CYTOLOGICAL STUDIES OF FIVE CHINESE SPECIES OF *SOLMS-LAUBACHIA*[1] (BRASSICACEAE)

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Abstract. The karyotypes of five species of *Solms-laubachia* (Brassicaceae) from Hengduan Mountains (Sichuan and Yunnan provinces) are investigated for the first time. The karyotype formulas of *S. minor, S. eurycarpa*, *S. pulcherrima,* and *S. linearifolia* are the same and all have 2n=14=6m+6sm+2st, whereas that of *S. retropilosa* is 2n=28=12m+12sm+4st. The first four species are diploids, the last is tetraploid, and the base number for *Solms-laubachia* is x=7.

Keywords: Brassicaceae, cytological studies, *Desideria*, karyotypes, *Leiospora,* Matthioleae, *Parrya, Solms-laubachia*, somatic chromosome numbers.

Solms-laubachia Muschler (Brassicaceae) consists of nine species eight of which are endemic to China, and one, *S. platycarpa* (J. D. Hooker & Thomson) Botschantsev also occurs in Bhutan and Sikkim (Schulz, 1936; Botschantsev, 1955; Lan and Cheo, 1981; Wu, 1984; Lan, 1987; Wang, 1993; Yin et al., 1993; Li, 1995; Al-Shehbaz and Yang, 2001). Species of *Solms-laubachia* are highly restricted to the alpine and subalpine areas of northwestern Yunnan, western Sichuan and eastern Tibet, where nearly all grow on scree slopes. They have attractive blue to purplish flowers, and some species have long been used in traditional medicine by Tibetans (Anonymous, 1991, 1993).

Although Schulz (1936) placed *Solms-laubachia* in the tribe Matthioleae, the phylogenetic relationships of the genus remain unclear. On the basis of fruit morphology and readily detachment of the fruits from fruiting pedicels, Al-Shehbaz (2001) and Al-Shehbaz and Yang (2001) suggested a closer relationship of *Solms-laubachia* to *Desideria* Pampanini and *Leiospora* (C. A. Meyer) Dvorák, respectively. However, these studies are based strictly on the gross morphology of the plants, and except for the incomplete data on pollen morphology of two species (Yin et al., 1993) and phytochemistry of one, *S. eurycarpa* (Maximowicz) Botschantsev (Hu, 1995), nothing else is known about the genus. No molecular studies have been conducted, and the present paper reports the first cytological data on five species of the genus. All counts in the present paper are based on material collected from the Hengduan Mountains, a region well known as one of the worlds "hot spots" of biodiversity (Myers, 1988; Myers et al., 2000; Boufford and van Dijk, 1999).

MATERIALS AND METHODS

Localities from which seed materials were collected are listed in Table 1. Voucher specimens and permanent slides have been deposited in the herbarium of Kunming Institute of Botany (KUN).

All cytological observations were made from root tips. Seeds were stored for 20 days at 4˚C in the refrigerator. They were soaked overnight in distilled water at room temperature and were allowed to germinate on wet filter papers in petri dishes. The germination ratio of each species was over 90%. Fresh root tips about 1.5 cm long were cut, pretreated in 0.002M 8-hydroxyquinoline at 23˚C for 3-3.2 hours, then fixed with Carnoy fluid (1:3 glacial acetic acid/absolute alcohol) at 4˚C for 30 minutes. They were then rinsed in distilled water several times then stored in 70% ethanol for about 10 minutes. Prior to staining, the root tips were

This study was supported by grants-in-aid of research from the National Natural Science Foundation of China (30270121), the Innovation Program of the Chinese Academy of Science (KSCX2-SW-123) and the Yunnan Natural Science Foundation (2002C0060M). The first author is grateful to Mr. Zelong Nie of the Kunming Institute of Botany for his valuable help.

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Harvard Papers in Botany, Vol. 7, No. 2, 2003, pp. 467–473.

hydrolyzed in 1:1 1N HCL: 45% acetic acid at 60˚C for 30 seconds, and then were squashed and stained in 1% aceto-orcein. Permanent slides were made by using the standard liquid nitrogen method.

