

## Phylogenetic relationships among *Roegneria amurensis*, *R. ciliaris* and *R. japonensis* (Poaceae: Triticeae)

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To evaluate the phylogenetic relationships among *Roegneria amurensis*, *R. ciliaris* and *R. japonensis*, hybridizations among them were carried out. In the hybrids of *R. amurensis* × *R. ciliaris*, *R. japonensis* × *R. amurensis* and *R. ciliaris* × *R. japonensis*, average chromosome associations were 0.38 I + 13.83 II, 0.18 I + 13.90 II and 0.15 I + 13.91 II, respectively. All the hybrids were partially fertile with stainable pollen grains and the fertility was 82%, 88% and 95%, respectively. The results indicated that the three species share the same basic genomes, StStYY and they are closely related. Combined with morphology and geographic distribution, *R. amurensis* and *R. japonensis* are treated as a subspecies and a variety of *R. ciliaris*, respectively.

Key words: chromosome pairing, genome, morphology, Poaceae, *Roegneria amurensis*, *R. ciliaris*, *R. japonensis*, Triticeae

### Introduction

*Roegneria* is a very large and widely distributed genus in the tribe Triticeae (Poaceae). It includes about 120 species worldwide and nearly 80 species in China (Keng & Chen 1963, Kuo 1987, Baum *et al.* 1991, Cai 1997), and many subspecies or varieties are recognized. The morphological limits among some species are not clear, and as such the taxonomy within the genus is very difficult. *Roegneria* was once included in *Elymus* (Tzvelev 1976, Dewey 1984, Löve 1984), but many taxonomists consider it a valid genus (Keng & Chen 1963, Kuo 1987, Baum *et al.* 1991, Cai 1997, Zhou *et al.* 1999a).

*Roegneria amurensis*, *R. ciliaris* and *R. japonensis* are three tetraploid ( $2n = 4x = 28$ ) taxa (Dewey 1984, Lu *et al.* 1988a, Wei *et al.* 2005). Both *R. ciliaris* and *R. japonensis* contain StStYY genomes (Dewey 1984, Lu *et al.* 1988b, Wang *et al.* 1994), while the genome constitution of *R. amurensis* is still unknown. Morphologically, *R. amurensis* differs slightly from *R. ciliaris* and *R. japonensis* in its pubescent leaves and leaf sheaths. *Roegneria ciliaris* differs slightly from *R. japonensis* in its pubescent glumes and erect spikes (Kuo 1987, Lu *et al.* 1988a). Cytologically, Wei *et al.* (2005) reported that the chromosomes of the three taxa are mainly metacentric and share similar karyotypes.

It is difficult to classify the three species due to the fact that their morphology differs only slightly. Tzvelev (1976) treated *R. amurensis* as *Elymus ciliaris* ssp. *amurensis*. Similarly, Löve (1984) treated *R. amurensis* and *R. japonensis* as subspecies of *Elymus ciliaris*. Based on cytological studies, Lu *et al.* (1988b) described *R. japonensis* as *Roegneria ciliaris* var. *japonensis*. Cai (1997) considered *R. amurensis* a valid species, and described *R. japonensis* as *R. ciliaris* var. *hackeliana* based on morphological comparison. Therefore, the taxonomic treatment and phylogenetic relationships of these three taxa are still in dispute. The main question is whether *R. amurensis* and *R. japonensis* are valid species, subspecies of *R. ciliaris*, or varieties of *R. ciliaris*.

The objectives of this study are: (1) to investigate the genomic constitution of *R. amurensis*; (2) to evaluate the phylogenetic relationships among *R. amurensis*, *R. ciliaris* and *R. japonensis* based on morphological comparison, meiotic study and fertility analysis; and (3) to provide a taxonomic treatment of the three taxa in *Roegneria*.

