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Production and cytogenetics of hybrids of *Triticum aestivum* × *Leymus innovatus*

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Summary. Hybrid plants were obtained between *Triticum aestivum* (2n = 6x = 42, AABBDD) and *Leymus innovatus* (2n = 4x = 28, JJNN) at a frequency varying from 0.4% to 1.2% of the pollinated florets. Improvement of the embryo culture medium resulted in a higher frequency of embryo rescue. Eight of ten hybrids had the expected chromosome number of 35 (ABDJN). Meiotic analysis indicated that there was no homology between the genomes of the two species. Two hybrids had only 28 chromosomes. Comparison of chromosome pairing between the two types of hybrids suggested that *Leymus innovatus* carries genes that affect chromosome pairing and behavior. The relatively high occurrence of spontaneous doubling in the meiocytes of these hybrids may indicate that backcrossing of the hybrids to wheat should be possible, although frequent chromosome irregularities observed in the meiocytes of the hybrids may decrease the probability of success of this step, which is essential to the process of gene transfer from *L. innovatus* to wheat.

Key words: Intergeneric hybrids – Wheat – *Leymus* – Chromosome pairing – Meiosis

Introduction

Leymus is a genus of about 30 species which are long-lived perennials. They are distributed from the coastal regions of the North Sea (*L. arenarius*) across Central Asia (*L. racemosus, L. angustus, L. secalinus*) to East Asia (*L. chinensia*) into Alaska (*L. mollis*) and western North America (*L. innovatus, L. triticoides, L. cinereus*). Growing in diverse environmental conditions, they exhibit interesting attributes for wheat improvement such as resistance to diseases, perennial habit and tolerance to salinity, drought and alkalinity (Dewey 1984).

Since the early 1940s, considerable work has been done in the field of intergeneric hybridization between species of the genus *Triticum* and the alien species of *Leymus*. However, out of the 30 species belonging to this genus, only 7 (*L. racemosus, L. arenarius, L. mollis, L. cinereus, L. triticoides, L. angustus* and *L. multicaulis*) have been successfully hybridized to wheat (Bodrov 1960; Petrova 1960; Mujeeb-Kazi and Rodriguez 1980, 1981a, b; Thomas et al. 1981; Mujeeb-Kazi et al. 1984; Comeau et al. 1985; Plourde et al. 1989). Although no commercial cultivar carrying *Leymus* genes has yet been released, the agronomic potential of wheat × *Leymus* amphiploids and derivatives in a wheat improvement program has been established (Fatih 1983; Maslova et al. 1985). Because intergeneric gene transfer between *Leymus* and *Triticum* seems possible and because this genus represents a gene pool of great interest, it is worthwhile to extend the investigation to other species of the genus.

This paper presents the production and cytogenetics of a new wheat × *Leymus* combination involving the hexaploid wheat species *Triticum aestivum* (2n = 6x = 42, AABBDD) and a tetraploid *Leymus* species native to North America, *Leymus innovatus* (2n = 4x = 28, JJNN).

Materials and methods

The three strains of *L. innovatus* (synonym *Elymus innovatus*) used as male parent in this experiment were accessions RS-3-1,5, Pi 387918, Pi 387921 and Pi 387923. All accessions originated in Canada. The strains were established in 1980 in the perennial species nursery at Laval University.

The female parents used were the spring wheat *Triticum aestivum* cultivars Asakaze, Fukuho and Chinese Spring, selected for their good crossability (Falk and Kasha 1981; Inagaki

Table 1. Production of F_1 hybrids between *Triticum aestivum* cultivars Fukuho, Asakaze and Chinese Spring and four accessions of *Leymus innovatus* in 1985, 1986 and 1987 at Laval University under field conditions

Wheat parent	*L. innovatus* accession	Year of crossing	Florets pollinated	Seeds obtained	Embryos excised	Plantlets produced	% of pollinated florets
Fukuho	RS-3-1,5	1985	104	1	1	1[a]	0.9
		1986	200	5	1	1[a]	0.5
		1987	168	2	2	2	1.2
	Pi387921	1986	530	5	3	0	0
		1987	2,234	14	12	9	0.4
	Pi387923	1987	471	0	0	0	0
Asakaze	Pi387918	1985	96	0	0	0	0
	Pi387921	1985	124	0	0	0	0
Chinese Spring	RS-3-1,5	1986	487	10	2	0	0
	Pi387921	1986	92	0	0	0	0

[a] Hybrid plants died at 1- to 2-leaf stage

and Snape 1982; Comeau et al. 1985). Seeds were sown in flats. Plantlets of Chinese Spring were vernalised for 6 weeks at 4 °C. At the 4- to 5-leaf stage, plantlets were transplanted in the field in 1985. In 1986 and 1987, they were transplanted into pots, 15 cm wide, and grown in a screen-covered shelter. Crosses were made in the summer of 1985, 1986 and 1987 using the approach method as described by Comeau et al. (1985).