Observations were made on nuclei at the somatic mitotic interphase and metaphase, and measurements of chromosome arms were taken from at least ten well-spread metaphases of five or more different root tips of each species. The karyomorphological classification of the resting and mitotic prophase chromosomes follows Tanaka (1971, 1977), the designation of the centromere position as median (m), submedian (sm), and subterminal (st) follows Levan et al. (1964), and the symmetry of karyotypes follows Stebbins (1971).

Results

The interphase nucleus of all species showed many dark-stained heteropycnotic bodies of irregular shapes, light-stained chromatin threads, and scattered chromomeric granules. According to Tanaka (1971, 1977), this morphology of the resting nuclei could be categorized as the simple chromocenter type (Fig. 1A). At the mitotic prophase, hetero- and euchromatin segments were distinguishable, with the heterochromatic segments distributed in the interstitial and proximal regions. Therefore, based on Tanaka (1977) the prophase chromosome is an interstitial type (Fig.1B). Selected photographs of chromosome morphology from the metaphase of each species are shown in Fig. 1, and their detailed parameters are listed in Tables 2 and 3.

Solms-laubachia minor Handel-Mazzetti has the karyotype formula 2*n*=14=6m+6sm+2st. The chromosomes with centromeres at the median position are the 1st, 4th, and 7th pair respectively. The 2nd pair is st-type chromosomes, and the centromere positions of the other chromosomes belong to sm-type. The ratio of the longest to the shortest chromosome is 1.6, the mean arm ratio is 2.1, and based on Stebbins (1971), the asymmetry of the karyotype is categorized as type 2A (Figs. 1C, 2C').

Solms-laubachia pulcherrima Muschler has the same karyotype as *S. minor,* but the ratio of the longest to the shortest chromosome is 1.97, the mean arm ratio is 2.1, and the asymmetry of the karyotype is 3A (Figs. 1E, 2E').

Solms-laubachia linearifolia (W. W. Smith) O. E. Schulz has the karyotype formula 2*n*=14=6m+6sm+2st. The ratio of the longest to the shortest chromosome is 1.80, the mean arm ratio is 2.0, and the asymmetry of the karyotype is 3A (Figs. 1D, 2D'). One or two supernumerary B-chromosomes were found in some individuals.

Solms-laubachia eurycarpa (Maximowicz) Botschantsev has the karyotype formula 2*n*=14=6m+6sm+2st. The ratio of the longest to the shortest chromosome is 1.79, the mean arm ratio is 2.27, and the asymmetry of the karyotype is 3A (Figs. 1G, 1H, 2G'). In some individuals, one B-chromosome is occasionally present (Figs. 1G, 2G').

Solms-laubachia retropilosa Botschantsev is a tetraploid with the karyotype formula 2*n*=28=12m+12sm+4st. The ratio of the longest to the shortest chromosome is 1.80, the mean arm ratio is 1.9, and the asymmetry of the karyotype is 2A (Figs. 1F, 2F').

Discussion

Species of *Solms-laubachia* are characterized by being perennials with entire leaves, simple trichomes or glabrous, latiseptate flattened siliques or silicles, entire capitate stigma, mature fruits readily detached basally from the pedicel, and rounded replum concealed by strongly angled valve margins (Lan and Cheo, 1981; Al-Shehbaz and Yang, 2001). This combination of characters readily distinguish *Solms-laubachia* from the related genera. Karyotypes of the five species studied in this paper are quite similar. In the four diploids, the metacentric chromosomes (m-type) are the 1st, 2nd, 7th, 8th, 13th, 14th. The 3rd and 4th chromosomes are subtelocentric (st-type), and the others are sm-type chromosomes. The tetraploid *S. retropilosa* has the same chromosome types as the diploid species.

Although the cytological data on *Solms-laubachia* is incomplete, it is safe to conclude that the base chromosome number for the genus is x=7. In order to achieve a better understanding of the karyotype morphology and evolution in the genus, efforts will be made to collect seeds of the remaining four species, as well as of additional populations of the five investigated here.