Material and methods

The material used in this study is listed in Table 1. *Roegneria amurensis* that originated in the Russian Federation was kindly provided by American National Plant Germplasm System (Pullman, Washington, USA). Other accessions were collected by the authors of this paper. All species are currently growing at Triticeae Research Institute, Sichuan Agricultural University, China (SAUTI).

The procedures of hybridization, fixation and staining of the cytological material and

meiotic preparation followed those reported by Zhou *et al.* (1999a). Chromosome pairing behavior at meiotic metaphase-I (MI) of parents and F<sub>1</sub> hybrids was observed in at least 50 pollen mother cells (PMCs). The count of mean pairing frequency (*C* = the mean frequency with which two related chromosome arms pair) was calculated according to Alonso and Kimber (1981). When mean *C* > 0.50, the two genomes in the hybrids are considered identical (Wang 1992). Microphotographs were taken from permanent meiosis preparations with an Olympus BX-51 camera. Pollen grains from mature anthers were stained in an aqueous I<sub>2</sub>-KI solution for pollen fertility studies.

Results

Intraspecific cross

The crosses *R. amurensis* (♀) × *R. ciliaris* (♂), *R. japonensis* (♀) × *R. amurensis* (♂) and *R. ciliaris* (♀) × *R. japonensis* (♂) produced hybrids without the aid of embryo rescue (Table 2). In *R. amurensis* × *R. ciliaris* and *R. japonensis* × *R. amurensis*, the seed sets were 22% and 20%, respectively. The seed set of *R. ciliaris* × *R. japonensis* was 50%, thus much higher than that of the hybrids including *R. amurensis* (Table 2). Most of the seeds from each cross germinated easily and developed into vigorous hybrid plants. Six plants of the eleven seeds in *R. amurensis* × *R. ciliaris*, three plants of the nine seeds in *R. japonensis* × *R. amurensis* and two plants of the two seeds in *R. ciliaris* × *R. japonensis* were obtained. All the hybrid plants developed vigorously and set spikes later.

Table 1. Material used in this study.

| Taxa                        | 2n | Genomes | Origin                       | Accession no. |
|-----------------------------|----|---------|------------------------------|---------------|
| <i>Roegneria amurensis</i>  | 28 | —       | Russian Federation           | PI 547303     |
| <i>Roegneria ciliaris</i>   | 28 | StY     | Wugong, Shanxi, China        | 88-89-227     |
|                             |    |         | Yaan, Sichuan, China         | 88-89-229     |
| <i>Roegneria japonensis</i> | 28 | StY     | Yibin, Sichuan, China        | 88-89-253     |
|                             |    |         | Haerbin, Heilongjiang, China | 88-89-257     |

# Morphological comparisons among the parental species and the hybrids

Twenty-one morphological characters of the parents and their hybrids were measured (Table 3). Only few characters distinguished the three *Roegneria* taxa. The leaves and leaf sheaths of *R. amurensis* were pubescent, while they were glabrous in *R. ciliaris* and *R. japonensis*. The mean length of internodes is clearly longer and the number of florets per spikelet in *R. amurensis* is higher than in *R. ciliaris* and *R. japonensis*. *Roegneria amurensis* had smooth lemma tops, while *R. ciliaris* and *R. japonensis* had toothed ones. *Roegneria ciliaris* is readily distinguished from *R. amurensis* and *R. japonensis* by its longer top internodes. The general appearances of the  $F_1$  hybrids were intermediate between their parents or near to one of the parental species. There was a tendency toward female parents in *R. amurensis*  $\times$  *R. ciliaris* and *R. japonensis*  $\times$  *R. amurensis*, especially in the number of spikelets per spike, number of florets per spikelet and length of spikelet (Table 3 and Fig. 1).

# Meiosis in parents and hybrids

Chromosome pairing at MI of PMCs in the three parental species and their  $F_1$  hybrids is listed in Table 4. The meiotic pairing of the parents was quite normal with 14 ring bivalents (Fig. 2A–C). Univalents were observed only occasionally and multivalent was not found in parents.

