Cytologia **88**(4): 295–300

Karyotype Report

Comparative karyotype analysis of six native *Passiflora* species (Passifloraceae) from SW China

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Received March 8, 2023; accepted June 2, 2023

Summary Six *Passiflora* species from SW China were studied: *Passiflora menghaiensis* X.D.Ma, L.C.Yan & J.Y.Shen, *P. xishuangbannaensis* Krosnick, *P. siamica* Craib, *P. altebilobata* Hemsl., *P. henryi* Hemsl. and *P. papilio* Li. Chromosomes squashes could be obtained by using the conventional tableting method and the karyotypes were analyzed. The results showed that all six species of passion flower were diploid and the chromosome number was $2n=12$. The karyotype formula of *P. menghaiensis* was $2n=2x=12=8m+2sm+2sm(sat)$, *P. xishuangbannaensis* was $2n=2x=12=8m+4sm$, *P. siamica* was $2n=2x=12=10m+2sm$, *P. altebilobata* was $2n=2x=12=10m+2sm$, *P. henryi* was $2n=2x=12=8m+4sm$, *P. papilio* was $2n=2x=12=12m$. The karyotypes of all six species are reported for the first time. This study provides comprehensive cytological data for the conservation and genetic study of *Passiflora* in Asia. The evolutionary relationships between the six species were discussed at the cytological level and compared with published classifications based on morphological and molecular data.

Keywords Passifloraceae, Karyotype analysis, Cytogenetics, Cluster analysis.

Passion flowers (*Passiflora*) belong to the Passifloraceae family, with more than 500 species distributed in tropical and subtropical climate regions of the world (Feuillet and MacDougal 2003). *Passiflora* is the largest genus in Passifloraceae, and is divided into five main subgenera: *Passiflora, Decaloba* (DC.) Rchb., *Deidamioides* (Harms) Killip, *Astrophea* (DC.) Mast., and *Tetrapathea* (DC.) P. S. Green (Ulmer and Macdougal 2004). The subgenus *Decaloba* contains approximately 230 species with a basic chromosome number of $x=6$ ($2n=12$, 24, 36) (De Melo and Guerra 2003, Krosnick 2005, Ma *et al.* 2019). The chromosomes of species in subgenus *Decaloba* show secondary contractions with metacentric and submetacentric chromosomes (Beal 1973, Vieira and Carneiro 2005). The subgenus *Passiflora* contains approximately 250 species with a basic chromosome number of $x=9$ ($2n=18$, 36, 72) or $x=10$ ($2n=20$) (Sader *et al.* 2019). *Astrophea, Dedamioides*, and *Tetrapathea* subgenera, have a basic chromosome number of $x=12$ ($2n=24$) (Melo *et al.* 2001).

Most passion flower species are distributed in South America, and there are only 20 native species in China (Wang *et al.* 1999). Yunnan is the center of diversity for passion flowers in China. In addition to the edible fruits of some species, many passion flowers also have an ornamental value. To date, there is a lack of research on the native Asian species of *Passiflora* as well as their conservation, phylogenetics, and breeding for fruit production.

Karyotyping is a technique used in ploidy level studies and hybridization studies, and the morphological characteristics of chromosome provide helpful data for revealing the interspecific genetic relationships within *Passiflora*. The number and position of secondary contraction, the number and length of chromosome, and the position of centromeres is characteristic of some subgenera and subgroups. Plant chromosomes may have evolved from symmetry to asymmetry; the higher the symmetry coefficient is, the less derived the plant could be (Stebbins 1971). In breeding, cytological knowledge can help in planning interspecific hybridization, and it also provides relevant information about the effect of individual genomes on hybrid pairing (Souza *et al.* 2008). Cytological studies in several *Passiflora* taxa have been published (Beal 1969, 1971, De Melo *et al.* 2001, Li *et al.* 2022); however, detailed cytogenetic studies on Asian species are scarce, and analyses of chromosome number and karyotype structure are not yet available for these six species. Hence, the present study that aims at producing karyotypes of six native *Passiflora* species (Passifloraceae) from SW China and to explore their