In 1985, hybrid seeds were collected at 15–20 days following pollination. The embryos were aseptically dissected and cultured in the dark on a modified Norstog II (NII) medium (Norstog 1973) adjusted to pH 5.4. The modifications consisted in using 10 mg/l of Fe-Na EDTA and 6 mg/l of ferric ammonium citrate instead of 10 mg/l of Fe-citrate.

In 1986, embryo rescue was done as previously described, except that the dissected embryos were cultured on the modified NII medium supplemented with 10 mg/l of L-serine, L-proline and L-tryptophane, and 1 mg/l of Accel, product DPX-CI504, a synthetic cytokinin (N-(phenylmethyl)-9-(tetrahydro-2H-pyran-2yl)-adenine) from Dupont (Wilmington, Delaware), supplied to us in acetone. In addition, the malic acid concentration was reduced to 0.5 g/l and the agar concentration was increased to 7.0 g/l.

For the 1987 production, hybrid seeds were collected at 7–10 days following pollination. Embryos and proembryos were grown using novel media and methods (A. Comeau, unpublished results).

The cultured embryos were maintained at room temperature in the dark until germination. They were then transferred to B5 medium (Gamborg 1982) and exposed to fluorescent lighting until they developed chlorophyll. At the 1- to 2-leaf stage and when the roots were well developed, plantlets were transplanted into peat pellets («Jiffy-7») soaked with distilled water containing 1 g/l of 20-20-20 N-P-K and 0.2 g/l of NH_3, and placed in a plastic bag. They were kept in a growth chamber maintained at a day/night temperature of 20°/15 °C and a photoperiod of 16 h, supplied by a combination of fluorescent and incandescent lamps providing energy at 600 $\mu E/m^2/s$. They were watered when needed and later on transplanted into 15-cm pots. Plants were grown in a greenhouse in conditions set as for the growth chamber.

Root tips for somatic chromosome counts were prepared according to the standard Feulgen technique and squashed in acetocarmine. Spikes of hybrid plants at the pollen mother cell (PMC) stage were fixed in Carnoy's solution (6:3:1 ethanol-chloroform-acetic acid) and the anthers were squashed in aceto-carmine for meiotic studies.

The c value, which is the mean chromosome arm pairing frequency, was calculated based on the formula $c = Xt/3 \times B$ for the tetraploid hybrids (Kimber and Alonso 1981) and $c = Xt/4 \times B$ for the pentaploid hybrids (Espinasse and Kimber 1981), where Xt is the mean number of chiasmata per cell and B, the basic chromosome number of the species.

Results

Production

Details on the number of embryos excised and number of wheat × *L. innovatus* hybrid plantlets produced in the field at Laval University, Sainte-Foy, Québec, in the summer of 1985, 1986 and 1987, are given in Table 1.

In the summer of 1985, a total of 324 wheat florets were pollinated with *L. innovatus*. One hybrid seed, containing a differentiated embryo, was produced from the cross Fukuho × *L. innovatus* accession RS-3-1,5 (0.96% of pollinated florets). The embryo was successfully rescued but died at the 1-to 2-leaf stage, possibly from a phenomenon similar to hybrid necrosis (Pfeffer and Zeller 1987).

In 1986, two wheat cultivars, Fukuho and Chinese Spring, were used as female parents in crossing with two accessions of *L. innovatus*. Out of the 1309 florets pollinated, 20 set seeds. The frequency of seed set for the wheat cv Fukuho was 2.5% and 0.9% and for the cultivar Chinese Spring, 2.0% and 0%, in crosses with *L. innovatus* accessions RS-3-1,5 and Pi 387921, respectively. On average, only 30% of the hybrid seeds had embryos that could be cultured on the modified NII medium. All the embryos were at the globular stage and only one was successfully rescued, but the plantlet died at the 1- to 2-leaf stage, similarly as in the previous year.

In 1987, 16 hybrid seeds were obtained from 2,873 pollinated florets, and 7 of these contained well-differentiated embryos that could possibly have grown on NII medium; however, these were grown using novel media

and methods (A. Comeau, unpublished results). Some endosperm development was found in these grains. Two hybrid seeds contained no visible embryo or endosperm. From the remaining grains, which were no larger than an ovary, 6 plantlets were rescued with new media and methods (A. Comeau, unpublished results). In total, 11 plantlets were produced (Table 1). Two of the plantlets apparently came from proembryos, this event being a novelty. The morphology of these plants was initially somewhat similar to tissues grown from 80-cell barley proembryos (Norstog 1961). Cytogenetic studies were made on 10 of the plantlets.

