Chromosome Numbers and Ploidy Levels of Chinese *Curcuma* Species

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Abstract. *Curcuma* L. is an economically important genus in the family Zingiberaceae. Many species are grown as medicinal, culinary, and ornamental crops. As a result of their high morphological diversity and small chromosome sizes, chromosome numbers and species relationships of Chinese *Curcumas* remain debated. This study examined chromosome numbers of 15 populations representing 11 species of *Curcuma* from China. Results showed that only *Curcuma flaviflora* S. Q. Tong was diploid with $2n = 2x = 42$ and *C. kwangsiensis* S. G. Lee & C. F. Liang was tetraploid with $2n = 4x = 84$. The other species were triploid ($2n = 3x = 63$). The study indicated that the basic chromosome number of *Curcuma* from China could be $x = 21$. The diploid *C. flaviflora* produced viable seeds, which was the main means for propagation. The tetraploid and the triploids produced no seeds and relied on rhizomes for propagation. Chromosome sizes of all species were small, ranging from 0.5 to 2.1 μm , which prevented karyotype analysis. The fact that nine of 11 species studied were triploid indicates that triploidy may have some type of competitive advantage over the diploid and tetraploid. In addition, the triploids are popular commercially because of abundant rhizome production and this may contribute to their wide distributions.

The genus *Curcuma* L. is a member of the family Zingiberaceae consisting of ≈ 80 species distributed mainly in South and Southeast Asia with outposts in India and southern China (Larsen et al., 1998). Some species are also found in Australia and the South Pacific (Wu and Larsen, 2000). *Curcuma* species are important specialty crops produced as medicinal, culinary, and ornamental plants (Chen and Xia, 2011). For example, *C. aromatica* Salisb., *C. kwangsiensis* S. G. Lee & C. F. Liang, *C. phaeocaulis* Valetton, *C. sichuanensis* X. X. Chen, *C. wenyujin* Y. H. Chen & C. Ling, and *C. zanthorrhiza* Roxb. (Liu, 1985) are widely used as medicinal plants. *C. long* L. produces highly aromatic and antiseptic turmeric, which has been extensively used as a spice, a beauty care agent, and traditional medicine (Aggarwal et al., 2007). Some others are used as ornamental plants such as *C. elata* Roxb., which is popular as cut flowers (Škorničková et al., 2007).

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Despite their economic importance, disagreements on chromosome numbers of *Curcuma* species still exists (Table 1). Somatic chromosome numbers of $2n = 40$, 42, and 77 were documented for *C. oligantha* Trim. by Saensouk and Chantaranonthai (2003), Eksomtramage et al. (2002), and Leong-Škorničková et al. (2007), respectively. The chromosome number of *C. aromatica* was reported to be $2n = 42$ (Leong-Škorničková et al., 2007; Raghavan and Venkatsubban, 1943), 63 (Islam, 2004; Leong-Škorničková et al., 2007; Liu, 1985; Ramachandran, 1961), or 86 (Ramachandran, 1961). Variation also occurs in reports of the basic chromosome number, including $x = 21$ (Raghavan and Venkatsubban, 1943); $x = 7$ or 8 (Sato, 1960); and $x = 7$ (Leong-Škorničková et al., 2007). Thus far, $x = 21$ appears to be considered acceptable as the basic chromosome number (Eksomtramage et al., 2002; Islam, 2004; Joseph et al., 1999; Ramachandran, 1961, 1969). The ambiguity in chromosome numbers may be partially explained by potential misidentification of species. *Curcuma* species identification has been difficult as a result of the lack of a comprehensive taxonomic revision and the existence of numerous closely related species (Chen and Xia, 2011). So far, the number of species has been estimated at 50 (Wu and Larsen, 2000), 80 (Larsen et al., 1998), and 120 (Leong-Škorničková et al.,

Table 1. A summary of somatic chromosome numbers studied in *Curcuma* species.