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DOI: 10.1508/cytologia.88.295

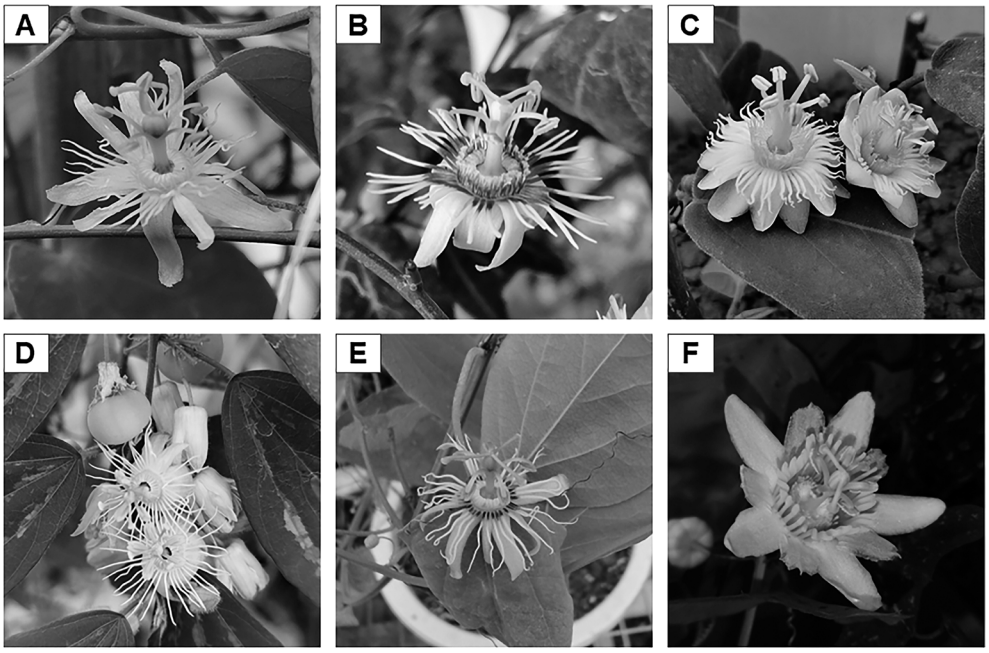

Fig. 1. Flower of studied *Passiflora*. (**A**) *P. menghaiensis*; (**B**) *P. xishuangbannaensis*; (**C**) *P. siamica*; (**D**) *P. altebilobata*; (**E**) *P. henryi*; (**F**) *P. papilio.*

