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Intermeiocyte connections and cytomixis in intergeneric hybrids. II. *Triticum aestivum* × *Psathyrostachys huashanica* Keng

Genlou Sun, Chi Yen and Junliang Yang

Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan city, Sichuan, China

Summary

The intergeneric F_1 hybrid of *Psathyrostachys huashanica* Keng with *Triticum aestivum* L. cv J-11 was produced and studied cytologically. Chromatin transfer through conjugation openings among microsporocytes was observed. The chromatin migration occurred at the chromonema stage of the prophase of meiotic PMCs and stage of resting nuclei of young pollen grain. The consequential, unusual nuclear behavior frequently occurred, such as coenocytism, high level chromosome multiplication, multipolar division, variation in size of pollen grain and appearance of aneuploid. These events could lead to the doubling of spontaneous chromosome number. The possible origin of autoallopolyploid and aneuploid plants by these processes is discussed in the paper.

Introduction

We had previously studied the intermeiocyte connections and cytomixis in intergeneric hybrids of *Roegneria ciliaris* (Trin.) Nevski with *Psathyrostachys huashanica* Keng. It was found that chromatin transferred through conjugation tube or conjugation opening before, during and after meiosis in the hybrid. Consequently, unusual nuclear behavior frequently observed included coenocytism, cell size variation and abnormal chromosome or nucleus number, non-synchronous or multipolar division, and delayed chromatin condensation (Yen et al. 1993).

In order to study biosystematic relationships between *Triticum aestivum* and *Psathyrostachys huashanica* Keng, we studied the microsporogenesis of their F_1 hybrid. The variations in cell size and chromosome number per cell, the chromatin material migration among microsporocytes, and the formation of conjugation tube and opening were observed in *Roegneria ciliaris* × *Psathyrostachys huashanica*. In *Triticum aestivum* × *P. huashanica* F_1 hybrid, all these phenomenona were also observed except the conjugation tube.

This paper reports descriptions of nuclear material transferring, coenocytism, chromosome number diminution and multiplication, and multipolar division in the F_1 hybrids of *Psathyrosachys huashanica* with *T. aestivum*.

Materials and methods

Psathyrostachys huashanica Keng (2n=14, N^hN^h) used in this study was collected from the Huashan Mt., Shaanxi province, China. *Triticum aestivum L. cv.* J-11 (2n=42, AABBDD) is a selected strain of a landrace native to Sichuan province.

Hybridization was made by pollinating hand-emasculated spikes of *T. aestivum* cv J-11 with pollen of *P. huashanica*. Immature hybrid embryos were cultured on N$_6$ and C$_{17}$ media 16-17 days after pollination. The hybrid seedlings obtained were transplanted into soil pots when the seedlings reached the three leave stage.

For cytological observations, young spikes of the F$_1$ hybrid were fixed in Carnoy's fluid. Chromosome numbers were counted at metaphase I of the PMCs. Microsporogenesis was studied on slides prepared by standard acetocarmine squashing.

Results and discussion

The intergeneric F$_1$ hybrids of *P. huashanica* with *T. aestivum* cv. J-11 are theoretically expected to have 28 chromosomes. The majority of the PMCs observed met the expectation (Table 1, Fig. 1 (1)). The meiotic data of the normal F$_1$ hybrids and their parents indicate that *P. huashanica* shares no common genome with *T. aestivum* (Sun et al. 1992a). However, as shown in Table 1, unusual chromosome numbers were observed. The chromosome number was fewer than 28 in 18.8% of the PMCs observed; and it was more than 28 in 7.2% of the PMCs. The highest chromosome number was more than one hundred (Fig. 1 (8)).

