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# Hybridization between *Triticum aestivum* L. and *Agropyron michnoi* Roshev.

## 1. Production and cytogenetic study of $F_1$ hybrids

**L. H. Li and Y. S. Dong**

Institute of Crop Germ Plasm Resources, Chinese Academy of Agricultural Sciences, Beijing 100081, China

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**Summary.** Intergeneric hybrids between *Triticum aestivum* cv 'Chinese Spring' (2n = 6x = 42, AABBDD) and *Agropyron michnoi* Roshev. (2n = 4x = 28, PPPP) were obtained by embryo culture. Their spike characteristics were similar to those of common wheat but, unlike their parents, they were long-awned. The average meiotic chromosome pairing at MI of $F_1$ hybrids was: 6.39 I + 3.75 rodII + 8.64 ringII + 0.81 III + 0.30 IV + 0.04 V, the bivalent and multivalent formation of which was much higher than expected from the genomic formulae. It is especially worthwhile to note that the $F_1$ hybrids were self-fertile, self set being 0.15%, and seeds were easily obtained from the backcross of $F_1$ plants with hexaploid and tetraploid wheats; here the seed set was more than 20.0%. The polyploid taxa and the position of *A. michnoi* in *Agropyron* are discussed.

**Key words:** *Triticum aestivum* – *Agropyron michnoi* – Intergeneric hybrid – Chromosome pairing – Self-fertile

## Introduction

*Agropyron* Gaertn. is a small genus of no more than ten species, which constitute what is known as the "crested wheatgrass complex," in accordance with the terminology of many modern botanists (Melderis 1980; Tzvelev 1983; Dewey 1984; Love 1984). *Agroypron* species occur at three ploidy levels – 2n = 14, 2n = 28, and 2n = 42, with the tetraploid being the most common. Hybridization between the various crested wheatgrass taxa clearly indicates that the polyploid taxa are autoploid or near autoploid and that *Agropyron* is founded on one basic genome – P (Dewey 1984).

Four *Agropyron* species – *A. cristatum* (L.) Gaertn., *A. desertorum* (Fisch.) Schult., *A. michnoi* Roshev., and *A. mongolicum* Keng – are found in China. Although *A. michnoi* is rather similar to *A. cristatum* in morphology, the plants of *A. michnoi* have creeping underground shoots and their spikelets are slightly apart with spike rachis.

In addition to their interest as forage plants, the *Agropyron* form a vast genetic reservoir which might be used to improve wheat. Only recently have successful hybrids of *Triticum aestivum* cv 'Chinese Spring' (CS) with *A. cristatum* (2n = 4x = 28, PPPP) and *A. desertorum* (2n = 4x = 28, PPPP) been reported (Chen et al. 1989; Li and Dong 1990) after numerous failures (White 1940; Smith 1943; Dewey 1984). Hybridization of wheat whith the various species in *Agropyron* is important for studying the genetic characteristics of the P genome itself as well as the gene transfer to wheat.

The purpose of this paper is to report some data on the morphology and cytology of wheat × *A. michnoi* $F_1$ hybrids and their further progenies $F_2$ and $BC_1$.

## Material and methods

In this experiment, *A. michnoi* 4x (PPPP) accession nos. Z611 and Z618 were collected in 1986 in Xilingol Grassland, Inner Mongolia, during an expedition organized by the Institute of Crop Germ Plasm Resources, Chinese Academy of Agricultural Sciences, with support from the International Board for Plant Genetic Resources (IBPGR).

Crosses were made in the field during the summer of 1988, using 'Chinese Spring' (CS) as female parent and *A. michnoi* as male. The CS spikes were pollinated 1–2 days after emasculation and treated with 75 ppm GA3 the next day. Hybrid seed development was monitored and 12-day-old seeds were dissected under sterile conditions. Embryos were placed on the CS endosperm from which CS embryos wer removed, and then