The patterns of chromosome pairing at MI were different in the three combinations. In the hybrid of *R. amurensis*  $\times$  *R. ciliaris*, the mean pairing configuration was 0.38 I + 13.83 II and the chiasma frequency was 24.99 with  $C = 0.89$ . In *R. japonensis*  $\times$  *R. amurensis*, the average

meiotic configuration was 0.18 I + 13.90 II and the chiasma frequency was 25.95 with  $C = 0.93$ . As for the hybrid of *R. ciliaris*  $\times$  *R. japonensis*, the mean pairing configuration was 0.15 I + 13.91 II and the chiasma frequency was 25.98 with  $C = 0.93$ . The frequencies of 14 bivalents observed in MI cells of the three combinations were 83%, 91% and 93%, respectively. In the three combinations, the lagging chromosomes were found at anaphase-I cells and micronuclei were also observed in the tetrads of the three crosses (Fig. 2H, I).

# Fertility

The pollen fertilities and seed sets of the parental species and their hybrids were examined (Table 5). Most pollen grains of the parental species as well as their hybrids were viable. The average pollen fertilities of *R. amurensis*, *R. ciliaris* and *R. japonensis* were 89%, 95% and 92%, respectively. In the hybrids of *R. amurensis*  $\times$  *R. ciliaris*, *R. japonensis*  $\times$  *R. amurensis* and *R. ciliaris*  $\times$  *R. japonensis*, the mean pollen fertilities were 82%, 88% and 95%, respectively. The seed set of the parental plants was higher than that of their  $F_1$  hybrids. Under open pollination, the respective average seed sets of *R. amurensis*, *R. ciliaris* and *R. japonensis* were 91%, 91% and 92%. In the three hybrids of *R. amurensis*  $\times$  *R. ciliaris*, *R. japonensis*  $\times$  *R. amurensis* and *R. ciliaris*  $\times$  *R. japonensis*, the mean opening seed sets were 53%, 64% and 84%, respectively.

# Discussion

There is slight disparity among *R. ciliaris*, *R. japonensis* and *R. amurensis* in morphology.

