New occurrence of B chromosomes in *Rheum tanguticum* Maxim. ex Balf. (Polygonaceae)

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Abstract — A cytogenetic study was conducted on *Rheum tanguticum* populations from Huangnan Tibetan Autonomous Prefecture, China. The results revealed the chromosome numbers 2n=22 and the karyotype formulae are 2n=22=22m or 2n=22=2sm+20m, belonging to Stebbins 1A type. However, it was interesting to observe metaphases with variable B chromosomes (1, 2, 3, 4, 6, 7) in a few specimens. For the first time, the occurrence of B chromosomes was reported in natural populations of *R. tanguticum*. The number of B chromosomes in *R. tanguticum* varied not only among individuals but also within the same root tip.

Key words: B chromosomes; karyotype; *Rheum tanguticum* Maxim. ex Balf.

INTRODUCTION

The genus *Rheum*, a member of the family Polygonaceae, including about 60 species, is mainly distributed in the mountainous and desert regions of the Qinghai-Tibetan Plateau areas and subtropical Asia (Li 1998). *Rheum tanguticum* Maxim. ex Balf., the root and rhizome of which is commonly called rhubarb, is a widely used traditional Chinese medicinal herb and one of the important ingredients in Chinese traditional prescriptions (Yang *et al.* 2001; Wang and Ren 2009). Rhubarb has been used to treat various syndromes caused by the obstruction of blood circulation (such as dysmenorrhoea, hypermenorrhea, hematemesis, lower abdominal pain, etc), jaundice, diarrhea, as well as constipation (Komatsu *et al.* 2006; Li *et al.* 2006; Zhang *et al.* 2009; Chinese Pharmacopoeia Commission 2010).

Cytological data are fundamental to studies of plant taxonomy, evolution, diversification and breeding (Stebbins 1971; Stace 2000). The B-chromosome, which is called the supernumerary chromosome, is additional and mostly dispensable chromosome found in nearly 1300 plants and over 500 animals, as well as 10 species of fungi (Jones 1975; Jones and Rees 1982; Miao *et al.* 1991; Jones 1995; Leclair *et al.* 1996; Camacho *et al.* 2000; Jones and Diez 2004; Camacho 2005; S-Garmaroodi and Taga 2007; Voltolin *et al.* 2010). B chromosomes share a number of common features as follows (Beukeboom 1994; Jones and Houben 2003; Jones *et al.* 2008): (1) they are often morphologically distinct, usually smaller than the autosomes (standard A chromosomes); (2) they are generally heterochromatic and vary in number among individuals from the same population; (3) their inheritance is non-Mendelian; (4) they are regarded as inert material, although a few active genes in some B chromosomes have actually been found, especially for ribosomal genes (Miao *et al.* 1991; Jamilena *et al.* 1994; Vanetten *et al.* 1994; Donald *et al.* 1995; Jones 1995; Covert *et al.* 1996; Leclair *et al.* 1996; Funnell and Vanetten 2002); (5) they rarely share any linkage homology with A chromosomes; (6) they are rich in highly repetitive sequences.

Previous chromosome studies on *Rheum* showed a consistent basic chromosome number (X=11) (Darlington and Wylie 1955; Hu *et al.* 2007; Liu *et al.* 2010), without any B chromosomes reported. In this paper, the karyotypes of

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wild *R. tanguticum* in their genuine producing area were carried out and B chromosomes were firstly reported.

MATERIAL AND METHODS

Seeds of *R. tanguticum* were collected from two populations (MX: 101°56′38.1″E, 35°16′35.6″N; NX: 100°51′6.4″E, 35°11′27.3″N) of Huangnan Tibetan Autonomous Prefecture, Qinghai, China. They were germinated in petri dishes lined on moist filter paper. The root tips were pretreated in distilled water at 0°C for 24h when they grow up to a length of 1cm, then fixed in Carnoy (absolute alcohol: glacial acetic acid 3:1) at 4°C for 24h, and transferred to 70% ethanol for storage at 4°C until required. The fixed roots were hydrolyzed in 1mol/L hydrochloric acid at 60°C for 10 min, then washed with distilled water. Finally, samples were stained with carbol fuchsin and squashed in 45% acetic acid for observation. The chromosome number was determined under a light microscope (Nikon Eclipse 50i) and photomicrographs were made with an optical camera (Nikon DS-U2). The metaphase chromosomes of at least 5 cells of different plant roots were measured. Karyotype analysis was followed by LI and CHEN (1985), and karyotype classification was performed as the method described by STEBBINS (1971).