Table 1. Chromosome number per cell and its frequency at MI of pollen mother cells of *Triticum aestivum* with *Psathyrostachys huashanica* F$_1$ hybrid

Chromosome number	No. cells observed (%)
15	1 (0.37)
17	1 (0.37)
20	2 (0.75)
21	1 (0.37)
22	5 (1.89)
24	10 (3.77)
25	5 (1.89)
26	16 (6.04)
27	9 (3.39)
28	196 (73.96)
29	2 (0.75)
30	2 (0.75)
36	1 (0.37)
42	1 (0.37)
47	1 (0.37)
49	1 (0.37)
51	1 (0.37)
54	1 (0.37)
56	1 (0.37)
62	1 (0.37)
66	1 (0.37)
67	1 (0.37)
70	1 (0.37)
84	1 (0.37)
100	4 (1.51)

Microspore formation was observed in intergeneric hybrids of *T. aestivum* with *P. huashanica*. Under certain circumstances, chromonemata were migrating from one PMC into immediate neighboring PMCs through small openings, which might be just common plasmodesmata reported in many literatures. In some cases, chromonemata were seen passing from one PMC to another through a big opening (Fig. 1 (3)). If microsporocytes were arranged closely together, conjugation openings were formed at these conjoined points. The nucleus, chromatin mass or chromonemata (Fig. 1 (3, 5, 6)) could migrate through the opening into immediate neighbouring microsporocytes. The conjugation opening observed in this hybrid was similar to that in *Roegneria ciliaris* × *P. huashanica* hybrid (Yen et al. 1993). In that F₁ hybrid of *R. ciliaris* × *P. huashanica*, meiocytes were arranged very loosely. Outgrowth of bud-like structure came into contact with other pollen mother cells and became fused together, forming conjugation tube between cells (Yen et al. 1993). In this hybrid, meiocytes were arranged very closely. Thus, the cells came into contact with other so closely that there was no space for conjugation tube formation. A conjugation opening had the same function as a conjugation tube, but they differed in morphology (Yen et al. 1993). If the chromatin mass migrates through an opening as small as plasmodesma, this is cytomixis, which has been reported by many authors. In this hybrid, we observed that the chromatin material of pre-meiotic PMCs could migrate through the conjugation opening (Fig. 1 (2, 3)) and from coenocytes (Fig. 1 (7)). It has been observed that the chromatin of the pollen grains migrates through the conjugation opening after meiosis (Fig. 1 (4, 5, 6)). Fig. 1 (4) shows that the walls of young pollen grains (a) and (b) are dissolving, the nucleus of pollen grain (a) is elongating toward pollen grain (b), the young pollen grain walls of (c) and (d) have dissolved and formed a giant pollen grain. The nucleus migration is taking place between pollen grains (c) and (d), and the two nuclei are fused together.

Coenocytism in microsporogenesis is not rare in intergeneric hybrids in *Triticeae* (Kagawa 1929; Kihara and Lilienfeld 1934; Villax and Mota 1953; Nakajima 1954a, b). According to Price (1956), coenocytes were formed: i) by the failure of cytokinesis in the mitotic division preceding to meiosis, ii) through the passage of a nucleus from one PMC into another, and iii) through the fusion of PMCs as reported also by Mehra and Kalia (1973). Our observation showed that the coenocytes were formed through the nucleate materials transferring of PMCs at different stages of meiosis. Wang (1988) reported coenocytism in the *P. huashanica* × *Secale montanum* hybrids and observed an average of 4.44 nuclei, ranging from 2 to 25 per PMC. Yen et al. (1993) reported that 19.92% of the PMCs observed in the *R. ciliaris* × *P. huashanica* hybrids were found to be coenocytes containing 2 to 11 nuclei. In the present study, 52.0% of the PMCs observed were coenocytes containing 2 to 4 nuclei (Table 2).

Table 2. Coenocytes in *Triticum aestivum* with *Psathyrostachys huashanica* F₁ hybrids

Nuclei per cell	No. cell (%)
1	308 (48.0)
2	212 (33.1)
3	93 (14.5)
4	28 (4.4)

