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Ploidy level of Chinese cherry (*Cerasus pseudocerasus* Lindl.) and comparative study on karyotypes with four *Cerasus* species

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ABSTRACT

Chinese cherry [*Cerasus pseudocerasus* (Lindl.) G. Don] is a fruit tree species within the family Rosaceae, with high economic and ornamental values. To verify the ploidy level of this species and compare the karyotypes with relative species, the chromosome number and karyotype characterization of representative wild and cultivated Chinese cherry samples from sixteen natural populations in four Provinces in China, and four *Cerasus* relative species were investigated. No aneuploids were found in all samples studied and no diploids or triploids were observed among Chinese cherry by root tips from both seedlings and softwood cutting. The chromosomes were quite small in size and mainly composed by median-centromere (m) and submedian-centromere (sm) chromosomes. Fourteen wild and cultivated Chinese cherry samples were all tetraploid with main karyotype formula of $2n = 4x = 32 = 28m + 4sm$, samples from Yingjing County as $2n = 5x = 40$ and Fengxian County as $2n = 6x = 48$, respectively. The ratio between the longest and shortest chromosome ranged from 2.05 to 2.32. All sixteen Chinese cherry samples had karyotypes of "2B" type. *Cerasus avium* (L.) Moench, *C. campanulata* (Maxim.) Yü et Li, *C. serrulata* G. Don var. *lannesiana* (Carr.), and *C. tomentosa* (Thunb.) Wall. were all diploid with $2n = 2x = 16$, whose karyotypes were "2A", "1A", "2B", and "1B" type, respectively. By comparison of the karyotype formula and characterization, we indicated that *C. pseudocerasus* showed close relationship with *C. serrulata* var. *lannesiana* rather than *C. avium*. The present study provided powerful important references for the confirmed ploidy level and the possible genome composition of *C. pseudocerasus*.

1. Introduction

Chinese cherry [*Cerasus pseodocerasus* (Lindl.) G. Don] is an important fruit tree species, belonging to the section *Lobopetalum*, subgenus *Cerasus*, genus *Cerasus* of the family Rosaceae (Yü and Li, 1986). It originates from Southwest China, which is widely distributed in the temperate zone of North Hemisphere, especially on sunny mountain slops or on the sides of ravines (Li and Bartholomew, 2003; Chen et al., 2016). As one of the four economically important cultivated cherry species: Chinese cherry (*C. pseudocerasus*), sweet cherry (*C. avium*), sour cherry (*C. vulgaris*), and downy cherry (*C. tomentosa*), the cultivation history can be date back to 3000–4000 years ago (Liu and Liu, 1993; Dong and Liu, 2008). During subsequent cultivation, rooting sucker and scion grafting gradually became two main reproduction modes, and a large number of desirable lines/individuals were selected out and maintained indefinitely in landraces, leading to locally adapted populations (Chen et al., 2016). Chinese cherry has also long been used as the rootstock for sweet cherry ever since the latter's introduction into China (Zhang and Gu, 2016). In general, *C. serrulata* is usually chosen as the rootstock for Chinese cherry. These facts indicate that Chinese cherry is likely to be closely related to these *Cerasus* relative species.

In the previous studies, the chromosome number and karyotype characterization of *C. pseudocerasus* and *Cerasus* relative species have been rarely reported (Oginuma, 1987a,b; Wang et al., 1992; Chen et al., 1993; Gu et al., 2014). The basic number of this genus was proved to be $x = 8$. Wang et al. (1992) reported Chinese cherry as a tetraploid by root tips from seedlings, while Chen et al. (1993) found that Chinese cherry collected from Changli County, He'nan Province had both diploid and tetraploid. Oginuma (1987a,b) and Gu et al. (2014) suggested that diploid, triploid and tetraploid all existed within *C. pseudocerasus* populations by seed germination. What we felt confused that *C. pseudocerasus* was tetraploid or had various ploidy levels from diploid to

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tetraploid. If it was tetraploid, what was the chromosomal composition? It was hard to judge whether or not the tetraploid *C. pseudocerasus* was of the autopolyploid or segmental allopolyploid or amphidiploid from its meiotic chromosome configurations at metaphase I (Oginuma, 1988). These questions were not resolved yet based on above studies. Therefore, it is necessary to focus on comprehensive wild and cultivated samples from different natural populations in China by using root tips from both seed germination and softwood cutting. We believe that it is helpful to deeply understand the origin of Chinese cherry if we clarify these key issues.

Here we used wild and cultivated Chinese cherry resources from sixteen populations in four Provinces in China, and four *Cerasus* relative species to conduct chromosome number and karyotype analyses. Our objectives are (i) to determine the ploidy level of *C. pseudocerasus*; (ii) to analyze the genetic relationship between this species and four relative species by comparing the karyotype characterization; and (iii) to try to explore the chromosomal homology and origin of polyploid Chinese cherry. This study will provide cytological evidence for the confirmed ploidy level and the possible genome composition of *C. pseudocerasus*.