Morphology

L. innovatus is a cross-pollinating species. Its spikes are rather dense, 5–10 cm long, and the rachis is very flexible. The lemmas are densely purplish-villose. The spikelets are usually present in pairs at each node of the rachis. It has relatively short, slender rhizomes and the spike morphology is somewhat variable (Scoggan 1978).

The *T. aestivum* cv Fukuho has an average spike length of 10 cm. Its spikelets are single on each node. The spikelets and the rachillas are glabrous.

The F_1 hybrid plants were all much taller than both parents. They were generally vigorous, tillered profusely, were not rhizomatous and did not express the purple lemma color of the alien species. The spikes were either equal to or longer than those of the parents (Fig. 1). Seven of the hybrids (INN-2, INN-4, INN-5, INN-6, INN-7, INN-8, INN-9) expressed the pubescent character of *L. innovatus* at different degrees, from sparsely pubescent to densely villous. Hybrids INN-1, INN-3 and INN-10 were glabrous as 'Fukuho'. The hybrids had a spike and spikelet morphology intermediate between both parents except for hybrids INN-3 and INN-10, which somewhat resembled wheat cv Fukuho, although they had longer spikes (Fig. 1 a and b). Hybrid vigor was also generally obvious in the root system.

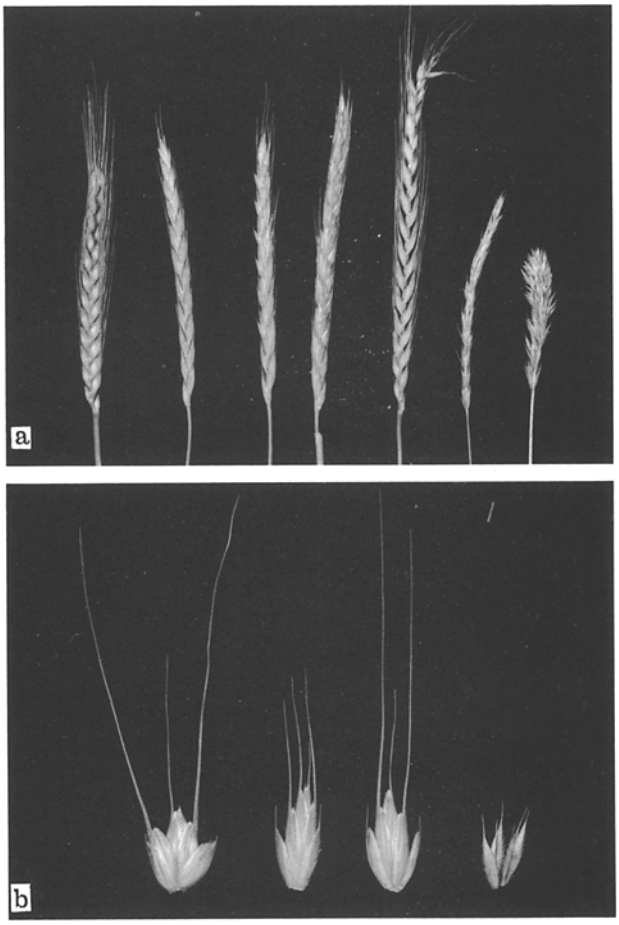

Fig. 1 a and b. Spike and spikelet structure of wheat × *L. innovatus* hybrids and their parents. **a** Spikes of *Triticum aestivum* cv Fukuho, hybrids INN-7, INN-8, INN-9 and INN-10, and two heads of *Leymus innovatus* (left to right, respectively). **b** Spikelet structure of *T. aestivum* cv Fukuho, hybrids INN-9 and INN-10, and *L. innovatus* (left to right, respectively)

Chromosome number and chromosome pairing relationships

The somatic chromosome counts revealed that all of the F_1 hybrids but two had the expected chromosome number of 35 from the expected ABDJN genomic constitution. The somatic chromosome number of hybrid INN-3 and INN-10 was 28. The parental *L. innovatus* lines were investigated by somatic counts and all were found to possess 28 chromosomes as expected; this rules out that a putative 14-chromosome *L. innovatus* line had been the male parent.