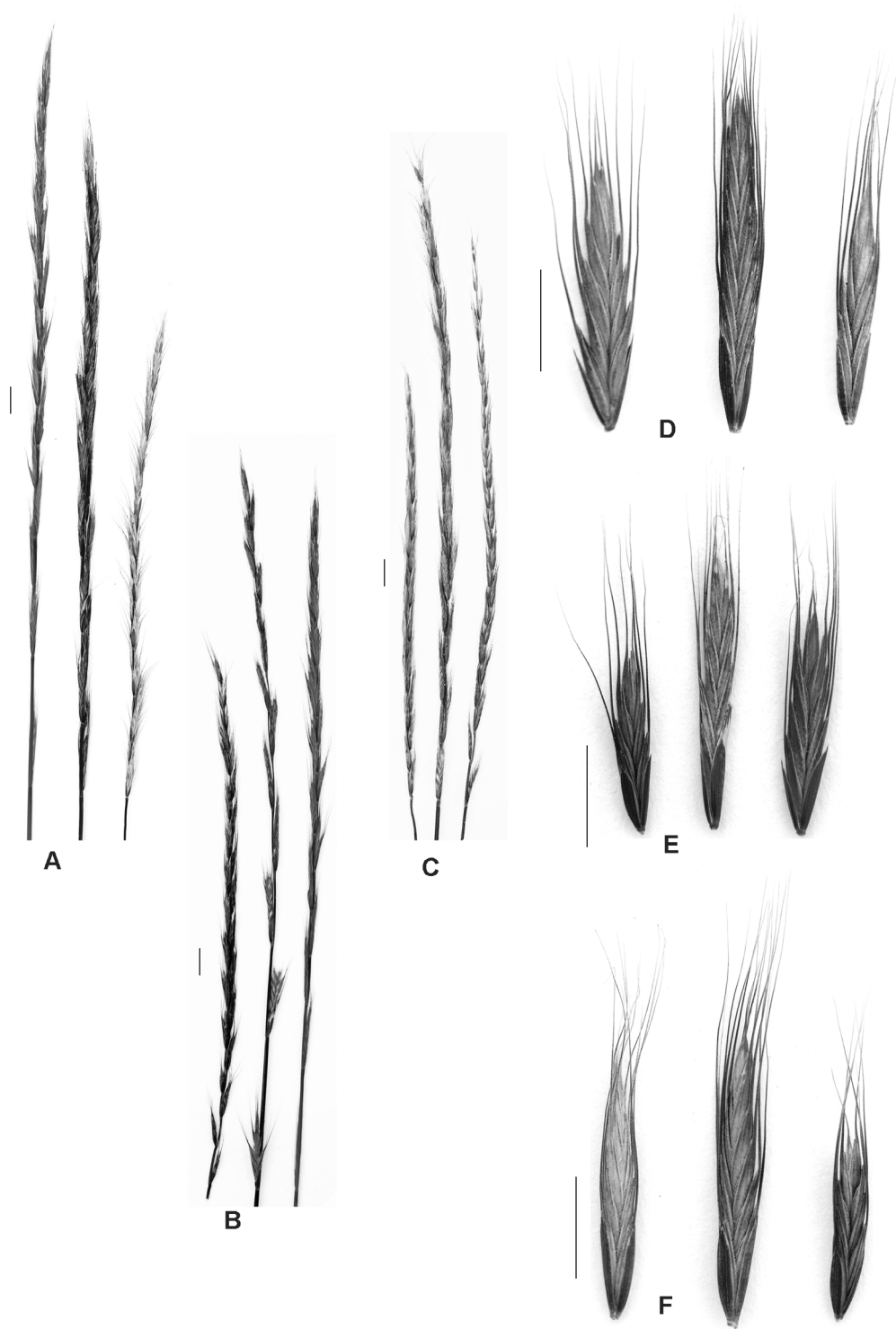
**Table 2.** The cross combinations carried out in this study

| Combinations (♀ $\times$ ♂)   | Number of emasculated florets | Seed set |            | Plants |            |
|---|-------------------------------|----------|------------|--------|------------|
|   |                               | Number   | Percentage | Number | Percentage |
| <i>R. amurensis</i> (PI 547303) $\times$ <i>R. ciliaris</i> (88-89-227)   | 50                            | 11       | 22         | 6      | 54.55      |
| <i>R. japonensis</i> (88-89-257) $\times$ <i>R. amurensis</i> (PI 547303) | 45                            | 9        | 20         | 3      | 33.33      |
| <i>R. ciliaris</i> (88-89-229) $\times$ <i>R. japonensis</i> (88-89-253)  | 4                             | 2        | 50         | 2      | 100.00     |

**Table 3.** Comparison of morphological characters in *Roegneria amurensis*, *R. ciliaris*, *R. japonensis* and their  $F_1$  hybrids (cm, mean  $\pm$  S.D)