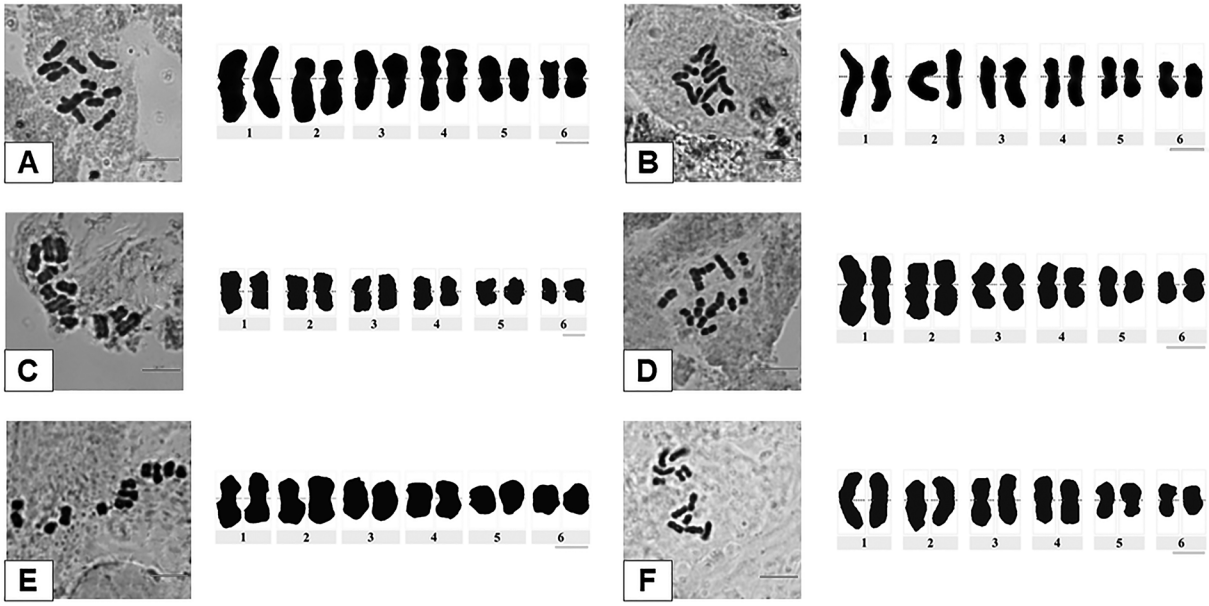

Fig. 2. Karyotypes during mitotic metaphase of six *Passiflora* species. (**A**) *P. menghaiensis*; (**B**) *P. xishuangbannaensis*; (**C**) *P. siamica*; (**D**) *P. altebilobata*; (**E**) *P. henryi*; (**F**) *P. papilio*. In the karyotype figure, scale bar=5 μm. In the ordered karyotype figure, scale bar=2 μm.

evolutionary significance in *Passiflora*.

Materials and methods

Sample collection

Passiflora menghaiensis X.D.Ma, L.C.Yan & J.Y.Shen, *P. xishuangbannaensis* Krosnick, *P. siamica* Craib, *P. altebilobata* Hemsl., *P. henryi* Hemsl. and *P. papilio* Li were collected from the nursery of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, longitude 101°25′E and latitude 101°25′N, which has a tropical monsoon type climate (Fig. 1).

Karyotype preparation

Young healthy shoots were collected and placed on moist soil for rooting. When the roots grew to 5–10 cm long, 1–2 cm long tips were cut and placed in 0.2 mol L^{-1} colchicine for 5–6 h at 4°C. The roots were then fixed for 6–24 h in Carnoy's fixation solution (glacial acetic acid : anhydrous ethanol=1 : 3). The experimental materials were then rinsed and transferred to 1 M HCl for 3–8 min. The samples were dyed with carbol fuchsin

solution, placed on a glass slide and pressed under the cover slide using the tableting method before observation under a microscope (Nicon ECLIPSE Ci-L and Oplenic Digital Camera PSC603-20S).

Chromosome number and karyotype study

Five cells during the mitotic phase and with clear chromosomes spreads were selected for karyotype analysis (Li and Chen 1985). The homologous chromosomes were paired by the automatic karyotype analysis system Beckman Beion 4.20/Multisizer, and the lengths of the long and short arms of the chromosomes were measured (Fig. 2). According to the Levan's method (Levan *et al.* 1964), the relative length and arm ratio (r) of chromosomes were calculated, and the chromosome types were described. Karyotype analysis was carried out according to Li and Chen (1985), and the karyotype asymmetry coefficient (As.k%) was calculated (Arano 1964). Relative chromosome length (RL%), arm ratio (L/S), centromeric indices (CI%), coefficient of relative length (CR%) (Table 1), total form percent (TF%), mean centromeric asymmetry (M_{CA}) of each pair of chromosomes were also measured (Paszko 2006). Homologous chromosome pairs were adjusted according to sizes, and the karyotypes were drawn (Table 2) (Guo *et al.* 2022, Zarabizadeh *et al.* 2022). In order to study the phylogenetic relationship among species a cluster analysis of the karyotype similarity coefficients was carried out using the UPGMA method (Ergin *et al.* 2022, Tan and Wu 1993). Fifteen karyotype characters; relative length, range and variance of relative length, centromere index, average and variance of centromere index, arm ratio, average and variance of arm ratio, average centromere asymmetry, karyotype asymmetry coefficient, total morphological percentage, presence of satellites, chromosome number and the karyotype resemblance-near coefficient, were used to calculate the similarity coefficients of the above several species (Li *et al.* 2022).