Chromosome pairing data in normal cells observed at metaphase I in the F_1 hybrids is presented in Table 2. The overall average chromosome pairing at metaphase I in the meiocytes of the 35-chromosome hybrids was 31.27 I + 1.70 rod II + 0.077 ring II + 0.046 III + 0.006 IV, but hybrids INN-4 and INN-6 showed a bivalent frequency about twice that observed in the others [3.29 and 2.635 II, respectively (Fig. 2 a and b)]. In the normal cells of the 28-chromosome hybrids, pairing was very low, with a mean bivalent frequency of 0.25 and 0.26 for hybrids INN-3 and INN-10, respectively (Fig. 2 c). The mean arm pairing frequency (*c* value) was 0.012 for hybrids INN-3 and INN-10, and varied from 0.042 (hybrid INN-5) to 0.134 (hybrid INN-4) in the 35-chromosome hybrids.

Some chromosome irregularities were noted in the meiocytes of the F_1 hybrids. These were lagging chromosomes, dividing univalents at anaphase I, unequal and irregular cytokinesis leading to the formation of a vari-

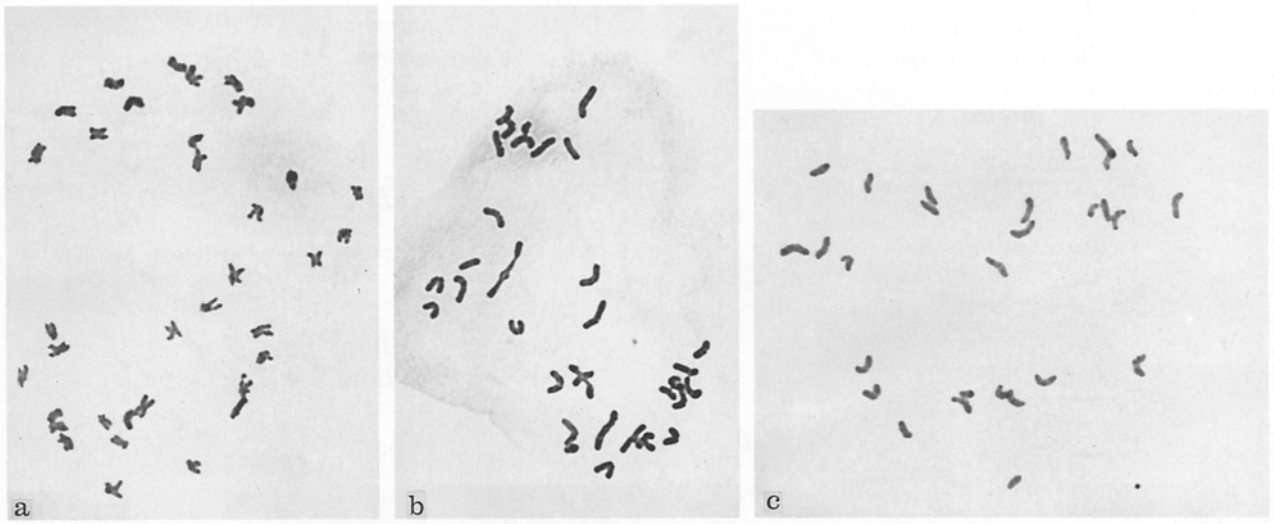

Fig. 2a–c. Meiosis in hybrids of *Triticum aestivum* × *Leymus innovatus*. **a** Diakinesis with 35 undivided chromosomes in hybrid INN-4. **b** Metaphase I of hybrid INN-1 showing 33 I + 1 II. **c** Metaphase I of hybrid INN-10 showing 28 I

Table 2. Meiotic behavior in the normal meiocytes of wheat × *Leymus innovatus* F_1 hybrids

Hybrid designation	Somatic chromosome no.	Chromosome pairing at metaphase I					Chiasmata/cell	c[b]	No. of cells
		Mean chromosome associations							
		I	rod II	ring II	III	IV			
INN-1	35	31.47 (21–35)[a]	1.62 (0–7)	0.02 (0–1)	0.07 (0–1)	0	1.82	0.065	58
INN-2	35	32.57 (27–35)	1.20 (0–4)	0.02 (0–1)	0	0	1.23	0.044	61
INN-3	28	27.50 (24–25)	0.25 (0–2)	0	0	0	0.25	0.012	111
INN-4	35	28.06 (18–35)	3.07 (0–8)	0.22 (0–1)	0.12 (0–1)	0	3.75	0.134	107
INN-5	35	32.66 (27–35)	1.11 (0–4)	0	0.04 (0–1)	0	1.19	0.042	74
INN-6	35	29.56 (22–35)	2.42 (0–6)	0.21 (0–3)	0.05 (0–1)	0	2.95	0.105	79
INN-7	35	32.00 (21–35)	1.43 (0–7)	0.02 (0–1)	0.03 (0–1)	0	1.53	0.055	95
INN-8	35	31.93 (25–35)	1.36 (0–5)	0.02 (0–1)	0.05 (0–2)	0.04 (0–1)	1.62	0.058	45
INN-9	35	31.92 (23–35)	1.43 (0–4)	0.11 (0–2)	0	0	1.65	0.059	71
INN-10	28	27.48 (22–28)	0.26 (0–3)	0	0	0	0.26	0.012	97