| Characters                  | <i>R. amurensis</i><br>(88-89-227) | <i>R. ciliaris</i><br>(88-89-227) | <i>R. japonensis</i><br>(88-89-257) | <i>R. amurensis</i> $\times$ <i>R. ciliaris</i> | <i>R. japonensis</i> $\times$ <i>R. amurensis</i> | <i>R. ciliaris</i> $\times$ <i>R. japonensis</i> |
|-----------------------------|------------------------------------|-----------------------------------|-------------------------------------|---|---|--|
| Height                      | 72.38 $\pm$ 5.33                   | 77.86 $\pm$ 14.49                 | 70.10 $\pm$ 4.47                    | 67.24 $\pm$ 6.38                                | 62.34 $\pm$ 8.39                                  | 71.40 $\pm$ 7.75                                 |
| Length of flag leaf         | 19.32 $\pm$ 3.66                   | 17.82 $\pm$ 3.15                  | 14.86 $\pm$ 2.97                    | 19.24 $\pm$ 3.21                                | 16.27 $\pm$ 3.10                                  | 15.44 $\pm$ 2.03                                 |
| Width of flag leaf          | 0.87 $\pm$ 0.11                    | 1.14 $\pm$ 0.22                   | 0.92 $\pm$ 0.08                     | 1.02 $\pm$ 0.22                                 | 0.91 $\pm$ 0.09                                   | 0.87 $\pm$ 0.06                                  |
| Length of top internode     | 37.58 $\pm$ 1.95                   | 46.86 $\pm$ 6.36                  | 32.88 $\pm$ 4.25                    | 40.12 $\pm$ 5.37                                | 33.28 $\pm$ 2.04                                  | 36.26 $\pm$ 3.25                                 |
| Length of internode         | 3.42 $\pm$ 0.69                    | 2.22 $\pm$ 0.38                   | 1.82 $\pm$ 0.16                     | 3.06 $\pm$ 0.42                                 | 2.26 $\pm$ 0.23                                   | 1.65 $\pm$ 0.12                                  |
| Length of spike             | 20.36 $\pm$ 1.26                   | 18.22 $\pm$ 4.10                  | 16.44 $\pm$ 1.51                    | 19.22 $\pm$ 2.13                                | 18.43 $\pm$ 1.87                                  | 16.61 $\pm$ 4.46                                 |
| No. of spikelets per spike  | 19.60 $\pm$ 2.97                   | 13.60 $\pm$ 3.65                  | 14.00 $\pm$ 1.22                    | 19.40 $\pm$ 2.58                                | 14.20 $\pm$ 1.24                                  | 15.80 $\pm$ 3.00                                 |
| No. of florets per spikelet | 12.00 $\pm$ 1.41                   | 9.60 $\pm$ 1.14                   | 8.60 $\pm$ 0.55                     | 11.00 $\pm$ 2.18                                | 9.80 $\pm$ 0.62                                   | 14.40 $\pm$ 0.50                                 |
| Length of spikelet          | 4.06 $\pm$ 0.34                    | 3.16 $\pm$ 0.36                   | 3.24 $\pm$ 0.13                     | 4.01 $\pm$ 0.22                                 | 3.28 $\pm$ 0.26                                   | 4.15 $\pm$ 0.15                                  |
| Length of lower glume*      | 0.61 $\pm$ 0.05                    | 0.74 $\pm$ 0.05                   | 0.67 $\pm$ 0.03                     | 0.65 $\pm$ 0.08                                 | 0.64 $\pm$ 0.05                                   | 0.70 $\pm$ 0.11                                  |
| Length of upper glume*      | 0.70 $\pm$ 0.07                    | 0.84 $\pm$ 0.05                   | 0.78 $\pm$ 0.03                     | 0.78 $\pm$ 0.05                                 | 0.76 $\pm$ 0.05                                   | 0.75 $\pm$ 0.12                                  |
| Length of lemma**           | 0.86 $\pm$ 0.19                    | 0.80 $\pm$ 0.26                   | 0.84 $\pm$ 0.41                     | 0.85 $\pm$ 0.21                                 | 0.90 $\pm$ 0.22                                   | 0.90 $\pm$ 0.01                                  |
| Length of lemma awn         | 1.46 $\pm$ 0.22                    | 1.42 $\pm$ 0.26                   | 1.58 $\pm$ 0.39                     | 1.41 $\pm$ 0.24                                 | 1.48 $\pm$ 0.22                                   | 1.48 $\pm$ 0.23                                  |
| Length of palea             | 0.57 $\pm$ 0.06                    | 0.68 $\pm$ 0.02                   | 0.68 $\pm$ 0.03                     | 0.64 $\pm$ 0.04                                 | 0.62 $\pm$ 0.03                                   | 0.67 $\pm$ 0.03                                  |
| Lemma vestiture             | rough                              | rough                             | glabrous                            | rough   | rough   | rough  |
| Lemma top                   | smooth                             | toothed                           | toothed                             | toothed   | toothed   | toothed  |
| Palea top                   | obtuse                             | obtuse                            | obtuse                              | obtuse  | obtuse  | obtuse   |
| Hairs on leaf               | +++                                | —                                 | —                                   | —   | —   | —  |
| Hairs on leaf sheath        | ++                                 | —                                 | —                                   | —   | —   | —  |
| Hairs on glume              | +                                  | +                                 | —                                   | +   | +   | +  |
| Hairs on lemma              | +                                  | ++                                | +                                   | +   | +   | +  |

