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月季种质资源的倍性变异及核型多样性研究

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摘　要:采用常规压片法对包括5个蔷薇野生种、8个古老月季及9个现代月季品种共22份月季种质资源进行核型分析。供试材料共有3种不同倍性:5个野生种中粉团蔷薇为三倍体(2n=3x=21),其他4个材料为二倍体(2n=2x=14);9个现代月季品种均为四倍体(2n=4x=28);而古老月季品种中包含二、三、四倍体3种倍性。22份材料中共发现4种不同类型的核型,在野生种中为1A、2A;古老月季中为1A、2A、1B;现代月季中为1A、1B、2B。另外,供试材料在核不对称系数、着丝粒指数、随体数目及位置、染色体相对长度组成等方面差异较大,说明在月季种质资源中存在着丰富的核型多样性。

关键词:月季;核型多样性;染色体;倍性

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Ploidy Variation and Karyological Diversity in Rose Germplasm

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Abstract: A karyological study of 22 *Rosa* taxa, including 5 wild species, 8 old garden roses and 9 modern cultivars, was performed using traditional squashing and pressing methods. There are three ploidy levels among the taxa, *R. multiflora* var. *cathayensis* is triploid with 21 chromosomes (2n=3x=21), the other wild taxa are diploid with 14 chromosomes (2n=2x=14), and the modern rose taxa are all tetraploid with 28 chromosomes (2n=4x=28), while the old garden roses contain all three ploidies. Four karyotypes (1A, 2A, 1B and 2B) have been found among the taxa, with 1A and 2A in wild species, 1A, 2A and 1B in old garden roses, and 1A, 1B and 2B in modern roses. Also, the studied taxa differ from each other in the asymmetry index, centromere index, satellite position and number and constitution of relative lengths. The results indicate that karyological diversity is abundant in *Rosa* germplasm resources.

Key words: rose; karyological diversity; chromosome; ploidy

The genus *Rosa* L. is comprised of about 200 species and 30 000 cultivated varieties[1-4], but only 7 species have contributed to modern roses: *R. chinenesis*, *R. gigantea*, *R. multiflora*, *R. moschata*, *R. wichuraiana*, *R. gallica* and *R. foetida*[5]. Old garden roses play a significant role in the breeding of modern roses[6]; however, these germplasm resources are not always sufficiently used.

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The genetic background of modern roses is relatively narrow compared to the abundant genetic diversity of wild species and old garden roses[7-8]. China was considered as the home of *Rosa*[6,9]. Between the late 18th century and early 19th century, wild species and old garden roses native to China were introduced into western countries, such as Great Britain and France. The European breeders used these materials to repeatedly hybridize with indigenous species and finally obtained 'La France' in 1867, the first Hybrid Tea rose. At present, more efficient breeding strategies are needed due to the increasing demand for new modern roses. Many species and old garden roses have desirable traits, such as superior disease resistance, higher fragrance levels, winter-hardiness and the absence of thorns[10-11]. Breeders can be innovative in the production of new varieties, provided that the desirable traits from wild species and old garden roses can be used in the breeding of modern roses[12].

Chromosomes, as the carriers of genes, dominate the reproductive process and breeding behavior of plants through their structure and behavior. Therefore, studies of the number, structure and cytological behavior of chromosomes are of great significance for understanding the genetics of plants. *Rosa* was among the first genera of garden flowers to attract the attention of cytologists and molecular biologists because of the importance of cultivated roses as horticultural plants, the small chromosome sizes and the small nuclear genomes[13-15]. Cytological studies in *Rosa* included chromosome numbers, karyotypes and meiotic configuration frequencies using traditional squashing and pressing methods[16-27], C-banding[28], FISH[29-32], chromosome doubling[33] and flow cytometry[34]. The genus *Rosa* is characterized by a typical polyploid chromosome series based on multiples of seven. The chromosome numbers of wild species range from 2n=2x=14 to 2n=10x=70[22,35-37], old garden roses are 2n=2x, 3x and 4x=14, 21 and 28, respectively[21,26,34,38], and the modern cultivars are mostly tetraploid[4].