[a] Values in parentheses are the ranges in each case
[b] Mean arm pairing frequency calculated as described by Espinasse and Kimber (1981) and Kimber and Alonso (1981)

able number of cells and micronuclei at the tetrad stage (Fig. 3a), chromatid fragments and chromosome disintegration.

Five hybrids (INN-1, INN-3, INN-4, INN-7, INN-10) were analysed at the dyad and quartet stages (Table 3). On average, 13% of the cells observed at the dyad stage were triads which led to the production of quintads and hexads at the telophase II stage at about the same frequency (16%). The average number of micronuclei in the 4-, 5- and 6-cell quartets was 4.2, with a range of

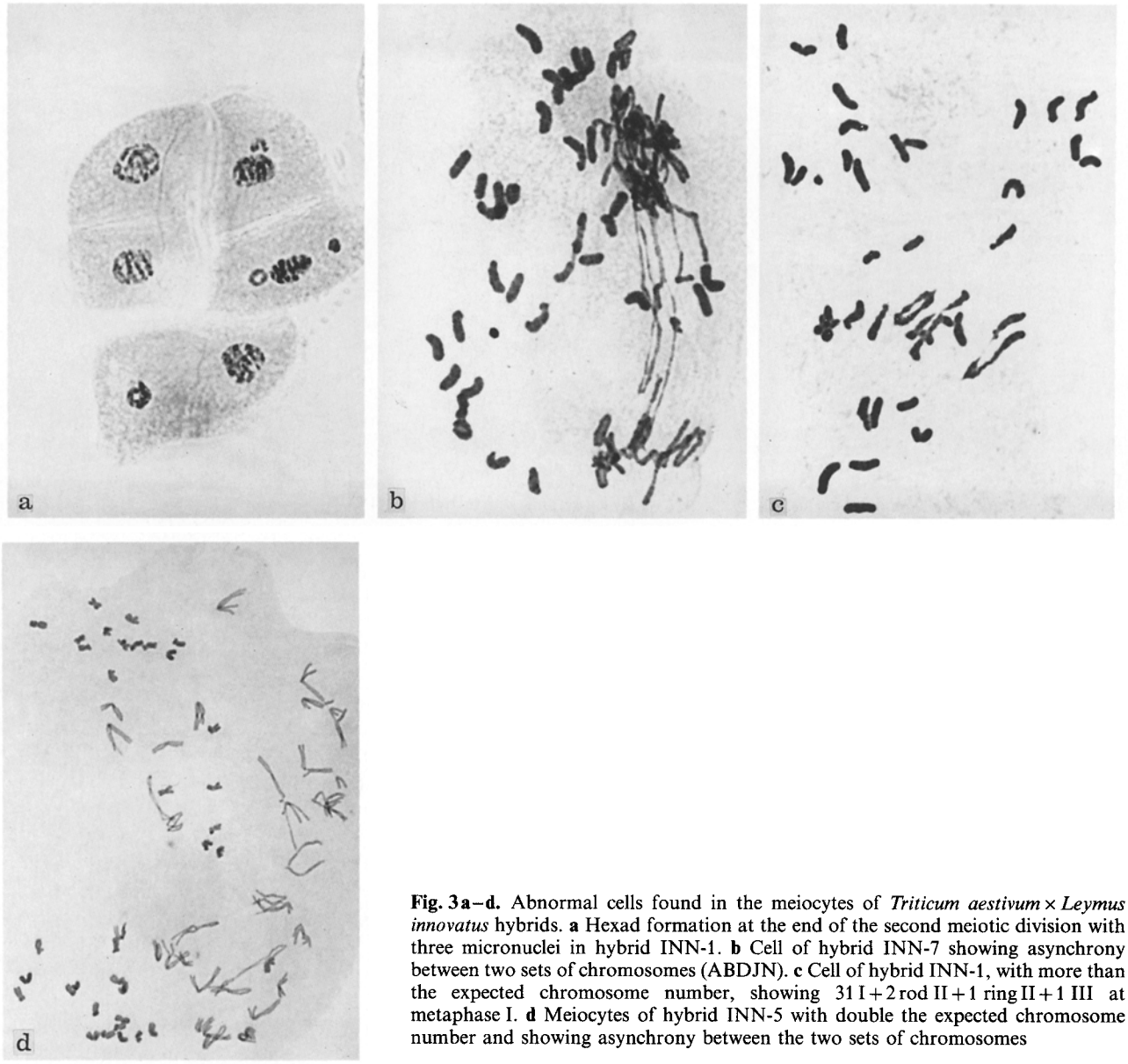

Fig. 3a–d. Abnormal cells found in the meiocytes of *Triticum aestivum* × *Leymus innovatus* hybrids. **a** Hexad formation at the end of the second meiotic division with three micronuclei in hybrid INN-1. **b** Cell of hybrid INN-7 showing asynchrony between two sets of chromosomes (ABDJN). **c** Cell of hybrid INN-1, with more than the expected chromosome number, showing 31 I + 2 rod II + 1 ring II + 1 III at metaphase I. **d** Meiocytes of hybrid INN-5 with double the expected chromosome number and showing asynchrony between the two sets of chromosomes

Table 3. Frequencies of dyads and polyads observed at the telophase I and II stages, respectively, and mean number per cell and range of micronuclei observed at the quartet stage in the meiocytes of five F_1 hybrids of wheat × *Leymus innovatus*

Hybrid designation	Telophase I			Telophase II									
	Dyad	Triad	No. of cells (%)	Tetrad			Quintad			Hexad			No. of cells
	Cells (%)	Cells (%)		Cells (%)	No. of micronuclei		Cells (%)	No. of micronuclei		Cells (%)	No. of micronuclei		
					Mean	Range		Mean	Range		Mean	Range	
INN-1	89	11	155	90	3.3	0–18	2.5	4.0	3–5	7.5	3.7	1–7	79
INN-3[a]	85	15	127	77	3.8	0–9	9	4.4	2–8	14	2.9	0–6	150
INN-4	88	12	131	89	3.9	0–18	3	8.0	3–16	8	4.9	1–8	115
INN-7	87	13	149	78	3.6	0–18	5	3.4	0–9	17	4.5	0–9	152
INN-10[a]	85	15	167	85	4.6	0–9	5	3.6	1–6	10	4.0	0–8	133

