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Cytogenetic study in seven species of Zingiberaceae family from Bueng Kan Province, Thailand

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Abstract. *Saensouk P, Saensouk S, Phechphakdee T, Ragsasilp A. 2022. Cytogenetic study in seven species of Zingiberaceae family from Bueng Kan Province, Thailand. Biodiversitas 24: 68-77.* Bueng Kan province is located in the Northeast Thailand. The family Zingiberaceae in Bueng Kan Province was found to be a highly diverse plant. The aim of this study was to study the chromosome numbers, fundamental number (NF), karyotype form and ideogram analysis of seven species in the family Zingiberaceae from Bueng Kan province, Northeast Thailand. The seven species of Zingiberaceae family-namely *Boesenbergia rotunda* (L.) Mansf., *Curcuma aeruginosa* Roxb., *Etlingera elatior* (Jack) R.M.Sm., *Kaempferia elegans* Wall., *Kaempferia pulchra* Ridl., *Kaempferia rotunda* L. and *Zingiber montanum* (J.Koenig) Link ex A.Dietr. in this study have been recognized the cytogenetic study including the chromosome number from root tips, karyology, NF, symmetrical karyotype, RL, CI, and ideogram. The chromosome numbers of all species were reported between 2n = 20 and 68. The NF of six species was reported for the first time. The symmetrical karyotype formula has been recognized as a symmetrical karyotype (four species) and an asymmetrical karyotype (three species). The karyotype formula of five species was reported for the first time. The ideogram of all species was provided for the first time. The cytogenetic data of all species in this study can be used for identification.

Keywords: Bueng Kan Province, chromosome number, ideogram, karyotype, Thailand, Zingiberaceae

INTRODUCTION

Zingiberaceae family or ginger family belongs to the order Zingiberales, monocotyledons (Larsen and Larsen 2006; Saensouk P and Saensouk S 2021a; Lin et al. 2022). It is an herbaceous plant that grows well in tropical and subtropical regions (Picheansoonthon 2010). The distribution center of this family is located in Southeast Asia. Worldwide, there are approximately 1,600 species of the 57 genera, and the largest genus is the genus Alpinia (Kew Science 2022; Ragsasilp et al. 2022). Thailand has a ginger family, about 30 genera, and more than 300 species (Saensouk P and Saensouk S 2021a). This family is unique in that every part of the plant smells of essential oils (Phokham et al. 2013; Ragsasilp et al. 2022). The ginger family has been used for many purposes, especially in food, ingredient, beverage, medicinal plants, ornamental plants, cosmetics, dyes, ritual plants, and economic plants, which was reported by several botanists—Khumkratok et al. (2012), Leong-Škorničková et al. (2015, 2020), Wongsuwana et al. (2015), Nopporncharoenkul and Jenjittikul (2017), Boonma et al. (2020), Jenjittikul and Ruchisansakun (2020), Soonthornkalump et al. (2020), Saensouk P and Saensouk S (2021a), Saensouk and Saensouk (2021a), Ragsasilp et al. (2022), Chumroenphat and Saensouk (2022),

Bueng Kan Province is located along the Mekong river in northeastern Thailand and has many forests, rich streams, beautiful natural scenery, and a variety of higher plants, especially the family Zingiberaceae. This province has found five ecosystem forest types, i.e. deciduous dipterocarp forest, mixed deciduous forest, dry evergreen forest, river basin, and cultivated in home gardens. Ragsasilp et al. (2022) studied three tribes, 13 genera, and 67 species (*Alpinia* (eight species), *Amomum* (six species), *Boesenbergia* (three species), *Caulokaempferia* (four species), *Curcuma* (12 species), *Etlingera* (one species), *Gagnepainia* (two species), *Globba* (10 species), *Hedychium* (one species), *Kaempferia* (seven species), *Meistera* (one species), *Wurfbainia* (one species), and *Zingiber* (11 species)) of the family Zingiberaceae in Bueng Kan Province. Many species were reported as rare species and endemic species in Bueng Kan Province, i.e. *Alpinia macrostaminodia, Amomum biphyllum, A. monophylum, A. wandokthong, Boesenbergia baimaii, Caulokaempferia jirawongsei, C. phutonkensis, C. phuwoaensis, C. phulangkaensis, Globba laeta*, and *Kaempferia siamensis*. Moreover, Ragsasilp et al. (2022) reported the traditional uses (medicine, food, spice,