In this study, 22 taxa from the genus *Rosa*, including 5 wild species, 8 old garden roses and 9 modern roses(Table 1), were analyzed using karyological techniques. The main aims of this study were: (1) detecting the role of wild species and old garden roses in modern rose breeding; (2) providing a cytological basis for directional hybridization and germplasm resource innovations in rose.

1 Material and methods

1.1 Plant materials

In total, 22 taxa, including 5 wild species, 8 old garden roses and 9 modern roses, growing in the rose germplasm resources nursery of Shenzhen Park Service(Guangdong, China) were selected for this study(Table 1). Among the nine modern roses, five miniature roses with unknown names were recorded as M_1, M_2, M_3, M_4 and M_5 in this paper. 'Mount Shasta × Kosai' and 'Charles de Gaull × Kardinal 85' were the F_1 of a hybridization, and 'Fragrant Butterfly' was bred by crossing climber 'Crimson Glory' with 'May Day'. The other plants were observed to confirm their identities using morphological traits and literary descriptions[2,4,11]. Tender branch cuttings were used in this study.

1.2 Pretreatment, dissociation and staining

All cytological observations were made from root tips. In the middle of April, appropriate numbers of central branches from each variety(species) were selected and cut into pieces(5－8 cm), and then the stem segments were cultivated on rice husk ash. Branches were immersed into 150 mg/L indolebutyric acid for 2－3 h to improve the rooting rate. Shoot tips were collected when the roots grew to 1－2 cm. They were then pretreated in 0.002 mol/L 8-hydroxyquinoline for 4 h at 20 ℃, then fixed with Carnoy's fluid(absolute ethyl alcohol:acetic acid=3∶1) at 4 ℃ for 24 h. The fixed tips were dissociated with 5 mol/L HCl for 30 min at room temperature, stained with carbol fuchsin for 2 h and squashed on glass slides for observation. Photos were taken under a Nikon E800 microscope(NIKON JAPAN).

1.3 Data analysis

Observations were made on nuclei at the somatic mitotic metaphase, and measurements of chromosome arms were taken from at least 30 well-spread metaphases of 5 root tips. The standards of Levan *et al*.[39] and Li and Chen[40] were adopted for the analysis of the relative lengths, arm ratios and chromosome patterns. The karyotype symmetry was in accordance with the classification of Stebbins[41]. The asymmetry index was calculated according to Arano[42] and the constitution of relative length of the genome was calculated according to Kuo *et al*.[43]. Ikaros software was used to analyze the chromosomes.

2 Results and analysis

The metaphase chromosomes, karyotypes and karyotype pattern for each studied material are shown in Fig. 1 and 2, respectively. Karyotype information for the studied materials are listed in Table 2.

2.1 Wild species

The five wild species studied were all diploid (2n=2x=14), except *R. multiflora* var. *cathayensis* (2n=3x=21), and all of the chromosomes of *R. multiflora* and *R. bracteata* contained median centromeres (2n=2x=14=14m), while the other four species consisted of median and sub-median centromeric chromosomes. The genome of *R. bracteata* was composed of all four types of chromosomes. Those of *R. multiflora* and *R. odorata* were composed of long chromosomes (L), medium long chromosomes (M_2) and medium short chromosomes (M_1), but not short chromosomes (S). The genome of *R. multiflora* var. *catheyensis* was composed of medium long chromosomes (M_2), medium short chromosomes (M_1) and short chromosomes (S), while that of *R. laevigata* was composed of only medium long chromosomes (M_2) and medium short chromosomes (M_1). Asymmetry indices ranged from 57.57% to 62.75%, and the ratio of the longest to the shortest chromosome varied from 1.60 to 1.85. The karyotype of *R. multiflora* var. *cathayensis* was 2A, and the rest were 1A.