Results and discussion

Photographs of the karyotypes are provided for the first time for all six species. All the species are diploid with a basic chromosome number of x=6 (Fig. 3). *P. menghaiensis* karyotype formula is $2n$=$2x$=12=8m+2sm+2sm(sat). Four pairs of chromosomes are metacentric, and two pairs are submetacentric, of which the 7th and 8th chromosomes have satellites. The relative length of the chromosome is 12.46–23.09%, the average arm ratio is 1.43, the range of the arm ratio is 1.01–2.08, the centromeric index is 32.51–49.64%, and the karyotype asymmetry coefficient As.K%=57.96%, indicating a 2A karyotype.

P. xishuangbannaensis karyotype formula is $2n$=$2x$=12=8m+4sm. Four pairs of chromosomes are metacentric, and two pairs are submetacentric. The relative length of the chromosomes was 11.88–21.86%, the average arm ratio was 1.51, the range of the arm ratio was 1.21–2.15, the centromere index was 31.76–45.25%, and the karyotype asymmetry coefficient was 59.89%, indicating a 2A karyotype.

P. siamica karyotype formula is $2n$=$2x$=12=10m+2sm. Five pairs of chromosomes are metacentric, and

Table 1. Karyomorphological results of six species of *Passiflora*.

Species	Chromosome pair	RL (%)	L/S	CI (%)	CR (%)	Type
P. menghaiensis	I	23.09	1.32	43.04	1.39	m
	II	18.46	2.08	32.51	1.11	sm
	III	17.82	1.14	46.77	1.07	m
	IV	14.26	1.94	34.01	0.86	sm(sat)
	V	13.91	1.07	48.37	0.84	m
	VI	12.46	1.01	49.64	0.75	m
P. xishuangbannaensis	I	21.86	2.15	31.76	1.31	sm
	II	19.32	1.23	44.91	1.16	m
	III	17.14	1.25	44.40	1.03	m
	IV	16.18	1.72	36.73	0.97	sm
	V	13.62	1.21	45.25	0.82	m
	VI	11.88	1.49	40.20	0.71	m
P. siamica	I	19.38	1.94	34.06	1.16	sm
	II	18.20	1.59	38.63	1.09	m
	III	18.72	1.22	45.05	1.12	m
	IV	16.94	1.22	45.08	1.02	m
	V	14.76	1.09	47.88	0.89	m
	VI	12.00	1.09	47.79	0.72	m
P. altebilobata	I	20.76	1.96	33.76	1.25	sm
	II	19.82	1.28	43.94	1.19	m
	III	17.30	1.10	47.60	1.04	m
	IV	16.14	1.38	42.07	0.97	m
	V	13.64	1.19	45.73	0.82	m
	VI	12.34	1.09	47.80	0.74	m
P. henryi	I	23.00	1.53	39.59	1.38	m
	II	20.78	1.83	35.37	1.25	sm
	III	17.68	1.84	35.26	1.06	sm
	IV	14.90	1.12	47.08	0.89	m
	V	12.80	1.17	46.06	0.77	m
	VI	10.84	1.13	46.86	0.65	m
P. papilio	I	20.18	1.10	47.71	1.21	m
	II	18.34	1.10	47.58	1.10	m
	III	17.36	1.15	46.55	1.04	m
	IV	16.14	1.13	47.01	0.97	m
	V	14.72	1.28	43.80	0.88	m
	VI	13.26	1.23	44.80	0.80	m

RL (%): Relative chromosome length; L/S: Arm ratio; CI (%): Centromeric indices; CR (%): Coefficient of relative length. m: Metacentric chromo-some; sm: Submetacentric chromosome; sat: Satellite. Satellites are not included in chromosome length calculations.

