Wheat Information Service
December 79: 24 –27 (1994)

Intermeiocyte connections and cytomixis in intergeneric hybrids III: *Roegneria tsukushiensis* × *Psathyrostachys huashanica*

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Summary

The F_1 intergeneric hybrid of *Roegneria tsukushiensi* (Honda) B.R.Lu et J.L. Yang with *Psathyrostachys huashanica* Keng was made and studied cytologically. Conjugation openings were observed. Intercellular migration of chromatin material led to the formation of coenocytism and variation in cell size. These events could lead to spontaneous variation in chromosome numbers. As a possible consequence of migration of chromatin materials, polyploid and aneuploid PMCs appeared. It may combine two different gene pools. There is possibility for transferring drought resistance character from *P. huashanica* into *R. tsukushiensis* by chromatin migration among cells. The observed result in this hybrid supports further that N^h genome of *P. huashanica* has a genetic system for controlling this process.

Key words: Cytomixis; Conjugation opening; Aneuploid; Polyploid; Variation in chromosome number, *Roegneria tsukushiensis*, *Psathyrostachys huashanica*

Introduction

Roegneria tsukushiensis is one of a few good forage grass in Triticeae in South Subtropic region of Far East, but it lacks resistance to drought. *Psathyrostachys huashanica* Keng is a diploid perennial species. It is restricted to a narrow region in the Huashan Mountain of central China where it occurs on mountain slopes, but is a hardened and drought resistant plant. The intergeneric cross of the *R. tsukushiensis* with *P. huashanica*, was made. The intergeneric F_1 hybrid was studied cytologically.

We had previously studied the intermeiocyte connections and cytomixis in intergeneric hybrids of *Roegneria ciliaris* (Trin.) Nevski, *Aegilops tauschii* Cosson and *Triticum aestivum* L. with *P. huashanica* (Sun et al. 1992, 1993b; Yen et al. 1993). It was found that chromatin was transferred through conjugation opening or tube before, during and after meiosis in these hybrids. Consequently, unusual nuclear behavior frequently occurred, such as coenocytism, high level chromosome multiplication, multipolar division, variation in size of pollen grain, aneuploid formation, non-synchronous, and delayed chromatin condensation

According to our observation, chromatin material migration among cells had been found in all the intergeneric hybrids derived from *P. huashanica*. We suggested that the N^h genome has a gene system for controlling this process. In order to prove this suggestion, we studied the microsporogensis of *R. tsukushiensis* × *P. huashanica* F_1 hybrid. The transfer of nucleate materials through

conjugation opening was also found. The results of observation are described and discussed in the present paper.

Materials and Methods

Three accessions of *Roegneria tsukushiensis* (Honda) B.R. Lu et J.L. Yang (2n=42, SSHHYY) used in this study were collected from Yaan and Yibin, Sichuan Province respectively. Two accessions of *Psathyrostachys huashanica* Keng (2n=14, N^hN^h) were collected from Mt. Huashan, Shaanxi Province, China. All these accessions were cultivated at Triticeae Research Institute, Sichuan Agricultural University in 1990.

Hybridization was made by pollinating hand-emasculated spikes of *R. tsukushiensis* with pollen of *P. huashanica*. Three F_1 seeds were germinated in petri-dish, then transplanted into pots.

For cytological study, young spikes of the three F_1 hybrids derived from three different accessions of *R. tsukushiensis* crossed with one accession of *P. huashanica* and their parents were fixed in Carnoy's fluid for 24 hours. They were transferred to 70% ethanol and stored at 4°C. Microsporogensis was studied on slides prepared by standard acetocarmine squashing.

Results and Dicussion

The intergeneric F_1 hybrid of *R. tsukushiensis* with *P. huashanica* is theoretically expected to have 28 chromosomes. Chromosome numbers counted at metaphase I of the pollen mother cells (PMCs) met the expectation (Fig. 1-1). The meiotic data indicated that *R. tsukushiensis* shared no common genome with *P. huashanica* (Sun et al. 1993a).

Abnormal microspore formation was observed in microsporogenesis of this intergeneric hybrid. Chromatin materials were transferred through conjugation opening among neighbouring cells from the time before meiosis until the young pollen grains stage (Fig. 1-2~7). The amount of chromatin materials transferred varied among cells. The process of chromatin migration among cells causes coenocytes, multiplication and diminution of chromosomes greatly, and variation in cell size. Fig. 1-2 shows PMC a and PMC b in contact with each other and forming a fused big opening. The numbers of anaphase chromosomes in these two cells are unequal. Anaphase chromosomes also show non-synchronous separation. In cell a, there are 9 anaphase chromosomes and 7 chromatids; in cell b there are 2 anaphase chromosomes and 3 chromatids. In Fig. 1-3, two PMCs at chromonema stage contacted each other, chromonemata migration takes place through the conjugation opening. In Fig. 1-4, cell a has two separate synchronous nuclei at chromonema stage; cell b with small cell size has only one chromosome and two chromatids. The chromosome diminution might be caused by uneven distribution of chromosomes before cytokinesis of two conjugated PMCs as shown in Fig. 1-2. After cytokinesis, this kind of small size PMC has occurred as a result a diminished number of chromosomes. In cell c, two chromonema stage nuclei are mixed together. A nucleus has twice as many chromosomes as the usual one. Polyploidy or aneuploidy will occur. A bud-like structure has a micronucleus (Fig. 1-4). The young pollen grains are shown

