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Hybrid seed development and viability in crosses of *T. aestivum* (cv. Chinese Spring) monosomic lines with *S. cereale*

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Summary. The diploid and tetraploid wheats have a crossing barrier that inhibits development and viability of the F_1 hybrid seeds which result from their pollination with rye. This barrier is ineffective in synthetic or natural hexaploid wheat × rye crosses. From the results obtained by crossing the Chinese Spring monosomic series to a diploid rye composite, it was concluded that the breakdown of this barrier in hexaploid wheats is determined by polygenes, but may also involve gene-dosage effects. While more than half of the hexaploid-wheat chromosomes may contribute to the breakdown of the barrier, chromosome 1D had the strongest effect. Its absence resulted in shrivelled and inviable hybrid kernels similar to those obtained when the *T. durum* cultivar Langdon was crossed with rye.

Key words: Wheat – Rye – Crossing barrier

Introduction

The Kr genes that regulate the frequency of kernels set in wheat × rye crosses are well documented (Lein 1943; Riley and Chapman 1967; Jalani and Moss 1980). However, little is known about a second, and apparently independent, crossing barrier which suppresses hybrid seed development in diploid and tetraploid wheat × rye crosses (Krolow 1970). Such hybrid kernels are characteristically shrivelled and inviable. The suppression of F_1 hybrid seed development was only partial when certain diploid and tetraploid wheats were crossed to the more primitive species of rye, e.g., *S. sylvestre* and *S. vavilovii* (Krolow 1964, 1970; Nakajima 1955). A synthetic tetraploid wheat (Tetra Canthatch) derived from hexaploid Canthatch (Kerber and Rowland 1974) produced only shrivelled kernel when crossed with rye (Marais and Pienaar 1977). In hexaploid wheat × rye crosses (Marais and Pienaar 1977) as well as synthetic hexaploid wheat × rye crosses, i.e., AABBDD × rye (Marais and Pienaar 1977) or AAAABB × rye (Krolow 1970, 1973) development of the hybrid kernels is fairly normal. Pollination of hexaploid triticale with diploid or tetraploid rye produced well-developed kernels (Pienaar 1985). Substitutions of D genome chromosomes for individual A and B genome homoeologues of the durum variety Langdon (Joppa 1985) did not improve the development of the F_1 seed obtained in crosses of these substitution lines with rye (Pienaar and Marais 1986).

Starvation of the endosperm and embryo as a secondary result of abnormal post-fertilization development may be a component of hybrid-seed failure (Brink and Cooper 1940, 1947). Aberrant endosperm and embryo growth were regular features in wheat × rye hybrids (Keyworth and Larter 1979), and the cellular- and nuclear-doubling times differed from that in normal wheat endosperm resulting from self-fertilization (Wojciekowska and Lange 1977). Hypotheses aiming to explain the causes of failure of hybrid seed development in interspecific crosses were considered by various authors, but presently none of these explanations seem to have a proven, general applicability (Brink and Cooper 1947; Nishiyama and Yabuno 1978; Johnston et al. 1980).

In this investigation, which aims to study the effects of individual wheat chromosomes on the expression of the seed developmental barrier in wheat × rye crosses, use was made of the Chinese Spring monosomic series developed by Sears (1953), because the $kr_1kr_1kr_2kr_2$ genotype of this cultivar does not prevent hybrid seed set. The development of the wheat female gametophyte is such that the egg cell and polar nuclei receive identical chromosomes (Briggs and Knowles 1967). Thus, meiosis in a monosomic plant results in the simultaneous absence or presence of the monosomic chromosome in both the egg cell and the polar nuclei. Regarding monosomic plants, Morris and Sears (1967) explained that chromosome lagging during meiosis on average produces 75% egg cells with 20-chromosomes and 25% with 21-chromosomes. The transmission rates differ for different monosomics (Tsunewaki 1963), and in Chinese spring 20-chromosome egg cells may range from approximately 57–82%, whereas 21-chromosome egg cells may range from 18–43%.

Materials and methods

Seeds of the complete Chinese Spring monosomic series (obtained from Prof. E. R. Sears, University of Missouri-Columbia, Missouri) were germinated, and the monosomic plants were identified and raised in a greenhouse. Up to eight plants per monosomic line were used in crosses with a diploid rye composite developed by the Department of Genetics, University of Stellenbosch. Euploid plants of Chinese Spring and Langdon (*T. durum*) were included to serve as indicators of the neutralized and fully operative effect of the seed developmental barrier, respectively. Hybrid kernels were harvested and graded visually as "filled" or "shrivelled". The thousand-kernel mass of each group of hybrid kernels was determined. After allowing for a three to four-month post-harvest resting period, the F_1 kernels were germinated in Petri dishes at 20 °C.