Taxa	No. ($2n$)	Origin	Reference
<i>C. aff. oligantha</i> Trim.	42	Thailand	Eksomtramage et al. (2002)
	40	Thailand	Saensouk and Chantaranonthai (2003)
<i>C. oligantha</i> Trim.	77	India	Leong-Škorničková et al. (2007)
<i>C. amarissima</i> Roscoe	63	Bangladesh	Islam (2004)
	63	India	Leong-Škorničková et al. (2007)
<i>C. aromatica</i> Salisb.	42	India	Raghavan and Venkatsubban (1943)
	63, 86	India	Ramachandran (1961)
	63	China	Liu (1985)
	63	Bangladesh	Islam (2004)
	42, 63	India	Leong-Škorničková et al. (2007)
<i>C. elata</i> Roxb.	63	Bangladesh	Islam (2004)
	63	India	Leong-Škorničková et al. (2007)
	63	Thailand	Soontornchainaksaeng and Abantgawat-Jónsson (2011)
<i>C. longa</i> L.	64	Unknown	Sugiura (1936)
	62	India	Raghavan and Venkatsubban (1943)
	32	Japan	Sato (1960)
	63	India	Ramachandran (1961)
	63	China	Liu (1985)
	63	Bangladesh	Islam (2004)
	63	India	Leong-Škorničková et al. (2007)
<i>C. kwangsiensis</i> S. G. Lee & C. F. Liang	64	China	Liu (1985)
	84	China	Chen et al. (1988)
	84	China	Huang et al. (2010)
<i>C. nankunshanensis</i> N. Liu, X. B. Ye & J. Chen	84	China	Ye et al. (2008)
<i>C. phaeocaulis</i> Valetton	63	China	Liu (1985)
	63	China	Zhao et al. (2011)
<i>C. rubrobracteata</i> Škorničk. Sabu & Prasanthk.	42	India	Leong-Škorničková et al. (2007)
<i>C. viridiflora</i> Roxb.	63	China	Chen and Xia (2010)
<i>C. wenyujin</i> Y. H. Chen & C. Ling	42	Bangladesh	Islam (2004)
<i>C. yunnanensis</i> N. Liu & C. Senjen	63	China	Chen and Chen (1984)
<i>C. zanthorrhiza</i> Roxb.	63	China	Liu (1985)
	63	China	Chen and Chen (1984)
	63	India	Leong-Škorničková et al. (2007)
<i>C. zedoaria</i> (Christm.) Roscoe	63	Bangladesh	Islam (2004)
	64	India	Venkatsubban (1946)

No. = chromosome number.

2007). High intra- and interpopulation variation also likely contributes to confusion in identification of *Curcuma* species (Chen and Xia, 2011).

There are ≈ 12 *Curcuma* species in China, mainly distributed in Yunnan, Guangxi, Sichuan, Guangdong, and Zhejiang provinces (Wu and Larsen, 2000). Somatic chromosome numbers of some Chinese *Curcumas* have been examined. Liu (1985) reported $2n = 63$ for *C. aromatica*, *C. longa* L., *C. phaeocaulis*, and *C. yunnanensis* N. Liu & C. Senjen and $2n = 64$ for *C. kwangsiensis*. In contrast, Chen et al. (1988) stated $2n = 84$ for *C. kwangsiensis*. Chromosome numbers of other Chinese *Curcumas* have not been documented such as *C. flaviflora* S. Q. Tong and *C. sichuanensis*.

Chromosome numbers and ploidy levels are important information for plant taxonomy, genetics, and evolution as well as essential for plant conservation and use (Bennett and Leitch, 2005). Cytogenetic characters have also been considered critical for defining intrageneric groups in *Curcuma* (Islam, 2004; Joseph et al., 1999; Leong-Škorničková et al., 2007; Ramachandran, 1961). To pursue a better understanding of Chinese *Curcuma* species, this study was intended to examine chromosome numbers of available *Curcuma* species in China, their ploidy levels, and their implications in distribution.

