THAI FOREST BULL., BOT. 48(1): 7–17. 2020.
DOI https://doi.org/10.20531/tfb.2020.48.1.02

Cytogenetic verification of *Curcuma candida* (Zingiberaceae) from Thailand and Myanmar

NATTAPON NOPPORNCHAROENKUL[1], THAYA JENJITTIKUL[1], NGARMNIJ CHUENBOONNGARM[1], KESARA ANAMTHAWAT-JÓNSSON[2] & PUANGPAKA UMPUNJUN[1,*]

ABSTRACT

Curcuma candida is a conservation-vulnerable species, rare and endemic to the Tenasserim Range (Thio Khao Tanaosri). This species was initially classified into the genus *Kaempferia*, based on morphology of specimen from Myanmar, and was given the name *K. candida* in 1830, but the first specimen from Thailand was only discovered in 2000. With the addition of molecular evidence, the species was transferred to *Curcuma*. The aim of the present study was therefore to find cytogenetic identity of *C. candida*, by investigating mitotic and meiotic divisions of five accessions obtained from the border districts of Thailand and Myanmar, in comparison with previous cytogenetic records of *Curcuma* and *Kaempferia*. The results show that *C. candida* is diploid with $2n = 42$ and there is no variation among accessions. All accessions showed meiotic figure representing 21 bivalents during microsporogenesis, indicating the secondary base number $x = 21$. These chromosomal characteristics are closely similar to those found in diploid *Curcuma* species. The present paper also includes field observation and description of the plant phenology. The work has underlined an urgent need to protect this species in nature. During the sample collection trips we witnessed an excessive collection of edible flowers for local consumption.

KEYWORDS: Chromosome number, *Curcuma*, cytotaxonomy, *Kaempferia*, meiosis, mitosis, Zingiberaceae

Accepted for publication: 17 December 2019. Published online: 29 January 2020

INTRODUCTION

The ginger family or Zingiberaceae comprises over 1,500 species of 53 genera worldwide. Geographically, Thailand is part of the Indochinese region that harbours the highest ginger genetic resources (Larsen & Larsen, 2006; Leong-Škorničková & Newman, 2015). A large number of Thai Zingiberaceous taxa are known as edible, ornamental or medicinal plants, from which commercial products beneficial to human can be developed. Several of these species are rare and endemic to Thailand (Saensouk, 2011). The present paper involves two ginger genera: *Curcuma* L. and *Kaempferia* L.

The genus *Curcuma* comprises about 80 accepted species distributed mainly in tropical Asia (Maknoi 2006; Leong-Škorničková *et al.*, 2007). Thirty-eight species are found in Thailand including eight endemic species (Maknoi, 2006). The genus is recognized for its economic value, a widely known example being turmeric, *C. longa* L., used as spice, dye, drug and cosmetic (Ravindran *et al.*, 2007). The genus is also known for its medicinal and ornamental value. Numerous *Curcuma* species are of great economic importance in Thailand, resulting in large scale cultivation in the region. Ornamental *Curcuma* species, such as *C. alismatifolia* Gagnep. (Siam tulip) and *C. parviflora* Wall. (Khumkratok *et al.*, 2012), are second only to orchids in being the nation's most widely exported cut flowers. Medicinal species, such as *C. comosa* Roxb., has received much attention in recent years for being a phytoestrogen producing plant (e.g. Soontornchainaksaeng & Jenjittikul, 2010; Thongon *et al.*, 2017). Products from rhizomes of *C. comosa* have been developed for use in Thai traditional medicine as an anti-inflammation remedy and for treatment of uterine abnormalities and ovarian hormone deficit (Tabboon *et al.*, 2019).

Kaempferia is a smaller genus compared to *Curcuma*. It comprises approximately 60 species distributed worldwide, and in Thailand at least 28

[1] Department of Plant Science, Faculty of Science, Mahidol University, Ratchathewi, Bangkok 10400, Thailand.

[2] Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, Reykjavík 101, Iceland.

* Corresponding author: puangpaka.ump@mahidol.ac.th

Kaempferia species have been recorded (Sirirugsa, 1989, 1992; Saensouk & Jenjittikul, 2001; Nopporn-charoenkul *et al.*, 2017 and references there in). Several rare and endemic species have been assigned a threatened conservation status. *Kaempferia* is also an economically important genus. The best-known example is *K. galanga* L., commonly known as kencur or aromatic ginger – it is one of the most popular, aromatic medicinal plants used in the tropics and subtropics of Asia (Bakkali *et al.*, 2007). Other *Kaempferia* species are cultivated in Thailand for use as medicinal plants, such as the Thai black ginger (*K. parviflora* Wall. ex Baker), which has been shown to have several pharmacological effects including anti-fungal/microbial and anti-cancer properties (Sookkongwaree *et al.*, 2006; Saokaew *et al.*, 2017; Paramee *et al.*, 2018). Rhizome extracts from *K. marginata* Carey ex Roscoe (peacock ginger) have been shown to have an anti-inflammatory and wound-healing effects (Kaewkroek *et al.*, 2013; Muthachan & Tewtrakul, 2019). Species such as *K. roscoeana* Wall., *K. marginata* Carey ex Roscoe, *K. elegans* (Wall.) Baker and *K. pulchra* Ridl. are also edible or ornamental plants (Chuakul & Boonpleng, 2003; Picheansoonthon & Koonterm, 2008).