\*including glume awn length, \*\*excluding lemma awn length. + pubescent along margin, ++ pubescent on both back and margin, +++ pubescent on both surfaces and margin, — hairless.



**Fig. 1.** Spikes and spikelets of *Roegneria amurensis*, *R. ciliaris*, *R. japonensis* and their  $F_1$  hybrids. — **A** and **D**: Left *R. amurensis*, middle *R. amurensis*  $\times$  *R. ciliaris*, right *R. ciliaris* (88-89-227). — **B** and **E**: Left *R. japonensis* (88-89-257), middle *R. japonensis*  $\times$  *R. amurensis*, right *R. amurensis*. — **C** and **F**: Left *R. ciliaris* (88-89-229), middle *R. ciliaris*  $\times$  *R. japonensis*, right *R. japonensis* (88-89-253). Bars = 1 cm.

**Table 4.** Chromosome pairing at MI of PMCs in the parental species of *Roegneria* and their  $F_1$  hybrids.

| Parents and hybrids                        | 2n | No. of cells observed | I          | Chromosome pairing II |            |              | Chiasma per cell | C    |
|--|----|-----------------------|------------|-----------------------|------------|--------------|------------------|------|
|  |    |                       |            | Total                 | Rod        | Ring         |                  |      |
| <i>R. ciliaris</i> (88-89-227)             | 28 | 68                    | 0.03 (0-2) | 13.99 (13-14)         | 0.60 (0-6) | 13.39 (8-14) | 27.38 (22-28)    | 0.98 |
| <i>R. amurensis</i> (PI 547303)            | 28 | 106                   | 0.08 (0-2) | 13.85 (13-14)         | 1.27 (0-6) | 12.58 (7-14) | 26.43 (20-28)    | 0.94 |
| <i>R. japonensis</i> (88-89-257)           | 28 | 136                   | 0.07 (0-2) | 13.97 (13-14)         | 1.10 (0-5) | 12.87 (9-14) | 26.84 (23-28)    | 0.96 |
| <i>R. amurensis</i> × <i>R. ciliaris</i>   | 28 | 126                   | 0.38 (0-4) | 13.83 (12-14)         | 2.67 (0-5) | 11.16 (9-14) | 24.99 (23-28)    | 0.89 |
| <i>R. japonensis</i> × <i>R. amurensis</i> | 28 | 100                   | 0.18 (0-2) | 13.90 (13-14)         | 1.85 (0-5) | 12.05 (9-14) | 25.95 (23-28)    | 0.93 |
| <i>R. ciliaris</i> × <i>R. japonensis</i>  | 28 | 123                   | 0.15 (0-4) | 13.91 (12-14)         | 1.84 (0-6) | 12.07 (8-14) | 25.98 (20-28)    | 0.93 |