Table 1　*Rosa* L. materials used in this study

Code	Taxon	Reference	Ploidy	Karyotype	Colour	Category
1	*R. multiflora* Thunb. var. *mutiflora*	Jian *et al*[24]	2x	2n= 2x=14=14 m	White	Wild species
2	*R. multiflora* Thunb. var. *cathayensis* Rehd. et Wils	Liu *et al*[16]	2x,4x	No report	Rosy pink	Wild species
3	*R. laevigata* Michx. var. *laevigata*	Hurst[35], Jian *et al*[24]	2x	2n=2x=14=10m+4sm	White	Wild species
4	*R. bracteata* Wendl. var. *bracteata*	Darlington *et al*[36], Jian *et al*[24]	2x	2n=2x=14=10m+4sm	White	Wild species
5	*R. odorata* (Andr.) Sweet	Hurst[35], Jian *et al*[23]	2x	2n=2x=14=12m+2sm	Light pink	Wild species
6	Chunshui Lübo	Jian *et al*[21]	3x	2n=3x=21=18m+3sm	White	Chinese old garden rose
7	Dafugui	Jian *et al*[21]	4x	2n=4x=28=26m+2sm	Dark pink	Chinese old garden rose
8	Huzhongyue	Jian *et al*[21]	3x	2n=3x=21=18m+3sm	Pink	Chinese old garden rose
9	Lü e(*R. chinensis* var. *viridiflora*)	Jian *et al*[21]	2x	2n=2x=14=12m+2sm	Green	Chinese old garden rose
10	Qinglian Xueshi	Zhang *et al*[38]	3x	2n=3x=21=13m+8sm	Lavender	Chinese old garden rose
11	Simianjing	Zhang *et al*[38]	3x	2n=3x=21=15m+6sm	Red	Chinese old garden rose
12	Yulinglong	Jian *et al*[21]	2x	2n=2x=14=12m+2sm	Light pink	Chinese old garden rose
13	Zihongxiang	Zhang *et al*[38]	4x	2n=4x=28=16m+12sm	Purple	Chinese old garden rose
14	M_1	No report		No report	White	Miniature rose
15	M_2	No report		No report	Red	Miniature rose
16	M_3	No report		No report	Pink	Miniature rose
17	M_4	No report		No report	Yellow	Miniature rose
18	M_5	No report		No report	Scarlet	Miniature rose
19	Mount Shasta×Kosai	No report		No report	Orange red	F_1 of hybridization
20	Charles de Gaull×Kardinal 85	No report		No report	Scarlet	F_1 of hybridization
21	Crimson Glory(Cl)	No report		No report	Dark red	Climber rose
22	Fragrant Buterfly	No report		No report	Pink blend	Climber rose

2.2 Chinese old garden roses

Marked differences in chromosome numbers were found among the eight Chinese old garden roses. 'Lüe' (*R. chinensis* var. *viridiflora*), 'Yuling-

