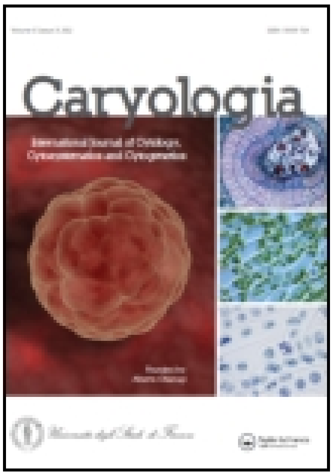

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Meiotic observations of eight taxa in the genus Salvia

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Meiotic observations of eight taxa in the genus *Salvia*

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Abstract — Observations were made on chromosome morphology and behavior during meiosis of PMCs (pollen mother cells) for eight taxa in the genus *Salvia*: *S. miltiorrhiza* Bunge, *S. miltiorrhiza* Bunge var. *miltiorrhiza* f. *alba* C. Y. Wu et H. W. Li, *S. cynica* Dunn., *S. flava* Forrest, *S. yunnanensis* C. H. Wright, *S. cavaleriei* Levl. var. *simplicifolia* Stib., *S. przewalskii* Maxim. and *S. brevilabra* Franch. Results show that the basic chromosome number in the genus *Salvia* is x=8. The chromosome configurations in *S. miltiorrhiza* (from Sichuan, Shandong, Zhejiang and Shanxi), *S. miltiorrhiza* f. *alba*, *S. cynica*, *S. flava*, *S. yunnanensis* and *S. cavaleriei* var. *simplicifolia* are formulated uniformly as 8II. In *S. miltiorrhiza* from Henan, the typical chromosome configuration observed at metaphase is 0.13 I +7.80 II+0.07 IV, whereas that in *S. przewalskii* is 0.33 I+15.50 II+0.16 IV and in *S. brevilabra* is 0.53 I+15.73 II. Configurations of the bivalents in all materials investigated are different. According to the chromosomes pairing in metaphase I, except that *S. przewalskii* and *S. brevilabra* are tetraploids, the others are diplonts. Chromosomes bridges or lagging chromosomes are appeared at anaphase II in *S. miltiorrhiza* from Henan, *S. przewalskii* and *S. brevilabra*. The abnormal behaviors of the chromosomes in meiosis observed may have effects on the development of pollen grains in these taxa.

Key words: bivalents, chromosome behavior, chromosomes bridges, meiosis, *Salvia*.

INTRODUCTION

The genus *Salvia* Linn., with about 900 species, is probably the largest member of the family Lamiaceae. It is distributed throughout the Old and New World, in subtropical and temperate areas (Standley and Williams 1973). Western Asia and the Mediterranean region are considered to be the two original centers of distribution (Wu and Li 1982).

In China, there are approximately 78 species, 43 varieties and 8 forms of *Salvia*, about 70 species of which are endemic to China, and most taxa are distributed in southwestern regions such as Sichuan, Yunnan and Guizhou provinces (Wu and Li 1990). The genus *Salvia* is quite well known for its horticultural as well as commercial importance (Bhattaharya 1978). Some species are commonly used in local folk medical practices and in cosmetics (Ella and Putiesky 1985), such as the root of *Salvia miltiorrhiza* Bunge, a Chinese traditional medicine in each version of Pharmacopoeia of People's Republic of China (2005). But the standards in local folk practices contain 36 taxa which have medical value in *Salvia*, such as *S. przewalskii*, *S. yunnanensis*, *S. cavaleriei* var. *simplicifolia* and so on (Xiao et al. 1997; Chen et al. 2005; Cheng et al. 2005; Cai et al. 2006).

There are several cytological observation reports on the genus *Salvia* at home and abroad, but most of these researches are on the chromosome counts (Stewart 1939; Cai et al. 1993; Yang et al. 2004; Zhao et al. 2006; Yang et al. 2009). Meiosis is a premise of sexual reproduction and the behaviors in the meiosis are correlation between the chromosomes and the genes (Swanson 1981). Meiotic and mitotic chromosomes of 13 species of *Salvia* from Argentina were studied and there were diploid, tetraploid, hexaploid and octoploid. The basic chromosome numbers were diverse as well (Alberto 2003). The most common basic chromosome numbers were x=6, 7, 8, according to the researches about 20 species of *Salvia* from

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the Himalayas (Gill 1971). However, there was no report on the meiosis of *Salvia* in China.