Schulz's (1936) placement of *Solms-laubachia* in the tribe Matthioleae was followed by Lan (1987), and both authors considered the genus to be closely related to *Parrya* R. Br. As indicated above, on the basis

TABLE 1. Species of *Solms-laubachia*, localities, and vouchers (all in KUN). All collections were made from the Hengduan Mountains in Yunnan (Y) and Sichuan (S) provinces, China.

SPECIES	LOCALITY	LATITUDE	LONGITUDE	ALTITUDE (M)	VOUCHERS
S. minor	Zhongdian (Y)	N27˚47'	E99˚35'	4330	*Yue 200156*
S. pulcherrima	Lijiang (Y)	N27˚03'	E100˚11'	4210	*Yue 200153*
S. retropilosa	Xiancheng (S)	N29˚06'	E100˚01'	4790	*Yue 200162*
S eurycarpa	Deqin (Y)	N28˚23'	E99˚01'	4650	*Yue 200158*
S. linearifolia	Deqin (Y)	N28˚23'	E99˚00'	4600	*Yue 200157*

TABLE 2. Measurements of somatic chromosomes at mid-metaphase of karyotypes of diploid *Solms-laubachia minor*, *S. linearifolia,* and *S. pulcherrima.* (RL=relative length; AR=arm ratio; PC=position of centromere; m=metacentric chromosome; sm=submetacentric chromosome; st=subterminal chromosome).

Chromesome Number	*S. minor* 2n=14=6m+6sm+2st			*S. linearifolia* 2n=14=6m+6sm+2st			*S. pulcherrima* 2n=14=6m+6sm+2st		
	RL	AR	PC	RL	AR	PC	RL	AR	PC
1	9.3	1.5	m	10.2	1.2	m	10.3	1.1	m
2	9.0	1.2	m	9.5	1.1	m	9.7	1.3	m
3	7.9	3.7	st	8.2	3.6	st	8.9	3.3	st
4	7.9	3.6	st	7.7	3.3	st	8.7	3.2	st
5	7.3	2.3	sm	7.3	2.8	sm	8.0	2.8	sm
6	6.6	2.9	sm	7.3	2.2	sm	6.7	2.9	sm
7	6.9	1.2	m	7.3	1.2	m	6.9	1.2	m
8	6.8	1.2	m	7.2	1.1	m	5.9	1.1	m
9	6.9	2.5	sm	6.5	2.0	sm	6.1	2.9	sm
10	6.6	2.4	sm	5.9	2.1	sm	5.9	2.3	sm
11	6.7	1.9	sm	5.8	2.0	sm	5.9	2.3	sm
12	6.1	2.1	sm	5.7	2.0	sm	5.9	2.5	sm
13	6.1	1.5	m	5.8	1.5	m	5.9	1.3	m
14	5.9	1.2	m	5.6	1.6	m	5.2	1.5	m

TABLE. 3. Measurements of somatic chromosomes at mid-metaphase of karyotype of diploid *Solms-laubachia eurycarpa* and tetraploid *S. retropilosa.* (Abbreviations as in TABLE 2.)

Chromesome Number	*S. eurycarpa* 2n=14=6m+6sm+2st			*S. retropilosa* 2n=28=12m+12sm+4st							
	RL	AR	PC	Chro.	RL	AR	PC	Chro.	RL	AR	PC
1	9.5	1.3	m	1	5.2	1.1	m	15	3.4	1.1	m
2	9.3	1.6	m	2	5.2	1.1	m	16	3.3	1.5	m
3	9.3	3.1	st	3	4.9	1.0	m	17	3.4	2.1	sm
4	8.5	4.6	st	4	4.7	1.1	m	18	3.2	1.9	sm
5	7.0	2.7	sm	5	4.1	3.0	st	19	3.2	2.3	sm
6	6.5	2.9	sm	6	3.9	3.0	st	20	3.1	2.4	sm
7	7.2	1.1	m	7	3.9	3.0	st	21	3.3	1.8	sm
8	6.2	1.3	m	8	3.8	3.3	st	22	3.0	2.4	sm
9	6.4	2.5	sm	9	3.5	2.4	sm	23	3.0	2.4	sm
10	6.4	2.5	sm	10	3.5	2.0	sm	24	2.9	2.3	sm
11	6.3	2.5	sm	11	3.4	2.1	sm	25	3.1	1.3	m
12	6.0	2.6	sm	12	3.3	2.3	sm	26	3.0	1.5	m
13	6.2	1.4	m	13	3.7	1.4	m	27	2.9	1.6	m
14	5.3	1.7	m	14	3.2	1.3	m	28	2.9	1.5	m