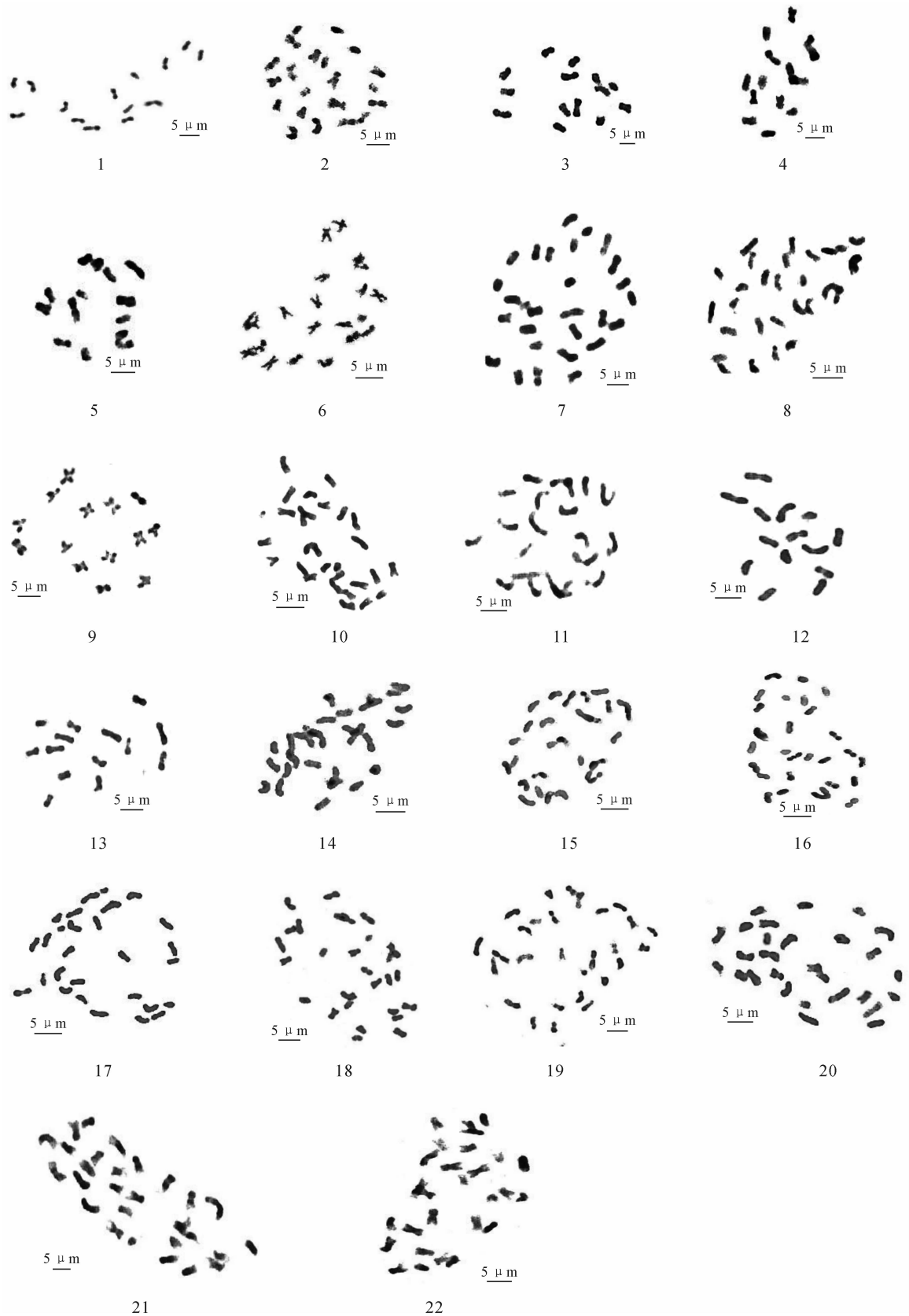

Fig. 1 Metaphase chromosome morphology of 22 *Rosa* L. materials

The numbers are provided in Table 1 next to the material names; The same as below