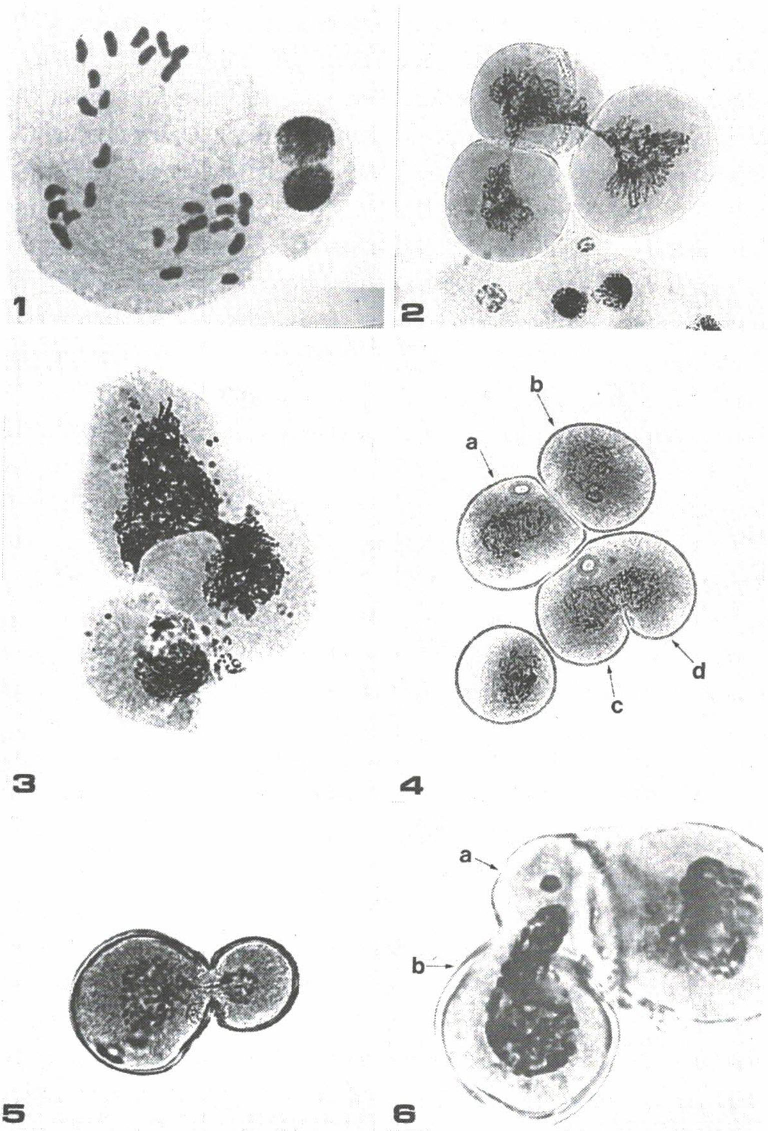
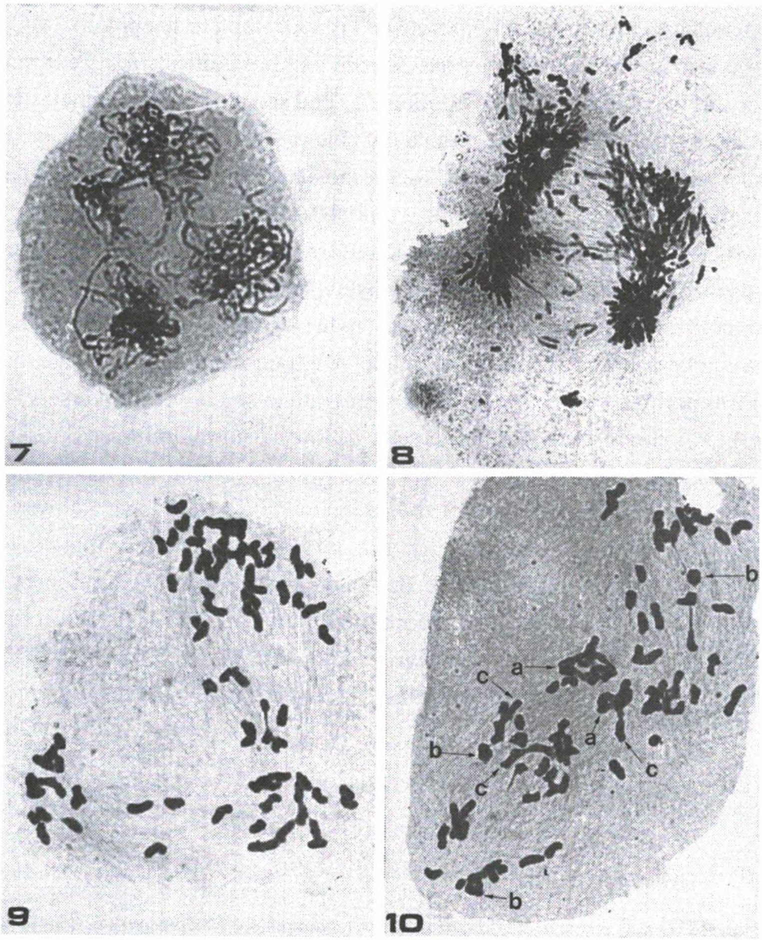