In the present study, observations were made on chromosome morphology and behavior during meiosis of PMCs in the genus *Salvia*: *S. miltiorrhiza* from 5 provinces, *S. miltiorrhiza* f. *alba*, *S. przewalskii*, *S. brevilabra*, *S. cynica*, *S. flava*, *S. yunnanensis* and *S. cavaleriei* var. *simplicifolia*. The main aims of this research are to observe and reveal differences among the species in chromosome behavior and morphology by studying the chromosome configuration during meiosis of PMCs, and to confirm the chromosome ploidy and basic number of *Salvia* in China, as well as to offer cytogenetical evidences for sterile mechanism of *Salvia.*

MATERIALS AND METHODS

Flower buds of native species were collected from wild populations and those of the introduced species from cultivated plants. The taxa are named after their localities and the herbarium voucher specimens are listed in Table 1. Herbarium voucher specimens are deposited in SAUT (Herbarium of Triticeae Research Institute, Sichuan Agricultural University, Dujiangyan, Sichuan, China).

For meiotic studies, flower buds were fixed in absolute ethanol and glacial acetic acid (3:1) for at least 24 h, and then transferred into 70% ethanol and stored at 4–5 in a refrigerator until required. Immature anthers were then squashed in carbolfuchsin as a mordant. Meiosis was studied using a minimum of 30 PMCs. Digital photographs were taken using an Olympus 3030 camera mounted on an Olympus microscope BX51.

RESULTS

The chromosome numbers (2n), levels of ploidy and chromosome configurations of the 12 *Salvia* materials studied are presented in Table 2. The basic number is x=8 for all of the studied materials, and except that *S. przewalskii* and *S. brevilabra* are tetraploids, the others are diplonts. In addition, *S. miltiorrhiza* from Henan and *S. brevilabra* show one B chromosome severally.

1. *S. miltiorrhiza* - Bunge and *S. miltiorrhiza* f. *alba* – Both the two species were diplonts and the chromosome numbers were 2n=2x=16. The chromosome configuration at metaphase I indicated that 8 bivalents were easily distinguished from each other in morphology and structure for *S. miltiorrhiza* f. *alba*, *S. miltiorrhiza* from Sichuan, Shandong, Zhejiang and Shanxi. The average chromosome configurations were 8 II for these taxa (Figs. 1-5). There were great differences of the proportion between ring bivalents and rod bivalents, especially *S. miltiorrhiza* from Shanxi. However, the observed meiosis of *S. miltiorrhiza* from Hennan showed 8 bivalents at diplotene and metaphase I generally (Fig. 6). The quadrivalent or two univalents occurred (Fig. 7), and the univalents stood outside of the equatorial plate. In some cells one B chromosome could be seen (Fig. 8). The average chromosome configuration was 0.13I+7.80II+0.07IV. Also the rod bivalents were

Table 1 —The origin of materials investigated

Taxon	Locality	Voncher
Salvia miltiorrhiza Bunge	Zhongjiang, Sichuan	L. Zhang 05002
S. miltiorrhiza Bunge	Nanyang, Henan	H. X. Zhao05006
S. miltiorrhiza Bunge	Taishan, Shandong	H. Q. Yu 05003
S. miltiorrhiza Bunge	Shengzhou, Zhejiang	Z. J. Yang 06007
S. miltiorrhiza Bunge	Xian, Shanxi	Z. J. Yang 05004
S. miltiorrhiza f. *alba* C. Y. Wu et H. W. Li	Laiwu, Shandong	H. Q. Yu 05001
S. przewalskii Maxim	Wenchuan, Sichuan	Z. J. Yang 06005
S. brevilabra Franch..	Kangding, Sichuan	Z. J. Yang 06008
S. cynica Dunn	Tianquan, Sichuan	Z. J. Yang 05005
S. flava Forrest	Yanyuan, Sichuan	X. Fan 04001
S. yunnanensis C. H. Wright	Luding, Sichuan	X. Fan 04002
S. cavaleriei Levl. var. *simplicifolia* Stib.	Nanchuan, Chongqing	Z. J. Yang 06006

Table 2 — The chromosome configurations at M1 in PMCS of materials investigated.