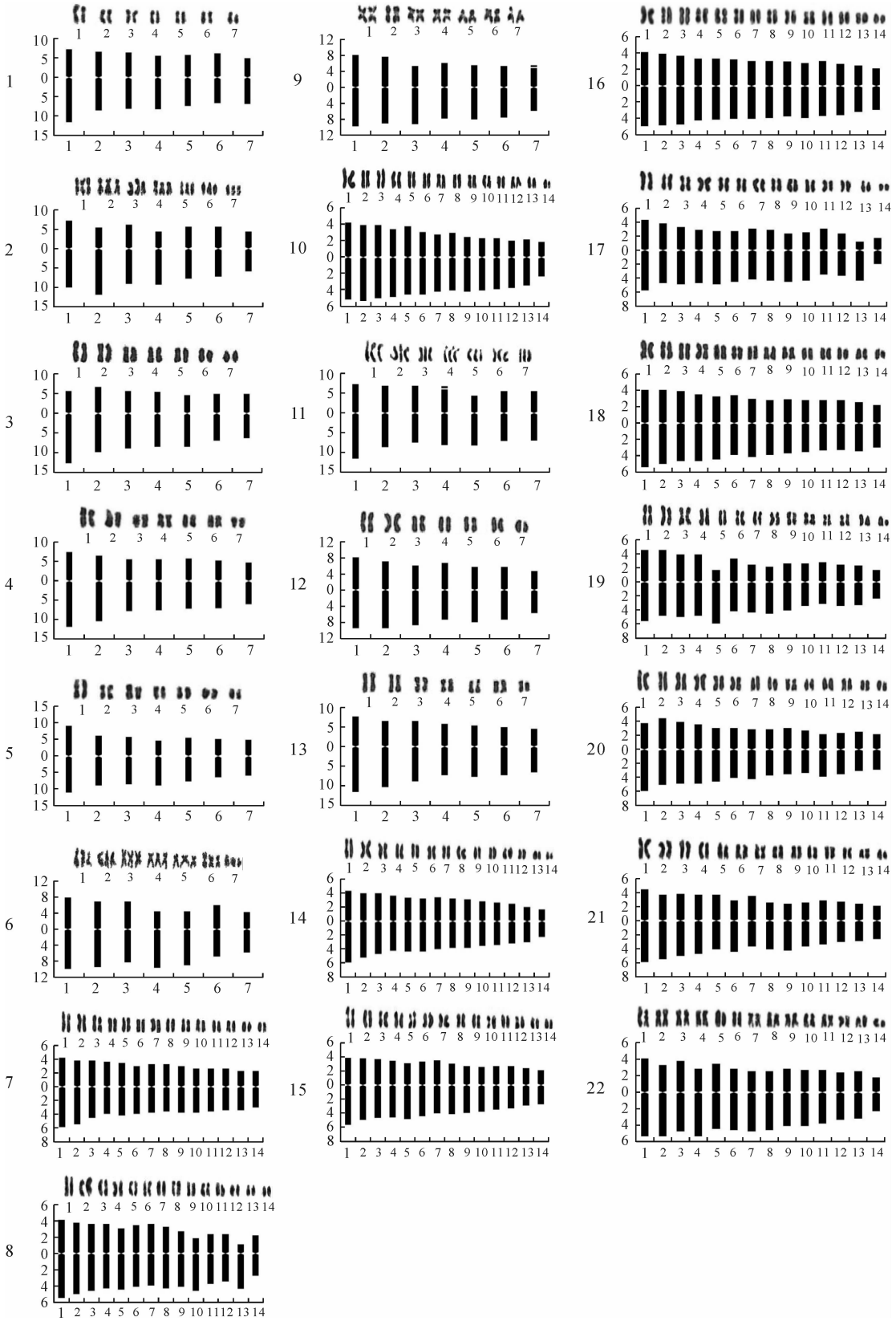

Fig. 2　Karyotypes and karyotype models of the 22 *Rosa* L. materials

The chromosomes of the tetraploid materials were paired in the same way as the diploid materials because it is unknown whether they are homologous or heterogenous

