



# Cytogenetics of Two *Kaempferia* L. Species in Thailand

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## Abstract

The chromosome numbers and karyotype analyses of *Kaempferia laotica* Gapnep and *K. rotunda* L. belonging to family Zingiberaceae in Thailand were studied from root tips. The results show that the chromosome number of *Kaempferia laotica* and *K. rotunda* was found the same numbers to be  $2n = 22$  and the different karyotype formula with different in position of satellite chromosome has been found in  $14m + 4sm + 4st$  (and one visible satellite chromosome) and  $12m + 10sm$  (and one visible satellite chromosome), respectively. Therefore, chromosome structure, karyotype formula and satellite chromosomes can be used for classification in each species. The satellite chromosomes of both species are the first time reported. The karyotype of *K. laotica* was studied for the first time.

**Keywords:** Chromosome, Kayotype, *Kaempferia laotica*, *Kaempferia rotunda*, Zingiberaceae

**Abbreviations:** PDB: Paradichlorobenzene; SM: Submetacentric; ST: Subtelocentric; RL: Relative Length; CI: Centromeric Index; SD: Standard Deviation.

## Introduction

The family Zingiberaceae or ginger family is perennial herbs with creeping horizontal or tuberous rhizomes. It is a large family in monocotyledons with about 50 genera of 1,600 species in worldwide. Ginger family is distributed in tropical zone and center diversity of the family is located in Southeast Asia. Moreover, the dominant characteristic of Zingiberaceae is found unique smell of essential oils in all parts of plant especially in rhizome Larsen and Larsen [1]. The knowledge of uses from ginger family is important foods, spices, ingredients, medicines, dyes, cosmetics and ornamental plants [2-4] Many species in several genera are important for medicinal uses [4,5]. In Thailand, Larsen and Larsen [1] recognized the diversity of Zingiberaceae

approximately 30 genera and more than 300 species.

The genus *Kaempferia* belongs to tribe Zingibereae, subfamily Zingiberoideae, family Zingiberaceae. Sixty species are reported in world wide. In Thailand, this genus was found more than 20 extant species Boonma, et al. [6] Several *Kaempferia* species in Thailand are well known for their ethnomedical uses by local people Larsen and Larsen [1], Saensouk, et al. [4] Saensouk and Saensouk [7] Boonma, et al. [6]. The aim of this study was to observe the chromosome number and karyology of *Kaempferia laotica* and *K. rotunda* from Thailand.

The chromosome numbers of family Zingiberaceae are studied in some genera by several scientists, namely Beltran and Kam [8], Chen, et al. [9], Chen and Chen [10], Newman [11], Chen, et al. [12], Das, et al. [13], Das, et al. [14], Nerle and Torne [15], Omanakumari and Mathew [16] and Saenprom, et al. [17] For chromosome numbers from root

tips of *Kaempferia* species to be  $2n = 22 - 54$  was studied by Omanakumari and Mathew [16] and Saenprom, et al. [17] Only Saenprom, et al. [17] studied karyotype and ideogram of *Kaempferia rotunda*. While, karyotype and ideogram of *Kaempferia laotica* never previously studied.

## Materials and Methods

### Plant Materials

The *Kaempferia laotica* Gagnep. (coll. no. S. Saensouk 2302) and *K. rotunda* L. (coll. no. S. Saensouk 2303) were collected from the field in Thailand and voucher specimens were deposited at Mahasarakham University Herbarium. All specimens were cultivated in a nursery at the Walai Rukhavej Botanical Research Institute and the Center of Excellence for Silk Innovation, Division of Research Facilitation and Dissemination, at Mahasarakham University, Maha Sarakham Province, Thailand. Root tips were collected for chromosome number analysis.

### Chromosome Number Analysis

Prepared the root tips of all specimens were pretreated with paradichlorobenzene (PDB) at 4°C for 6 h, fixed in ethanol acetic acid (3:1, v:v) at room temperature for 30 min and stored at 4°C or used immediately. Samples of the metaphase chromosomes in photomicrographs. The nomenclature used for the were washed in distilled water, hydrolyzed in 1M HCl for 5 min at 60°C, and washed again in distilled water, then were stained and squashed in 2% aceto-orcein, and observed under a microscope (Zeiss Axiostar Plus). The karyotype formulas were derived from measurements description of the chromosome morphology is that proposed by Levan, et al. [18]

### Results and Discussion

Chromosome numbers of all four species were determined. The chromosome numbers of *Kaempferia laotica* and *K. rotunda* were found to be  $2n = 22$  (Table 1).