RESULTS

Our results showed a diploid chromosome number equal to 2n=22 and the karyotype was mainly composed of metacentric (m) chromosomes and submetacentric (sm) chromosomes. Karyotypic formulae were 2n=22=22m for MX population (Figure 1A and 1A`) and 20=22=2sm+20m for NX population (Figure 1B and 1B`), both belonging to 1A type according to STEBBINS (1971). However, in some specimens

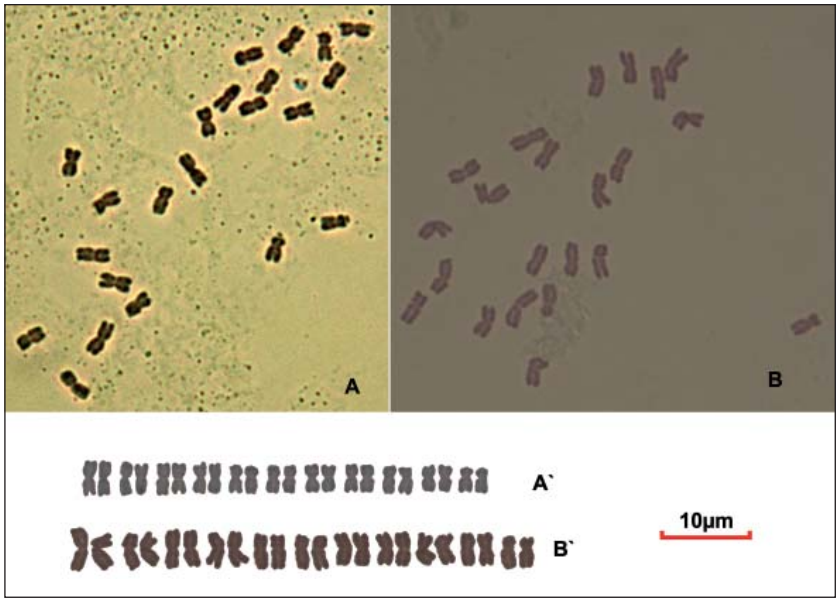

Fig. 1 — Cytological features of *R. tanguticum*. A-B, Chromosomes of metaphases. A′–B′, Karyotypes. A, A′, MX population. B, B′, NX population. Bars=10μm.

TABLE 1 — The frequency of B chromosomes in *R. tanguticum* from Huangnan Tibetan Autonomous Prefecture.

	0B	1B	2B	3B	4B	6B	7B	Analyzed cells
MX	67	43	54	7	9	2	1	183
NX	17	6	6	2	7	2	0	40
Total number of cells	84	49	60	9	16	4	1	223
%	37.67	21.97	26.91	4.04	7.17	1.79	0.45	100

it was also possible to observe metaphases with 1, 2, 3, 4, 6 or 7 B chromosomes (Table 1, Figure 2). Among all the cells that analyzed, the proportion of 1B and 2B chromosomes was similar and high (Table 1). Only one cell was found containing 7B chromosomes (figure 2C). Besides, most of the B chromosomes in *R. tanguticum* were small metacentric chromosomes.

DISCUSSION

B chromosomes have already been described in many other perennial plants, such as *Allium prattii* (XUE *et al.* 2000), *Aconitum tuguancunense* (YANG and GONG 1995), *Schismatoglottis irrorata* (OKADA 1992) and *Crepis capillaris* (BROWN and JONES 1976; MALUSZYNSKA 1997; LEACH *et al.* 2005). Additionally, in Polygonaceae, only two *Rumex* species including *Rumex thyrsiflorus* and *Rumex acetosa* were found with B chromosomes (ZUK 1969; PARKER and WILBY 1989). In *Rumex thyrsiflorus* the B chromosome was large, and equaled in size to the X sex chromosome (ZUK 1969). However, B chromosomes in *Rumex acetosa* were small telocentric and the number of B chromosomes varied between cells, tissues and organs of the same plant (PARKER and WILBY 1989). Nevertheless, there are no reports about B chromosomes in *Rheum* species, and this repre-

