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Karyotype Studies on *Pseudoroegneria gracillima* and *P. kosaninii* (Poaceae: Triticeae)

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Abstract: In order to obtain more cytological data, the karyotypes of *Pseudoroegneria gracillima* and *P. kosaninii* were investigated. Root tips of *P. gracillima* and *P. kosaninii* were pretreated in an ice bath, fixed in a mixture of 95% ethanol: glacial acetic acid and treated in 1 M HCl at 60°C in a water bath. Somatic cells were stained in Schiff at room temperature and the meristematic portions of the root tips were squashed in 45% acetic acid. The results show that: (1) *P. gracillima* is diploid with two pairs of satellites and *P. kosaninii* is octoploid with three pairs of satellites. The karyotypes of diploid *P. gracillima* and octoploid *P. kosaninii* are first reported, (2) the karyotype formulas of *P. gracillima* and *P. kosaninii* are 2n = 2x = 14 = 12 m (2sat)+2sm (2sat) and 2n = 8x = 56 = 42 m (6sat)+12sm+2st, respectively and (3) the karyotype of *P. gracillima* is 1A type, while *P. kosaninii* is 2B type. This demonstrated that there are great variations between the karyotypes of *P. gracillima* and *P. kosaninii*.

Key words: Karyotype, *P. gracillima*, *P. kosaninii*, diploid, octoploid, ploidy

INTRODUCTION

Pseudoroegneria is a genus in Triticeae (Poaceae) with *Pseudoroegneria strigosa* (M. Bieb.) Á. Löve as the type species (Löve, 1980). The genus contains a basic St genome, with diploid (2n = 2x = 14, StSt) and auto- and allo-polyploid species. St genome is one of the most important genomic components, present in more than half of the perennial Triticeae species (Löve, 1984; Dewey, 1984; Yen and Yang, 1990; Yen *et al*., 2005a, b). Morphologically, the species in this genus are caespitose, long-anthered and cross-pollinating perennials. They are distributed in the Northern Hemisphere, with its species occurring on open rocky hillsides from the Middle East and Transcaucasia across Central Asia and Northern China to Western North America (Löve, 1984). *Pseudoroegneria* grasses have exceptionally drought tolerant and excellent forage quality, which are precious germplasm resources in crop forage breeding (Dewey, 1984).

Pseudoroegneria gracillima (Nevski) Á. Löve and *Pseudoroegneria kosaninii* (Nabelek) Á. Löve are two species of *Pseudoroegneria* which distributed in Russian Federation and Turkey, respectively. Löve (1984) treated them into *Pseudoroegneria* based on the morphological study and he noted that the taxonomic status of the two species is temporary with lacking of cytological data. Ding *et al*. (2004) reported the karyotype of *P. gracillima*, whereas no cytological data about *P. kosaninii* are reported. *Pseudoroegneria kosaninii* is inferred to be tetraploid and the relationship of *P. gracillima* and *P. kosaninii* is close in the RAPD and RAMP analysis (Ding *et al*., 2005a, b). Based on genome

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specific RAPD markers, *P. gracillima* and *P. kosaninii* contained at least one St or slightly modified St genome (Ding *et al*., 2005c). Yu *et al*. (2008) suggested that *P. gracillima* and *P. kosaninii* contain one St genome in the analysis of the ITS data of the species in *Pseudoroegneria*. Therefore, the chromosome numbers and genomic constitutions of *P. gracillima* and *P. kosaninii* are still obscure. To obtain more cytological data, karyotypes of the two species were investigated. The aims of this study are (1) to report the karyotypes of *P. gracillima* and *P. kosaninii* and (2) to provide more cytological data for the appropriate taxonomic treatments of the two species.

MATERIALS AND METHODS

The study was conducted in July 2007 at Dujiangyan City, Triticeae Research Institute of Sichuan Agricultural University. The materials used in this study are shown in Table 1. Seeds of *P. gracillima* and *P. kosaninii* were kindly provided by American National Plant Germplasm System (Pullman, Washington, USA). The two species are currently growing at Triticeae Research Institute, Sichuan Agricultural University, China (SAUTI) and the mature plants were carefully identified and determined by Chi Yen, Junliang Yang and Yonghong Zhou.

Seeds were scarified and germinated in Petri dishes at 22°C on filter paper. Root tips were obtained from roots that were 1.0-1.5 cm in length and pretreated in an ice bath for 24 h before fixation in a mixture of 95% ethanol: glacial acetic acid (3:1, v/v) for 24 h. They were then treated for 8-10 min in 1 M HCl at 60°C in a water bath. Somatic cells were stained in Schiff at room temperature (20-25°C) for about 30 min. The meristematic portions of the root tips were squashed in 45% acetic acid. Microphotographs were taken from metaphase cells with a complete chromosome complement by the Olympus BX-51 camera system. Five metaphase cells were analyzed for each species (Li and Chen, 1985). Idiograms were constructed based on the chromosome lengths and relative arm ratios. Index of the karyotypic asymmetry and karyotype type analyses were basically the same as described by Arano (1963) and Stebbins (1971), respectively.