Curcuma candida (Wall.) Techapr. & Škorničk., the species under study, is a rare and endemic species of Myanmar and Thailand. It has a limited distribution within the Tenasserim Range (Thio Khao Tanaosri) bordering the two counties. The conservation status of *C. candida* has been assigned as vulnerable (Leong-Škorničková *et al.*, 2012). This species produces substantial and attractive inflorescences, which are traditionally used as a vegetable eaten with native food, as well as having the potential to be developed as an ornamental or medicinal plant (Jenjittikul & Larsen, 2000; Picheansoonthon & Koonterm, 2008). Taxonomically, this species was initially classified into the genus *Kaempferia*, based on morphology of specimen from Myanmar, and was given the name *K. candida* by Wallich (1830). In 2000, the species was discovered in Thailand for the first time, in Kanchanaburi province, which in its west borders Mon State and Tanintharyi Region of Myanmar (Jenjittikul & Larsen, 2000).

Further investigations did not support the placement of this species in *Kaempferia*. Based on nucleotide sequence polymorphisms of *psbA-trnH* and partial *petA-psbJ* spacers in chloroplast DNA, all *Kaempferia* species were phylogenetically clustered as a monophyletic genus, except for *K. candida* which was found to be separated from the *Kaempferia* clade (Techaprasan *et al.*, 2010). On the other hand, the phylogeny of *Curcuma* grouped *K. candida* within the *Curcuma* cluster. Consequently, the species was formally transferred to *Curcuma* (Techaprasan & Leong-Škorničková, 2011). The taxonomic status of *C. candida* was further elaborated, based on botanical and molecular analyses (Záveská *et al.*, 2012, 2016; Leong-Škorničková *et al.*, 2015).

Cytogenetic characters, including chromosome numbers ($2n$), base chromosome number (x), ploidy level, as well as karyomorphological information and genome size measurements, either together or separately, are effective classification criteria in the same manner as the morphological characters. Cytogenetic analysis has been extensively applied in the taxonomic classification and identification of numerous Zingiberaceous plants (Joseph, 2010).

The genus *Curcuma* contains chromosome numbers spanning the full range of the family Zingiberaceae, from $2n = 20$ to 105 (references here below), but are characterized by chromosomes of particularly small sizes, usually less than 2 μm. A large number of *Curcuma* species (at least 25 species) have the chromosome number $2n = 42$ (base number $x = 21$, see Discussion), several (ca 12) species have $2n = 63$, and other numbers such as 20, 24, 32, 34, 84 and 105 have also been reported (Ramachandran, 1969; Apavatjrut *et al.*, 1996; Eksomtramage *et al.*, 2002; Sirisawad *et al.*, 2003; Saensouk & Saensouk, 2004; Leong-Škorničková *et al.*, 2007; Soontornchai-naksaeng & Jenjittikul, 2010; Chen *et al.*, 2013; Puangpairote *et al.*, 2016). Moreover, different cytotypes are also found within species: for example, *C. comosa* ($2n = 42, 63$), *C. latifolia* Roscoe ($2n = 63, 84$) and *C. zeodaria* (Christm.) Roscoe ($2n = 64, 105$) (Rice *et al.*, 2015; Puangpairote *et al.*, 2016). Chromosome numbers of *Kaempferia* have also been reported. Most *Kaempferia* species are diploid having $2n = 22$ (base number $x = 11$), whereas other ploidy levels exist within the genus, such as $2n = 33$ (triploid), 44 (tetraploid) and 55 (pentaploid) (Raghavan & Venkatasubban, 1943; Chakravorti, 1948; Ramachandran, 1969; Mahanty, 1970; Eksomtramage & Boontum, 1995; Eksomtramage *et al.*, 1996; Saensouk & Jenjittikul, 2001; Eksomtramage *et al.*, 2002; Saensouk & Saensouk, 2004; Nopporncharoenkul

et al., 2017.) Ploidy level variation within species is also common in *Kaempferia*: for example, *K. angustifolia* Roscoe ($2n = 22$ and 33); *K. elegans* ($2n = 22$, 33 and 44); and *K. galanga* ($2n = 22$, 44 and 55) (Nopporncharoenkul *et al.*, 2017).

The chromosome number records cited above clearly indicate that the genomes of *Curcuma* and *Kaempferia* are different: while the most common number in diploid *Curcuma* is $2n = 42$ ($x = 21$), the diploid number in *Kaempferia* is $2n = 22$ ($x = 11$). For the species of the present study (*C. candida*), the only chromosome record existed is this *Kaempferia*'s $2n = 22$ (Rice *et al.*, 2015). To the best of our knowledge, no other cytogenetic information on *C. candida* was available to date. Therefore, in the present study we determined the somatic chromosome number of *C. candida* from samples collected in natural locations in Thailand and Myanmar, with an aim to find out if based on chromosome number would *C. candida* conform correctly to the genus *Curcuma* or not. In addition, we examined meiosis in flower buds of *C. candida* in order to confirm the somatic number and to evaluate the sexual viability of the species based on chromosome pairing behaviour at meiosis.

