Chromosome studies in four species of *Saxifraga* L. (Saxifragaceae) collected in Tuva and adjacent regions, Russia

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ABSTRACT. Four species of *Saxifraga* such as *S. aestivalis*, *S. hieracifolia*, *S. foliolosa* and *S. hirculus* were collected in Russia and observed their somatic chromosome numbers. *Saxifraga aestivalis* showed the somatic chromosome number of $2n=28$, *S. hieracifolia* showed that of $2n=114$, *S. foliolosa* showed that of $2n=60$, and *S. hirculus* showed that of $2n=16$. These chromosome numbers verified with the previous reports, except for *S. foliolosa*. The chromosome numbers of $2n=60$ for *S. foliolosa* was reported here for the first time. Chromosome sizes of four species varied in mean lengths with each others; *S. aestivalis* and *S. foliolosa* had small-sized mean chromosomes 1.31 μm and 1.47 μm long respectively, *S. hieracifolia* had medium sized, mean chromosome 2.75 μm long, and *S. hirculus* had large sized, mean chromosome 4.4 μm long. *Saxifraga aestivalis* had a bi-modality and decrease in chromosome size from the largest to the smallest chromosomes, while the other three species had mono-modal or gradual decrease in chromosome size from the largest to the smallest chromosomes.

KEYWORDS: Chromosome number, Russia, *Saxifraga*, Saxifragaceae, Tuva and adjacent regions

Saxifraga L., the representative genus in the family Saxifragaceae, is an annual or perennial herbaceous plants and consists of approximately 450 species. They are distributed in temperate to arctic regions in Eurasian and North and South American continents and grow in various habitats from stone to rock debris fields, damp and swampy meadows, brook banks, and bogged forests and dry meadows (altitude about 3–5,000 m above the sea level). One hundred and 32 species are found in Russia [Czerepanov (ed.) 1995] of which 14 species are found in Altai and Tuva Republics and Krasunoyarsk region (Malyshev 2004).

Chromosome studies of *Saxifraga* have been made in approximately 250 species and documented about 100 different chromosome numbers from $n=5$ for *S. tempestiva* Elvander *et* Denton (Elvander and Denton 1976) to $2n=206\text{--}220$ for *S. androsacea* L. (Kupfer 1974) include in aneuploid and polyploid [Fedrov (ed.) 1974; Ornduff (ed.) 1967, 1968; Moore (ed.) 1973, 1974, 1977; Goldblatt (ed.) 1981, 1984, 1985, 1988; Goldblatt and Johnson (eds.) 1990, 1991, 1994, 1996, 1998, 2000, 2003, 2006, 2010; Funamoto 2010; Funamoto *et al.* 2001].

Our team made a field trip to Altai, Tuva and adjacent regions, Russia in 2010, and collected four species of *Saxifraga*. This paper reports somatic chromosome characters and ecological conditions of them..

MATERIALS AND METHODS

During the field trip made in 2010, four species of *Saxifraga* were collected in five sites in four localities and were brought to Japan (Fig. 1; Table 1). Those plant materials were cultivated in pots in shade place in the

experimental garden of Showa Pharmaceutical University. Chromosome observations were made in meristematic cells of freshly growing root-tip cells. They were cut off 5–10 mm long and pretreated in 2mM 8-hydroxyquinoline at ca 20°C for 4 h, and then, fixed in 45% acetic acid at about 2°C for 10 min. Finally, they were macerated in a mixture of 45% acetic acid and 1N hydrochloric acid (1:1) for 20–23 sec at about 60°C, and were then stained in 2% aceto-orcin at room temperature in a moist chamber with 45% acetic acid for ca 30 min. Slides preparations were made by the conventional aceto-orcin squash methods. The slides were faintly heated under an alcohol frame for 1–2 sec before observation.

Measurements of chromosomes in the four species *Saxifraga* were made in each average length in several good metaphase cells. Classification of mitotic metaphase chromosomes followed Levan *et al.* (1964).