With respect to each monosomic line, the ratio of filled: shrivelled kernels and viable: inviable kernels expected if absence of the specific chromosome resulted in total reinstatement of the seed developmental barrier, were calculated. For this purpose the monosome transmission rate data given by Tsunewaki (1963), and the hybridization results obtained with Chinese Spring and Langdon in the present study, were used. The calculations were based on the assumption that the seed development and viability of a proportion of the wheat × rye F_1 kernels, which corresponds to the transmission rate of the monosome through the egg cell, equals that of euploid Chinese Spring. Seed development and viability of the balance of the F_1 seeds, i.e., those with 27 chromosomes, could be similar to that of the tetraploid wheats.

The seed classification and germination data were subsequently transformed to the form Arcsin $\sqrt{\text{percentage}}$, to allow for the execution of one-way analyses of variance (with unequal numbers of observations per entry) as outlined by Snedecor and Cochran (1967). Tests of significance (t) were done to ascertain whether the results produced by a specific monosomic line differed from that obtained on normal Chinese Spring.

These results were also plotted. The effectiveness in percentage, of the seed developmental barrier, was calculated as follows for both seed quality and viability in each Chinese Spring × rye cross.

$$\frac{(A-B)}{(C-B)} \times 100$$

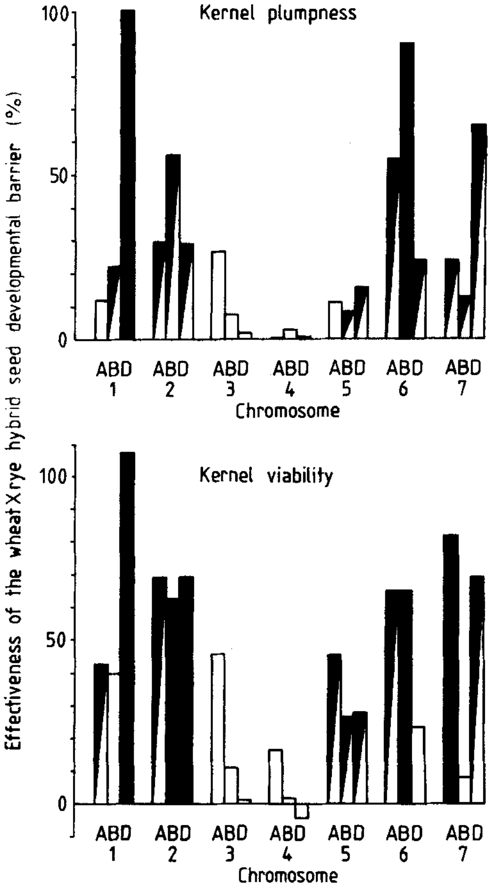

Fig. 1. Hybridization results. ■ : not significantly (5%) different from Langdon; ▨ : intermediate; □ : not significantly different from Chinese Spring

Where, A = Observed frequency of shrivelled/inviable kernels; B = Observed frequency of shrivelled/inviable kernels in Chinese Spring × rye crosses; C = Expected frequency of shrivelled/inviable seeds with the barrier fully operative.

Results and discussion

The crossing and germination results are given in Table 1, and the degree of shrivelling of the hybrid seeds obtained in each monosomic line × rye cross, as well as their viability, are graphically illustrated in Fig. 1. The base line in these graphs represents the level of kernel development/viability observed in the cultivar Chinese Spring, while a 100% expression of the barrier is comparable to the poor F_1 seed development/viability obtained in the *T. durum* × rye crosses. The relatively high 1,000-kernel mass of the shrivelled hybrid seeds resulting from the Langdon × rye cross

Table 1. Results of crossing the Chinese Spring monosomic series with a spring rye composite

Entry	No. of plants involved	No. of florets pollinated	Kernels set (% of florets pollinated)	Filled kernels			Shrivelled kernels			Total kernels	
				% of total kernels set	1,000-kernel mass (g)	Germin-ability (%)	% of total kernels set	1000-kernel mass (g)	Germin-ability (%)	1000-kernel mass (g)	Germin-ability (%)
Chinese Spring monosome:											
1A	2	826	68.2	83.7	15.5	59.4	16.3	5.8	1.1	13.9	49.9
2A	5	1,813	77.6	75.4	14.7	55.6	24.6	5.9	3.6	12.6	42.8
3A	4	1,082	84.5	75.3	16.3	67.0	24.7	5.7	3.1	13.7	51.2
4A	8	2,521	89.3	92.1	14.9	70.5	7.9	5.9	5.1	14.2	65.5
5A	4	2,200	82.4	84.2	13.7	56.1	15.8	5.1	7.0	12.3	48.3
6A	4	929	60.8	54.9	13.8	63.2	45.1	3.9	8.6	9.3	38.6
7A	1	935	87.0	77.1	12.4	39.6	22.9	4.7	8.6	10.6	32.5
1B	3	911	75.2	79.7	13.3	66.3	20.3	4.2	15.1	11.4	55.9
2B	2	893	72.8	58.0	16.9	68.4	42.0	6.5	11.0	12.5	44.3
3B	3	935	82.4	87.0	17.8	75.7	13.0	6.2	13.0	16.3	67.5
4B	3	1,759	83.7	90.3	13.1	77.8	9.7	5.0	26.6	12.3	72.8
5B	4	1,251	87.2	87.0	12.3	68.9	13.0	4.0	9.2	11.2	61.1
6B	3	1,090	81.6	47.0	13.1	72.0	53.0	3.0	26.3	7.8	47.8
7B	4	1,128	83.0	84.4	14.1	81.3	15.6	5.5	8.2	12.7	69.9
1D	4	955	61.3	22.1	14.8	62.8	78.0	1.6	0.2	4.5	14.0
2D	3	1,064	78.4	74.6	13.3	50.6	25.4	1.1	11.8	11.2	40.8
3D	3	1,263	71.5	90.7	15.6	78.6	9.3	5.4	22.6	14.6	73.4
4D	3	939	85.9	91.7	14.2	80.7	8.3	5.4	23.9	13.4	76.1
5D	5	1,232	79.0	82.1	13.2	70.3	17.9	3.7	12.1	11.5	59.9
6D	6	1,321	82.1	75.6	13.5	76.3	24.4	3.7	11.4	11.1	60.5
7D	4	1,027	75.8	53.3	11.6	70.4	46.7	2.8	6.6	7.5	40.6
Controls:											
Chinese Spring	14	2,511	86.4	92.1	15.5	78.6	7.9	6.7	19.8	14.8	73.9
Langdon (*T. durum*)	3	439	39.6	4.0	14.1	28.6	96.0	6.6	3.0	6.9	4.0

