广 西 植 物 Guihaia　22（4）：352－356　　2002 年 7 月

大鹅观草与阿拉善鹅观草杂种的形态学和细胞学研究

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摘　要：为探讨阿拉善鹅观草（*R. alashanica*（Keng）S. L. Chen）的染色体组组成，进行了大鹅观草（*R. grandis* Keng（2n＝24，**StStYY**））和阿拉善鹅观草种间杂交，对这两个种及其杂种 F_1 的形态学、繁育学和减数分裂染色体配对行为进行了研究。结果表明：杂种 F_1 的形态特征介于父母本之间，其花粉母细胞减数分裂染色体平均构型为：20.40 Ⅰ＋3.69 Ⅱ＋0.09 Ⅲ＋0.04 Ⅳ。表明阿拉善鹅观草含有一个修饰的 **St** 基因组，即 $\mathbf{St}^{a}$。

关键词：大鹅观草；阿拉善鹅观草；种间杂交；减数分裂；基因组；染色体配对

中图分类号：Q942　　**文献标识码**：A　　**文章编号**：1000-3142(2002)04-0352-05

Morphological and cytological studies on hybrid from *Roegneria grandis* ×*R. alashanica* (Poaceae: Triticeae)

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Abstract: Interspecific hybridization was carried out between *Roegneria alashanica* (Keng) S. L. Chen and *R. grandis* Keng (2n=24. **StStYY**). The morphology, fertility and chromosome pairing behavior in meiosis of the parents and their hybrid F_1 were studied to determine the genomic constitution of R. alashanica. The F_1 hybrid appeared intermadiate between the two parents. The meiotic configuration of the hybrid was 20.40 Ⅰ +3.69 Ⅱ +0.09 Ⅲ +0.04 Ⅳ. The results indicated that *R. alashanica* has one slightly modified **St** genome, be designated as $\mathbf{St}^{a}$.

Key words: *Roegneria grandis*; *R. alashanica*; interspecific hybrid; meiosis; genome; chromosome pairing

Roegneria C. Koch of the tribe Triticeae (Gramineae) is a very large and widely distributed genus which includes about 130 species in the world and nearly 70 in China[1]. Since this genus contains a large number of species, subspecies, and the morphological limits among some of them are not so clear, so taxonomy seems very difficult within the genus.

Roegneria grandis Keng and *R. alashanica*

收稿日期：2001-10-26

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基金项目：教育部骨干教师资助项目教技司[2000]63 号

(Keng) S. L. Chen are tetraploid ($2n=4x=28$) perennial species, awnless on the lemma and distributed in northwest China. *R. grandis* has two genomes, **StStYY**[2], while the genomic constitution of *R. alashanica* is still unknown[3]. Morphologically, *R. alashanica* has a solitary spikelet at each rachis node and have the disarticulation of the rachilla above the glumes and beneath the florets. All of these characters were considered to be similar to *R. elytrigioides* Yen *et* J. L. Yang. Considering the morphological similarity between *R. alashanica* and *R. elytrigioides*, Cai[4] treated it as *R. alashanica* var. *elytrigioides* (Yen *et* J. L. Yang) L. B. Cai. Lu[5] reported that *R. elytrigioides* contained two slightly modified **St** genomes - **St^{1}St^2**, and transferred it to *Psedoroegneria*. In order to investigate the genomic constitution of *R. alashanica* (Keng) S. L. Chen, intergeneric hybridization was carried out between *R. grandis* and *R. alashanica*. Morphological comparison and chromosome pairing behavior at metaphase I of pollen mother cells (PMCs) were observed in the parental species and their F_1 hybrid.

Materials and Methods

Roegneria grandis Keng was collected in Shanxi, China and *R. alashanica* (Keng) S. L. Chen was collected from Ningxia, China. Both of them were grown in the field at the Triticeae Research Institute, Sichuan Agricultural University.

For hybridization was: the spikes of *R. grandis* were emasculated and put in a cellulose bag. Two days after the maternal stigmas were brushed, the hand-emasculated spikes were pollinated with newly broken anthers of *R. alashanica*. The hybrid seeds were germinated on filter paper in petri-dishes and then the seedling were sowed in pots at two leaf stage.

Sixteen morphological characters were compared between the parents and their F_1 hybrid. For cytological observation, the young spikes were fixed in Carnoy's Ⅱ solution (6 alcohol: 3 chloroform: 1 acetic acid), and stored in a refrigerator. Chromosome pairing was observed at the metaphase I (MI) of the pollen mother cells (PMCs) using the acetic-carmine squash technique. Microphotographs were taken from permanent meiosis preparations. Pollen grains of parents and F_1 hybrid from mature anthers were stained in an aqueous I_2-KI solution for pollen fertility studies.