Taxon	Chromosome number(2n)	Ploidy level	Chromosome configuration			Bivalent configuration (%)	
			I	II	IV	Ring	Rod
S. miltiorrhiza (Sichuan)	16	2x		8		25.78	74.22
S. miltiorrhiza (Henan)	16+0-1B	2x	0.13	7.80	0.07	28.21	71.79
S. miltiorrhiza (Shandong)	16	2x		8		23.08	76.92
S. miltiorrhiza (Zhejiang)	16	2x		8		40.10	59.90
S. miltiorrhiza (Shanxi)	16	2x		8		14.77	85.23
S. miltiorrhiza f. *alba*	16	2x		8		24.38	75.62
S. przewalskii	32	4x	0.33	15.50	0.16	18.75	81.25
S. brevilabra	32+0-1B	4x	0.53	15.73		25.64	74.36
S. cynica	16	2x		8		34.38	65.62
S. flava	16	2x		8		63.33	26.67
S. yunnanensis	16	2x		8		52.50	47.50
S. cavaleriei var.*simplicifolia*	16	2x	0	8		57.37	42.63

dominant. Additionally, lagging chromosomes and chromosome bridges were occasionally found in some PMCs at anaphase I and at anaphase I(Figs. 9-11).

2. *S. przewalskii* - This species was tetraploid and the chromosome number was 2n=4x=32. PMCs of this species showed 16 bivalents at diplotene I and metaphase I, but occasionally one or two quadrivalents could be seen (Fig. 12-13). Two or four univalents were present (Fig. 14). In some cells these univalents and few bivalents were outside of the equatorial plate. The average chromosome configuration was 0.33I+15.50II+0.161IV and the proportion of rod bivalents was much more than that of ring bivalents, reaching 81.25%. However, abnormalities were observed, such as lagging chromosomes and chromosome bridges at anaphase I (Fig. 15).

3. *S. brevilabra* - In this species PMCs showed 16 II at diplotene and metaphase I (Fig. 16), but occasionally 15II + 2I or 14II + 4I could be observed (Fig. 17). Besides, in some cells one B chromosome was visible (Fig. 18). The average chromosome configuration was 0.53I+15.73II and the proportion of rod bivalents was high, reaching 74.36%. Chromosome bridges were occasionally found in some PMCs at anaphase I (Fig. 19).

4. *S. cynica* - This species was diplont and the chromosome number was 2n=2x=16. The chromosome configuration at diplotene and metaphase I showed that 8 bivalents were existed in all cells (Fig. 20). The average chromosome configuration was 8 II and the rod bivalents were dominant, reaching 65.62%.

5. *S. flava* - This species was diplont and the chromosome number was 2n=2x=16. The meiotic configurations observed in this plant were 8 II and the ring bivalents were dominant, reaching 63.33% (Fig. 21).