Table 2. Karyotype results of six species of *Passiflora*.

Species	$2n$	Ploidy level	Karyotype formula	As.k (%)	TF (%)	M_{CA}	K
P.menghaiensis	12	2x	8m+2sm+2sm(sat)	57.96	42.04	0.16	2A
P. Xishuangbanna ensis	12	2x	8m+4sm	59.89	40.11	0.20	2A
P. siamica	12	2x	10m+2sm	57.50	42.50	0.15	1A
P. altebilobata	12	2x	10m+2sm	57.12	42.88	0.14	1A
P. henryi	12	2x	8m+4sm	59.32	40.68	0.19	1B
P. papilio	12	2x	12m	53.59	46.41	0.07	1A

As.k (%): Asymmetrical karyotype coefficient; TF (%): Total form percent; M_{CA}: Mean centromeric asymmetry; K: Karyotype.

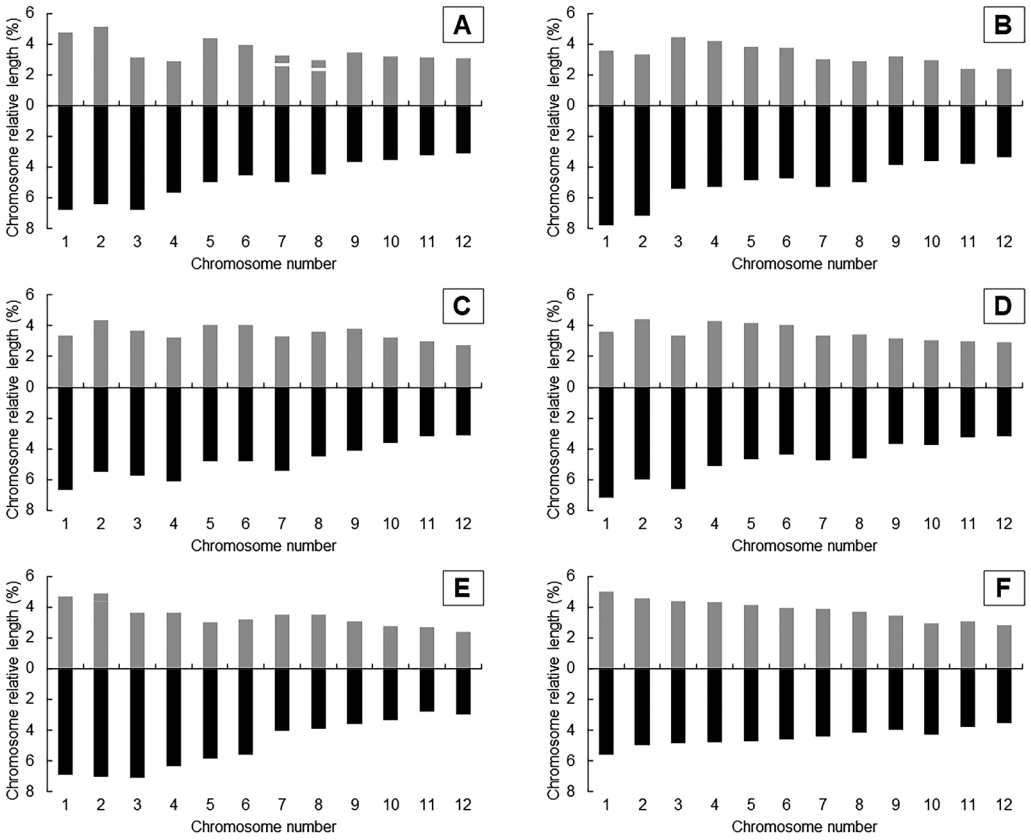

Fig. 3. Haploid idiograms of *Passiflora*. (**A**) *P. menghaiensis*; (**B**) *P. xishuangbannaensis*; (**C**) *P. siamica*; (**D**) *P. altebilobata*; (**E**) *P. henryi*; (**F**) *P. papilio.*

one pair is submetacentric. The relative length of the chromosomes was 12.00–19.38%, the average arm ratio was 1.36, the range of the arm ratio was 1.09–1.94, the centromere index was 34.06–47.88%, the karyotype asymmetry coefficient was 57.50%, indicating a 1A karyotype.