Voucher specimens of the parents and F_1 hybrid are deposited in Triticeae Research Institute, Sichuan Agricultural University.

Results

1. Interspecific cross

A total of 45 florets of *R. grandis* were pollinated with *R. alashanica* and 9 seeds were obtained. Seed set was 20.00%. Two of the nine seeds were germinated and developed into adult plants. The hybrid plants grew vigorously and set spikes later.

2. Morphological comparison among the parental species and the F_1 hybrid

Sixteen morphological characters of the parents and the F_1 hybrid were measured (Table 1). *R. grandis* was generally larger than *R. alashanica* in many aspects, e. g. length and width of leaf and flag leaf, length of spike, glume (first and second), palea and anther. *R. alashanica* had more spikelets per spike and florets per spikelet than those of *R. grandis*. Both of them had no awn on lemma, while the F_1 hybrid had an awn, around 1.36 cm. The general appearance of the F_1 hybrid was intermediate between their two parents or near to one of the parental species (Fig. 1).

3. Meiosis in the F_1 hybrid

The chromosome pairings at the MI of PMCs in the parental species and the F_1 hybrid are listed in Table 2. The meiotic chromosome pairings of the parents were normal and no univalents or multivalents were observed (Fig. 2. A-B). The hybrid has 28 chromosomes. A large number of univalents were found in this hybrid with 20.40 (from 14-26) per cell on average. The bivalents were 3.69 (from1-7) per cell. Trivalents and quadrivalents were infrequent, around 0.09 and 0.04 per cell, respectively. Of 57 F_1 PMCs examined, 25 (43.76%) showed 3 to 4 bivalents (Fig. 2. C). The chiasma frequency was 4.51 with the range of 1-9 and the C-value was 0.16. Chromosome bridges and the lagging chromosomes at anaphase I were found (Fig. 2. D-E), and micronuclei were also observed in tetrads of F_1 hybrid (Fig. 2. F).

Table 1 Comparison of morphological characters among *R. grandis*, *R. alashanica* and their F_1 hybrid

Characters	*R. grandis*	*R. grandis*×*R. alashanica*	*R. alashanica*
Height (cm)	50.05±4.68	83.37±13.65	51.34±3.94
Leaf length (cm)	30.89±1.95	32.75±2.62	24.33±3.65
Leaf width (cm)	0.85±0.08	2.46±0.05	0.32±0.04
Length of flag leaf (cm)	29.79±2.79	31.09±4.56	20.43±2.30
Width of flag leaf (cm)	0.86±0.07	2.48±0.13	0.49±0.01
Length of top internode (cm)	17.21±4.91	27.93±4.72	21.75±1.82
Spike length (cm)	19.33±2.77	25.38±2.03	11.73±6.42
No. of spikelets per spike	6.85±0.69	15.14±3.02	10.58±1.05
No. of florets per spikelet	8.56±0.62	7.25±1.06	9.33±0.58
Length of first glume (cm)	1.37±0.26	1.02±0.08	0.89±0.04
Length of second glume (cm)	1.12±0.23	0.60±0.05	0.68±0.06
Length of first lemma (cm)	1.48±0.04	2.39±0.15	0.96±0.05
Length of first lemma awn (cm)	—	1.36±0.11	—
Length of palea (cm)	0.92±0.05	0.94±0.04	0.80±0.02
Length of anther (cm)	0.42±0.03	0.48±0.05	0.38±0.01
Hairs on lemma	+	+	—