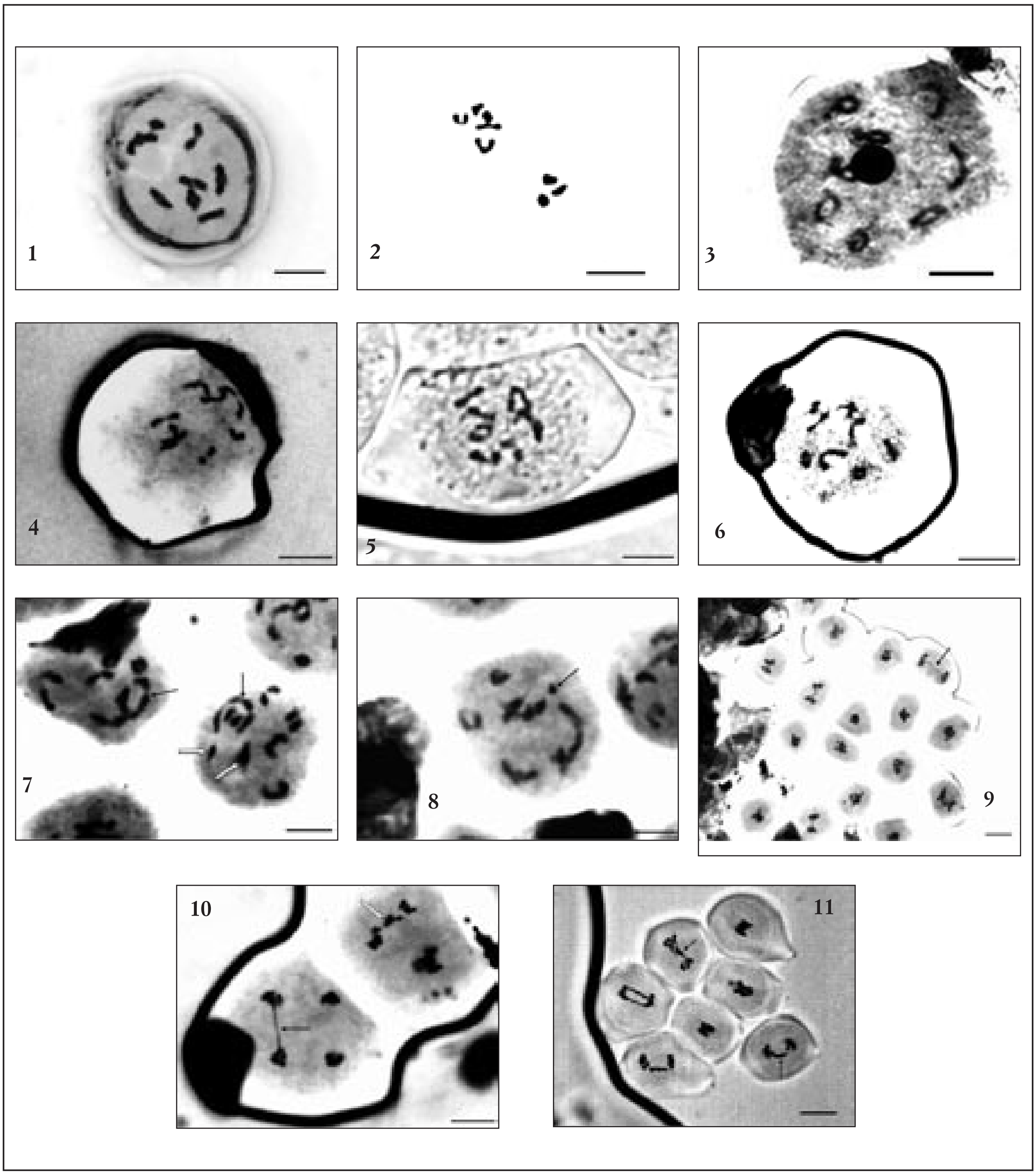
6. *S. yunnanensis* - This species was diplont and the chromosome number was 2n=2x=16. The average chromosome configuration was 8 II and the proportional difference between ring and rod bivalents wasn't conspicuous (Fig. 22).

7. *S. cavaleriei* var. *simplicifolia* - This species was diplont and the chromosome number was 2n=2x=16. The average chromosome configuration was 8 II and the ring bivalents were dominant slightly (Fig. 23).