2. Materials and methods

2.1. Plant materials

We used twenty representative samples including *C. pseudocerasus*, *C. avium*, *C. campanulata*, *C. serrulata* var. *lannesiana*, and *C. tomentosa* for this study. The former four species are assigned into four sections of subgen. *Cerasus*, while the latter one belongs to sect. *Amygdalocerasus* of subgen. *Microcerasus* (Yü and Li, 1986). For Chinese cherry, four wild accessions were collected from Sichuan and Shaanxi Provinces, and twelve cultivated accessions from Sichuan, Chongqing, He'nan and Shaanxi Provinces, respectively. Their detail information was listed in Table 1.

2.2. Chromosome preparation

Chromosome preparation and description were followed procedures of Wang et al. (Wang et al., 2010). Chromosome observations were made from adventitious root tip cells produced from canes of these populations. Using cutting propagation technique, canes with 3–4 nodes, without being induced by any rooting agents, were directly cuttinged in vermiculite media in plastic greenhouse where more than 80% humidity and about 25 °C temperature environments provided. Also, some root tips were from seed germination or tissue culture. About three weeks later, adventitious roots were harvested and pretreated in 0.002 mol L^{-1} 8-hydroxyquinoline at 4 °C for 24 h, and then fixed in Carnoy solution (absolute alcohol: acetic acid, 3:1, v/v) at 4 °C for 24 h. The fixed root tips were hydrolyzed in 1 mol L^{-1} HCl at 60 °C for 35–45 s, stained with Carbol Fuchsin, and squashed for viewing under magnification.

2.3. Karyotype analysis

The karyotypes of somatic chromosomes at metaphase were determined by measuring ten well-spread metaphases from five different root tips. Chromosome types, median centromeric (m) and submedian centromeric (sm), were defined as arm ratios of 1.0–1.7 (m) and 1.7–3.0 (sm), respectively (Levan et al., 1964). Following parameters were calculated: chromosome number (counter in each cell), MAR (mean of long arm length/short arm length ratio), and karyotypic formula referred to the standard of Levan et al. (1964); PCA (percentage of chromosomes with an arm ratio > 2 in chromosome set), As.K (index of the karyotypic asymmetry: total long arm length in chromosome set/total chromosome length in chromosome set), and karyotype (the classification of karyotype in relation to their degree of asymmetry) followed Stebbins' standard (1971) (Table 2).

3. Results

3.1. Chromosome number of Chinese cherry and Cerasus relative species

The metaphase chromosome size and morphology, and karyotypes of twenty samples were shown in Figs. 1, 2 and 3. Detailed parameters and karyotype formula were listed in Table 3. Fourteen Chinese cherry were all tetraploid with $2n = 4x = 32$ by using root tips from seed germination and softwood cutting (Figs. 1–2, A–N). The representative individual from Yingjing, Sichuan, and Fengxian, Shaanxi was

Table 1
The locality and chromosome number of Chinese cherry samples from sixteen natural populations in four Provinces and four *Cerasus* relative species.

Code	Locality	Altitude (m), Latitude (N)/Longtitude (E)	Chromosome number	Figs. 1–3
Cerasus pseudocerasus (Lindl.) G. Don				
Wild				
1	Beichuan, Sichuan	1000, 32°00.030′/104°38.578′	$32^{\alpha,\beta}$	A
2	Foping, Shaanxi	1782, 33°43.747′/107°58.331′	32^{α}	B
3	Qingxi, Sichuan	1285, 32°24.099′/104°50.035′	32^{α}	C
4	Yucheng, Sichuan	828, 29°54.256′/103°01.463′	32^{β}	D
Cultivated				
5	Fuling, Chongqing	390, 29°41.460′/107°23.038′	$32^{\alpha,\beta}$	E
6	Jianyang, Sichuan	677, 30°28.499′/104°18.219′	32^{α}	F
7	Luding, Sichuan	1366, 29°53.273′/102°12.990′	32^{α}	G
8	Luding, Sichuan	1311, 29°53.268′/102°13.164′	32^{α}	H
9	Luoyang, He'nan	263, 89°02.732′/110°06.303′	$32^{\alpha,\gamma}$	I
10	Miyi, Sichuan	2134, 27°00.821′/102°02.739′	32^{α}	J
11	Pengzhou, Sichuan	728, 30°98.052′/103°93.735′	32^{α}	K
12	Shimian, Sichuan	854, 29°23.420′/102°37.532′	32^{α}	L
13	Yucheng, Sichuan	615, 29°58.521′/102°57.258′	32^{β}	M
14	Yucheng, Sichuan	585, 30°05.018′/103°08.317′	32^{β}	N
15	Yingjing, Sichuan	459, 29°08.362′/102°85.176′	$40^{\alpha,\beta}$	O
16	Fengxian, Shaanxi	1093, 33°54.938′/106°30.740′	48^{α}	P
Relative species				
17 *C. avium* (L.) Moench	Sichuan Agricultural University		16^{β}	Q
18 *C. campanulata* (Maxim.) Yü et Li	Sichuan Agricultural University		16^{β}	R
19 *C. serrulata* G. Don var. *lannesiana* (Carr.)	Sichuan Agricultural University		16^{β}	S
20 *C. tomentosa* (Thunb.) Wall.	Sichuan Agricultural University		16^{β}	T

The classification system was followed by Yü and Li (1986). α, β, γ represented root tips from seed germination, softwood cutting, and tissue culture, respectively.