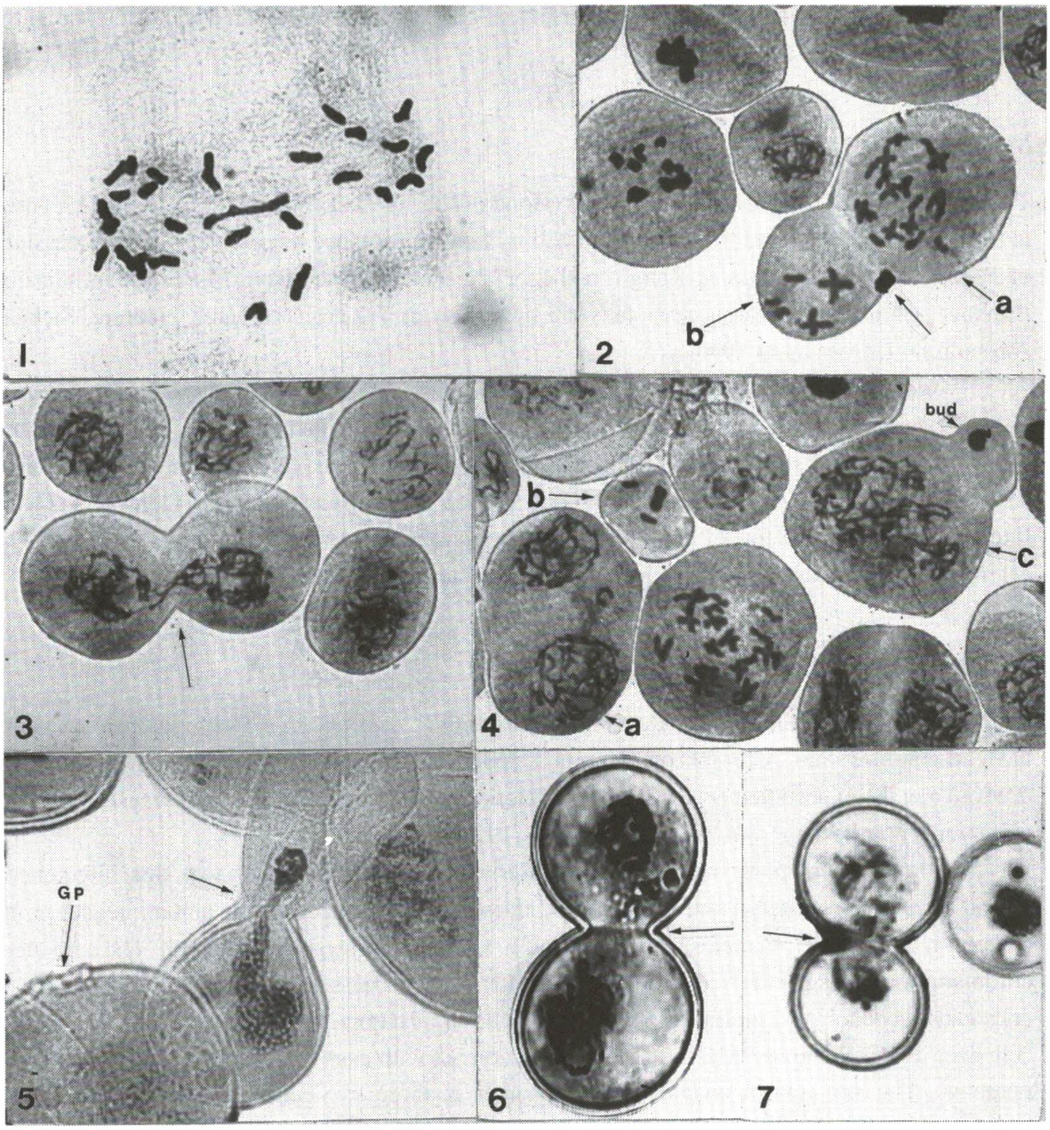

Fig. 1. PMC's and young pollen grains showing intermeiocyte connections and cytomixis

1. A normal PMC of *R. tsukushiensis* × *P. huashanica* hybrird has 28 chromosomes.
2. PMC a and PMC b contacting each other and forming a big opening or being somewhat fused. The number of anaphase chromosomes distributed in these two cells is unequal. Anaphase chromosomes also show non-synchronous separation. In cell a, there are 9 anaphase chromosomes with 7 chromatids; in cell b, there are 2 anaphase chromosomes with 3 chromatids.
3. Two chromonema stage PMCs contacting each other, and chromonemata migration just taking place through conjugation opening.