MATERIALS AND METHODS

Field study and sample collection

Field studies were conducted during the period from April 2013 to May 2017 in three locations within two Thai provinces near the border between Thailand and Myanmar, the area that covers most of the distribution of *C. candida*: Kanchanaburi and Tak (Figure 1; Table 1). Sangkhla Buri and Thong Pha Phum districts were the two collection sites of *C. candida* in Kanchanaburi, the western province of Thailand not far from the type locality described in Wallich (1830). Mae Sot district in Tak province was the other locality where *C. candida* was collected in Thailand. From each accession (natural study site), five individual plants with rhizomes and at least twenty young flower buds (inflorescences) were collected for somatic and meiotic chromosome analyses. In addition, harvested young inflorescences for meiotic study were obtained from local markets: in Dan Singkhon Myanmar border market near the minor cross-border points in Prachuap Khiri Khan province (the accession originally collected from Tanintharyi Division of Myanmar) and in Thong Pha Phum local market in Kanchanaburi province (Figure 1, Table 1).

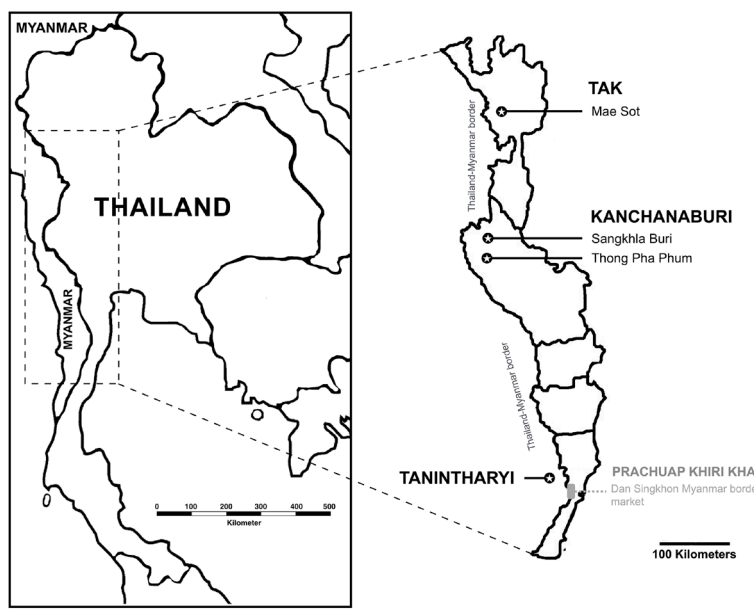

Figure 1. Origin of *Curcuma candida* (Wall.) Techapr. & Škorničk. in the present study. Samples from Tak and Kanchanaburi were collected in the field, in the plant's natural habitat. Fresh samples (inflorescences only) from Prachuap Khiri Khan were obtained from a Thai local market near the border with Myanmar but were originally collected in Myanmar.

Table 1. Cytogenetic characters of *Curcuma candida* (Wall.) Techapr. & Škorničk. from Thailand and Myanmar.

Collection locality	Accession numbers	Chromosome number (2n)	Meiotic figure (II = bivalent)	Ploidy level	Viable seeds	Note
Thong Pha Phum, Kanchanaburi, Thailand	NNSB327	42	21II	diploid	present	
	NNSB528	42[a]	21II	diploid		sold in local market
Sangkhla Buri, Kanchanaburi, Thailand	NNSB147	42	21II	diploid	present	
Mae Sot, Tak, Thailand	NNSB323	42	21II	diploid	present	
Tanintharyi, Myanmar (from market in Prachuap Khiri Khan)	NNSB153	42[a]	21II	diploid		Sold in local market

[a] Chromosome number derived from only meiotic division of pollen mother cell

Samples for mitotic chromosome analysis were collected from living plants, which were grown from rhizomes originally collected from natural habitats and maintained as living collection at Department of Plant Science, Mahidol University, Phayathai Campus, Bangkok. All plant specimens were identified based on morphological characters according to the taxonomic description by Jenjittikul & Larsen (2000), in comparison with their respective herbarium specimens that were kept at Bangkok Herbarium (BK), Forest Herbarium (BKF), Queen Sirikit Botanic Garden Herbarium (QBG) and Suan Luang Rama IX Herbarium. Voucher specimens: NNSB327, 528, 147, 323 and 153 (NN = Nattapon Nopporncharoenkul) were deposited at Suan Luang Rama IX Herbarium, Suan Luang, Bangkok.

Somatic chromosome investigation

The chromosome number (2*n*) was determined from mitotic metaphase chromosomes prepared using the Feulgen squash method described in Soontornchainaksaeng & Jenjittikul (2010), with modifications regarding the ice-water pre-treatment (Anamthawat-Jónsson & Sigurdsson, 1998). Five to twenty actively growing root tips from each plant, and five individual plants from each accession, were collected from newly germinating rhizomes. They were immediately pre-treated in ice-water at 4°C for 25 h, after which the root tip samples were fixed and stored in acetic alcohol (3:1v/v of absolute ethanol and glacial acetic acid) at 4°C until use. To macerate the cell wall, the fixed samples were washed in distilled water, followed by hydrolysing in 1 N hydrochloric acid (HCl) at 60°C for 10 min. Then, each hydrolysed root tip was placed on an acid-cleaned microscopic slide and squashed in 2% w/v of aceto-orcein stain. Chromosome number of each plant was determined from images of at least 20 metaphase cells taken at 1000× magnification using an Olympus DP50 digital camera operated with Olympus BX50 light microscope.