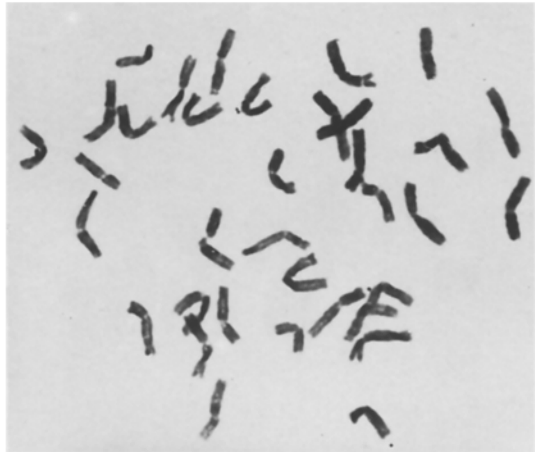


**Fig. 1.** Spike characteristics (*left to right*) of *A. michnoi*, three spikes of $F_1$ hybrid CS × *A. michnoi*, Chinese Spring

**Fig. 2.** Root-tip cell in the $F_1$ hybrids, $2n=35$

cultured on N6 medium. When the plantlets had three or four tillers, they were cut into two parts and separately subcultured, in order to recover more samples of the same hybrid plants. All four tube seedlings were transplanted into pots at the end of September. In the last 10-day period of November, two out of four of the pots were put in a normal greenhouse, and only the morphology of these $F_1$ plants could be observed due to adverse environmental conditions. Th remaining two pots were transferred to a cold greenhouse (< 10 °C), and taken out in the spring of the following year, so that $F_1$ plants could ear and flower under the natural conditions. Their spikes were bagged for selfing and backcrossing with wheat.

For mitotic and meiotic studies, the methods used were those described in detail by Li and Dong (1990).

## Results

### *Production of $F_1$ hybrids between CS and A. michnoi and their morphology*

Six seeds were obtained from the cross CS × *A. michnoi*, accession nos. Z611 and Z618, after making 3,345 pollinations. The seed set was rather low 0.18% (see Table 1).

Among six $F_1$ seeds dissected, only three from the cross CS × *A. michnoi* accession no. Z618 were slightly torpedo-shaped, whith little or no scutellum. Only one $F_1$ hybrid plant grew after in vitro culture. Before being transplanted into pots, four samples of this plant had been obtained from in vitro, sequential asexual multiplication.

All plantlets were transplanted into pots at the end of September and survived up to maturity. Root tips from $F_1$ hybrid plants were examined to determine the chromosome count. All of them had $2n=35$ (Fig. 2), as expected.