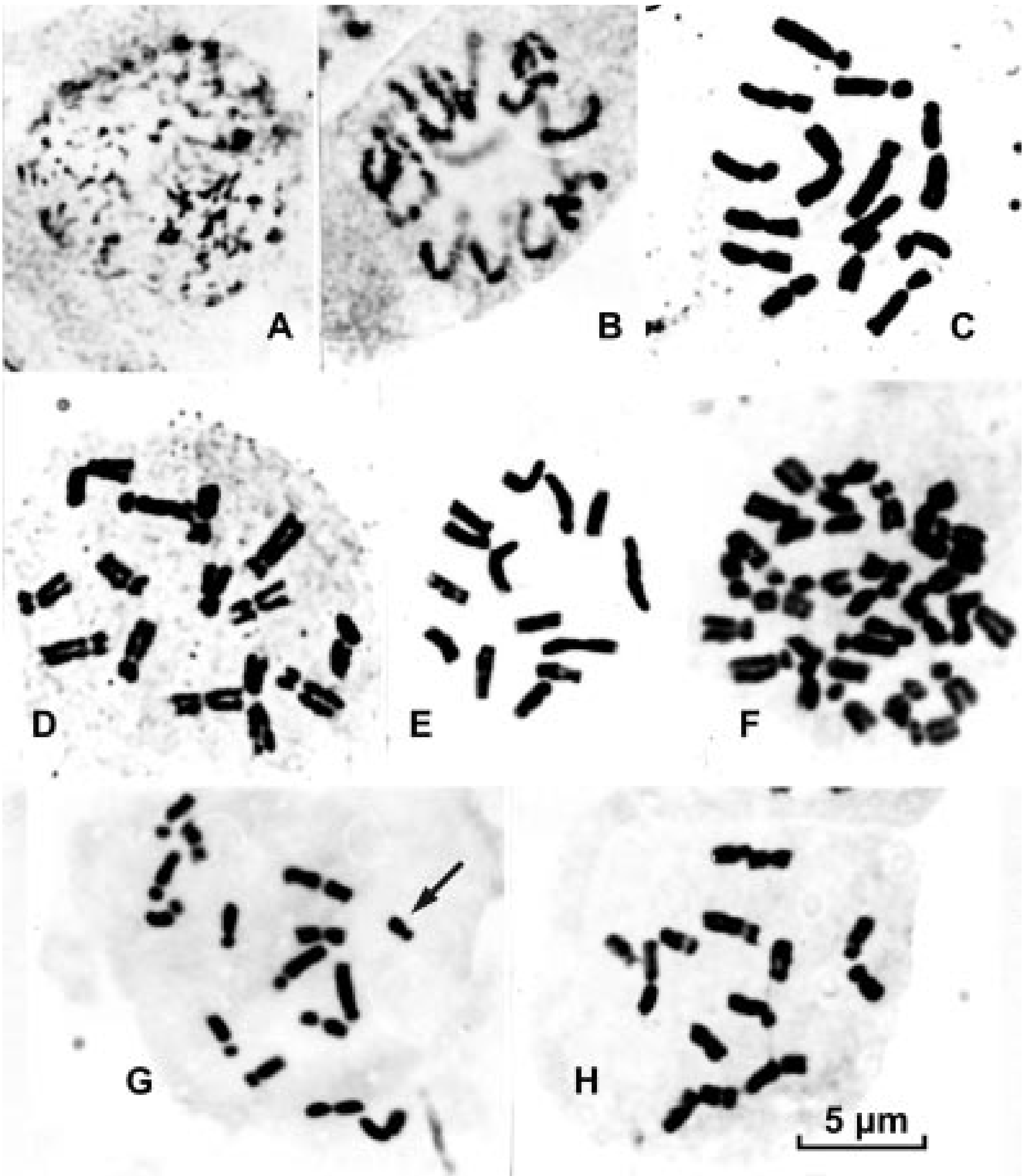

FIGURE 1. Micrographs showing mitosis in root tips of five *Solms-laubachia* species. A=interphase of *S. minor*; B=prophase of *S. minor*; C=metaphase of *S. minor*; D=metaphase of *S. linearifolia*; E=metaphase of *S. pulcherrima*; F=metaphase of *S. retropilosa*; G=metaphase of *S. eurycarpa* (arrow pointing to B-chromosome); H=metaphase of *S. eurycarpa* (without B-chromosome). Ideograms in C′, D′, E′, F′, and G′ of Fig. 2 correspond to the same species of C, D, E, F, and G in the micrographs above.

of gross morphology, *Solms-laubachia* appears to be closer to *Desideria* and *Leiospora* than to *Parrya* (Al-Shehbaz, 2001; Al-Shehbaz and Yang, 2001). However, the tribal assignment of these genera would have to wait for comprehensive phylogenetic studies that also include their presumed relatives. Schulz's (1936) tribal classification of the Brassicaceae has been shown to be highly artificial on morphological (Hedge, 1976; Al-Shehbaz, 1984) and molecular grounds (Price et al., 1994; Koch et al., 1999, 2001).