[a] These hybrids had 28 chromosomes instead of the expected 35 chromosomes (2n = 35, ABDJN)

Table 4. Frequency of abnormal cells and proportion of each type of anomaly in the meiocytes of the F_1 hybrids of wheat × *Leymus innovatus*

Hybrid no.	Somatic chromosome no. (2n)[a]	Abnormal/total cells (%)	Frequency of each type of anomaly (%)			
			Hypoploid	2n with additional groups	Hyperploid with or without additional groups	4n[b]
INN-1	35	40/98 (41)	3.1	16.3	19.4	2.1
INN-2	35	54/115 (47)	2.6	37.4	4.4	2.6
INN-3	28	5/116 (4)	1.7	0.86	0	1.7
INN-4	35	41/148 (28)	6.7	14.2	6.1	0.7
INN-5	35	52/126 (41)	7.9	19.1	12.7	1.6
INN-6	35	36/115 (31)	2.6	13.9	13.9	0.9
INN-7	35	37/132 (28)	3.0	17.4	6.8	0.8
INN-8	35	66/111 (59)	9.0	25.2	25.2	0
INN-9	35	33/104 (32)	1.9	11.5	17.3	1.0
INN-10	28	3/100 (3)	0	0	2.0	1.0

[a] These plants were haploid and expected to have 2n = 35 chromosomes; the 28-chromosome hybrids apparently had chromosome elimination

[b] A most unusual feature of all these doubled cells was that expected chromosome pairing was not observed, except at very low frequencies

0–18. No differences in the ratio of dyads to triads were observed between the 35- and 28-chromosome hybrids.

Cells with unexpected chromosome number were observed in the meiocytes of the wheat × *L. innovatus* hybrids. In the 35-chromosome hybrids, their frequency was high (average of 38%) and varied from 28% to 59%, whereas the two 28-chromosome hybrids were almost completely stable [mean frequency of 3.5 (Table 4)]. Four types of abnormal cells were observed; cells with less than the expected chromosome number (hypoploid), cells with the expected chromosome number plus additional groups of chromosomes at different prophase stages (Fig. 3 b), cells with more than the expected chromosome number (hyperploid), with or without additional group(s) of chromosomes at different prophase stages (Fig. 3 c) and cells with double the expected chromosome number (Fig. 3 d). The frequencies of each type of anomaly for each F_1 hybrid are presented in Table 4.

