



## Karyotype analysis of *Rheum palmatum*

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**ABSTRACT.** *Rheum palmatum*, one of the source plants of the traditional Chinese medicine rhubarb, is an endemic and endangered species. To our knowledge, this is the first report on the chromosome number and karyotype of this species. Sectioning combined with micrography was used to analyze the karyotype. The following results were obtained: *R. palmatum* had a stable chromosome number  $2n = 22$ ; the basic number of chromosomes was 11; karyotype formula is  $2n = 22 = 20 \text{ metacentric} + 2 \text{ submetacentric}$ , belonging to Stebbins' 1A type; and karyotype asymmetry index was 55.39%. The present study showed that *R. palmatum* has a primitive type of karyotype.

**Key words:** *Rheum palmatum*; Chromosome number; Karyotype

## INTRODUCTION

Rhubarb is a very important traditional Chinese medicine and is widely used as a purgative and anti-inflammatory agent (Chinese Pharmacopoeia Committee, 2010). Three closely related species [*Rheum palmatum* L., *Rheum tanguticum* (Maxim. ex Regel) Maxim. ex Balf., and *Rheum officinale* Baill.] are considered as important sources of rhubarb; all the plants belong to section *Palmata* A. Los., and are endemic to China (Bao and Grabovskaya-Borodina, 2003). Because of the excessive exploitation of these plants for rhubarb, the wild varieties of these species have been decreasing annually and are now considered endangered. Our field survey suggested that very few natural populations of these species are found in areas such as Chongqing and Guizhou Provinces. Of the three plant species, *R. palmatum* shows the widest distribution range in China and is mainly found in Hebei, Shanxi, Shaanxi, Gansu, Sichuan, Qinghai, and Tibet Provinces of China. This species can be found at the forest edges on hills, in shrubs, or in the valleys near rivers.

Our research group has investigated the distribution and genetic variation of the section *Palmata* (Wang et al., 2010, 2012a,b), and many previous studies on this section have focused on components analysis (Zheng and Zhang, 1993; Zhang and Liu, 2004; Ye et al., 2007) and pharmacological properties (Xiao et al., 1984; Yu et al., 2005; Tseng et al., 2006). However, the chromosome number and karyotype of this section are not yet known. *Rheum* has been reported to have two ploidy levels,  $2n = 22$  and  $4n = 44$  (Darlington and Janaki-Amal, 1945; Darlington and Wylie, 1955). The chromosome number and karyotype have been reported for *R. tanguticum* (Hu et al., 2007) and were determined for *R. officinale* in 1928 by Jaretzky. Chromosome number, size, and karyotype are known to be good taxonomic characters, and cytological data are essential in studies focusing on diversification (Stebbins, 1971). Further, polyploidy has long been recognized as a prominent factor responsible for plant diversification and speciation (Stebbins, 1971; Grant, 1981; Levin, 2002). Our previous studies on the distribution pattern (Wang et al., 2010) and morphological characters (unpublished data) of the three species of rhubarb suggest that they might not be different species and might actually form a species complex (*R. palmatum*). On the other hand, *R. tanguticum* was initially published by Regel (1874) as a variety of *R. palmatum*, and Grabovskaya-Borodina indicated that *R. tanguticum* was a synonym of *R. palmatum* (Bao and Grabovskaya-Borodina, 2003). Therefore, the present study aimed to expand the current knowledge on the chromosome number and karyotype variation of the source plants of rhubarb and determine whether the karyological data of *R. palmatum* and *R. tanguticum* support the recognition of a single species or suggest that they are in fact two species. The results might provide a basis for the future studies on the species differentiation, speciation, and diversification of the section *Palmata*.

## MATERIAL AND METHODS

### Material

Seeds of *R. palmatum* were collected from Yala in Kangding County, Sichuan Province, China, on September 2, 2010. Voucher (altitude, 3692m; 30°14.782'N, 101°51.833'E; Yu-qu Zhang 1008090218) specimens are deposited in the Herbarium of Shaanxi Normal University (SANU). Somatic chromosomes were studied using samples from the root meristems of germinating seeds.