Materials and Methods

For this study, nine populations across eight species were collected from the wild in southwestern China. Six individuals of four species from Guangxi Botanical Garden of Medicinal Plants and South China Botanical Garden, Chinese Academy of Sciences, were examined (Table 2). Collected plants were grown outdoors in the ginger garden or were cultivated in containers in a greenhouse at the South China Botanical Garden. The plant specimens were identified with the help of Dr. De-Lin Wu at the South China Botanical Garden. Herbarium acronyms followed Index Herbariorum (Holmgren et al., 1990). Taxonomic names were in accordance with the classifications of Wu and Larsen (2000).

Actively growing root tips of the collected plants were taken and pretreated with 2.0 mM 8-hydroxyquinoline for 6 h, fixed in Carnoy I (three parts absolute ethanol and one part glacial acetic acid), macerated in 1 M HCl at 60 °C for 5 min, and stained with Carbol fuchsin. The root tips were squashed with forceps in a drop of 45% acetic acid on a glass slide, covered with a cover glass, and squashed again. Cover glasses were removed by freezing glass slides in liquid nitrogen. Slides were dried at room temperature and passed through three solutions: absolute ethanol; 1:1 ethyl

ethanol:xylene; and xylene. Slides were sealed with permount mounting medium. Metaphase chromosomes were observed and photographed were taken under the OLYMPUS BX41 microscope (Olympus, Tokyo, Japan). Eight individual root tips from different plants of each *Curcuma* collection were studied. Chromosome numbers of a minimum of 20 cells of each collection were counted at the well-spread metaphase stage. Ploidy levels were determined based on $x = 21$ (Eksomtramage et al., 2002; Joseph et al., 1999; Ramachandran, 1961, 1969). All microscope slides were deposited in the South China Botanical Garden.

Reproductive strategies of different species were closely monitored and recorded, including seed propagation and asexual rhizome propagation. Seeds, if present, were collected and sowed. Rhizomes were also used for propagation.

Results

Chromosome numbers of 11 *Curcuma* species were determined (Table 2). Among them, *C. amarissima* Roscoe, *C. aromatica*, *C. gulingensis* N. H. Xia & J. Chen, *C. elata*, *C. sichuanensis*, *C. phaeocaulis*, *C. rubrobracteata*, *C. wenyujin*, and *C. zanthorrhiza* had the chromosome number $2n = 63$

Table 2. Chromosome numbers and vouchers of 11 species of *Curcuma* from China.

Scientific name	Habitat	Voucher	No. ^z (2n)	Ploidy level (x)	LR ^y (μm)	RS ^x
<i>Curcuma rubrobracteata</i> Škorničková, M. Sabu & Prasanthk.	Wild, growing in sand soil along the road in Menghai, Yunnan	J. Chen 0843 (IBSC)	63	3	0.5–1.3	A ^w
<i>C. aromatica</i> Salisb.	Cultivated in dry soil in Guangxi Botanical Garden of Medicinal Plants, Guangxi	J. Chen 0812 (IBSC)	63	3	0.8–1.8	A
<i>C. sichuanensis</i> X. X. Chen	Wild, growing beside streams in Menghai, Yunnan	J. Chen 0850 (IBSC)	63	3	0.8–1.8	A
<i>C. elata</i> Roxb.	Cultivated beside the road at South China Botanical Garden, Guangdong	J. Chen 0813 (IBSC)	63	3	1.0–1.8	A
<i>C. wenyujin</i> Y. H. Chen & C. Ling	Cultivated beside the road at South China Botanical Garden, Guangdong	J. Chen 0806 (IBSC)	63	3	0.8–1.8	A
<i>C. phaeocaulis</i> Valetton	Wild, growing beside streams in Wenshan, Yunnan	J. Chen 0828 (IBSC)	63	3	0.8–1.8	A
<i>C. gulingensis</i> N. H. Xia & J. Chen	Wild, growing beside streams in Wenshan, Yunnan	J. Chen 0826 (IBSC)	63	3	0.6–1.3	A
<i>C. zanthorrhiza</i> Roxb.	Collected from the wild field, Wenshan, Yunnan	J. Chen 20101 (IBSC)	63	3	0.5–1.2	A
<i>C. flaviflora</i> S. Q. Tong	Wild at the margin of the forest in Menghai, Yunnan	J. Chen 0885 (IBSC)	42	2	0.5–2.1	S, ^v A
<i>C. amarissima</i> Roscoe	Wild, growing in sand soil along the road in Menghai, Yunnan	J. Chen 0881 (IBSC)	63	3	0.5–1.5	A
<i>C. kwangsiensis</i> S. G. Lee & C. F. Liang	Wild, growing in sand soil along the road in Menghai, Yunnan	J. Chen 0848 (IBSC)	84	4	0.5–2.1	A
<i>C. kwangsiensis</i> S. G. Lee & C. F. Liang	Collected from the wild field, Baoshan, Yunnan	J. Chen 0849–1 (IBSC)	84	4	0.5–2.1	A
<i>C. kwangsiensis</i> S. G. Lee & C. F. Liang	Cultivated in dry soil in Guangxi Botanical Garden of Medicinal Plants, Guangxi	—	84	4	0.5–1.8	A
<i>C. kwangsiensis</i> S. G. Lee & C. F. Liang	Cultivated in dry slope at South China Botanical Garden	J. Chen 0816 (IBSC)	84	4	0.5–1.8	A
<i>C. wenyujin</i> Y. H. Chen & C. Ling	Cultivated in dry soil in Guangxi Botanical Garden of Medicinal Plants, Guangxi	J. Chen 0890 (IBSC)	63	3	0.8–1.8	A