**Table 1.** Seed set in the cross common wheat cv CS × *A. michnoi*

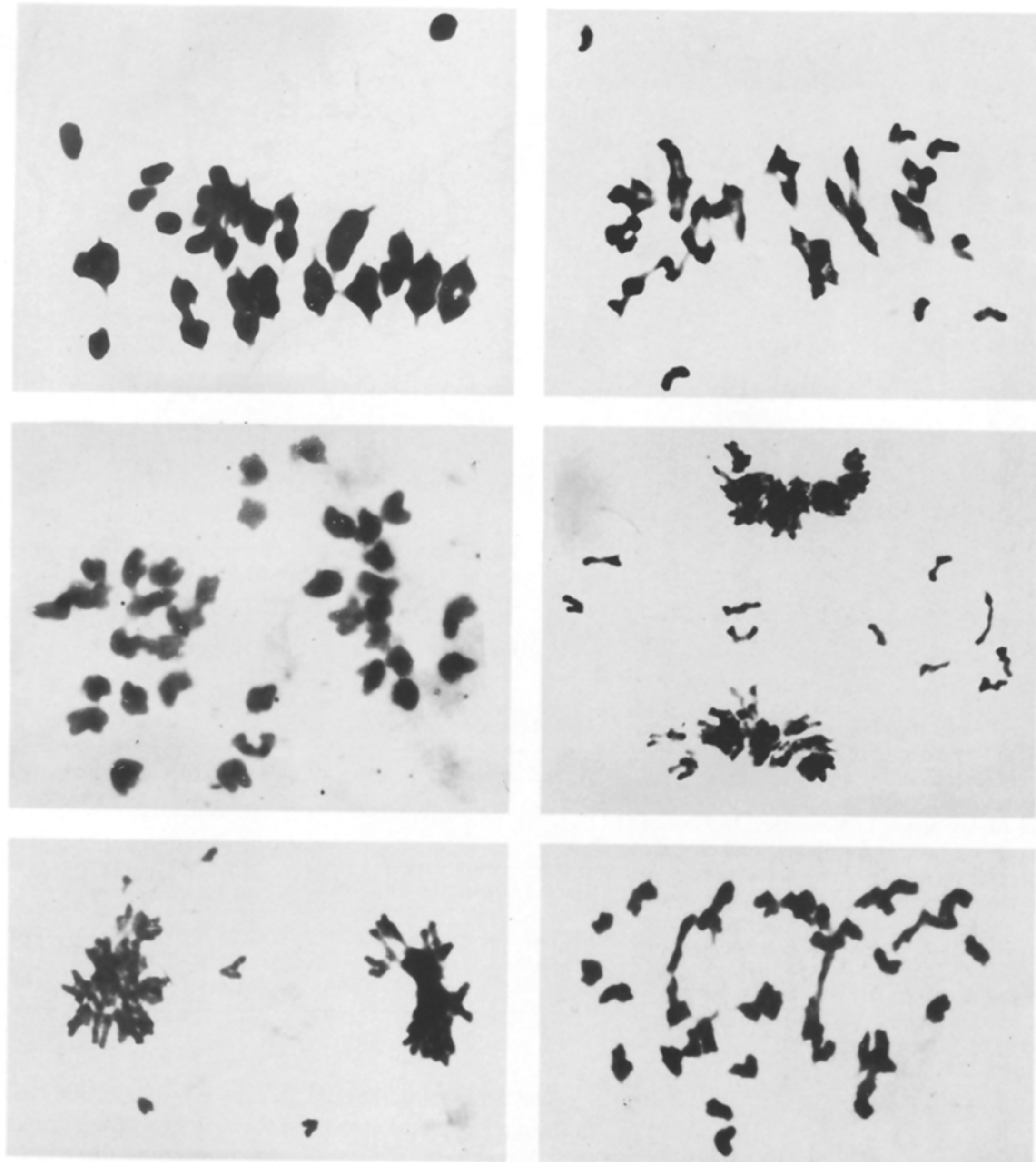
| Hybrid combination | No. of pollinated florets | Grains obtained | | Embryos cultured | |
|---|---|---|---|---|---|
| | | No. | % | No. | % |
| CS × *A. michnoi* Z611 | 458 | 1 | 0.22 | 0 | 0 |
| CS × *A. michnoi* Z618 | 2887 | 5 | 0.17 | 3 | 0.10 |
| Total | 3345 | 6 | 0.18 | 3 | 0.09 |

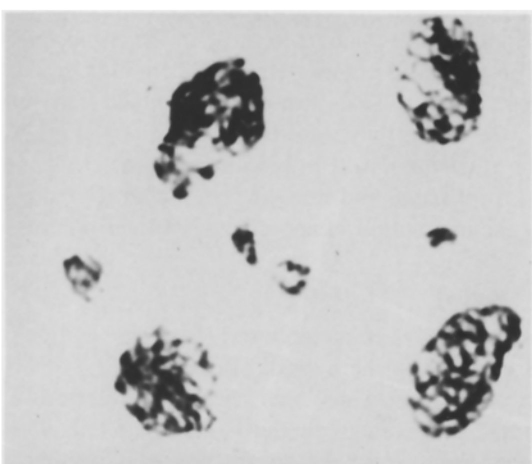
The two pots in the cool greenhouse were moved out in spring. The plantlets in these showed vigorous tillering; their leaves were wide and long, resembling the wheat parent, but they developed slowly and eared in the last 10-day period of May, similar to those of the *A. michnoi* parent. The $F_1$ hybrid plants were 72–82 cm high. Spike and spikelet characteristics, however, resembled those of common wheat, i.e., they were long and square (Fig. 1). The glume and lemma were pubescent, a character inherited from *A. michnoi*, and long-awned, a character produced by the interaction of genes between their parents.

### *Cytogenetic study of $F_1$ hybrid*

Chromosome pairing at metaphase I (MI) was analyzed in the parents and in the hybrids (Table 2).

In CS and *A. michnoi* it was regular. Formation of bivalents and multivalents in the $F_1$ hybrid plants was much higher than expected from the genomic formulae.