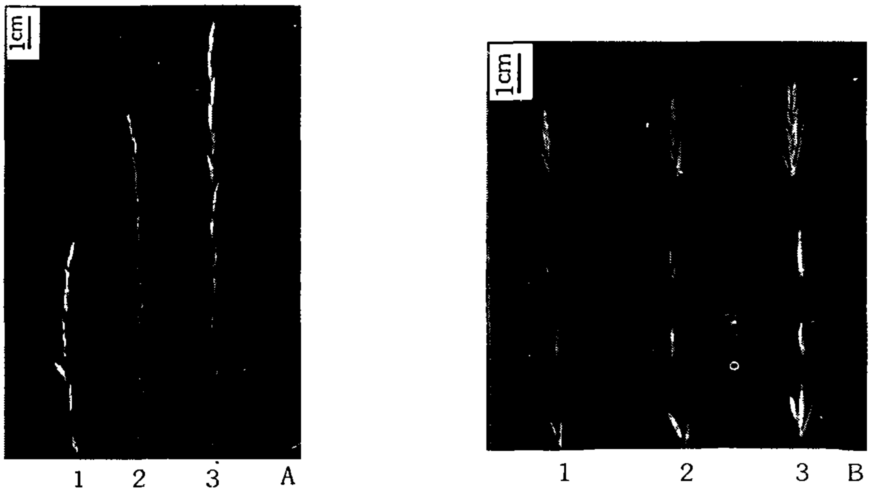

Fig. 1 Spikes of *R. alashanica*, *R. grandis* and the F_1 hybrid A. Spikes; B. Spikes, glumes, lemmas and paleas.
1. *R. alashanica*; 2. *R. grandis*×*R. alashanica*; 3. *R. grandis*.

Table 2 Chromosome pairing at MI of PMCs in *R. grandis*, *R. alashanica* and their F_1 hybrid

Parents and combinations	2n	No. of cells observed	Chromosome pairing I	Ⅱ Total	Ⅱ Rods	Ⅱ Rings	Ⅲ	IV	Chiasma per cell	C-value
R. grandis	28	50	—	14.00(14)	0.58(0-5)	13.42(9-14)	—	—	25.70(23-28)	0.92
R. alashanica	28	50	—	14.00(14)	2.30(0-5)	11.70(9-14)	—	—	26.11(23-28)	0.93
R. grandis × *R. alashanica*	28	57	20.40 (14-26)	3.69(1-7)	0.47(0-4)	3.22(1-7)	0.09 (0-2)	0.04 (0-1)	4.51 (1-9)	0.16

Table 3　Pollen fertility and seed set in *R. grandis*, *R. alashanica* and their F_1 hybrid

Parents and hybrids F1	No. of pollen grains observed	Pollen No.	Fertility (%)	No. of florets	Seed No.	Set (%)
R. grandis	350	330	(94.29)	150	125	(83.33)
R. grandis	360	337	(93.61)	170	155	(91.18)
R. alashanica × *R. alashanica*	500	0	(0.00)	2 000	0	(0.00)

4. Fertility

The pollen fertility and seed set of the parental species and F_1 hybrid were examined (Table 3). Most pollen grains of the parental species were viable, while those of F_1 hybrid were not. The seed set of the parental plants were 83.33% and 91.18%, respectively. But no seed was formed in F_1 hybrid after being carefully examined a great number of florets.

Discussion

Interspecific hybridization between *R. grandis* and *R. alashanica* in this study was comparatively easy to perform without the assistance of embryo rescue technique, indicating that crossing barriers are not very strong between these two species. However, pollens of F_1 hybrid were completely sterile while those of the parental species were quite normal. Based on the morphology and hybridization data, it is evident that *R. grandis* and *R. alashanica* are two dependent biological species.

The degree of chromosome pairing in an interspecific or intergeneric hybrid can be used as an index of chromosome homology as well as specific or generic evolutionary relationship[6]. Homologous chromosomes are defined as having the same nucleotide base sequences, whereas homoeologous chromosomes have only residual homology from the originally homologous chromosomes[7]. In the meiosis of F_1 hybrid from the cross of *R. grandis* and *R. alashanica*, the number of chromosome pairing varied from 1-7, showed an average of 3.69 bivalents per cell. This suggests that *R. alashanica* has one genome which is homoeologous to one genome of *R. grandis*. **St** genome is the basic genome in *Roegneria*, *Elymus* and *Elytrigia*, originating from the genus *Psedoroegneria*, while **Y** is mostly found in Asian species of *Roegneria*, which come from an unknown diploid progenitor. Zhang[3] observed that the hybrid of *R. alashanica* and *R. ciliaris* (Trin.) Nevski had a higher amount of chromosome pairing, up to 8.17 bivalents per cell and drew conclusion that *R. alashanica* shared at least one set of **St** genome of *R. ciliaris*. Therefore, we could conclude that *R. alashanica* has one slightly modified St genome, be designated as **St**^{a}.

Lu[5] made genome analysis of *R. elytrigioides*, and concluded that *R. elytrigioides* contains two slightly modified **St** genomes, $\mathbf{St_1St_1St_2St_2}$. Cai[4] treated morphologically *R. elytrigioides* as a variety of *R. alashanica*. Therefore, *R. alashanica* is related closely to *R. elytrigioides*. The genome of *R. alashanica* is in our further persuit. If *R. alashanica* has two **St** genomes as *R. elytrigioides*, according to the point of view of Dewey[8], Löve[9,10] and Baum[11], it should be transferred to *Psedoroegneria*.

