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A novel intergeneric hybrid in the Triticeae: *Triticum aestivum* × *Psathyrostachys juncea*

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Summary. Two hybrid embryos of intergeneric origin between *Triticum aestivum* cv Fukuho ($2n=6x=42$, AABBDD) and *Psathyrostachys juncea* ($2n=2x=14$, NN) were successfully rescued. One hybrid plant had the expected chromosome number of 28 (ABDN), whereas the second plant had 35 chromosomes. The average meiotic chromosome pairing in the 35-chromosome hybrid was 21.87 univalents + 6.38 bivalents + 0.11 trivalents + 0.009 quadrivalents, which indicates that two copies of the N genome were present. Chromosome pairing in the 28-chromosome hybrid was low (1.35 bivalents), and pointed out the lack of homology between the wheat genomes and the *P. juncea* genome. These new hybrids showed some necrosis and chlorosis, which caused severe floral abortion in the plant that had 35 chromosomes. These problems became gradually less severe after 18 months.

Key words: Russian wild rye – Wheat – Chromosome pairing – Meiosis

Introduction

In the 1970s, there was a renewed interest in wide hybridization in the Triticeae with the advances in hybridization techniques (Kruse 1973) and embryo culture (Murashige 1974). These improvements widened the possibility of successfully exploiting the vast genetic reservoir constituted by the perennial Triticeae to improve the annual cereals.

The genus *Psathyrostachys* is of particular interest in spite of its limited number of species, because it has been identified as the source of the N genome (Dewey 1984a). Six of its ten species are known to be diploid. Genome N was also found in combination with the J genome of *Thinopyrum* in the species belonging to the genus *Leymus*. The N genome is also one of the genomes constituting *Pascopyrum* (Dewey 1984a).

Species of *Psathyrostachys* occur in Asia, ranging from the Middle East to Mongolia. *Psathyrostachys juncea* (Russian wild rye) is an important forage crop for revegetating rangeland in western North America (Dewey 1984b). This species also shows tolerance to alkalinity and drought. Its apparent immunity to the most important virus in cereals, barley yellow dwarf virus (BYDV), made it of particular interest for cereal improvement (Comeau and Plourde 1987).

Crossing wheat with *P. juncea* had two objectives: to obtain a new hybrid as a first step towards the transfer of BYDV resistance into wheat, and to gain cytological information on the effect of the N genome on homoeologous chromosome pairing in a wheat background. This paper presents the successful production of two hybrid plants between *Triticum aestivum* L. ($2n=6x=42$, AABBDD) and *Psathyrostachys juncea* (Fisch.) Nevski ($2n=2x=14$, NN) and the meiotic chromosome relationships between genomes of the two species.

Materials and methods

All crosses were made in the summer of 1987 in the field using the approach method as described by Comeau et al. (1985). The female parent used was the spring wheat *Triticum aestivum* cv Fukuho. Seeds were sown in flats and the plantlets were subsequently transplanted into pots, 15 cm wide, and grown in a screen-covered shelter on the site of the wild perennial Triticeae nursery.

Three strains of *Psathyrostachys juncea* (Fisch.) Nevski (synonym *Elymus junceus* Fischer) were used as male parent. They were accession PI 370672, obtained from the USDA Regional Plant Introduction Station at Pullman, Washington, and accessions Sc 180113 and Sc 180114, provided by Dr. T. Lawrence, Swift Current, Canada. The strains were established in 1980 in the perennial Triticeae nursery at Laval University.

The hybrid seeds were collected 7–10 days following pollination. The seeds were surface sterilized in a 10% Javex solution (0.06% sodium hypochlorite) and dissected under sterile conditions. The embryos and proembryos were rescued on various modifications of the Norstog II medium, using methods similar to those previously reported (Norstog 1973; Plourde et al. 1989). They were initially cultured at room temperature in the dark and the plantlets were transferred to B5 medium (Gamborg 1982) under fluorescent lighting after germination. At the 1- to 2-leaf stage, the gelified medium was washed away from the roots and the 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. For acclimatization, they were placed in a closed plastic bag and kept in a growth chamber for 1 week at day-night temperatures of 20°–15 °C, with 16 h of light provided by a combination of fluorescent and incandescent lamps at 300 $\mu E/m^2/s$; after this, they were transplanted into 15-cm pots. The hybrid designated Fjuncea-1 showed some signs of necrosis at the tillering stage and was then cultured for a 2-week period at 28 °C.

Somatic chromosome counts in the root-tip cells were obtained by the standard Feulgen technique and immature spikes for meiotic chromosome studies in pollen mother cells (PMCs) were fixed in Carnoy's solution (alcohol-chloroform-acetic acid 6:3:1, v/v) and squashed in acetocarmine.

The mean chromosome arm pairing frequency or *c* value was calculated as described by Kimber and Alonso (1981) and Espinasse and Kimber (1981) for the tetraploid and pentaploid hybrids, respectively. The haploid plants of the maternal wheat cultivar Fukuho were obtained by means of crossing with sorghum as part of another study (F. Ahmad and A. Comeau, unpublished results).