Meiotic configuration study

Preparation of meiotic metaphase chromosomes from microsporocytes was performed using the aceto-carmine smear technique described in Nopporncharoenkul *et al.* (2017). Samples of at least twenty young flower buds from each accession were collected in the field and from local markets. They were immediately fixed in the Carnoy's solution (6:3:1 v/v of absolute ethanol, chloroform and glacial acetic acid) for 48 h before transferring to 70% ethanol for storage at 4°C until use. To prepare meiotic chromosomes, fixed flower buds were briefly washed in distilled water. Individual anthers were then separated under a stereo microscope and placed onto microscopic slide. An anther was stained with 2% w/v of aceto-carmine and gently smeared.

Meiotic figures were determined from the pattern of homologous chromosome pairing from late prophase to metaphase stages of the first meiotic division in microsporogenesis at 1000× magnification, using the same microscope and digital camera as in the mitotic study. For each accession, images were captured from at least 50 cells. Identification of meiotic configurations followed Sharma & Sharma (1980).

RESULTS

Curcuma candida and its phenology

Curcuma candida is a perennial herb with 70–90 cm tall in the wild and produces the largest white-yellow flower in the genus *Curcuma* (Fig. 2). It usually grows in deciduous forest with bamboos at moderately high elevations and at foothills along the roadsides to the hills where forest fires annually occur in March to early April. During our visits to the collection sites, numerous inflorescences with white flowers emerged directly from rhizomes every April, well before leafy shoots appearing (Fig. 2A–C). Much to our surprise, fresh inflorescences from the Burmese accession were sold in the Myanmar-Thailand border markets in late February, indicating that *C. candida* in Myanmar flowered earlier than the Thai plants and this may have been due to an earlier rainy season and high precipitation on the Myanmar side of the Tenasserim Range that forms an effective geographical barrier between Thailand and Myanmar. Mature fruits and viable seeds of the Thai accessions were observed around May, when the leafy shoots were expanding (Fig. 2D–G). Rapid growth of leaves and pseudostems occurred in May at the beginning of the rainy season, continued until late October and died off around November, as rhizomes went into dormancy. Unlike a large number of *Curcuma* species, *C. candida* cannot continually propagate asexually by its rhizomes. Sexual reproduction, with seed dispersal, is the main mechanism for generating progeny.

For traditional use, inflorescences are extensively collected from its natural habitats and sold by local people in Myanmar-Thailand border markets and along roadsides in provinces of Kanchanaburi (Thong Pha Phum and Sangkhla Buri districts), Tak (Mae Sot and Tha Song Yang districts) and Prachuap Khiri Khan (Fig. 2H–I). Young inflorescences are eaten as a fresh vegetable, or cooked, and the tuberous roots are boiled and also eaten. Such an extensive harvest of the flowers can severely hamper population expansion by sexual reproduction, which is the main mechanism of propagation of *C. candida*.

Cytogenetic characters, mitotic and meiotic

The somatic chromosome number $2n = 42$ was the only number shown by *C. candida*, from all three collection sites in Kanchanaburi and Tak provinces (Table 1, examples in Fig. 3A–B). Regular meiotic figure consisting of 21 bivalents (21-II) at the first metaphase of microsporogenesis was found in all samples (Table 1), with examples shown in Fig. 3C–D. This type of meiotic figure indicated $x = 21$ as base chromosome number of this species. According to these cytogenetic results, *C. candida* is a diploid *Curcuma* species with $2n = 2x = 42$.

DISCUSSION

The current study is the first to report the somatic chromosome number of $2n = 42$ for *Curcuma candida*. In addition, the results of this report are based on samples from different locations, from at least five individual plants each, therefore the study covers a variation that may exist within the species' distribution in the region. On the Thailand side of the Tenasserim Range, the somatic number $2n = 42$ and the meiotic figure comprising 21 bivalents (type 21-II) were obtained from all three locations (four accessions). But from the Myanmar location, we could only find flower samples for meiotic study from the border market; no rhizomes were available for mitotic study. Nevertheless, the normal 21-II meiotic figure of the Myanmar accession indicates that the somatic number is also $2n = 42$. The overall results show conclusively that there is no variation in chromosome number of this species and no variation in the chromosome pairing at meiosis either. This somatic chromosome number is exactly the same as that obtained from Thai diploid species of *Curcuma* (e.g. Soontornchainaksaeng & Jenjittikul, 2010; Puangpairote *et al.*, 2016) and from other regions (Ramachandran, 1969; Leong-Škorničková *et al.*, 2007; Chen *et al.*, 2013).

