present study is similar to that obtained from colchicine-treated wheat-rye amphihaploid plants (Lelley & Taira, 1979; Oettler, 1982; Taira et al., 1991). Hence, it is clear that the use of genes for meiotic restitution can successfully overcome the sterility of wheat-rye amphihaploids. The

alternative solution of doubling the chromosome number with colchicine is expensive, laborious and potentially hazardous to health.

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