in Figures 1-5~7. We can see that the resting stage nuclei are just migrating through the conjugation opening between two young pollen grains. They have germ pore on their thick walls. In Figure 1-6, the focus section of the conjugated pollen grains shows the structure of opening very clearly. Figure 1-7 shows the same conjugation opening as in Figure 1-6, but the chromonema is just migrating through the conjugation opening. It is suggested that a gene system of *P. huashanica* also controls conjugation opening formation in this hybrid as well as in those previously reported hybrids of *R. ciliaris* × *P. huashanica* (Yen et al. 1993), *T. aestivum* × *P. huashanica* (Sun et al. 1993b) and *Ae. tauschii* × *P. huashanica* (Sun et al. 1992). The result indicates that the N^h genome of *P. huashanica* has a gene system for controlling this process, because this kind of behavior appears in all the hybrids which are derived from *P. huashanica.*

The chromatin mirgation may cause coenocytes. If a coenocyte has synchronized nuclei, 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. This might be a way in which spontaneous chromosome doubling occurs. It is one way of natural polyploid formation, especially high level alloautopolyploid. 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 possibilities: firstly extremely deficient gametes will not survive and they will be eliminated; and secondly, those gametes which contain chromosome numbers different from the normal and are able to survive. In hybrid descendants of *R. tsukushiensis* with *P. huashanica*, polyploids and aneuploids which contain two different gene pools of *R. tsukushiensis* and *P. huashanica* may be obtained. Thus, it is possible to transfer drought resistance from *P. huashanica* into *R. tsukushiensis* by backcrossing F_1 hybrid with *R. tsukushiensis*.

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4. Cell a has two separate synchronous nuclei at chromonema stage; cell b with small cell size has only one chromosome and two chromatids; cell c, two chromosonema stage nuclei mixed together. A nucleus has twice as many chromosomes as the usual one; a bud-like structure has a micronucleus.
5. The resting stage nuclei are just migrating through conjugation opening (arrow) between two young pollen grains, and they have germ pore on their thick walls.
6. The focus section of the conjugated pollen grains shows the structure of opening (arrow) very clearly.
7. Chromonemata is just migrating through conjugation opening(arrow) at young pollen grain stage.

Wheat Information Service
December 79: 28 – 32 (1994)

The ineffectiveness of the *ph1b* gene on chromosome association in the F_1 hybrid, *Triticum aestivum* × *Psathyrostachys huashanica*

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Summary

An intergeneric cross was made between Chinese Spring *ph1b* mutant and perennial *Psathyrostachys huashanica* Keng. The meiotic chromosome pairing in the hybrid was 27.14 univalents and 0.43 bivalents. The result indicated that the *ph1b* gene did not induce homoeologous chromosome pairing between common wheat and *P. huashanica*, as well as among the common wheat chromosomes. Therefore, the presence of a *Ph1* or *Ph1*-like gene in *P. huashanica* was suggested.

Introduction

The evolutionary success of many polyploid species is largely due to their diploid-like cytological behavior, which is expressed by the virtually exclusive formation of bivalents, rather than multivalents, at the first metaphase of meiosis. Common wheat, *Triticum aestivum* L., contains several unlinked diploidizing gene systems (Sears 1976). The *ph1b* gene mutant was obtained by Sears (1977). This mutant allows homoeologous pairing in common wheat and in its hybrids.

The transfer of alien genetic material to common wheat through homoeologous recombination is an importment step in the efforts to increase the genetic variation. The previous studies indicated that the *ph1b* gene has strong effect on inducing homoeologous pairing in the hybrids between bread wheat and *Aegilops variabilis*, *Ae. triuncialis*, *Ae. turcomenica*, *Ae. triaristata*, *Ae. cylindrica*, *Ae. colmnaris*, and *Ae. ovata* (Kushnir et al. 1982; Sharma et al. 1986; Fan et al. 1992, 1993). However, the effectiveness of the *ph1b* gene inducing chromosome pairing in the hybrid between common wheat and *Psathyrostachys huashanica* has not been demonstrated yet.

This paper reports the first production of an intergeneric hybrid between Chinese Spring *ph1b* mutant and *P. huashanica*, and the meiotic analysis of the hybrid. The possible presence of a *Ph1*-like gene in *P. huashanica* is discussed in connection with the result of the meiotic analysis.

Materials and methods

The *ph1b* mutants of a common wheat cultivar Chinese Spring (abbreviated to CS *ph1b*) was kindly provided by the Cytogenetic Laboratory of Sichuan Agricultural University. Professor Z.L. Ren proved the authenticity of the mutant *ph* by cytogenetic method (personal communciation). The euploid Chinese Spring (CS) was kept at the Triticeae Research Institute, Sichuan Agricultural