RESULTS

Chromosomal characteristics and chromosomal parameters are shown in Table 2 and 3, respectively. The morphology of somatic chromosomes and karyotypes are shown in Fig. 1A-D. The idiograms are shown in Fig. 2A and B. Results of karyotype analysis of two species studied are presented below.

P. gracillima

The karyotype formula is 2n = 2x = 14 = 12 m (2sat)+2sm (2sat), which belongs to 1A type. The average arm ratio is 1.40, with a longest chromosome/shortest chromosome ratio of 1.62. Percentage of chromosomes with arm ratio >2 is 0 and index of the karyotypic asymmetry is 58.20. The relative length of chromosomes ranges in size from 11.07 to 17.94. All the chromosomes are metacentric with the exception of chromosome 3, which is submetacentric. One pair of minute satellites and one pair of large satellites are located on the short arms of chromosome 3 and 7, respectively (Fig. 1A, 2A).

Table 1: Materials used in the karyotype analysis

Species	Accession No.	Genome	Geographic origin
Pseudoroegneria (Nevski) Á. Löve			
P. gracillima (Nevski) Á. Löve	PI 440000	St	Russian Federation
P. kosaninii (Nabelek) Á. Löve	PI 237636	----	Turkey

Table 2: Chromosomal parameters of two species in the karyotype analysis

Species	Chromosome No.	Relative length	Arm ratio	Chromosome type
P. gracillima	1	10.03+7.91 = 17.94	1.27	m
	2	8.91+7.92 = 16.43	1.18	m
	3*	10.03+5.01 = 15.04	2.00	sm
	4	7.67+5.57 = 13.24	1.38	m
	5	7.80+5.29 = 13.09	1.47	m
	6	6.69+5.85 = 12.54	1.14	m
	7*	6.69+5.01 = 11.07	1.34	m
P. kosaninii	1	3.16+2.11 = 5.27	1.50	m
	2*	2.63+2.37 = 5.00	1.11	m
	3*	2.50+1.55 = 4.05	1.61	m
	4	2.63+1.37 = 4.00	1.92	sm
	5	2.11+1.84 = 3.95	1.15	m
	6	2.50+1.45 = 3.95	1.72	sm
	7	2.32+1.58 = 3.90	1.47	m
	8	2.05+1.79 = 3.84	1.15	m
	9	2.37+1.37 = 3.74	1.73	sm
	10	1.90+1.79 = 3.69	1.06	m
	11	1.98+1.71 = 3.69	1.16	m
	12*	2.11+1.58 = 3.69	1.34	m
	13	2.37+1.32 = 3.69	1.80	sm
	14	2.11+1.53 = 3.64	1.38	m
	15	1.92+1.71 = 3.63	1.12	m
	16	1.98+1.58 = 3.56	1.25	m
	17	2.37+1.19 = 3.56	1.99	sm
	18	2.11+1.34 = 3.45	1.57	m
	19	1.84+1.58 = 3.42	1.16	m
	20	1.79+1.58 = 3.37	1.13	m
	21	1.84+1.45 = 3.29	1.27	m
	22	1.63+1.58 = 3.21	1.03	m
	23	1.63+1.32 = 2.95	1.23	m
	24	1.58+1.32 = 2.90	1.20	m
	25	1.90+0.92 = 2.82	2.07	sm
	26	1.53+1.26 = 2.79	1.21	m
	27	1.40+1.29 = 2.69	1.09	m
	28	1.71+0.53 = 2.24	3.23	st

*Satellite chromosome, with the satellite length included in the chromosome length

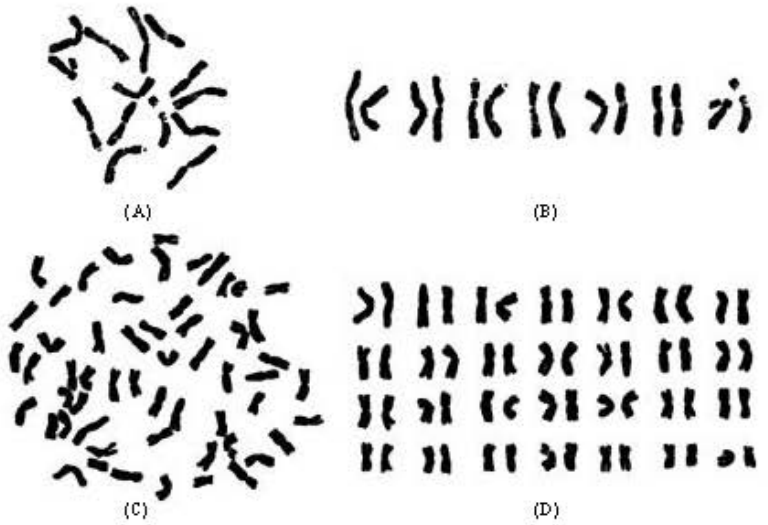

Fig. 1: The morphology of somatic chromosomes and karyotypes of two species in *Pseudoroegneria* (A, B) *P. gracillima* and (C, D) *P. kosaninii*. Bar = 10 μm