Chromosome elimination (cells with 2n-1 to 2n-7 chromosomes) was found, on an average, in 4.6% of the meiocytes of the 35-chromosome hybrids. However, the hybrids differed in the frequency of this type of anomaly, which varied from 1.9% (INN-9) to 9% (INN-8). Cells with 28 chromosomes were encountered in the meiocytes of hybrids INN-1, INN-5 and INN-8 but their frequency was very low (1 cell only). Chromosome elimination was not found in the 28-chromosome hybrid INN-10, but two cells out of the 116 cells observed in hybrid INN-3 had 2n-1 chromosomes.

The more frequent types of abnormal cells observed for all hybrids except hybrid INN-3 were those with the expected chromosome number plus distinct chromosomes and/or additional group(s) of chromosomes at the prophase stage (from 2% for hybrid INN-10 to 50.4% for hybrid INN-8). About 75% of these cells had one or more groups of chromosomes at different prophase stages, making it impossible to count with certainty the chromosome number involved.

A low frequency of meiocytes (0%–2.6%) had the doubled (amphiploid) chromosome number of 2n = 70. Unexpectedly, the mean chromosome pairing in the 70-chromosome cells was slightly less (65.0 I + 2.365 II + 0.09 III) than twice the mean chromosome pairing observed in the normal 35-chromosome cells. Moreover, some of the 70-chromosome cells, although apparently synchronous, behaved as if there were two metaphase plates within a unique cell, and some unexplained mechanism prevented homologous chromosome pairing. A lack of pairing was also observed in the three 56-chromosome cells found in the hybrids INN-3 and INN-10. Therefore, two abnormal phenomena were observed: asynchrony between groups of chromosomes (two sets of hybrid chromosomes, ABDJN) and near-total inhibition, by some unknown mechanism, of otherwise expected pairing between homologous chromosomes.

Discussion

Based upon the overall performance recorded in 1985, 1986 and 1987 for the intergeneric hybrid production of many species (unpublished data), the environmental conditions prevailing in 1985 were adequate. In 1986, it was noted that the hybrid seeds contained, at variable frequency, smaller embryos than those obtained in 1985. Although the modifications made to the NII medium in

1986 represented a significant improvement over the medium used in 1985, it was still not suitable to rescue the very tiny undifferentiated embryos. The relatively cold temperature conditions prevailing in the summer of 1986 may have been responsible for the slow embryo development rate observed.

In 1987, significant improvements in the frequency of hybrid production were realized. First, the environmental conditions allowed the production of a higher percentage of hybrid seeds containing embryos. Secondly, the new media and methods used led to the successful rescue of 75% – 100% of the embryos collected at a younger age (7 versus 15 days).

Therefore, the results obtained on hybrid seed set and production for the various hybrid combinations and years of crossing suggested that two factors – the age at which hybrid seeds were collected for embryo rescue, and the composition of the culture medium and rescue methods – are as important as the crossability factors in the parents in determining the success rate in certain hybrid combinations. This appears to be especially true for hybrid combinations yielding the most tiny embryos.

The hybrids, especially those with 35 chromosomes, expressed to various degrees the combined morphological characters of both parents. The variations observed between the hybrids were most probably due to genetic differences resulting from the genetic heterozygosity of *L. innovatus* (an outcrossing species). The codominant spike phenotype observation was similar to that reported for the *T. aestivum* × *E. giganteus* (*L. racemosus* 2n = 28) F_1 by Mujeeb-Kazi and Rodriguez (1981 a).

The 28-chromosome hybrids (INN-3 and INN-10), however, strongly resembled the wheat parent in morphology. This may have been due to a dosage effect of the *Leymus* genome, with probably one alien genome present in the 28-chromosome hybrid as opposed to two in the 35-chromosome counterpart. Whether the alien genome is a single J or N or a combination of the two has not as yet been established. In terms of effects of alien genomes on plant morphology, Mujeeb-Kazi and Bernard (1985) have stated that in hybrids between diploid alien species and *Triticum aestivum* (6 ×), the phenotype of the latter predominates in the F_1 hybrids.

Chromosome elimination, at a low frequency, was observed in the meiocytes of the 35-chromosome hybrids. Elimination has possibly occurred during premeiotic mitosis, as somatic chromosome number in the root meristems was stable. These differences in chromosome stability are difficult to explain genetically, but one can question the possible effects of the various media used to rescue the embryos, as tissue culture may promote chromosome instability (Nakamura and Keller 1982; Karp and Maddock 1984).