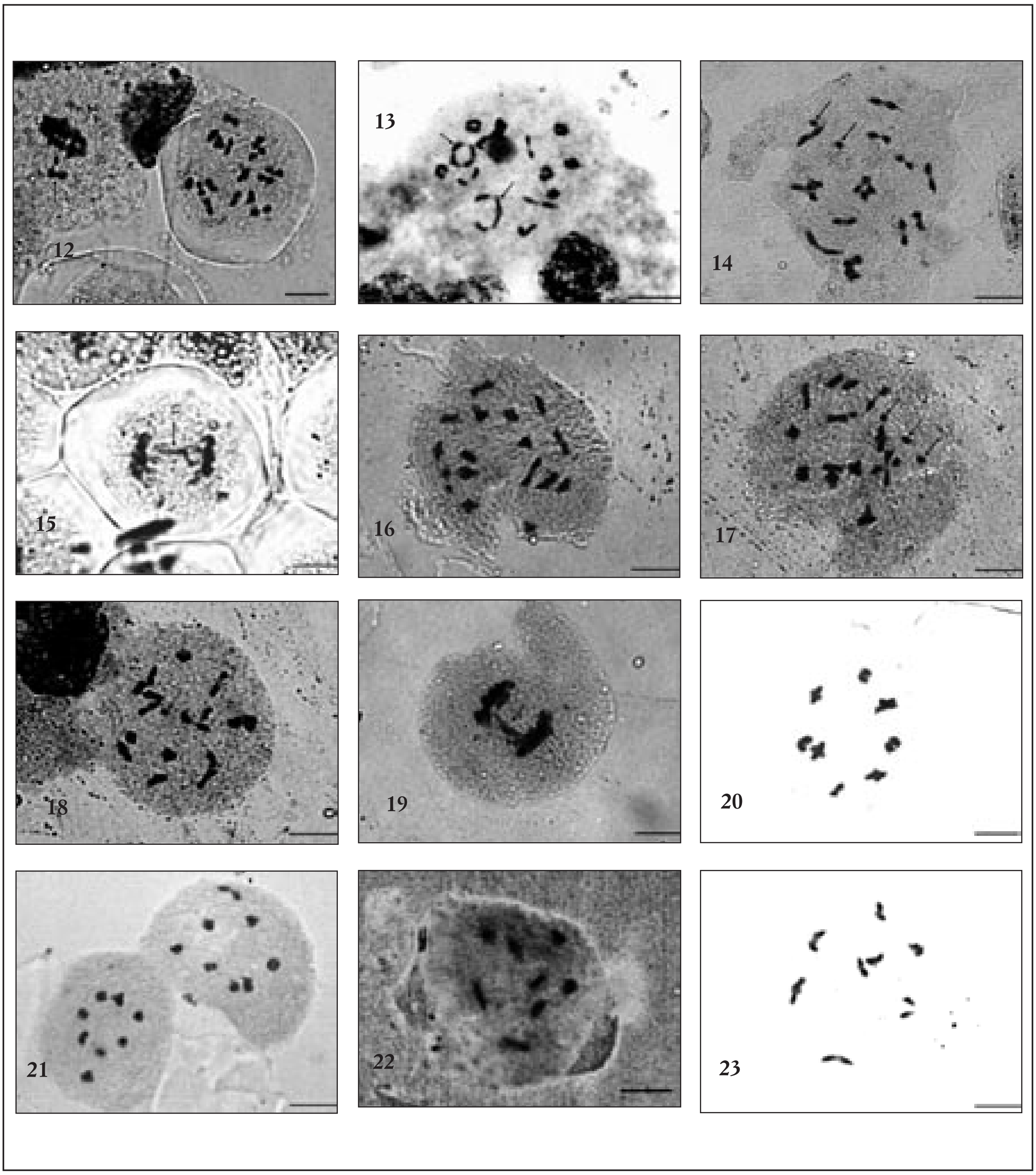
DISCUSSION

It is helpful to confirm the levels of ploidy by studying the meiosis of the PMCs. As in polyploidy individuals non-homologous chromosomes couldn't be synapsis at the meiosis. As a result the unmatched chromosomes show themselves as univalents at metaphase I and move to the two poles randomly or become the lagging chromosomes at anaphase I. The univalents, unmatched chromosomes and lagging chromosomes in the species of *S. przewalskii* and *S. brevilabra* give proofs to identify that they are polyploids.