P. altebilobata karyotype formula is $2n=2x=12=10m+2sm$. Five pairs of chromosomes are metacentric, and one pair is submetacentric. The relative length of the chromosomes was 12.34–20.76%, the average arm ratio was 1.33, the range of the arm ratio was 1.09–1.96, the centromere index was 33.76–47.80%, the karyotype asymmetry coefficient was 57.12%, indicating a 1A karyotype.

P. henryi karyotype formula is $2n=2x=12=8m+4sm$. Four pairs of chromosomes are metacentric, and two pairs are submetacentric. The relative length of the chromosomes was 10.84–23%, the average arm ratio was 1.44, the range of the arm ratio was 1.12–1.84, the centromere index was 35.26–47.08%, and the karyotype asymmetry coefficient was 59.32%, indicating a 1B karyotype.

P. papilio karyotype formula is $2n=2x=12=12m$. All six pairs of chromosomes are metacentric. The relative length of the chromosomes was 13.26–20.18%, the average arm ratio was 1.16, the range of the arm ratio was 1.10–1.28, the centromere index was 43.80–47.71%, and the karyotype asymmetry coefficient was 53.59%, indicating a 1A karyotype. The karyotype data of 9 passion flower species, including six native passion flower from China and three exotic species *P. ferruginea*, *P. micropetala* and *P. coriacea* (Ferreira de Melo *et al.* 2014) was used. *P. henryi* and *P. xishuangbannaensis* as well as *P. ferruginea* and *P. papilio* seem more closely related but no clear pattern could be observed (Fig. 4).

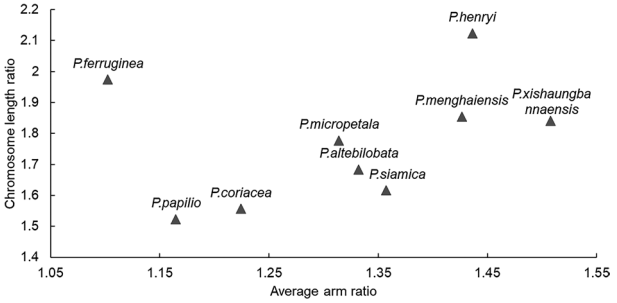

Fig. 4. Scatter plot of karyotype asymmetry of nine species of *Passiflora*.

According to the results of the UPGMA clustering, the nine species of passion flower were divided into four clades (Fig. 5). Clade I including *P. ferruginea*. Clade II including *P. xishuangbannaensis*. Clade III including *P. menghaiensis, P. siamica, P. altebilobata* and *P.henryi*. Clade IV including *P. coriacea, P. micropetala* and *P. papilio*. Among which *P. ferruginea* and *P. papilio* had the lowest degree of homology.