Results

Production

Hybrid seeds were obtained by crossing two accessions of *Psathyrostachys juncea* onto the spring wheat cultivar Fukuho (Table 1). The cross of Fukuho with *P. juncea* accession Sc 180114 was, however, unsuccessful as no hybrid seed was obtained from over 480 florets of wheat pollinated. Using the accessions PI 370672 and Sc 180113 as male parent, two seeds were obtained for each hybrid combination, representing a frequency of 0.45 and 1.28 seed per 100 florets pollinated, respectively. Although hybrid seeds were produced at a higher frequency with the latter accession, no plant could be rescued from it. One of the seeds contained no dissectable embryo, while the second seed contained only a proembryo and no endosperm.

Table 1. Production of F_1 hybrids between *Triticum aestivum* cv Fukuho and three accessions of *Psathyrostachys juncea* in 1987 at Laval University under field conditions

P. juncea accession	Florets pollinated	Seeds obtained	Embryos excised	Plantlets produced	% of pollinated florets
PI 370672	444	2	2	2	0.45
Sc 180113	156	2	1	0	0
Sc 180114	480	0	0	0	0

The pollination of wheat with *P. juncea* accession PI 370672 yielded hybrid seeds containing a bigger embryo and no endosperm. One embryo had reached the stage of differentiation and was rescued easily. This hybrid was named Fjuncea-1. The second embryo was rather large with no sign of normal differentiation but an apparent tendency toward callusing. Through an arbitrary sequence of transfer on many media and dissection to remove abnormal, rhizogenous tissue, differentiation of this abnormally shaped embryo into a normal plantlet was achieved. This hybrid was named Fjuncea-2.

Root-tip counts showed that the hybrid Fjuncea-1 had 35 chromosomes, namely 7 chromosomes more than the anticipated chromosome number of 28 from the expected ABDN genomic consitution, whereas the second hybrid, Fjuncea-2, had the normal somatic chromosome number of 28.

Morphology

In terms of plant morphology, Fjuncea-1 showed more Russian wild rye features than did Fjuncea-2. The wild rye was awnless and had two flowers per spikelet. Fjuncea-1 had awns only 5–6 mm long and three flowers per spikelet, whereas Fjuncea-2 had awns 10–15 mm long and four flowers per spikelet. Fukuho wheat had awns 31–36 mm long and four or five flowers per spikelet. Typical heads of the hybrids are shown in Fig. 1.

Fjuncea-1, the 35-chromosome line, had a necrosis problem which was somewhat similar to the hybrid necrosis-chlorosis reported in certain *T. aestivum*-*T. durum* hybrids (Hermsen 1963). The leaf chlorosis was more severe at 20 °C than at 28 °C. The severity of its chlorosis problem prevented the plant from reaching the heading stage for 18 months at 20 °C. However, heading was finally achieved after 20 months at 20 °C. On the other hand, Fjuncea-2 headed readily after 3 months at 20 °C despite moderate chlorosis.

Chromosome pairing

The mean chromosome pairing observed in the pentaploid Fjuncea-1 was 21.87 univalents + 6.38 bivalents + 0.11 trivalents + 0.009 quadrivalents (Table 2). A typical meiocyte is shown in Fig. 2a. Chromosome irregularities in meiotic cells were found at a frequency of 12.7%. Irregularities included hypoploidy (20–33 chromosomes), hyperploidy (36–60 chromosomes) and normal chromosome number plus additional non-synchronous chromosomes at different prophase stages. Some mitotic types of cells with odd chromosome number (6–15) were also observed.

Chromosome pairing in the tetraploid Fjuncea-2 was low, with an average bivalent frequency per cell of 1.35

Table 2. Mean and range of chromosome associations at metaphase I in the normal meiocytes of two hybrids between *Triticum aestivum* and *Psathyrostachys juncea* and also in *Triticum aestivum* cv Fukuho haploid

Plant designation	Somatic chromosome no.	Chromosome pairing at metaphase I						Chiasmata/cell	*c* value [b]	No. of cells
		Mean chromosome associations								
		I	Rod II	Ring II	Total II	III	IV			
Fjuncea-1	35	21.87 (13–29) [a]	4.65 (1–10)	1.73 (0–6)	6.38	0.11 (0–2)	0.009 (0–1)	8.36	0.30	211
Fjuncea-2	28	25.29 (18–28)	1.29 (0–4)	0.06 (0–2)	1.35	0	0	1.41	0.07	147
Fukuho haploid	21	18.86	0.99 (0–3)	0.02 (0–1)	1.01	0.04 (0–1)	0	1.11	0.08	100

[a] Values in parentheses are the ranges in each case

[b] Mean arm-pairing frequency was calculated as described by Espinasse and Kimber (1981) and Kimber and Alonso (1981)

Fig. 1. Spikes of the pentaploid hybrid (Fjuncea-1, ABDNN genome) of *Triticum aestivum* × *Psathyrostachys juncea*, the tetraploid hybrid (Fjuncea-2, ABDN genome) and the wheat parent Fukuho

(Table 2, Fig. 2b). Chromosome irregularity frequency was about half (5.7%) that found in the pentaploid hybrid. Two cells had less than the expected chromosome number (21 and 26), one cell had 35 chromosomes (29 I + 3 II) and the remaining cells had 28 chromosomes, plus one chromosome at diakinesis or a group of chromosomes at an early prophase stage. Two 28-chromosome cells had a heteromorphic bivalent.