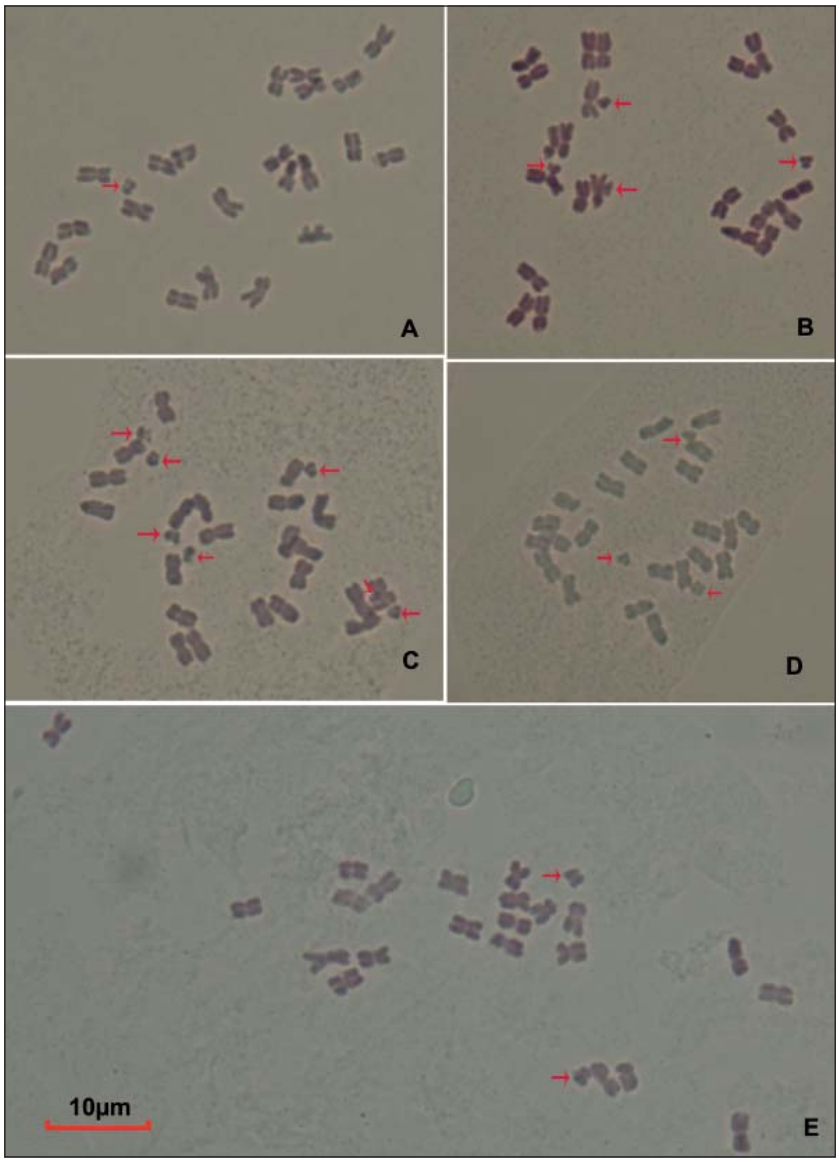

Fig. 2 — Metaphases of *R. tanguticum* with B chromosomes. Arrows indicate B chromosomes. Bars=10μm.

sents the first occurrence reported for this genus.

According to JONES (1995), B chromosomes are restricted to outbreeders and the probable reason for this is that selfing would not facilitate the spread and accumulation in populations by mitotic drive. Therefore, the appearance of B chromosomes in *R. tanguticum* further confirmed the outcrossing breeding system of *R. tanguticum*. This was consistent with our previous studies by molecular markers (HU *et al.* 2010).

The number of B chromosomes in *R. tanguticum* varied not only among individuals but also within the same root tip. This variation of B chromosomes was also found in other species like *Aconitum tuguancunense* (YANG and GONG 1995), *Schismatoglottis irrorata* (OKADA 1992), *Allium mairei* (CHEN *et al.* 2005) and *Ornithogalum Caudatum* (CAI *et al.* 2007).

Although the presence of B chromosomes has been discovered in different populations of *R. tanguticum*, further studies on B chromosomes is still required in order to provide a better understanding of chromosomal evolution in *Rheum* species.

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