Table 3: Chromosomal characteristics of two species in the karyotype analysis

Species	Karyotype formula	AAR	Lc/Sc	PCA	As.k (%)	Karyotype type
P. gracillima	2n = 2x = 14 = 12 m (2sat)+2sm (2sat)	1.40	1.62	0.00	58.20	1A
P. kosaninii	2n = 8x = 56 = 42 m (6sat)+12sm+2st	1.45	2.35	0.07	57.98	2B

AAR: Average arm ratio; Lc: Longest chromosome; Sc: Shortest chromosome; PCA: Percentage of chromosomes with arm ratio >2; As.k (%): Index of the karyotypic asymmetry

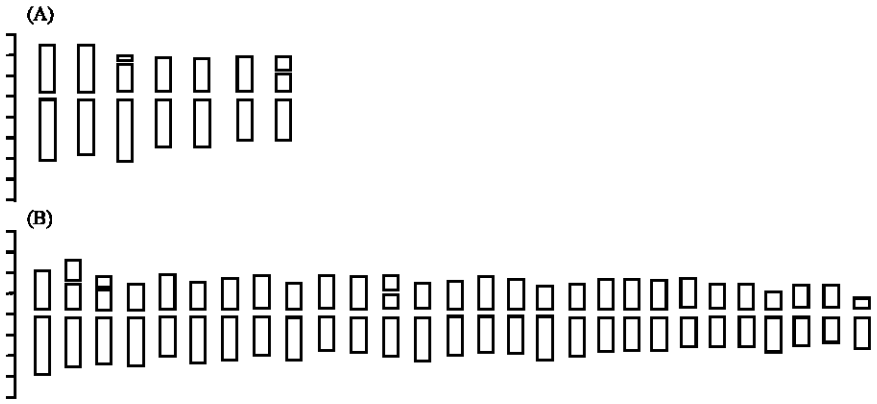

Fig. 2: Idiograms of two species in *Pseudoroegneria* (A) *P. gracillima* and (B) *P. kosaninii*

P. kosaninii

The karyotype formula is 2n = 8x = 56 = 42 m (6sat)+12sm+2st, which belongs to 2B type. The average arm ratio is 1.45, with a longest chromosome/shortest chromosome ratio of 2.35. Percentage of chromosomes with arm ratio >2 is 0.07 and index of the karyotypic asymmetry is 57.98. The relative length of chromosomes ranges in size from 2.24 to 5.27. All the chromosomes are metacentric or submetacentric, with the exception of chromosome 28, which is subtelocentric. Three pairs of large satellites are located on the short arms of chromosomes 2, 3 and 12, respectively (Fig. 1B, 2B).

DISCUSSION

Based on the cytological data, Dewey (1984) pointed out that some *Pseudoroegneria* species may have diploidy and tetraploidy in different populations, such as the diploid (2n = 2x = 14, StSt) *P. strigosa* and tetraploid (2n = 2x = 28, StStStSt) *P. strigosa*. The previous analysis indicated that *P. gracillima* is tetraploid with one pair of satellites (Ding *et al*., 2004). *Pseudoroegneria gracillima* and *P. kosaninii* is closely related and *P. kosaninii* is inferred to be tetraploid based on molecular data (Ding *et al*., 2005a, b). However, the ploidy of *P. kosaninii* is lack of cytological evidence. In the present study, the karyotypes of diploid *P. gracillima* and octoploid *P. kosaninii* were first reported. Combined with the earlier study, *P. gracillima* have diploidy and tetraploidy in different populations. The results added new cytological data of *Pseudoroegneria* species.

Stebbins and Pun (1953) indicated that different versions of the St genome exist in diploid *Pseudoroegneria* species, which suggested the differentiations of the St genome. Hsiao (1986) reported that five diploid *Pseudoroegneria* species have two pairs of satellites. One pair of large satellites are on the short arms of chromosomes 5, the other pair of small satellites are on the short arms of chromosomes 1, 2, or 3. In this study, diploid *P. gracillima* have one pair of small satellites on the short arms of chromosomes 3 and one pair of large satellites on the short arms of chromosomes 7, which is different to the reported five *Pseudoroegneria* species. Satellites on different chromosomes

displayed the variations in karyotype formulas among diploid *Pseudoroegneria* species. It is valid that different versions of the St genome have different karyotype formulas. The karyotype of diploid *P. gracillima* is 1A type and most of the chromosomes are metacentric or submetacentric and the similar results were obtained in diploid *Pseudoroegneria* species and tetraploid *P. gracillima* (Hsiao *et al*., 1986; Ding *et al*., 2004).

The karyotype analyses indicated that *P. kosaninii* is octoploid species, which is a higher ploidy than that of other *Pseudoroegneria* species (Dewey, 1984; Hsiao *et al*., 1986). Also, there are great variations between the karyotypes of *P. kosaninii* and other *Pseudoroegneria* species, especially in sizes and positions of the satellites (Hsiao *et al*., 1986; Liu and Wang, 1993; Ding *et al*., 2004). Therefore, more evidence needs to be obtained to make clear the taxonomic status and phylogenetic relationships of *P. kosaninii* in *Pseudoroegneria*.

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