Taxonomical treatments followed Czerepanov (ed.) (1995).

The voucher specimens of the plants used in this study were deposited in Funamoto's private herbarium in Show Pharmaceutical University.

RESULTS AND DISCUSSION

Total 29 plants of four species of *Saxifraga* were collected in five sites in four localities in Altai Republic, Tuva Republic and Krasunoyarsk region, Russia, and were studied their somatic chromosomes (Fig. 1; Table 1):

1. *Saxifraga aestivalis* Fisch. *et* C. A. Mey. [= *S. nelsoniana* D. Don ssp. *aestivalis* (Fisch. *et* C. A. Mey.) D. A. Webb] was distributed in Russia and Mongolia. Five

plants of this species were collected in a locality in forest mountains stream side meadows about 1,510 m above the sea level in Krasunoyarsk region, Russia (Fig. 1; Table 1), and had commonly the chromosome number of $2n=28$ (Fig. 2a). This chromosome number was verified with the previous reports (Devyatov *et al.* 1997; Funamoto 2010), while the other three different chromosome numbers of $2n=30$, 56 and ca 60 were reported previously by somebody else (Belaeva and Siplivinsky 1976; Zhukova and Petrovsky 1987; Devyatov *et al.* 1997). Chromosome sizes of this species ranged from 2.1-1.0 μm long and bimodal decrease in length from the largest chromosome to the smallest chromosome; the largest chromosome and

the second largest chromosome were 1.3-1.5 times as large as the 3rd largest chromosome, and the other 26 chromosomes from the 3rd to the 28th chromosomes showed a gradual decrease in chromosome alignment. This form verified with Funamoto (2010). The chromosome complement had total 36.8 μm long and average 1.31 μm long. Chromosome complements in centromeric positions, a pair of the longest chromosome had sub-median-centromeric position and the other 26 chromosomes had median-centromeric positions (Fig. 3a; Table 2). Small satellite was rarely observed on short arm of the smallest chromosome. These chromosome characters and ecological conditions in this species

Fig. 1. Collection sites in four species of *Saxifraga* in Altai, Tuva and adjacent regions. 1. Ojskii pass (alt. 1,510 m); 2 and 3. Tsagan-Shibelyk River Valley (alt. 2,030-2,240 m); 4. Dzhety-Tei river (alt. 2,130 m); 5. Buguyun pass (alt. 2,600 m). *Saxifraga aestivalis* (*); *S. hieracifolia* (★); *S. foliolosa* (■); *S. hirculus* (●).

Table 1. Collection sites, sample numbers and somatic chromosome numbers in four species of *Saxifraga* collected in Altai, Tuva and adjacent regions, Russia

Species	Collection site	Sample number	Chromosome number (2n)
<i>S. aestivalis</i> Fisch. et C.A. Mey.	Krasnoyarsk Region, Ermakovsk Dist., Ojskii pass, 52° 51' 11" N, 093° 15' 63" E, alt. 1,510 m	5	28
<i>S. hieracifolia</i> Waldst. & Kit.	Altai Terr., Altai Rep., Kosh-Agacz. Dist., Buguyun pass, 50° 11' 12" N, 089° 24' 59" E, alt. 2,600 m	3	114
<i>S. foliolosa</i> R. Br.	Altai Terr., Altai Rep., Kosh-Agacz. Dist., Buguyun pass, 50° 11' 12" N, 089° 24' 59" E, alt. 2,600 m	6	60
<i>S. hirculus</i> L.	Tuva Rep., Mongun-Tajga Dist., W Tuva, Tsagan-Shibelyk River Valley, 50° 29' 16" N, 090° 54' 36" E, alt. 2,240 m	3	16
	50° 27' 80" N, 090° 50' 19" E, alt. 2,030 m	4	16
	Tuva Rep., Mongun-Tajga Dist., W Tuva, Dzhety-Tei River, 50° 17' 57" N, 089° 38' 91" E, alt. 2,130 m	5	16
	Altai Terr., Altai Rep., Kosh-Agacz. Dist., Buguyun pass, 50° 11' 12" N, 089° 24' 59" E, alt. 2,600 m	3	16

verified with Funamoto (2010).