## Measurement and analysis of karyotype

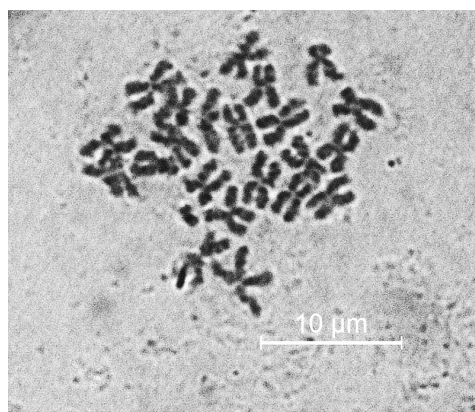
The cut roots were first pretreated with 0.2% colchicine for 2h at room temperature, and then pretreated with distilled water at 0°C for 24 h; subsequently, they were fixed with Carnoy's fluid (absolute alcohol:glacial acetic acid, 3:1) at 4°C for at least for 30 min and stored in 70% aqueous ethanol until required. The root tips were hydrolyzed with 1 M hydrochloric acid at 60°C for 16 min, and then rinsed in distilled water, stained, and squashed with carbol fuchsin. The chromosome number was determined from fifty well-spread chromosomal cells from five different root tips. Five cells with equivalent degree of chromosome contraction were used for karyotypic analyses. Photographs of the well-spread chromosomes were enlarged, and homologous chromosomes were paired by their similarity in size. Each chromosome was designated according to the position of the centromere (Levan et al., 1964) as follows: m = metacentric ( $r = 1.00-1.69$ ); sm = submetacentric ( $r = 1.70-2.99$ ); st = subtelocentric ( $r = 3.00-6.99$ ); and t = telocentric ( $r = 7.00$  and above).

Karyotypic analyses were performed according to the method described by Li and Chen (1985) and the classification of Stebbins (1971) was used. Karyotype asymmetry was determined as per Arano (1963). The following parameters were calculated: 1) chromosome relative length; 2) arm ratio; 3) chromosome length ratio; 4) centromeric index; 5) relative index of chromosome length (IRL, the ration of chromosome length to the average length of a haploid set of chromosomes); 6) karyotype asymmetry index (AsK%, the ration of the sum of the long arms of a haploid set of chromosomes to the total length of the haploid set of chromosomes x 100%).

## RESULTS

### Chromosome number

Fifty cells were observed under a fluorescence microscope (Leica DM5000B, German); of the 50 cells, 48 had 22 chromosomes, whereas 2 had 20 chromosomes. The chromosome number,  $2n = 22$  was represented by 96% of the total cells (Figure 1). The number of somatic chromosomes of *R. palmatum* can be thought to be  $2n = 22$ . No satellite markers and polyploid chromosomes were observed.



**Figure 1.** Cytological features of the somatic metaphase spread of *Rheum palmatum*.

## Karyotypes

Somatic chromosome karyotypes were measured in five cells, and the mean karyotype parameters were obtained (Table 1). The individual chromosome length ranged from 1.72 to 2.78  $\mu\text{m}$ . The total haploid chromatin length (TCL) was 24.41  $\mu\text{m}$ . The relative chromosome length was between 7.05 and 11.40%. The arm ratios ranged from 1.10 to 1.73, with an average of 1.27, and none of the chromosomes had an arm ratio of more than 2. The largest chromosome length ratio was 1.62. Karyotypes belonged to the Stebbins 1A category of asymmetry (Stebbins, 1971). The AsK% was 55.39%, and the mean centromeric index was 44.35. The haploid complement of *R. palmatum* consisted of 10 metacentric chromosomes and 1 submetacentric chromosome; therefore, the karyotype formula of *R. palmatum* is  $2n = 2x = 22 = 20m + 2sm$  (Figures 1-3).

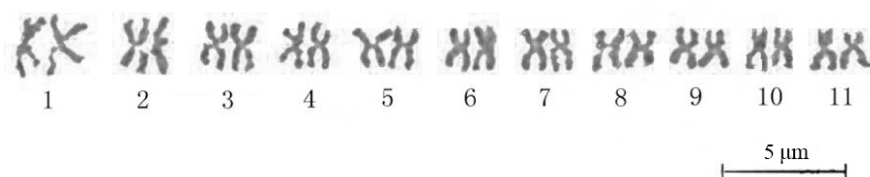


Figure 2. Idiogram of *Rheum palmatum* chromosomes.

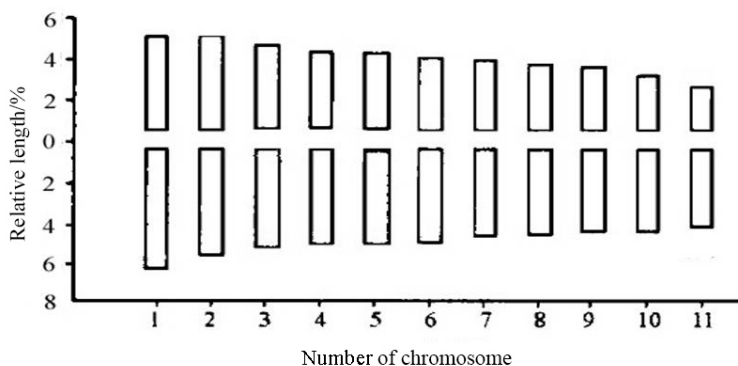


Figure 3. Karyogram of *Rheum palmatum* chromosomes.