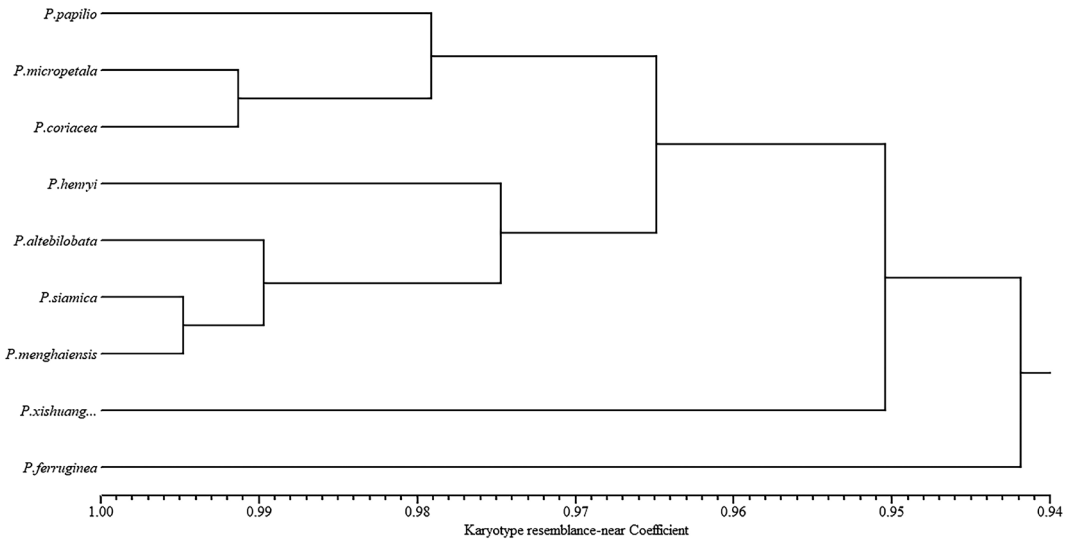

Fig. 5. Cluster analysis of morphological parameters of chromosomes of nine species of *Passiflora*. *P. xishuang* means *P. xishuangbannaensis.*

The number of passion flower species with known chromosome number and ploidy level is less than 30% (Meletti *et al.* 2005). Among the few species studied, *Passiflora pentagona* Mast. chromosome number is 2*n*=24 (De Melo *et al.* 2001); *P. caerulea* L., *P. nitida Kunth, P. elegans* Mast., *P. actinia* Hook., and *P. ligularis* Juss. have a chromosome number of 2*n*=18 (Beal 1971); *P. aurantia* G. Forst., *P. warmingii Masters, P. capsularis* L. and *P. rubra* (Beal 1971), have a chromosome number of 2*n*=12. This study has shown that *P. menghaiensis, P. xishuangbannaensis, P. siamica, P. altebilobata, P. henryi* and *P. papilio* have a chromosome number of 2*n*=12. This conclusion is consistent with recent publication on the phylogeny of *Passiflora* (Krosnick *et al.* 2013).

From the scatter plot, it can be seen that *P. henryi* and *P. xishuangbannaensis* are sister to the rest of the species, whereas *P. altebilobata* and *P. papilio* are more derived (Fig. 4).

Decaloba and *Passiflora* are the two subgenera with the most species in the *Passiflora* genus. Of the six species studied here, *P. xishuangbannaensis, P. siamica, P. altebilobata* and *P. henryi* in subgenus *Decaloba* have submetacentric chromosomes. *P. coccinea* and *P. bahiensis* in subgenus *Passiflora* also have submetacentric chromosomes (Ulmer and Macdougal 2004). Submetacentric chromosomes were found in species of both subgenera, suggesting that sm chromosomes are not restricted to a specific subgenus. *P. menghaiensis* have satellites. Satellites were found in several other species within *Decaloba*, which indicates that the number of satellites is not directly related to the number of basic chromosomes *x*=6 or *x*=9 (Melo *et al.* 2001).

Most edible passion flower varieties available for cultivation in the market, are the offspring of the cross breeding between purple-fruited passion flower and yellow-fruited passion flower, with some varieties developing poor resistance to disease and reduced genetic diversity. Wild native species seem more resistant to disease than commercial cultivars, and some genes from wild relatives could be used to improve existing cultivars. Cytological studies of passion flower species could be beneficial for the use of wild species in breeding and crop improvement in order to increase cultivated varieties and resistance to diseases of widely cultivated crops.

Author contributions

Lizhu Qian was the main contributor to the article, participating in the entire process of experiments and writing. Sven Landrein participated in writing and editing the article. Chunhui Hao participated in experimental design. Fuchuan Wu provided the experimental materials and funding, and participated in the review of the article.

Acknowledgments

This study is gratefully supported by the selection and introduction of plants suitable for the dry-hot valley of the Jinsha River (E0HX051B01).

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