2. *Saxifraga hieracifolia* Waldst. et Kit. is widely distributed in Europe, Russia, Mongolia and North America. Three plants of this species was collected in a locality in high mountains wet moist meadows (alt. 2,600 m) in Altai side the border pass between Altai Republic and Tuva Republic (Fig. 1; Table 1). They had commonly the chromosome number of $2n=114$ (Fig. 2b). This chromosome number verified with the previous report (Funamoto 2010), while the other eight different chromosome numbers such as $2n=56$, 63, >100, more than 100, c100, c110, 112 and 120 were previously reported by many researchers (e.g., Hamel 1953; Zhukova *et al.* 1973; Zhukova and Petrovsky 1987; Packer and McPherson 1974; Devyatov *et al.* 1997). These differences in chromosome number would be caused by high polyploidy and aneuploidy. Many more investigations are necessary to clarify and justify origin and reason why these different chromosome numbers occurred in this species. Chromosome sizes of this species ranged from 3.9-1.6 μm big to form a mono-modal (gradual) decrease in length from the largest to the smallest chromosomes and total 285.5 μm long and average 2.51 μm long. Their chromosome complement in centromeric positions consisted of median- and sub-median-centromeric chromosomes (Fig. 3b; Table 2). Small dot shaped satellite was observed. These chromosome characters and ecological conditions in this species verified with

Funamoto (2010).

3. *Saxifraga foliolosa* R. Br. is distributed in northern Europe, West, Central to East Siberia and Far East Russia and North America. This species was collected six plants in one locality in high mountains wet moist meadows (alt. 2,600 m) in Altai side the border pass between Altai Republic and Tuva Republic (Fig. 1; Table 1). They had commonly the chromosome number of $2n=60$ chromosomes (Fig. 3c). This chromosome number was reported here for the first time. The other four different chromosome numbers found in this species such as $2n=40$, 48, 56 and 64 (commonest one was $2n=56$) were previously reported by many researchers (e.g., Harmsen 1939; Löve and Löve 1951; Mosquin and Hayley 1966; Knaben and Engelskjön 1967; Johnson and Packer 1968; Löve *et al.* 1971; Packer and McPherson 1974; Zhukova 1980, 1982; Zhukova and Petrovsky 1976, 1987; Zhukova *et al.* 1973; Devyatov *et al.* 1997). These differences in chromosome numbers would be caused by polyploidization and aneuploidization. Many more investigations are necessary to clarify and justify origin and development of chromosome number in this species. Chromosome sizes of this species ranged from 2.5-0.9 μm large with mono-modal (gradual) decrease in size from the largest to the smallest chromosomes and total 87.8 μm long and average 1.47 μm long. The chromosome complement according to centromeric position showed commonly the 1st to the 10th chromosomes being sub-median-centromeric chromosomes and

Fig. 2. Somatic metaphase chromosomes in four species of *Saxifraga* observed. a: *S. aestivalis* ($2n=28$); b: *S. hieracifolia* ($2n=114$); c: *S. foliolosa* ($2n=60$); d: *S. hirculus* ($2n=16$). Arrows point satellites. Scale bar=5 μm .

the other 50 chromosomes had median-centromeric ones (Fig. 3b; Table 2). No satellited chromosome was observed.