Table 2 Karyological characteristics of the materials

Code[a]	Karyotype formula	CRL[b]	As. K%[c]	Lt/St[d]	Karyotype
1	2n=2x=14=14m	$2L+4M_2+8M_1$	57.57	1.60	1A
2	2n=3x=21=15m+6sm	$6M_2+6M_1+2S$	61.91	1.68	2A
3	2n=2x=14=10m+4sm	$6M_2+8M_1$	62.75	1.65	1A
4	2n=2x=14=14m	$2L+2M_2+8M_1+2S$	58.13	1.78	1A
5	2n=2x=14=12m+2sm	$2L+4M_2+8M_1$	58.29	1.85	1A
6	2n=3x=21=15m+6sm	$9M_2+9M_1+3S$	58.67	1.75	2A
7	2n=4x=28=28m	$4L+6M_2+16M_1+2S$	56.33	1.89	1A
8	2n=4x=28=24m+2sm+2st	$2L+14M_2+10M_1+2S$	58.96	1.93	2A
9	2n=2x=14=12m(2SAT)+2sm	$6M_2+6M_1+2S$	57.21	1.66	1A
10	2n=4x=28=24m+4sm	$4L+8M_2+14M_1+2S$	58.91	2.22	1B
11	2n=3x=21=18m(3SAT)+3sm	$3L+3M_2+15M_1$	57.63	1.51	1A
12	2n=2x=14=14m	$6M_2+6M_1+2S$	56.04	1.68	1A
13	2n=2x=14=14m	$2L+4M_2+8M_1$	58.94	1.72	1A
14	2n=4x=28=28m	$4L+10M_2+10M_1+4S$	55.84	2.59	1B
15	2n=4x=28=28m	$2L+12M_2+10M_1+4S$	57.16	1.96	1A
16	2n=4x=28=28m	$2L+10M_2+14M_1+2S$	55.72	1.78	1A
17	2n=4x=28=22m+4sm+2st	$2L+14M_2+10M_1+2S$	60.28	2.76	2B
18	2n=4x=28=28m	$2L+10M_2+14M_1+2S$	55.94	1.81	1A
19	2n=4x=28=22m+4sm+2st	$4L+8M_2+14M_1+2S$	58.88	2.50	2B
20	2n=4x=28=26m+2sm	$4L+6M_2+16M_1+2S$	57.60	1.90	1A
21	2n=4x=28=26m+2sm	$4L+8M_2+12M_1+4S$	56.88	2.19	1B
22	2n=4x=28=22m+6sm	$2L+12M_2+12M_1+2S$	59.37	2.30	1B

Note: a. The code was provided in Table 1 next to the material names; b. CRL constitution of relative length; c. As. K% asymmetry index; d. Lt/St ratio of the longest to shortest chromosome; The length of satellites was not included in the chromosome length.

long' and 'Zihongxiang' are diploid with 14 chromosomes (2n = 2x = 14), 'Chunshui Lübo' and 'Simianjing' are triploid with 21 chromosomes (2n =3x=21), 'Dafugui', 'Huzhongyue' and 'Qinglian Xueshi' are tetraploid with 28 chromosomes (2n =4x=28). The genomes of 'Chunshui Lübo' and 'Yulinglong' were composed of three types of chromosomes, medium long chromosomes (M_2), medium short chromosomes (M_1) and short chromosomes (S). Those of 'Simianjing' and 'Zihongxiang' were composed of long chromosomes (L), medium long chromosomes (M_2) and medium short chromosomes (M_1). The genomes of the other cultivars were composed of all four types. Asymmetry indices ranged from 56.04% to 58.96%, and the ratio of the longest to the shortest chromosome varied from 1.66 to 2.22. Satellites were found in the seventh chromosome in 'Lü e' and the fourth chromosome in 'Simianjing'. One pair of sub-terminal centromeric chromosomes were found in 'Huzhongyue' (2n = 4x = 28 = 24m + 2sm + 2st), whereas the other cultivars all consisted of median and sub-median centromeric chromosomes. The karyotype of 'Qinglian Xueshi' was 1B, those of 'Chunshui Lübo' and 'Huzhongyue' were 2A, and the rest were 1A.

2.3 Modern roses

The nine modern roses were all tetraploid with 28 chromosomes (2n = 4x = 28). Genomes of 'M_4' and 'Mount Shasta × Kosai' were composed of median, sub-median and sub-terminal centromeric chromosomes, and they shared a karyotype formula (2n = 4x = 28 = 22m + 4sm + 2st). The chromosomes of the other four miniature roses contained median centromeres (2n = 4x = 28 = 28m). The other three cultivars were composed of median and sub-median centromeric chromosomes. The genomes of these nine cultivars were comprised of all four types of chromosomes. Asymmetry indices ranged from 55.72% to 60.28%, and the ratio of the longest to the shortest chromosome varied from 1.78 to 2.76. The karyotypes of 'M_4' and

'Mount Shasta×Kosai' were 2B, 'M_1', 'Crimson Glory' and 'Fragrant Butterfly' were 1B, and the others were 1A.