Chromosome numbers of *Kaempferia* are, on the other hand, in the multiples of 11 (base number $x = 11$), i.e. from diploid number $2n = 22$ to pentaploid number $2n = 55$ (e.g. Nopporncharoenkul *et al.*,

Figure 2. *Curcuma candida* (Wall.) Techapr. & Škorničk. (A) Natural habitat, Thong Pha Phum, Kanchanaburi. (B–C) Inflorescences and flowers of accessions from Thong Pha Phum, Kanchanaburi, NNSB327 and Mae Sot, Tak, NNSB323, respectively. (D–E) Samples from Sangkhla Buri, NNSB147 and Thong Pha Phum, NNSB327, in Kanchanaburi, respectively. (F) Inflorescences and fruits, NNSB147. (G) Viable seeds, NNSB147. (H) Inflorescences of wild plants collected in Tanintharyi Division, Myanmar, sold in Dan Singkhon border market, Prachuap Khiri Khan, NNSB153. (I) Inflorescences of wild plants sold in Thong Pha Phum local market, Kanchanaburi, NNSB528. Scale bars represent 5 cm (A–F), 1 cm (G) and 10 cm (H–I).

2017). Therefore, the only previous record of $2n = 22$ for the species under study (as *Kaempferia candida* in the record) provided by the CCBD Chromosome Counts Database (Rice *et al.*, 2015) has become questionable. The Data Source of this chromosome number record is Flora of China (eFloras, 2019), whereby the primary location of this species is stated as being the mountainous area of SW Yunnan. Perhaps, this Chinese species with $2n = 22$ is different from *Curcuma candida* in the present study.

The result of meiotic investigation supports the use of "secondary" base number $x = 21$ for *Curcuma*. During the first meiotic metaphase in microsporocytes, chromosomes of *C. candida* paired completely with their homologs, forming 21 bivalents (meiotic figure type 21-II regular synapsis). The result is in full agreement with that found in sexually reproducing diploid *Curcuma* taxa with $2n = 2x = 42$ (Puangpairote *et al.*, 2016). Evidence from meiotic pairing in triploid *Curcuma* also supports $x = 21$. Numerous triploid accessions of *C. comosa* with $2n = 3x = 63$ showed complete formation of 21 trivalents, indicating autotriploidy (Puangpairote *et al.*, 2016), leading to severe reduction of plant sexual fertility. A high percentage of trivalent associations were also found in (sexually sterile) turmeric (*C. longa*) with $2n = 3x = 63$ (Ramachandran, 1961). Inferring from these patterns of meiotic pairing behaviour, the base number for *Curcuma* should be $x = 21$, the same as its haploid number $n = 21$. On the other hand, other triploid *Curcuma* taxa showed irregular meiotic pairing indicating allopolyploidy (Puangpairote *et al.*, 2016), presumably resulting from interspecific hybridization. Furthermore, our molecular cytogenetic mapping of the ribosomal genes has indicated hybrid origin of some diploid accessions of *C. comosa* (Soontornchainaksaeng & Anamthawat-Jónsson, 2011), meaning that "primary" or "ancestral" base numbers exist in the genus. To understand chromosomal variability, and to infer phylogenetic relationship among closely related plant species from chromosome data, it is a common practice to distinguish the different base numbers of an evolutionary route as primary, secondary and so on (Stebbins, 1971; Guerra, 2008). Different base chromosome numbers of *Curcuma* have been reported. Based on direct chromosome counting, the base chromosome number of $x = 16$ was reported by Sharma & Bhattacharya (1959),

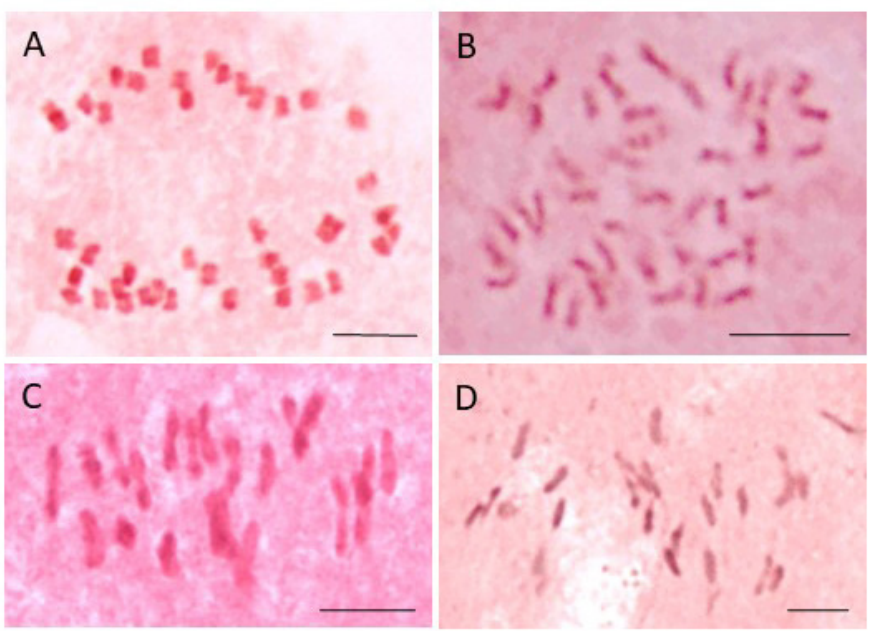

Figure 3. Mitotic and meiotic chromosomes of *Curcuma candida* (Wall.) Techapr. & Škorničk. (A–B) Somatic chromosomes in root tip cells showing 2n = 42. (A) Thong Pha Phum, Kanchanaburi, NNSB327. (B) Sangkhla Buri, Kanchanaburi, NNSB147. (C–D) Meiotic chromosomes during metaphase I of the pollen mother cells showing 21 bivalents. (C) Thong Pha Phum, Kanchanaburi, NNSB528. (D) Mae Sot, Tak, NNSB323. Scale bars represent 5 μm.