4. *Saxifraga hirculus* L. is widely distributed in European and Eurasian continents and North America. Fifteen plants of this species were collected in four sites in three localities in high mountain wet moist meadows and moss-covered river banks, alt. 2,130-2,600 m in Altai Republic and Tuva Republic (Fig. 1; Table 1), and had $2n=16$ chromosomes in all plants (Fig. 3d), and verified with previous reports (e.g., Mosquin and Hayley 1966; Zhukova 1980; Zhukova and Petrovsky 1987; Funamoto 2010). The other chromosome number of $2n=32$ was previously reported by many researchers (e.g., Zhukova

and Petrovsky 1987; Petrovsky and Zhukova 1981; Funamoto *et al.* 2001). These chromosome numbers seemed to be diploid and tetraploid respectively, if the basic chromosome number of $x=8$ was accepted. Chromosome sizes of this species ranged from 6.0-3.4 μm long with mono-modal (gradual) decrease in length from the longest to the shortest chromosomes and total 70.3 μm long and average 4.4 μm long. Chromosome complement in centromeric positions, the 13th and the 14th chromosomes consisted of sub-median-centromeric chromosomes and the other 14 chromosomes had median-centromeric ones (Fig. 3d; Table 2). No satellited chromosome was observed.

Fig. 3. Karyotype comparison in four species of *Saxifraga* observed in this study. a: *S. aestivalis*; b: *S. hieracifolia*; c: *S. foliolosa*; d: *S. hirculus*. Arrows point satellites. Scale bar=5 μm .

Table 2. Karyotype characters in four species of *Saxifraga* observed

Species	Chromosome number	Cell number	Modality	Chromosome length (μm)				Form
				Longest Mean $\pm$ SD (Range)	Smallest Mean $\pm$ SD (Range)	Total Mean $\pm$ SD (Range)	Average Mean $\pm$ SD (Range)	
<i>S. aestivalis</i>	$2n=28$	7	bi-modal	2.1 $\pm$ 0.19 (2.3-1.8)	1.0 $\pm$ 0.08 (1.1-0.9)	36.8 $\pm$ 2.12 (39.9-34.1)	1.31 $\pm$ 0.08 (1.43-1.22)	m+sm
<i>S. hieracifolia</i>	$2n=114$	4	mono-modal (gradual)	3.9 $\pm$ 0.29 (4.3-3.6)	1.6 $\pm$ 0.13 (1.7-1.4)	285.5 $\pm$ 23.05 (313.0-256.5)	2.51 $\pm$ 0.2 (2.75-2.25)	m+sm
<i>S. foliolosa</i>	$2n=60$	2	mono-modal (gradual)	2.5 (2.5-2.5)	0.9 $\pm$ 0.07 (0.9-0.8)	87.8 $\pm$ 2.26 (89.4-86.2)	1.47 $\pm$ 0.04 (1.49-1.44)	m+sm
<i>S. hirculus</i>	$2n=16$	5	mono-modal (gradual)	6.0 $\pm$ 1.05 (7.6-5.0)	3.4 $\pm$ 0.49 (4.2-3.0)	70.3 $\pm$ 10.36 (86.8-62.4)	4.4 $\pm$ 0.65 (5.43-3.9)	m+sm

SD: Standard Deviation; m: median-centromeric chromosome; sm: submedian-centromeric chromosome

These chromosome characters and ecological conditions in this species verified with previous report (Funamoto 2010).

It was concluded that these four species had different chromosome numbers in each other, and *Saxifraga aestivalis* had bi-modal chromosome alignment, while the other three species had mono-modal (gradual) ones. *Saxifraga aestivalis* and *S. foliolosa* had small sized chromosomes, *S. hieracifolia* had medium sized chromosomes and *S. hirculus* had large sized chromosomes. In ecological conditions in Altai Republic and Tuva Republic and Krasunoyarsk region, included in the previous report (Funamoto 2010), *Saxifraga aestivalis* was found in forest mountains stream side meadows 1,250-1,510 m above the sea level, *S. hieracifolia* and *S. foliolosa* were found in high mountains wet meadows 2,450-2,600 m above the sea level, and *S. hirculus* was found in high mountains wet moist meadows and moss-covered river banks from 2,030-2,600 m above the sea level.

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