For the introduction of desirable alien variation into *T. aestivum*, it becomes essential to know the genomic relationships of the alien species and their influence or expression in the hybrids. The average pairing frequency observed (1.97 chiasmata/cell) in the 35-chromosome hybrids suggested the occurrence of homologous or homoeologous pairing, as the frequency is higher than what was reported for several polyhaploids of wheat (ABD) by Gupta and Fedak (1985). However, the differences may be attributed to the pairing control system of the wheat cv Fukuho, which may be weaker compared to that of the wheat cv Chinese Spring. It was reported that the genomes J and N of *Leymus* are not homologous with each other or with the A, B or D genomes of *Triticum* (Dewey 1984), and they failed to exhibit pairing in the wheat × *E. giganteus* (*L. racemosus*) (JJNN) hybrid and its backcross to wheat. The mean chromosome pairing frequency obtained confirmed the non-homology between the chromosomes of the two species, as the *c* values were smaller than 0.2 (Espinasse and Kimber 1981; Kimber and Alonso 1981). Introduction of alien variation in hybrids of this type could be achieved either by irradiation or centromeric break-and-fusion of chromosomes in derivatives of their amphiploids with wheat.

Our data raise the possibility that the genes of *Leymus innovatus* have a certain effect on the pairing system of the wheat cultivar, which could explain the higher frequency of pairing observed in the hybrids. The suggestion of the existence of a diploidizing genetic system in *Leymus*, similar to the *Ph* gene system found in *Triticum aestivum* was made by Wang and Hsiao (1984). It may have originated from *Thinopyrum*, the putative donor of the J genome of *Leymus*. Indeed, Dvorak (1981) demonstrated that some species of *Thinopyrum* (*Elytrigia*) promoted heterogenetic pairing in hybrids involving *T. aestivum*. The existence of a similar chromosome pairing control system in *Psathyrostachys* (the proposed donor of the N genome of *Leymus*) has not been verified yet, as no hybrids with wheat have been reported (Dewey 1984). Such a hypothesis would explain the differences observed between the 35-chromosome hybrids for the mean arm pairing frequency, as the strength of the pairing control would be reduced by hemizygosity. On the other hand, pairing was very low in both of the 28-chromosome hybrids, suggesting that the *Leymus* chromosomes conserved did not affect the pairing system of 'Fukuho' which, in this case, appeared to be as strong as that reported for 'Chinese Spring'. It is possible that these chromosomes, eliminated early during the embryogenic developmental process, were those which affected pairing in the 35-chromosome hybrids. This possible effect of one of the genomes of *Leymus innovatus* has never been suggested for other members of the genus *Leymus*.

Successful gene transfer from *Leymus innovatus* to wheat is now dependent on the production of backcross or amphiploid derivatives. Successful backcrossing of the wheat × *Leymus* hybrids to wheat was reported as being

often dependent on the occurrence of unreduced gametes in the F_1 hybrid (Mujeeb-Kazi and Rodriguez 1980). The good frequency of spontaneous doubling in the meiocytes of the hybrids might give an indication of the good probability of producing BC progenies, providing spontaneous chromosome doubling occurs equally during megasporogenesis. However, two abnormal phenomena were observed: asynchrony between groups of chromosomes and near-total inhibition of otherwise expected pairing between homologous chromosomes, which may preclude the success of backcrossing. It is possible that these cells underwent spontaneous doubling at premeiotic mitosis, after which synchrony of meiosis between the two sets (ABDJN) was lost. There were also some indications that these meiocytes behaved as having two distinct spindle apparatuses. Irregularities in spindle function have previously been implicated in abnormal chromosome pairing, anaphase divisions and cytokinesis stages in intergeneric hybrids involving wheat and barley (Fedak 1980) and barley and *Agropyron* (Fedak 1985). Less-than-expected chromosome pairing in doubled cells has been reported in callus culture regenerants of a *Triticum crassum* × *H. vulgare* hybrid by Fedak and Grainger (1986), who attributed this to media components.

Other chromosome irregularities, such as multipolar migration and irregular cytokinesis, were observed and lead to the production of unbalanced microspores. Such irregularities have been reported by Petrova (1970) in hybrids of *T. aestivum* × *Elymus giganteus* (*L. racemosus*) and are responsible for the production of sterile pollen grains.

Since the hybrids in the present study showed normal mitotic chromosomes, it appears possible that the meiotic chromosome fragments were the result of genetic interaction, causing imbalance in the regulation of enzymes critical to the integrity of meiotic chromosomes (Sadasivaiah and Weijer 1981). Our observations focus on the hypothesis that *L. innovatus* has some genes that affect the process of meiosis in a wheat background. Analysis of the chromosome behavior in amphiploids and backcross progenies will be necessary to support or refute this hypothesis.

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