whereas Sato (1960) suggested that it could be $x = 7$ and 8. Leong-Škorničková *et al.* (2007) proposed, based on genome size data, that $x = 7$ should be considered a "primary" base chromosome number in *Curcuma*. In contrast, $x = 21$ has been deduced in the present study and other studies of mitosis and meiosis (as stated above), at least for taxa in certain subgenera. Since we are working on cytogenetics of this genus, mostly on the subgenus *Curcuma*, we prefer using $x = 21$ to represent the (secondary) base number for *Curcuma*, until more in-depth investigations, especially using genome-based phylogeny analysis, can reveal clear patterns of chromosome evolution in this diverse genus of Zingiberaceae.

Curcuma candida produces numerous viable seeds in its natural habitat (Fig. 2G). This species is therefore a sexually reproducing, wild diploid species ($2n = 2x = 42$). The result of the meiotic investigation shows regular bivalent pairing of homologous chromosomes, meaning that meiosis can proceed normally, and subsequent pollen production will be normal, and the plants are able to produce seeds. Thus, biologically, *C. candida* has sufficient fertility to be able to distribute and form a large population. But, in reality, the species has little chance of expanding by means of sexual reproduction. Early in the flowering season the flowers are collected and removed from natural habitats for consumption and ethno-medicinal usage by local people, hence the flowers are not available for fruit and seed production. Consequently, it is likely that the natural seed supply is drastically reduced which puts this plant at risk on its extinction. The species is only safe in the more remote area, where it is less accessible. However, as its best habitats are limited to deciduous forests usually with bamboos, they are seriously diminished by the agricultural expansion. These, and other factors, are contributing to a rapid decline of the species. During field studies from April 2013 to May 2017, we found that some ecological niches of *C. candida* were changed to agricultural fields and some populations had disappeared, and many locals had introduced this plant to cultivate domestically.

The present paper therefore serves as a wake-up call for conservation of *C. candida*. Cytogenetic results, especially those regarding ploidy determination and evaluation of sexual fertility via meiosis, are useful for both *in situ* and *ex situ* conservation strategies, as well as for a sustainable utilization in the near future. Therefore, relevant authorities should be proactive in both direct and indirect conservation projects, such as providing knowledge to, or tutoring, local people on how to conserve this plant as well as how to use it sustainably. This will help to create awareness on conservation, set up a sustainable exploitation system with accurate information on botanical principles, and also be practical for environmental policy and the sustainable management of natural resources. Consequently, this plant may then be harvested in a sustainable manner such as leaving some to set seeds for sexual propagation of the species and also to retain the genetic variability of the species in the long run.

ACKNOWLEDGEMENTS

This research was conducted using the facilities at the N305 Plant Cytogenetic laboratory, Department of Plant Science, Faculty of Science, Mahidol University, Bangkok. We would like to thank Dr Tidarat Puangpairote from Prince of Songkla University for helpful comments and encouragement throughout the long period of the research project. We thank all the reviewers who have made constructive comments and suggestions that make this manuscript significantly improved.

Funding

Science Achievement Scholarship of Thailand (SAST), Thailand Center of Excellence on Biodiversity (CEB), The National Research Council of Thailand and Mahidol University.

Author contributions

The first author conducted field studies, analysed the cytogenetic information and drafted the earliest version of the manuscript. The second author helped plan the field studies, guided a taxonomic identification and provided important taxonomic literatures. The third author supervised field collection of samples. The fourth author took active part in the interpretation of cytogenetic results, the discussion and in the manuscript writing. The last (corresponding) author initiated and designed the research project, guided all lab work, discussed and revised the manuscript. All authors approved the final manuscript.

REFERENCES

Anamthawat-Jónsson, K. & Sigurdsson, V. (1998). Chromosome number of Icelandic *Populus tremula*. Nordic Journal of Botany 18: 471–473.

Apavatjrut, P., Sirisawad, T., Sirirugsa, P., Voraurai, P. & Suwanthada, C. (1996). Studies on chromosome number of seventeen Thai *Curcuma* species. In: S. Kasemsup (ed.), Proceeding of the 2nd Thailand National Ornamental Meeting, pp. 86–99. National Research Council of Thailand.

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2007). Riological effects of essential oils – A review. Food and Chemical Toxicology 46: 446–475.

Chakravorti, A.K. (1948). Multiplication of chromosome numbers in relation to speciation in Zingiberaceae. Science and Culture 14: 137–140.

Chuakul, W. & Boonpleng, A. (2003). Ethnomedical uses of Thai Zingiberaceous plants. Thai Journal of Phytopharmacy 10: 33–39.

Chen, J., Xia, N., Zhao, J., Chen, J. & Henny, R. (2013). Chromosome numbers and ploidy levels of Chinese *Curcuma* species. Horticultural Science 48: 525–530.

eFloras (2019). Published on the internet http://www.efloras.org [accessed 12 August 2019] Missouri Botanical Garden, St. Louis, MO & Harvard University Herbaria, Cambridge, MA.