Attempts to double the chromosome number with colchicine were not successful. The line Fjuncea-2 could be backcrossed to wheat with reasonable success, but necrosis was an obstacle to the backcrossing of Fjuncea-1 to Fukuho wheat.

Discussion

The morphology of the tetraploid Fjuncea-2 and pentaploid Fjuncea-1 hybrids was somewhat different, the tetraploid being more wheat-like. Hybrids derived from crosses between hexaploid wheat and diploid wild species often have a plant morphology predominantly resembling that of the *Triticum* parent. In the pentaploid hybrid, more *P. juncea* traits are expressed, most probably because there is a closer balance (3:2) of *Triticum*:*Psathyrostachys* genomes than in the tetraploid hybrid. The presence of a double dosage of the N genome has probably resulted from the fertilization of the wheat ovule by an unreduced male gamete of *P. juncea*. An alternative explanation is the simultaneous fertilization of an egg cell by two pollen grains. The latter phenomenon is known to occur at a low frequency (M. D. Bennett, personal communication).

Plourde et al. (1989) have reported the possible effect of parental genome dosage on the morphology of the hybrids. Mujeeb-Kazi and Bernard (1985) have also stated that the phenotype of the hexaploid *Triticum* species predominates in the F_1 hybrids between wheat and diploid alien species. Differential expression as a result of relative genome balance in the hybrids was also reported in hybrids between *Hordeum* and *Triticum* species (Comeau et al. 1988).

The presence of two copies of the N genome in Fjuncea-1 was confirmed by the level of chromosome pairing (6.38 bivalents) found in the meiocytes of this plant. The mean arm-pairing frequency (0.30) obtained for this hybrid is quite high and is obviously due to the presence of two copies of the N genome. The bivalent frequency of Fjuncea-2 was only slightly higher than that

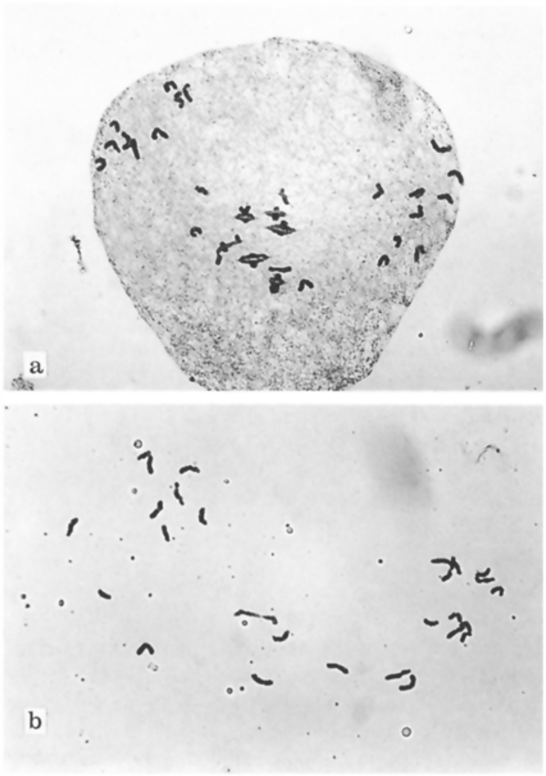

Fig. 2a and b. Meiosis in hybrids of *Triticum aestivum* × *Psathy*rostachys juncea. **a** Metaphase 1 of the pentaploid hybrid showing 23 univalents and 6 bivalents; **b** Metaphase 1 of the tetraploid hybrid showing 26 univalents and 1 bivalent

observed in the meiocytes of Fukuho haploid. This low pairing frequency and the low mean arm-pairing frequency resulting from it confirmed the lack of homology between the wheat genomes A, B, D and the alien genome N. This indicates that gene transfer through homoeologous pairing promoted by changes in the systems regulating chromosome pairing would have a low probability of success. However, the multivalent associations (quadrivalent) observed in the 35-chromosome hybrid suggest that some pairing between the wheat and *P. juncea* genomes may occur. If so, it may be possible to transfer genes from *Psathyrostachys* to *Triticum* by meiotic crossing-over. The probability of such an event is, however, too low to be reasonably reliable. Therefore, as indicated by Kimber (1984), irradiation or centric break-and-fusion in derived aneuploids remains the optimum method to achieve gene transfer.

Hybridization of wheat with *P. juncea* is difficult, and others tried it without successfully rescuing the embryos produced (Mujeeb-Kazi et al. 1987). We do not know yet if the hybrids of *T. aestivum* with *P. juncea* that we produced were due to some unusual crossability factors present in line PI 370672, but other lines should be test-crossed with wheat to settle this question. Our next goal will be to observe the expression of the disease resistance genes present in the N genome of *P. juncea* when introduced in the wheat background. The novel intergeneric hybrids produced should permit the development of a complete set of addition lines in the near future.

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