**Figs. 3–9.** Meiosis in the $F_1$ hybrid. **3** MI, showing 7I + 1 rodII + 13 ringII; **4** MI showing 7I + 11 ringII + 2III; **5** anaphase I showing 15-5-15 disjunction; **6** anaphase I showing five laggards tending to the equatorial plate by chromatids; **7** anaphase I showing eventual tendency of the five laggards in the equatorial plate; **8** anaphase I showing two chromosome bridges; **9** quartets showing micronuclei

**Table 2.** Mean chromosome pairing and range values of parents and CS × *A. michnoi*

| Material | Genome | No. of cells scored | Chromosome pairing | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | I | ringII | rodII | Total II | III | IV | V |
| CS | AABBDD | 21 | 0.26 (0–2) | 19.49 (18–21) | 1.38 (0–3) | 20.87 (19–21) | | | |
| *A. michnoi* | PPPP | 25 | 0.06 (0–1) | 6.25 (2–14) | 1.24 (0–2) | 7.49 (3–14) | | 3.24 (0–5) | |
| CS × *A. michnoi* | ABDPP | 52 | 6.39 (2–11) | 8.64 (4–15) | 3.75 (1–8) | 12.39 (4–16) | 0.81 (0–4) | 0.30 (0–2) | 0.04 (0–1) |

**Table 3.** Seed set in the selfed or backcrossed hybrid

| Hybrid combination | No. of chromosomes in male parent | No. of pollinated florets | Grain obtained | |
|---|---|---|---|---|
| | | | No. | % |
| $F_1$ selfed | 35 | 1328 | 2 | 0.15 |
| $F_1$ × *T. aestivum* cv 'Fuko' | 42 | 14 | 3 | 21.5 |
| $F_1$ × *T. persicum* | 28 | 10 | 2 | 20.0 |

The mean meiotic chromosome pairing of all hybrid plants was 6.39 I + 8.64 ringII + 3.75 rodII + 0.81 III + 0.30 IV + 0.04 V. The 12–16 bivalents per cell at MI occurred at a frequency of 81.2% (Fig. 3), and the overall percentage of cells with multivalents was 70.0% (Fig. 4). At anaphase I, 15-5-15 separations were observed in most cells (Fig. 5, 6); some of the laggards eventually reached the poles, while the others formed micronuclei (Fig. 7). Bridges were present in many cells (Fig. 8). At telophase II, the mean of micronuclei per sporocyte was 1.4 (Fig. 9).

*Production of $F_2$ and $BC_1$ progenies*

When $F_1$ plants headed in the last 10-day of May, only two of their spikes were backcrossed with those of *T. aestivum* cv 'Fuko' ($2n=6x=42$, AABBDD) and *T. persicum* ($2n=4x=28$, AABB), respectively, because of the different flowering stages of the parents – that of wheat was earlier than that of the $F_1$ plants. Most of the $F_1$ hybrid spikes were bagged for self-pollination. Thus, two selfed and five backcrossed seeds have been obtained. Seed set in self-pollinated spikes was rather low – 0.15%. However, seeds were easily obtained from the backcross of $F_1$ plants with hexaploid or tetraploid wheat, and seed set was 21.5 and 20.0%, respectively.

No seeds were different in their germinating ability, although $F_2$ seeds were obviously larger than the $BC_1$ seeds. Moreover, some immature influorescences were cultured for the $F_1$ plant regeneration, and many regenerated plants were recovered. One selfed and 85 seeds backcrossed with common wheat were obtained from those regenerants in the following year.

The variation of chromosome number in $F_2$ and $BC_1$ derivatives was quite large: from $2n=36$ to 41 in $BC_1$ plants, and from $2n=34$ to 36 and 56 in three $F_2$ plants, respectively. These will be reported on in another paper because the differences in their morphology and cytology are rather wide.

## Discussion

Attempts by many scholars have shown that hybridization of *Triticum* L. with *Agropyron* Gaertn. is very difficult (White 1940; Smith 1943; Dewey 1984). However, hybrids of *T. aestivum* with *A. cristatum* ($2n=4x=28$, PPPP) and *A. desertorum* have recently been reported (Chen et al. 1989; Li and Dong 1990), although only after scientists had gone to great lengths. In addition to the above-mentioned two hybrids, the hybrid between *T. aestivum* and *A. michnoi*, one of the most common species in *Agropyron*, was obtained in this experiment. Compared to the hybrids of *T. aestivum* with *A. cristatum* and *A. desertorum*, hybridization of *T. aestivum* with *A. michnoi* is more difficult, seed set only 0.09%.