Table 1. Chromosome parameters of *Rheum palmatum*.

Chromosome pair	Relative length (%)	Long arm ( $\mu\text{m}$ )	Short arm ( $\mu\text{m}$ )	Arm ratio	Index chromosome length (I.R.L)	Centromeric index (%)	Type
1	11.40 = 6.23+5.17	1.52	1.26	1.21	1.25	45.32	m
2	10.87 = 5.70+5.17	1.39	1.26	1.10	1.19	47.55	m
3	9.80 = 5.17+4.63	1.26	1.13	1.12	1.08	47.33	m
4	9.22 = 5.00+4.22	1.22	1.03	1.18	1.01	45.87	m
5	9.18 = 5.00+4.18	1.22	1.02	1.20	1.01	45.55	m
6	9.02 = 4.96+4.06	1.21	0.99	1.22	0.99	45.03	m
7	8.77 = 4.84+3.94	1.18	0.96	1.23	0.96	44.94	m
8	8.57 = 4.80+3.77	1.17	0.92	1.27	0.94	44.06	m
9	8.36 = 4.63+3.73	1.13	0.91	1.24	0.92	45.62	m
10	7.83 = 4.63+3.20	1.13	0.78	1.45	0.86	41.84	m
11	7.05 = 4.47+2.58	1.09	0.63	1.73	0.78	36.60	sm
Total	-	13.52	10.89	-	-	-	-
Mean	-	1.23	0.99	1.27	-	44.35	-

## DISCUSSION

In general, karyotypes show a transition from symmetry to asymmetry in higher plants (Stebbins, 1971). Primitive or primordial species mostly have symmetric karyotypes, whereas derived, specialized, and advanced plant taxa show asymmetric karyotypes. When the karyotype asymmetry index is close to 50%, the karyotype symmetry is considerably higher, and the karyotype is considered more primitive. The karyotype asymmetry index and karyotype analysis of *R. palmatum* showed an AsK% of 55.39%, which is considerably closer to 50%. Therefore, the karyotype symmetry is high. Further, the karyotypes belonged to Stebbins' 1A category, which also indicates that the karyotypes of *R. palmatum* are symmetric. The karyotype of *R. palmatum* was similar to that of *R. tanguticum* (Hu et al., 2007). Unlike that in *R. officinale*, no polyploids were found in *R. palmatum*. Thus, our findings suggest that *R. palmatum* might be a primitive or primordial species.

Cytological data have been widely used to elucidate the intraspecific and/or interspecific relationships and delimit specific or generic circumscriptions (Stebbins, 1971; Raven, 1975; Hong and Zhang, 1990), and karyotypes are one of the parameters that allow the authentic identification of a specimen. In the present study, besides the similarity in the karyotype, the relative chromosome length, arm ratio, and other parameter values of each chromosome pair were not significantly different ( $P < 0.05$ ) between *R. palmatum* and *R. tanguticum*, confirming that they belong to the same species and have a close relationship. The main morphological characteristics that can be used to distinguish between *R. palmatum* and *R. tanguticum* are the degree of leaf blade dissection and shape of the leaf lobes (Bao and Grabovskaya-Borodina, 2003): the leaf blades of *R. palmatum* are lobed, and the lobed parts are narrowly triangular, whereas the leaf blades of *R. tanguticum* are dissected, and the lobed parts are narrow, triangular, and lanceolate. However, the transitional morphs between these two species can often be found in the field, and they cannot be easily distinguished morphologically. Further, both the species cannot be discriminated at the chromosomal level. In our previous study on the genetic variation between *R. palmatum* and *R. tanguticum*, small molecular variation was detected between the two species (Wang et al., 2012b). Chen et al. (2008) also revealed that *R. palmatum* and *R. tanguticum* closely related more than any of other species of rhubarb source. Taken together, these findings indicate that *R. palmatum* and *R. tanguticum* might have a common ancestor, and that they might be a single species, i.e., *R. palmatum*. In other words, karyotypic differentiation has contributed relatively slightly to the interspecific morphological divergence within the source plants of rhubarb. Further chromosomal studies on more populations of the source plants of rhubarb are needed to reveal the diploid differentiation during plant speciation and diversification.

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