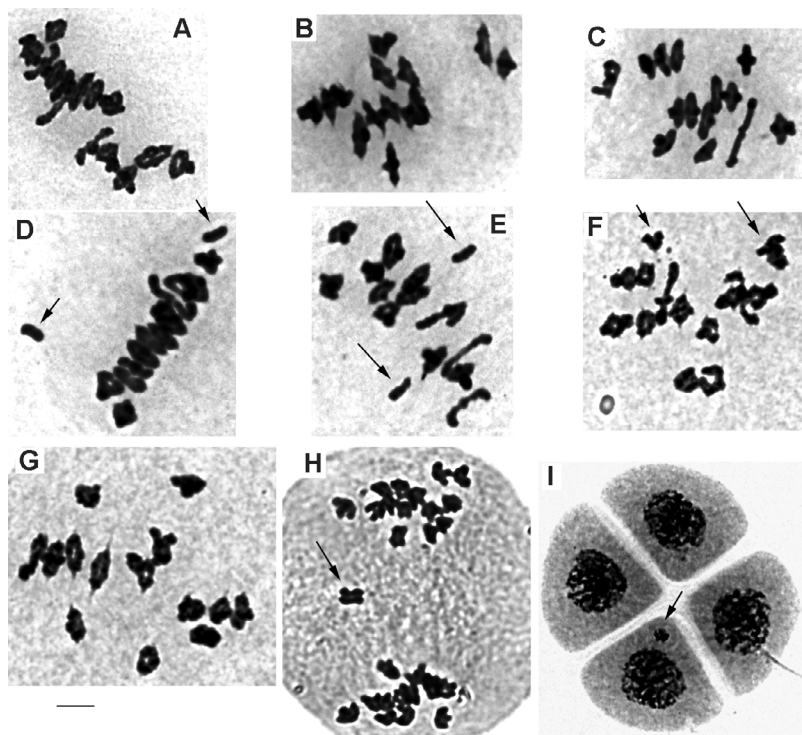
Traditionally, they were distinguished from each other by three morphological characters: the hairs on glumes, the erect or slightly vertical spikes and the hairs on leaves and leaf sheaths. In fact, the variation of hairs on glumes is continuous and the erect spikes become vertical as the plants are growing up and seeding (Lu *et al.* 1988a).

The karyotype formula of *R. ciliaris* and *R. japonensis* was  $2n = 4x = 28 = 18m$  (4SAT) + 6sm as shown by Lu *et al.* (1988a). Sun *et al.* (1992) suggested that the karyotype formula of *R. ciliaris* was  $2n = 4x = 28 = 22m$  (2SAT) + 6sm (2SAT). Wei *et al.* (2005) reported that the karyotype formula of *R. amurensis* was  $2n = 4x = 28 = 24m$  (2SAT) + 4sm and the karyotype formula slightly varied in different populations of *R. ciliaris* and *R. japonensis*. Therefore, the variations of karyotype within different populations of one species also made a sound taxonomy difficult.

The level of chromosome pairing in inter-specific or intergeneric hybrids has played an important role in the study of biosystematic relationships among genera or species in Triticeae (Kihara & Nishiyama 1930, Sakamoto & Muramatsu 1966, Kimber 1983, Dewey 1984, Jauhar & Crane 1989, Lu *et al.* 1990, Wang 1992, Zhou *et al.* 1999a, 1999b). Lu *et al.* (1988b) reported that *R. ciliaris* and *R. japonensis* were closely related and had the same StStYY genomes. Combining morphological comparison with chromosome pairing study, Lu *et al.* (1988b) treated *R. japonensis* as *Roegneria ciliaris* var. *japonensis*.

In the present study, chromosome pairings in *R. amurensis* × *R. ciliaris*, *R. japonensis* × *R. amurensis* and *R. ciliaris* × *R. japonensis* were quite high. The bivalents observed per cell varied between 12-14, 13-14 and 12-14 and the chiasma frequencies per cell were 23-28, 23-28 and 20-28, respectively. It indicated that *R. amurensis* contains a genome similar to that of *R. ciliaris* and *R. japonensis*, i.e. StStYY. The frequency of univalents is higher in both *R. amurensis* × *R. ciliaris* and *R. japonensis* × *R. amurensis*, while C, fertility and especially seed set are lower than in *R. ciliaris* × *R. japonensis* (Tables 4 and 5). Therefore, it is concluded that the phylogenetic relationship between *R. amurensis* and *R. ciliaris* or *R. japonensis* is more