Species	2n	n	Karyotype formula	Location	Reference
<i>K. laotica</i>	22	-	-	Thailand	Chen[19]
	22	-	-	Thailand	Bunnag, et al. [20]
	22	11	-	Thailand	Nopporncharoenkul, et al.[21]
	22	-	14m+4sm+4st*	Thailand	Present study*
<i>K. rotunda</i>	33	-	-	India	Chakravorti [22]
	44	11	-	India	Ramachandran [23]
	33	-	-	UK	Mahanty [24]
	54	-	-	India	Nerle and Torne [15]
	44	-	-	China	[12]Chen, et al. [12]
	45	-	-	India	Omanakumari and Mathew [16]
	22	-	-	Thailand	Chen [19]
	33	-	-	Thailand	Eksomtramage and Boontum [25]
	22	-	-	Thailand	Bunnag, et al. [20]
	22	-	-	Thailand	Saensouk and Chantaranonthai
	22	-	-	Thailand	Khamtang, et al. [26]
	22	-	-	Thailand	Nopporncharoenkul, et al. [21]
	30	-	-	Thailand	Saenprom, et al. [17]
22	-	-	Thailand	Present study*	

\* = show that satellite

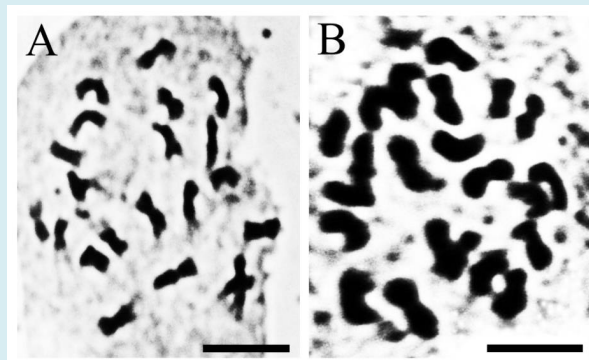
**Table 1:** A summary of previous reports in somatic chromosome numbers studied on *Kaempferia laotica* and *K. rotunda*.

Chromosome number and karyological analysis of *Kaempferia laotica* showed  $2n = 22$  (Figure 1A) and karyotype formula found to be  $14m + 4sm + 4st$  with one visible satellite chromosome (Figure 2A). The karyotype of this species

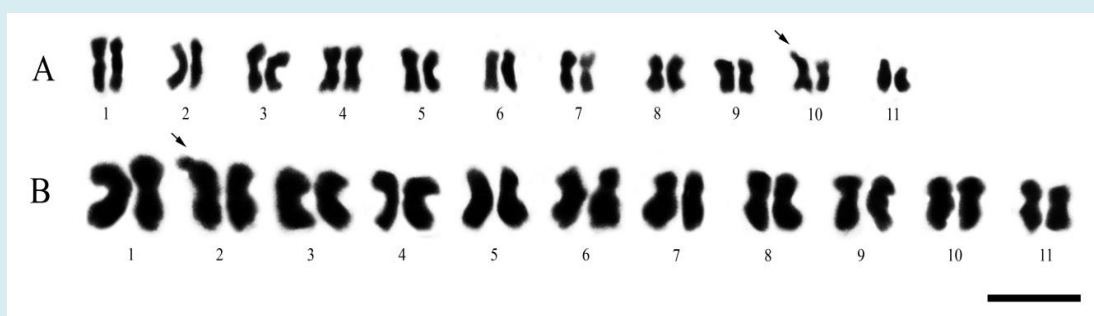
including 14 metacentric (m) pairs, four submetacentric (sm) pairs and four subtelocentric (st) pair with one visible satellite chromosome was asymmetrical. The relative length is presented a value between 6.34 to 12.26% and satellites

are presented on the one m chromosomes (Table 2). This results agreed with the previous chromosome number  $2n =$

22 and  $n = 11$  reported by Chen [19], Bunnag, et al. [20] and Nopporncharoenkul, et al. [21]



**Figure 1:** Microphotographs of somatic metaphase plate *Kaempferia laotica*  $2n = 22$  (A), *K. rotunda*  $2n = 22$  (B). Scale bars=10  $\mu\text{m}$ .



**Figure 2:** Karyotypes of *K. laotica*  $2n = 22$  (A), *K. rotunda*  $2n = 22$  (B) by conventional staining. Arrows in A and B indicate satellites. Scale bar = 10  $\mu\text{m}$ .