^zNo. = chromosome numbers.

^yLR = chromosome length range.

^xRS = reproduction strategies.

^vA = asexual reproduction.

^sS = sexual reproduction.

(Figs. 1A–H, 2J, and 2O), whereas *C. kwangsiensis* had $2n = 84$ (Fig. 2K–N), and *C. flaviflora* had $2n = 42$ (Fig. 2I). All plants examined were polyploids except for *C. flaviflora*, which was a diploid. The ploidy levels of all species fitted well with the primary number $x = 21$ (Figs. 1 and 2).

All chromosomes of the studied *Curcuma* were very small, ranging from 0.5 to 2.1 μm in length; *C. rubrobracteata* (Fig. 1A) and *C. zanthorrhiza* (Fig. 2H) had the smallest chromosomes (0.5–1.3 μm). As a result of the small chromosome sizes, no clear morphological differences were observed. Centromeres were difficult to detect; thus, karyotype analysis was not performed.

Our study showed that *C. flaviflora* produced viable seeds and rhizomes, but seeds were the main method for propagation (Table 2; Fig. 3A–C). Tetraploid *C. kwangsiensis* appeared not able to produce seeds in the South China Botanical Garden, so it was propagated through rhizomes (Table 2; Fig. 3F–G) as were all of the triploids (Table 2; Fig. 3D–E).

The only diploid in the study, *C. flaviflora*, was found only in Yunnan province where it inhabits mountains or mountain margins. The majority of the triploids occurred in Southern China with the center of distribution in the Yunnan province. The tetraploids also had a wide geographical distribution, occurring in

Yunnan, Guangxi, and Guangdong provinces (Table 2; Fig. 4).

Discussion

This is the first report of chromosome counts for *C. sichuanensis* (Fig. 1C) and *C. flaviflora* (Fig. 2I). Before this study, no diploid chromosome counts have been reported for Chinese *Curcumas* (Chen and Chen, 1984; Chen et al., 1988; Liu, 1985). *C. flaviflora* produced viable seeds, which was the main method for propagation. The identification of *C. flaviflora* as a diploid species provided additional evidence supporting the claim that the basic chromosome number of

Fig. 1. Somatic metaphase chromosomes of *Curcuma* species. (A) *Curcuma rubrobracteata* ($2n = 63$); (B) *C. aromatica* ($2n = 63$); (C) *C. sichuanensis* ($2n = 63$); (D) *C. elata* ($2n = 63$); (E) *C. wenyujin* ($2n = 63$); and (F) *C. phaeocaulis* ($2n = 63$). Scale bar = 10 μm .