**Fig. 2.** Chromosome pairings at MI in three parental species of *Roegneria* and their  $F_1$  hybrids. — **A:** *R. amurensis* with 14 bivalents (11 rings + 3 rods). — **B:** *R. ciliaris* (88-89-227) with 14 bivalents (14 rings). — **C:** *R. japonensis* (88-89-257) with 14 bivalents (12 rings + 2 rods). — **D:** *R. amurensis*  $\times$  *R. ciliaris* with two univalents (indicated with arrows) and 13 bivalents (11 rings + 2 rods). — **E:** *R. japonensis*  $\times$  *R. amurensis* with two univalents (indicated with arrows) and 13 bivalents (10 rings + 3 rods). — **F:** *R. ciliaris*  $\times$  *R. japonensis* with two univalents (indicated with arrows) and 13 bivalents (11 rings + 2 rods). — **G:** *R. ciliaris*  $\times$  *R. japonensis* with 14 bivalents (14 rings). — **H** and **I:** Lagging chromosomes at anaphase-I and micronuclei in tetrads; **H:** lagging chromosomes at anaphase-I of *R. japonensis*  $\times$  *R. amurensis* (indicated with an arrow). **I:** a tetrad of *R. amurensis*  $\times$  *R. ciliaris* showing micronuclei (indicated with an arrow). Bar = 5  $\mu$ m.

distant than that between *R. ciliaris* and *R. japonensis*.

*Roegneria amurensis* is distributed in the northeastern parts of China and eastern parts of the Russian Federation, while *R. ciliaris* and *R. japonensis* are mainly growing widely in China

as well as in Japan and Korea. According to the results of morphological comparison, chromosome pairing study, pollen fertility and seed set in the hybrids including *R. amurensis*, it is reasonable to treat *R. amurensis* as a subspecies of *R. ciliaris* and *R. japonensis* as a variety of *R.*

**Table 5.** Pollen fertility and seed set in the parental species of *Roegneria* and their  $F_1$  hybrids.

| Parents and hybrids                               | No. of pollen grains observed | No. of fertile pollen grains | Fertility (%) | No. of florets observed | Seed set |            |
|---|-------------------------------|------------------------------|---------------|-------------------------|----------|------------|
|   |                               |                              |               |                         | Number   | Percentage |
| <i>R. ciliaris</i> (88-89-227)                    | 663                           | 628                          | 95            | 128                     | 116      | 91         |
| <i>R. amurensis</i> (PI 547303)                   | 827                           | 739                          | 89            | 120                     | 109      | 91         |
| <i>R. japonensis</i> (88-89-257)                  | 786                           | 724                          | 92            | 132                     | 121      | 92         |
| <i>R. amurensis</i> $\times$ <i>R. ciliaris</i>   | 1035                          | 852                          | 82            | 300                     | 160      | 53         |
| <i>R. japonensis</i> $\times$ <i>R. amurensis</i> | 1100                          | 973                          | 88            | 280                     | 180      | 64         |
| <i>R. ciliaris</i> $\times$ <i>R. japonensis</i>  | 1287                          | 1218                         | 95            | 380                     | 320      | 84         |

*ciliaris*. This combination of *R. amurensis* as a subspecies of *R. ciliaris* has not previously been made.

***Roegneria ciliaris* (Trin.) Nevski ssp. *amurensis* (Drob.) Y.H. Zhou, H.Q. Yu & X. H. Wei, *comb. nov.***

*Agropyron amurensis* Drob., Tr. Bot. Muz. AN 12: 50. 1914. — *Roegneria amurensis* (Drob.) Nevski, Fl. SSSR 2: 606. 1934. — *Elymus ciliaris* (Trin.) Tzvelev ssp. *amurensis* (Drob.) Tzvelev, Nov. Sist. Vyssh. Rast. 9: 61. 1972.

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