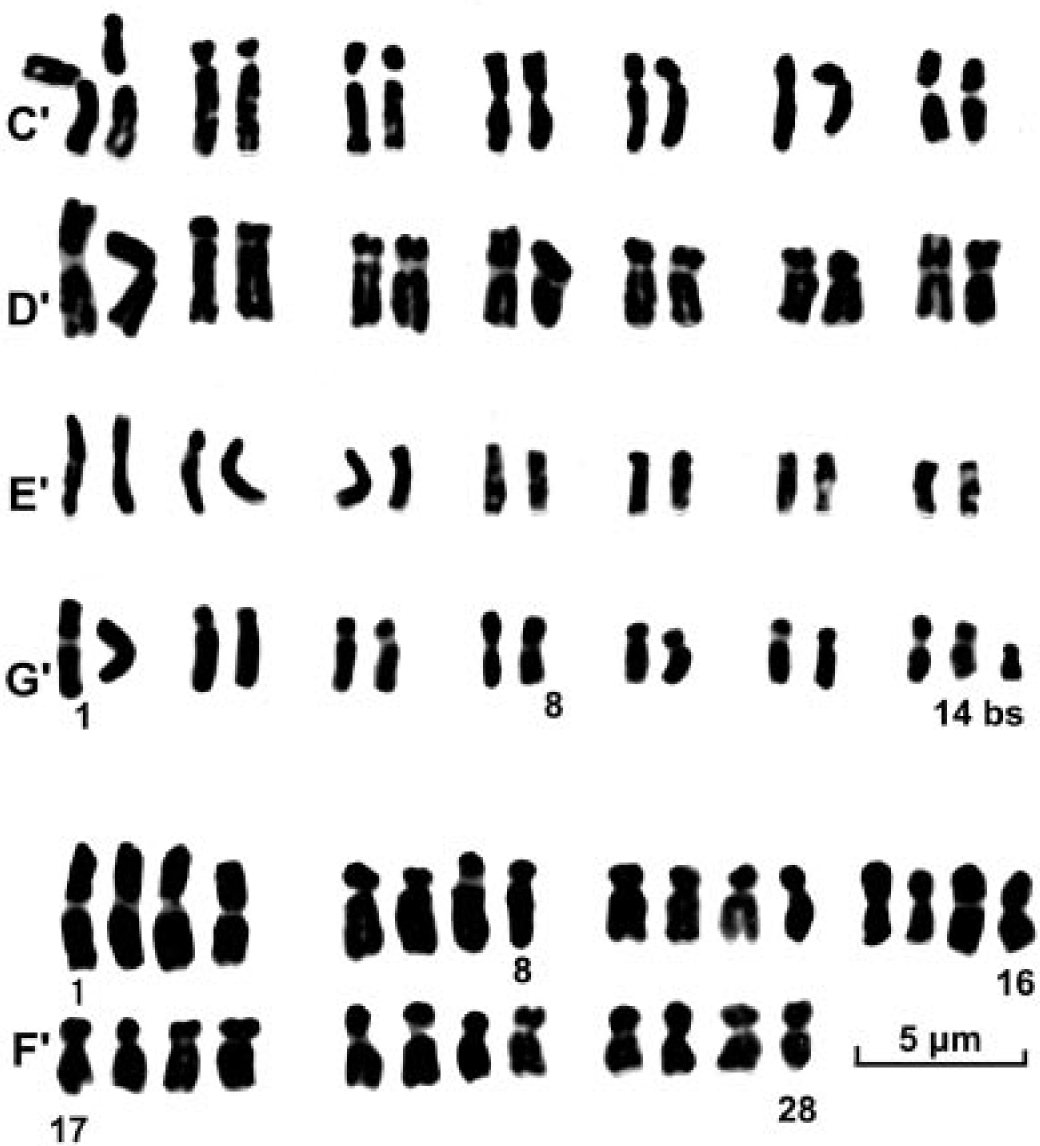

FIGURE 2. Ideograms of somatic metaphase chromosome of *Solms-laubachia.* C′. *S. minor*; D′. *S. linearifolia;* E′. *S. pulcherrima;* G′. *S. eurycarpa*; F′. *S. reteopilosa.* Scale = 5 um. (bs=B-chromosome).

Although initial cytological studies on the Brassicaceae by Manton (1932) and Jaretzky (1932) showed some patterns of potential taxonomic implications, a later review (Al-Shehbaz, 1984) clearly demonstrated that such data are not useful at the tribal level. The fragmentary cytological information available on *Desideria, Leiospora,* and *Parrya* does not allow meaningful comparisons to *Solms-laubachia*. Only one of the 11 species of *Desideria*, *D. flabellata* (Regel) Al-Shehbaz (listed as *Ermania flabellata* (Regel) O. E. Schulz), was reported to have 2*n*=14 by Yurtsev and Zhukova (1972). Two of the six species of *Leiospora*, *L. bellidifolia* (Danguy) Botschantsev and Pachomova and *L. eriocalyx* (Regel & Schmalhausen) Dvorák (listed as *Parrya eriocalyx* Regel & Schmalhausen), were reported to have 2*n*=14 by Zakharjeva (1990) and Yurtsev and Zhukova (1972), respectively. Of the 25 species of *Parrya*, the three counted for chromosome numbers are *P. arctica* R. Brown (2*n*=21 by Mosquin and Hayley, 1966), *P. schugnana* Lipschitz (2*n*=14 by Yurtsev and Zhukova (1972); 2*n*=28 by Matveeva and Tykhonova in Fedorov (1969)), and *P. nudicaulis* (Linnaeus) Regel (2*n*=14 by Hedberg (1967) and Knaben (1968); 2*n*=14, 28 by Zhukova and Petrovsky (1971, 1976), Yurtsev and Zhukova (1972), and Petrovsky and Zhukova (1983); 2*n*=28 by Zhukova (1965, 1980), Johnson and Packer (1968), Sokolovskaya (1968), Mulligan (1970), Zhukova et al. (1973), and Zhukova and Petrovsky (1977, 1980)). The finding each in *P. schugnana* and *P. nudicaulis* of both diploid and tetraploid populations raises the question as to whether or not similar situations occur in *Solms-laubachia*, especially the tetraploid *S. retropilosa*. This also points out the need to make new chromosome counts from additional populations of the five species reported here. From the limited counts above, it appears that *x*=7 might be the base chromosome number in this generic alliance. It is hoped that with additional counts on species of the four genera discussed above, a better understanding of the chromosomal evolution can be achieved.

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