Eksomtramage, L. & Boontum, K. (1995). Chromosome counts of Zingiberaceae. Songklanakarin Journal of Science and Technology 17: 291–297.

Eksomtramage, L., Sirirugsa, P. & Mayakul, S. (1996). Chromosome numbers of some Thai Zingiberaceae. Songklanakarin Journal of Science and Technology 18: 153–159.

Eksomtramage, L., Sirirugsa, P., Jivanit, P. & Maknoi, C. (2002). Chromosome counts of some Zingiberaceae species from Thailand. Songklanakarin Journal of Science and Technology 24: 311–319.

Guerra, M. (2008). Chromosome numbers in plant cytotaxonomy: concepts and implications. Cytogenetics and Genome Research 120: 339–350.

Jenjittikul, T. & Larsen, K. (2000). *Kaempferia candida* Wall. (Zingiberaceae), a new record for Thailand. Thai Forest Bulletin (Botany) 28: 45–49.

Joseph, R. (2010). Karyomorphometrical analysis and exploration of major essential oil constituents in Zingiberaceae. PhD Dissertation, Mahatma Gandhi University, India.

Kaewkroek, K., Watanapiromsakul, C., Kongsairee, P. & Twetrakul, S. (2013). Nitric oxide and tumor necrosis factor-alpha inhibitory substances from the rhizomes of *Kaempferia marginata*. Natural Product Communications 8: 1205–1208.

Khumkratok, S., Boontiang, K., Chutichudet, P. & Pramaul, P. (2012) Geographical distributions and ecology of ornamental *Curcuma* (Zingiberaceae) in Northeastern Thailand. Pakistan Journal of Biological Sciences 15: 929–939.

Larsen, K. & Larsen, S. (2006). Tribe Zingibereae: *Kaempferia* L. In: K. Larsen & S. Larsen (eds), Gingers of Thailand, pp. 55–61. Queen Sirikit Botanic Garden, Chiang Mai.

Leong-Škorničková, J. & Newman, M. (2015). Gingers of Cambodia, Laos and Vietnam. Singapore Botanic Gardens, Singapore, 229 pp.

Leong-Škorničková, J., Šída, O., Jarolímová, V., Sabu, M., Fér, T., Trávníček, P. & Suda, J. (2007). Chromosome numbers and genome size variation in Indian species of *Curcuma* (Zingiberaceae). Annals of Botany 100: 505–526.

Leong-Škorničková, J., Šída, O., Záveská, E. & Marhold, K. (2015). History of infrageneric classification, typification of supraspecific names and outstanding transfers in *Curcuma* (Zingiberaceae). Taxon 64: 362–373.

Leong-Škorničková, J., Tran, H.D., Newman, M., Lamxay, V. & Bouamanivong, S. (2012). *Curcuma candida*. Data from: The IUCN red list of threatened species 2012.

Mahanty, H.K. (1970). A cytological study of the Zingiberales with special reference to their taxonomy. Cytologia 35: 13–39.

Maknoi, C. (2006). Taxonomy and phylogeny of the genus *Curcuma* L. (Zingiberaceae) with particular reference to its occurrence in Thailand. PhD Thesis, Prince of Songkla University.

Muthachan, T. & Tewtrakul, S. (2019). Anti-inflammatory and wound healing effects of gel containing *Kaempferia marginata* extract. Journal of Ethnopharmacology 240: article no. 111964.

Nopporncharoenkul, N., Chanmai, J., Jenjittikul, T., Anamthawat-Jónsson, K. & Soontornchainaksaeng, P. (2017). Chromosome number variation and polyploidy in 19 *Kaempferia* (Zingiberaceae) taxa from Thailand and one species from Laos. Journal of Systematics and Evolution 55: 466–476.

Picheansoonthon, C. & Koonterm, S. (2008). Notes on the genus *Kaempferia* L. (Zingiberaceae) in Thailand. Journal of Thai Traditional and Alternative Medicine 6: 27–51.

Paramee, S., Sookkhee, S., Sakonwasun, C., Na Takuathung, M., Mungkornasawakul, P. & *et al.* (2018). Anti-cancer effects of *Kaempferia parviflora* on ovarian cancer SKOV3 cells. BMC Complementary and Alternative Medicine 18: 178.

Puangpairote, T., Maknoi, C., Jenjittikul, T., Anamthawat-Jónsson, K. & Soontornchainaksaeng, P. (2016). Natural triploidy in phytooestrogen producing *Curcuma* species and cultivars from Thailand. Euphytica 208: 47–61.

Raghavan, T.S. & Venkatasubban, K.R. (1943). Cytological studies in the family Zingiberaceae with special reference to chromosome number and cyto-taxonomy. Proceedings of the Indian Academy of Science, Section B 17: 118–132.

Ramachandran, K. (1961). Chromosome numbers in the genus *Curcuma* Linn. Current Science India 30: 194–196

_____ . (1969). Chromosome numbers in Zingiberaceae. Cytologia 34: 213–221.

Ravindran, P.N., Nirmal, B.K. & Sivaraman, K. (2007). Turmeric: the genus *Curcuma* (medicinal and aromatic plants-industrial profiles). Taylor and Francis Group, Abingdon, 484 pp.