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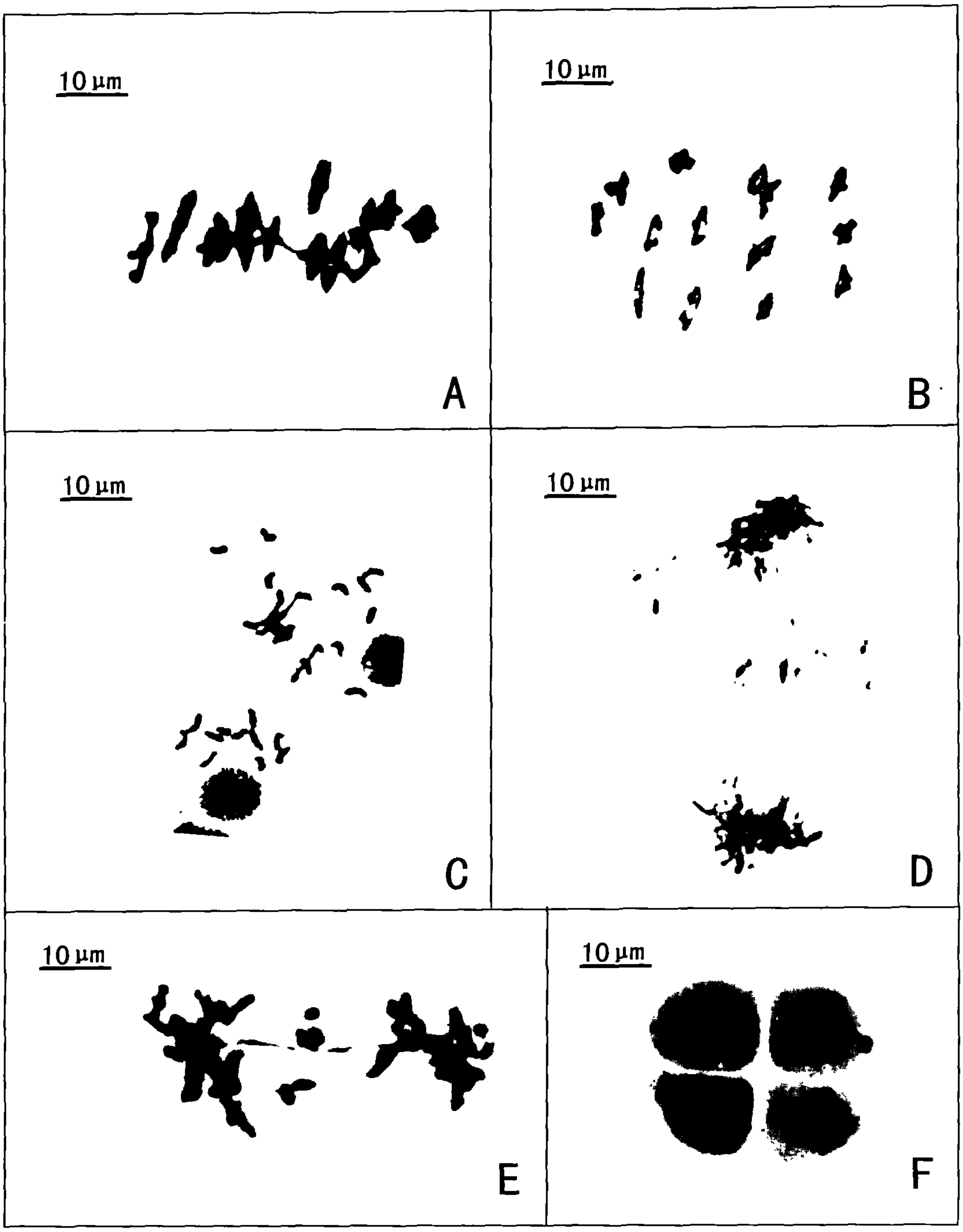
ZHANG Hai-qin, *et al.*: Morphological and cytological studies on hybrid from *Roegneria grandis* × *R. alashanica* (Poaceae: Triticeae)

图版 I
Plate I



Chromosome pairings at MI, AI of PMCs in the parents and their F_1 hybrid
A. *R. alashanica* with 14 bivalents; B. *R. grandis* with 14 bivalents; C. *R. grandis* × *R. alashanica* with 20 univalents and 4 bivalents; D. showing lagging chromosomes at AI; E. showing chromosome bridge at AI; F. showing tetrads with micronuclei