3 Discussion

Li *et al*[44] noted that it was difficult to observe chromosomes using root somatic cells from *Rosa* because the roots regenerated from the cutting are thin and solid. Moreover, colchicine and aqueous solutions of saturated paradichlorobenzene were worth recommending for processing the materials. Shoot tips and aqueous solution of saturated paradichlorobenzene are often chosen for pretreatment when observing the chromosomes of *Rosa*[16,18-19,21-25,38]. However, in our experiment, we found that, it is also a good method to pretreat root tips regenerated from cuttings with 0.002 mol/L 8-hydroxyquinoline, coupled with proper sampling times and temperatures.

It is generally acknowledged that the basic chromosome of Rosoideae is x=7－9[45]. The first report that *Rosa* was x=7 was made by Täckholm in 1920[46]. Later, Hurst[35] performed a statistical analysis of the chromosome numbers of thousands of species, mutations and varieties, and achieved the same conclusion. On the basis of both arm ratio and chromosome length, the authors concluded that karyotypes of *Rosa* were largely symmetrical among the genus[18-27]. Our study confirmed those previous reports. The materials were composed of a basic chromosome number of seven. Also, the karyotypes of most of the studied materials contained median and sub-median centromeric chromosomes.

Many species occurred at only one ploidy level, but a few formed a polyploid series[3,24,36]. According to Liu and Li[16], there were diploids and tetraploids in *R. multiflora* var. *cathayensis* (2n=2x=14 or 2n=4x=28, respectively). In this study, where more than 30 cells were observed, however, the chromosome number of the genome was determined to be 2n = 3x = 21. This intraspecific polyploidy had been reported in *R. laxa*[27], several species (*R. macrophylla*, *R. sertata* and *R. webbiana*) of Section Cinnamomeae[24], *R. odorata* var. *erubescens*[23] and *R. platyacantha*[2,27]. Polyploidy has long been recognized as a prominent force in evolutionary diversification and is an important cytogenetic mechanism in plant evolution and rapid speciation[41]. Thus, a comprehensive study would require a greater number of population samples, representing all the variations and the full range of habitats. What is more, the results indicated that different ploidy levels were associated with the different regions and habitats where the germplasm resources grew[27].

The chromosomes of the tetraploid materials were paired in the same way as the diploid materials because it is unknown whether they are homologous or heterogenous. Different ploidy levels were also observed among three old garden roses. According to Jian *et al*[21] and Zhang *et al*[38], 'Huzhongyue' and 'Qinglian Xueshi' were triploid (2n=3x=21), and 'Zihongxiang' was tetraploid (2n=4x=28). In our study, 'Huzhongyue' and 'Qinglian Xueshi' were tetraploid, whereas 'Zihongxiang' was diploid (2n=2x=14). Further studies combining materials and cultivation locations are necessary to determine whether these results indicate a polyploidy of cultivars or another situation. Additionally, there were also several slight differences in karyotypes and karyotype formulae among some materials that had already been studied[21,26,38], which may be due to different degrees of chromosome contraction caused by the various conditioning fluids and/or pretreatment times

It is believed that wild species, old garden roses and modern roses exhibit karyological diversity and lack sub-terminal centromeric chromosomes[18-27]. Our study confirmed these beliefs. Four karyotypes (1A, 2A, 1B and 2B) were found among the accessions in our research, and all of the karyotypes contained median and sub-median centromeric chromosomes, except three accessions (sample no. 9, 18 and 20). Levizky[47] first suggested the concepts of karyotype symmetry and asymmetry, with the basic trend of karyotype evolution in plants progressing from symmetry to asymmetry[41]. The accessions used in this study were consistent with that view-

point. The karyotypes of wild species were 1A and 2A, old garden roses were 1A, 2A and 1B, and modern roses were 1A, 1B and 2B.

The ploidy levels in wild species ranged from 2n=2x=14 to 2n=10x=70[22,35-37]. However, genomes with more than a tetraploid chromosome number were rare in old garden roses and modern roses. Several authors predicted that high-ploidy hybrids must have emerged during the distant hybridization process and may have been discarded by breeders[16,48]. What the appearances of high-ploidy cultivars and whether they are worth selecting require further study in the future.

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