(Table 1) is due to the fact that the caryopsis of Langdon is much longer than that of Chinese Spring.

A large percentage of the F_1 hybrid kernels produced by 13 different monosomic lines were markedly inferior to those produced by the Chinese Spring disomic × rye cross. Absence of each of the eight remaining chromosomes had no detectable effect on hybrid seed quality.

The most striking result was obtained with the monosomic 1D × rye cross. As is evident from Table 1, this cross resulted in 78% of the F_1 seeds containing virtually no endosperm. These seeds in fact closely resembled the shrivelled hybrid seeds produced by the Langdon × rye cross, and probably do not possess chromosome 1D. The absence of chromosome 6B likewise resulted in a proportion of F_1 seeds with poor endosperm development which is not significantly different from that in the Langdon × rye cross. However, the degree of shrivelling observed was not as great as that of the Langdon × rye F_1 seeds or that of the F_1 seeds without chromosome 1D.

While the monosomic 2D line also produced some 25% rather shrivelled F_1 hybrid seeds, the degree of shrivelling in the hybrid seeds produced by the other monosomic lines was less pronounced. In the case of chromosome 3A, one of the three plants crossed produced results quite different from that of the other two. Due to a large number of florets pollinated on this plant, the overall proportion of shrivelled kernels is biased in its favour. Since the tests of significance were based on the transformed proportions obtained for individual plants, the transformed mean does not show up as significantly different from zero. The same situation applies to a lesser extent to some of the other crosses, while the unequal numbers of plants per cross also influenced the outcome of tests of significance.

It is evident from Table 1 and Fig. 1 that a high proportion of the reasonably well developed F_1 kernels resulting from crosses involving monosomic lines 1A, 5A and 7A did not germinate. Conversely, especially in the case of monosomic line 6B it was observed that a fairly high proportion of the badly shrivelled kernels germinated normally. Homoeologous groups 3 and 4, as well as the monosomic lines 1B, 6D and 7B had little, if any, effect on hybrid seed viability. The individual absence of at least five chromosomes (viz. 1D, 2B, 2D, 6B and 7A) severely reduced hybrid seed viability, the effect of chromosome 1D being the most marked. Thus, the results appear to indicate that more than half of the chromosomes of the hexaploid wheat complement are cumulatively involved in the development of the hybrid seeds resulting from the hexaploid wheat × rye crosses. It must be realized, however, that the absence of individual wheat chromosomes from the hybrid embryo (ABDR) may produce effects that are not totally related to the normal operation of the developmental barrier. In view of the results obtained by Krolow (1964, 1970), when he used different rye species to pollinate diploid and tetraploid wheats, it seems possible that the genotype of the rye pollen may also contribute to the course of hybrid seed development.

The present results implicate at least two chromosomes in five homoeologous groups as affecting development of the hybrid seeds resulting from wheat × rye crosses. Their effects range from weak responses predominantly affecting viability, or endosperm shrivelling, or both, to the very drastic effect associated with chromosome 1D, and it is likely that different gene products are involved. When these observations are considered in conjunction with the findings of Krolow (1970, 1973), i.e., that increased doses of the A genome can achieve the same effect as an additional D genome, it seems likely that the D genome does not produce a unique gene product(s) that causes failure of the barrier preventing seed development. It also seems likely that gene dosage effects may, at least in part, be important contributors to this end. These observations are strengthened by the fact that single D genome substitutions in *T. durum* did not alter the endosperm developmental barrier, even when Chinese Spring chromosome 1D was substituted into Langdon (Pienaar and Marais 1986). It therefore seems likely that a certain production level of a range of gene products is needed to overcome the barrier to seed development. This situation may be readily achieved in wheats with a higher ploidy level.

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