Fig. 2. Somatic metaphase chromosomes of *Curcuma* species. (G) *Curcuma gulinqingensis* ($2n = 63$); (H) *C. zanthorrhiza* ($2n = 63$); (I) *C. flaviflora* ($2n = 42$); (J) *C. amarissima* ($2n = 63$); (K) *C. kwangsiensis* 1 ($2n = 84$); (L) *C. kwangsiensis* 2 ($2n = 84$); (M) *C. kwangsiensis* 3 ($2n = 84$); (N) *C. kwangsiensis* 4 ($2n = 84$); and (O) *C. wenyujin* ($2n = 63$). Scale bar = 10 μm .

Curcuma was 21. Sharma and Bhattacharya (1958) first reported $x = 16$ in the genus *Curcuma*. Sato (1960) proposed $x = 7$ and $x = 8$, and recently Leong-Škornicková et al. (2007) suggested that $x = 7$ should be considered a primary basic chromosome number for at least the majority of Indian *Curcuma* species (subgenus *Curcuma*). This proposal was based on the chromosome count of $2n = 77$ in *C. oligantha* Trim by flow cytometry. On the other hand, the basic number $x = 21$ appeared too

high to be the primary one. Raghavan and Venkatasubban (1943), Ramachandran (1961), and Venkatasubban (1946) believed that this basic number might have been derived either by dibasic amphidiploidy (by combination of lower basic numbers of nine and 12 found in some genera in the family) or by secondary polyploidy. Nevertheless, the chromosome numbers of all 11 species in the present study can be explained by the basic chromosome number $x = 21$.

The present study, in conjunction with an earlier investigation (Joseph et al., 1999), has demonstrated small and similar chromosome sizes in the genus *Curcuma*. Similar chromosome size was also prevalent in other genera characterized by $x = 21$ such as *Caulokaempferia yunnanensis* (Gagnep.) Smith and *Hitchenia* (Chen et al., 1988; Leong-Škornicková et al., 2007; Ramachandran, 1969). The chromosomes of *Curcuma* were as small as those of other genera of the subfamily Zingiberoideae

Fig. 3. Reproductive strategies of *Curcuma* species. (A–C) Spikes, seeds, and rhizomes of diploid species *Curcuma flaviflora*; (D–E) spikes and rhizomes of triploid species *C. elata*; and (F–G) spikes and rhizomes of tetraploid species *C. kwangsiensis*.

such as *Globba*, *Hedychium* and *Cornu-kaempferia* (Eksomtramage et al., 2002; Ramachandran, 1969). Stebbins (1966) pointed out that variation in chromosome size was correlated with climatic adaptation: the genera having smaller chromosomes were predominantly tropical or subtropical, whereas the large chromosomes were found exclusively in temperate climates. The genus *Curcuma*, one of many genera of the Zingiberaceae

mainly occurring in tropical and subtropical habitats (Islam, 2004), may bear smaller chromosomes for adaptation to its habitat.

The triploids and tetraploids were located in areas either rich in water or influenced by human activities. Triploids probably originated by a fusion of reduced and unreduced gametes of diploids within or between species (Leong-Škornicková et al., 2007; Rieseberg and Willis, 2007). Given the continuum of traits in

polyploid taxa, hybridization may have also played an important role (Rehse, 2005). Nevertheless, the fact that the majority of species in China were triploid indicates that triploids may have some type of competitive advantage over diploids and tetraploids. The triploids are sterile and do not require seed production, their abundant rhizomes (Fig. 3D–E) make them popular in production, and subsequent selection may contribute to their wide distribution.

Fig. 4. The distribution of studied *Curcuma* in Guangdong, Guangxi, and Yunnan provinces, China. ★, ●, and ▲ represent the triploid, tetraploid, and diploid species, respectively.

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