Chromosome pair	Ls $\pm$ SD ( $\mu\text{m}$ )	Ll $\pm$ SD ( $\mu\text{m}$ )	LT $\pm$ SD ( $\mu\text{m}$ )	RL (%)	CI	Chromosome type
1	1.75 $\pm$ 0.10	3.58 $\pm$ 0.21	5.33 $\pm$ 0.31	12.26	0.67	Submetacentric
2	1.72 $\pm$ 0.10	3.12 $\pm$ 0.18	4.85 $\pm$ 0.28	11.16	0.64	Submetacentric
3	2.18 $\pm$ 0.13	2.55 $\pm$ 0.15	4.74 $\pm$ 0.27	10.9	0.54	Metacentric
4	1.75 $\pm$ 0.10	2.46 $\pm$ 0.14	4.21 $\pm$ 0.24	9.68	0.58	Metacentric
5	1.75 $\pm$ 0.10	2.29 $\pm$ 0.13	4.04 $\pm$ 0.23	9.29	0.57	Metacentric
6	0.87 $\pm$ 0.05	3.10 $\pm$ 0.18	3.97 $\pm$ 0.23	9.15	0.78	Subtelocentric
7	1.65 $\pm$ 0.10	2.32 $\pm$ 0.13	3.97 $\pm$ 0.23	9.14	0.58	Metacentric
8	1.47 $\pm$ 0.08	1.99 $\pm$ 0.11	3.45 $\pm$ 0.20	7.95	0.58	Metacentric
9	1.33 $\pm$ 0.08	1.93 $\pm$ 0.11	3.26 $\pm$ 0.19	7.5	0.59	Metacentric
10*	1.18 $\pm$ 0.07	1.70 $\pm$ 0.10	2.88 $\pm$ 0.17	6.62	0.59	Metacentric
11	0.73 $\pm$ 0.04	2.03 $\pm$ 0.12	2.75 $\pm$ 0.16	6.34	0.74	Subtelocentric

\* = satellite chromosome.

**Table 2:** Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI of *K. laotica*  $2n = 22$ , obtained from 10 metaphase plates.

Chromosome numbers of *K. rotunda* showed  $2n = 22$  (Fig. 1B) and karyotype formula presented to be  $12m + 10sm$  with one visible satellite chromosome (Fig. 2B). The karyotype of this species including 12 metacentric (m) pairs and 10 submetacentric (sm) pairs with one visible satellite chromosome was symmetrical. The relative length is found a value between 18.01 to 12.14% and satellites are present on the one m chromosomes (Table 3) [27-31]. Chromosome numbers  $2n = 22$  of this study differs from previously studied by several scientists, namely Ramachandran [23]

and Chen, et al. [12]  $2n = 44$ , Chakravorti [22], Mahanty [24] and Eksomtramage and Boontum [25]  $2n = 33$ , Nerle and Torne [15]  $2n = 54$ , Omanakumari and Mathew [16]  $2n = 45$  and Saenprom, et al. [17]  $2n = 30$ . Only Ramachandran [23] reported  $2n = 11$ . The karyotype formula with satellite chromosome of this study are presented which is differs from Saenprom, et al. [17] found that the karyotype formula without satellite because it might be variation morphology from both strains in both studies.

Chromosome pair	Ls±SD (µm)	Ll±SD (µm)	LT±SD (µm)	RL (%)	CI	Chromosome type
1	2.81±0.11	3.62±0.14	6.42±0.25	12.14	0.56	Metacentric
2*	2.42±0.09	2.76±0.10	5.18±0.19	9.78	0.53	Metacentric
3	2.24±0.08	2.87±0.10	5.12±0.19	9.67	0.56	Metacentric
4	2.22±0.09	2.90±0.10	5.11±0.19	9.66	0.57	Metacentric
5	1.67±0.07	3.00±0.11	4.66±0.18	8.81	0.64	Submetacentric
6	1.52±0.05	3.05±0.11	4.57±0.16	8.63	0.67	Submetacentric
7	1.46±0.06	3.06±0.11	4.52±0.17	8.55	0.68	Submetacentric
8	1.92±0.07	2.52±0.10	4.44±0.17	8.39	0.57	Metacentric
9	1.48±0.05	2.86±0.10	4.33±0.16	8.19	0.66	Submetacentric
10	1.56±0.05	2.76±0.10	4.32±0.15	8.17	0.64	Submetacentric
11	2.10±0.05	2.14±0.08	4.24±0.13	8.01	0.5	Metacentric

\* = satellite chromosome.

**Table 3:** Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI) and standard deviation (SD) of RL, CI of *K. rotunda*  $2n = 22$ , obtained from 10 metaphase plates.

From table 1 show that according to available literatures review; *K. rotunda* is widely distributed in the world that can be found in many environments. Therefore, variation morphological characteristics have been found many strains in this species such as green leaf strain, mosaic leaf strain and green with mosaic leaf strain. From the above reasons, the variation in chromosome numbers and chromosome structure including karyotype formula has been found.

Previously reports from table 1 show that the chromosome numbers of several *Kaempferia* species were recorded a wide range of chromosome numbers,  $n = 11$  and  $2n = 22, 30, 33, 44, 45$  and  $54$ . However, the result of the study show that *K. laotica* and *K. rotunda* were found the same chromosome numbers to be  $2n = 22$  which is indicating that these two species are diploid and the basic chromosome number should be  $x = 11$  based on the reported of Ramachandran [23].

This study, the karyotype of *Kaempferia laotica* was studied for the first time. The karyotype formula and structure of both species from genus *Kaempferia* in this study are not similar with different position of the satellite chromosomes.

Therefore, chromosome structure, karyotype formula and satellite chromosomes can be used for classification in each species.

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