Rice, A., Glick, L., Abadi, S., Einhorn, M., Kopelman, N.M., Salman-Minkov, A., Mayzel, J., Chay, O. & Mayrose, I. (2015) The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytologist 206: 19–26.

Saensouk, S. (2011). Endemic and rare plants of gingers family in Thailand. Khon Kaen University Research Journal 16: 306–330.

Saensouk, S. & Jenjittikul, T. (2001). *Kaempferia grandifolia* sp. nov. (Zingiberaceae) a new species from Thailand. Nordic Journal of Botany 21: 139–142.

Saensouk, S. & Saensouk, P. (2004). Chromosome numbers of some Zingiberaceae in Thailand. Khon Kaen University Research Journal 9: 3–9.

Sato, D. (1960). The karyotype analysis in Zingiberales with special reference to the protokaryotype and stable karyotype. College of General Education, University of Tokyo 10: 225–243.

Sharma, A.K. & Bhattacharyya, N.K. (1959). Cytology of several members of Zingiberaceae and a study of inconstancy of their chromosome complements. La Cellule 59: 299–346.

Sharma, A.K. & Sharma, A. (1980). Chromosome Techniques: Theory and Practice. Butterworth-Heinemann Ltd, Oxford, 711 pp.

Saokaew, S., Wilairat, P., Raktanyakan, P., Dilokthornsakul, P., Dhippayom, T. & *et al.* (2017). Clinical effects of Krachaidum (*Kaempferia parviflora*): A systematic review. Journal of Evidence-Based Complementary & Alternative Medicine 22: 413–428.

Sirirugsa, P. (1989). The genus *Kaempferia* (Zingiberaceae) in Thailand. Nordic Journal of Botany 9: 257–260.

_____. (1992). Taxonomy of the genus *Kaempferia* (Zingiberaceae) in Thailand. Thai Forest Bulletin (Botany) 19: 1–15.

Sirisawad, T., Sirirugsa, P., Suwanthada, C. & Apavatjrut, P. (2003). Investigation of chromosome numbers in 20 taxa of *Curcuma*. In: P. Chantaranothai, K. Larsen, P. Sirirugsa & D. Simpson (eds), Proceedings of the 3rd Symposium on the Family Zingiberaceae, pp. 54–62. Khon Kaen University, Thailand.

Sookkongwaree, K., Geitmann, M., Roengsumran, S., Petsom, A. & Danielson, U.H. (2006). Inhibition of viral proteases by Zingiberaceae extracts and flavones isolated from *Kaempferia parviflora*. Pharmazie 61: 717–721.

Soontornchainaksaeng, P. & Anamthawat-Jónsson, K. (2011), Ribosomal FISH mapping reveals hybridity in phytoestrogen producing *Curcuma* species from Thailand. Plant Systematics and Evolution 292: 41–49.

Soontornchainaksaeng, P. & Jenjittikul, T. (2010). Chromosome number variation of phytoestrogen-producing *Curcuma* (Zingiberaceae) from Thailand. Journal of Natural Medicines 64: 370–377.

Stebbins, G. L. (1971) Chromosomal Evolution in Higher Plants. Addison-Wesley, Reading, USA.

Tabboon, P., Tantiyasawasdikul, S. & Sripanidkulchai, B. (2019). Quality and stability assessment of commercial products containing phytoestrogen diaryheptanoids from *Curcuma comosa*. Industrial Crops and Products 134: 216–224.

Techaprasan, J., Klinbunga, S., Ngamriabsakul, C. & Jenjittikul, T. (2010). Genetic variation of *Kaempferia* in Thailand based on chloroplast DNA (*psbA-trnH* and *petA-psbJ*) sequences. Genetics and Molecular Research 9: 1957–1973.

Techaprasan, J. & Leong-Škorničková, J. (2011). Transfer of *Kaempferia candida* to *Curcuma* (Zingiberaceae) based on morphological and molecular data. Nordic Journal of Botany 29: 773–779.

Thongon, N., Boonmuen, N., Suksen, K., Wichit, P., Chairoungdua, A. & *et al.* (2017). Selective Estrogen Receptor Modulator (SERM)-like Activities of Diarylheptanoid, a Phytoestrogen from *Curcuma comosa*, in Breast Cancer Cells, Pre-osteoblast Cells, and Rat Uterine Tissues. Journal of Agricultural and Food Chemistry 65: 3490–3496.

Wallich, N. (1830). Plantae Asiaticae Rariores. Vol. 1, part 3. Treuttel and Würtz: Treuttel Jr. and Richter, London, pp. 47.

Záveská, E., Fér, T., Šída, O., Krak, K., Marhold, K. & Leong-Škorničková, J. (2012). Phylogeny of *Curcuma* (Zingiberaceae) based on plastid and nuclear sequences: Proposal of the new subgenus *Ecomata*. Taxon 61: 747–763.

Záveská, E., Fér, T., Šída, O., Marhold, K. & Leong-Škorničková, J. (2016). Hybridization among distantly related species: Examples from the polyploid genus *Curcuma* (Zingiberaceae). Molecular Phylogenetics and Evolution 100: 303–321.