A possible reason why the $F_1$ hybrids of CS with *A. michnoi* exhibited such a high degree of chromosome paring, which pattern of was characteristic of other $F_1$ hybrids of CS with *A. cristatum* and *A. desertorum*, was described previously (Chen et al. 1989; Li and Dong 1990). Although the production of the hybrids between CS and *A. michnoi* furnished novel and abundant evidence for the mechanism of advanced analysis, the relationship of the P genome of *Agropyron* to the A, B and D genomes of *Triticum* cannot be stated unequivocally, due to the effect of the *Ph* gene and the two P genomic doses in hybrids of common wheat with *Agropyron*. Perhaps use of diploid species in *Agropyron* in hybrids with *T. aestivum* and or other techniques for identifying chromosomes can provide more definitive answers towards explaining genomic relationships between *Triticum* and *Agropyron*.

It is especially worth noting that the $F_1$ hybrids between CS and *A. michnoi* were self-fertile, although self set was rather low – 0.15%; this was a rare case in which almost all intergeneric hybrids were totally sterile (Sharma and Gill 1983). In the reported hybrids of CS with *A. cristatum* ($2n=4x=28$, PPPP) and *A. desertorum*, no studies have been done on the $F_2$ or $BC_1$ progenies of the former; however, in the latter, our results showed that it was also self-fertile and that self set was higher (0.46%) than that in CS × *A. michnoi*. The reasons the hybrid is self-fertile have been described previously (Li and Dong 1990). For example, the $F_1$ plants had a much higher degree of chromosome pairing; the tillers of $F_1$ tube seedlings were cut into two parts and subcultured separately so that the plant numbers were increased. Their growth period was prolonged because they survived through the winter, thus increasing their genetic adaptability. The self set in CS × *A. michnoi* was much lower than that in CS × *A. desertorum*, which may be due to the fact that the former has more micronuclei (1.4) per sporocyte in quartet than the latter (0.6).

Hybridizing CS × *A. michnoi* has met with success, and has also furnished indirect data for discussing the homology of the P genome. The type of polyploidy in *Agropyron* has been studied by many scientists. Knowles (1955) and Schulz-Schaeffer et al. (1963) thought that the polyploid taxa were better described as segmental alloploids, while Dewey (1969) felt that they were autoploid or near autoploid. Comparing some characteristics among the obtained three hybrids – CS with *A. cristatum*, *A. desertorum*, and *A. michnoi* – it was found that all of them had a very high degree of chromosome paring. However, the $F_1$ hybrid between CS and *A. michnoi* exhibited long awns and spikes similar to those in common wheat, which characteristics appeared neither in the parents nor in the $F_1$ hybrid plants of CS with *A. cristatum* and *A. desertorum* (Chen et al. 1989; Li and Dong 1990). The crossability rate of CS with *A. cristatum* (1.91%), *A. desertorum* (0.426%), and *A. michnoi* (0.09%) and the self-fertility of each $F_1$ hybrid were greatly different. Moreover, the quadrivalent frequency of chromosome pairing at MI in *A. michnoi* (3.24 IV) was much higher than that in *A. cristatum* (2.18 IV) and in *A. desertorum* (2.45 IV). As for morphology, the plants of *A. michnoi* have creeping underground shoots, while those of *A. cristatum* and *A. desertorum* do not. In summary, the basic P genome in various species of *Agropyron* has indeed undergone some modifications or variations in the course of plant evolution, i.e., the tetraploid species are not strict autoploids, at least in certain genetic backgrounds. Furthermore, *A. michnoi* may be a more primitive tetraploid species in *Agropyron*.

It was believed in the past transfer from *Agropyron* to cereal might not be possible because intergeneric hybridization of *Agropyron* with other genera was rare in Triticeae (Dewey 1984). In this work, the wide variation of chromosome number in $F_2$ and $BC_1$ derivatives that has been obtained will provide great potential for creating alien addition, alien substitution, and even alien translocation lines.

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