Nakipoglu (1993a, 1993b) observed that *Salvia* species had B type chromosomes and Yang (2009) observed B chromosomes in *S. tricuspis*. Tomson (2005) reported that B chromosomes



Figs. 1-11 — Meiosis of PMCs in *S. miltiorrhiza* and *S. miltiorrhiza* f. *alba*. **1.** 8 bivalents in *S. miltiorrhiza* from Sichuan. **2.** 8 bivalents in *S. miltiorrhiza* from Shandong. **3.** 8 bivalents in *S. miltiorrhiza* from Zhejiang. **4.** 8 bivalents in *S. miltiorrhiza* from Shanxi. **5.** 8 bivalents in *S. miltiorrhiza* f. *alba*. **6-11** *S. miltiorrhiza* from Henan. **6.** 8 bivalents. **7.** 8 bivalents (Black arrow) and univalents (White arrow). **8.** B chromosome (Black arrow). **9.** Chromosome bridge at anaphase I (Black arrow). **10.** Chromosome bridge (Black arrow) and lagging chromosome (White arrow) at anaphase II. **11.** Lagging chromosome at anaphase I (Black arrow). Bar =5μm.



Figs. 12-23 — Meiosis of PMCs in 6 *Salvia* taxa. **12-15.** *S. przewalskii*. **12.** 16 bivalents and univalents (Black arrow). **13.** Quadrivalent (Black arrow). **14.** Univalents (Black arrow). **15.** Chromosome bridge at anaphase I (Black arrow). **16.** 16 bivalents in *S. brevilabra*. **17.** Univalents (Black arrow) in *S. brevilabra*. **18.** B chromosome (Black arrow) in *S. brevilabra*. **19.** Chromosome bridge at anaphase I (Black arrow) in *S. brevilabra*. **20.** 8 bivalents in *S. cynica*. **21.** 8 bivalents in *S. flava*. **22.** 8 bivalents in *S. yunnanensis*. **23.** 8 bivalents in *S. cavaleriei* var. *simplicifolia*. Bar =5μm.

were found in cytotype of *S. coccinea* naturalized in Western Ghats of India which was the probable cause of the essential oil scarcity in the plant. In our studies, we observed B chromosomes in *S. miltiorrhiza* from Henan and *S. brevilabra*, which were the new reports of B chromosomes for the species from China. Following TOMSON's idea we need further research to confirm whether there is influence or not on the composition in these plants.

Bivalent configurations coincide with the interchromosomal crossing-over frequencies. From the bivalent configurations of the studied materials at metaphase I, the rod bivalents, only single crossing-over happens in the two chromosome arms, were in predomination, and also there were ring bivalents whose crossing-over happened in the two chromosome arms twice or more. In all materials investigated the frequencies were different. The highest was *S. flava* and the lowest was *S. miltiorrhiza* from Shanxi. Interchromosomal crossing-over is one of the important sources of intraspecific variation, although the crossing-over frequencies are not too high in each PMC.

SHU (2006) reported that the forms were similar apparently between the fertile and sterile plants except the great differences at the shape or size of anthers. Abnormal phenomena in meiosis have close relationship with the fertility. There are fertile anthers and sterile anthers simultaneously in *S. miltiorrhiza* from Henan. There are mainly two reasons led the anthers to be sterile. On the one hand, when secondary sporogenous cells differentiate into microsporocytes and anther wall cells, for the abnormal differentiations, thick pollen sac walls are formed, which make the anther not crack to diffuse pollen and thus cause abortion at last. On the other hand, sterile sporules or pollen granules are formed at the time of PMCs, early stage of tetrad, monocaryophase and dikaryophase when the pollens develop. Thus sterile pollen could be caused by the abnormal meiosis, and this abortion is irreversible. The lagging chromosomes and chromosome bridges in the meiosis of *S. miltiorrhiza* from Henan probably influence the growth of the pollens to a certain extent. The abnormal phenomenon which appeared in the species of *S. przewalskii* and *S. brevilabra* at anaphase I and anaphase I showed that variations of chromosomes structure took place and the pollens were sterile partially. The reasons for variations may be due to the external environment, such as low temperature, continuous rain and so on. Also it may be caused by cross-pollinating. The specific factors need further studies.

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