Fig. 1. PMCs of the *T. aestivum* × *P. huashanica* hybrid. (1) A normal PMCs of the *T. aestivum* × *P. huashanica* F_1 hybrid, showing 28 univalents. (2) The PMCs of the *T. aestivum* × *P. huashanica* hybrid, chromonemata migration among PMCs through a very small opening (maybe a normal plasmodesmata) (3) Chromonemata transfer through conjugation opening observed in the *T. aestivum* × *P. huashanica*. (4) Walls of young pollen grain (a) and (b) are dissolving, the nucleus in pollen grain (a) is elongating toward pollen grain (b), the young pollen grain walls of (c) and (d) have dissolved and formed a giant pollen grain, the nuclei migration are taking place between (c) and (d). (5) The choromatin is transferring between pollen grains through conjugation opening. (6) The nucleus of cell (a) is transferring into cell (b) through conjugation opening.



(7) Coenocytism with three synchronized nuclei. (8) A PMC contains probably more than one hundred chromosomes (their high density make an accurate count impossible) (9) A PMC contains more than 28 chromosomes, showing multipolar division and lagging chromosomes. (10) A PMC of the *T. aestivum* × *P. huashanica* F_1 hybrid, showing 2 quadrivalents (arrow a), 3 ring bivalents (arrow b), 3 rod bivalents (arrow c) and 64 univalents.

If a coenocytes has synchronized nuclei (Fig. 1 (7)), the chromosome number could be doubled or redoubled by nuclei fusion and a unified high level polyploid nucleus could be formed, although in some cases nuclei may not remain fused in coenocytes (Fig.1 (7)). PMCs that contained probably more than one hundred chromosomes (the high density of chromosomes made an accurate count impossible) were observed (Fig. 1 (8)). The decaploid PMC (Table 1) could form a pentaploid tetrad after meiosis. This might be a way in which spontaneous chromosome number doubling occurs. Thus, high level autoallopolyploid *Leymus* and *Elytrigia* species might have evolved by this mechanism.

If the transfer of a nucleus into a neighboring cell is not complete, aneuploid PMCs will appear. A loss or gain of one or more chromosomes has two obvious possibities: firstly extremely deficient gametes will not survive and they will be eliminated; and secondly, those gametes which contain chromosome numbers different from the normal are able to survive. The latter may be responsible for producing aneuploids. Fig. 1 (9) shows a 70-chromosome PMC. If the transfer of a nucleus into a neighboring cell is complete, chromosome number would double. Fig. 1 (10) shows a 84-chromosome PMC, which contains two quadrivalents (arrow a), three ring bivalents (arrow b), three rod bivalents (arrow c) and 64 univalents. This may explain the process of polyploid formation.

Multipolar division, which occurred in this hybrid (Fig. 1 (9)) might be caused by the formation of multipolar zones of synchronized nuclei in a coenocyte. We speculate that this kind of PMCs cannot form normal tetrads and has to disintegrate eventually. Conversely, synchronized nuclei in a few PMCs might form normal tetrads, following normal bipolar division (Yen et al. 1993). If this is true, the spontaneous chromosome number doubling and redoubling might have occurred. It might be one of the pathways of speciation in *Triticeae*. Such a pathway could lead to the origin of a high level autoallopolyploid such as *L. angustus*. We believe that the mechanism of chromatin transfer through conjugation tube or opening is a kind of variation of fertilization. According to our observation, chromatin material migration among cells has been found in all the intergeneric hybrids which derived from *Psathyrostachys huashanica* (Sun et al. 1992b; Yen et al. 1993; and the present study). We suggest that the N genome has a gene system for controlling this process.

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