ornamental, rituals, cosmetics, perfume, and dyes) of Zingiberaceae family from Bueng Kan Province, i.e. *Alpinia galanga, A. conchigera, A. siamensis, Boesenbergia rotunda, Curcuma aeruginosa, C. angustifolia, C. longa, C. singularis*, *Etlingera elatior* (red form inflorescence), *Kaempferia elegans*, *Kaempferia pulchra*, *Kaempferia rotunda* and *Zingiber montanum.* Whereas, seven Zingiberaceae species (*B. rotunda*, *C. aeruginosa*, *E. elatior*, *K. elegans*, *K. pulchra*, *K. rotunda* and *Z. montanum*) can be found the most uses from villagers in this research area. The cytogenetic data of Zingiberaceae species, including these seven species, were few reported. While the chromosome numbers of these four species were studied by some scientists, i.e. Eksomtramage et al. (2002), Sirisawad et al. (2003), Saensouk and Saensouk (2004), Khamtang et al. (2014), Nopporncharoenkul et al. (2017), Saenprom et al. (2018), Mood et al. (2020), Nopporncharoenkul et al. (2020), Moonkaew et al. (2020), Ikeda et al. (2021), Saensouk and Saensouk (2021a, b, 2022). Two species (*B. rotunda* and *K. rotunda*) from these seven Zingiberaceae species were studied with the karyotype formula by Saenprom et al. (2018), Saensouk and Saensouk (2021b) and Saensouk and Saensouk 2022. At the same time, the ideogram of all these plants was never studied. Moreover, the information from this research will support future cytological studies to be more comprehensive.

Therefore, this study will be found many new knowledges. The aim of this study was to study the chromosome numbers, fundamental number (NF), karyotype forms and ideogram analysis of these seven species in the family Zingiberaceae from Bueng Kan Province, Northeast Thailand.

MATERIALS AND METHODS

Plant specimens examined

Seven species of the family Zingiberaceae comprised of *Boesenbergia rotunda* (L.) Mansf., *Curcuma aeruginosa* Roxb., *Etlingera elatior* (Jack) R.M.Sm., *Kaempferia elegans* Wall., *K. pulchra* Ridl., *K. rotunda* L. and *Z. montanum* (J.Koenig) Link ex A.Dietr. The specimens were collected from Bueng Kan Province, northeastern Thailand. All specimens in this study were grown in a nursery at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham Province, Thailand.

Chromosome numbers and karyology analysis

The chromosome numbers study was studied from root tips followed Saensouk et al. (2019). All root tips *c.* 1 cm long) were collected in the morning after sunrise. These root tips were pretreated with paradichlorobenzene (PDB) at 4°C for 6 h and then fixed in ethanol-acetic acid (3:1, v:v) at room temperature for 30 min after used immediately or stored these roots at 4°C. Root samples were washed in distilled water, then hydrolyzed in 1M HCl for 5 min at 60°C and washed again in distilled water, then they were stained and squashed in 2% aceto-orcein, and observed cells under a light microscope (Zeiss Axiostar Plus) (followed Saenprom et al. 2018 method) to capture the micrograph). Measure the chromosome length and rearrangement of chromosomes was used the software Adobe Photoshop CS3 Extended. The nomenclature of the chromosome morphology followed the method of Levan et al. (1964), Senavongse et al. (2018) and Saensouk and Saensouk (2020). The metacentric (m), submetacentric (sm), subtelocentric (st) or acrocentric (a) chromosomes had been classified. The chromosome number, chromosome length range, diploid chromosome length, arm ratio, relative length and karyotype formula were studied from 20 clearly metaphase cells in each species. Moreover, the karyotypes were calculated from the parameters for the average length of the short arm (Ls), average length of long arm (Ll), total length of each chromosome (LT), average relative length (RL = LT/∑LT, formula for relative length), chromosome index (CI = Ll/LT, the formula for chromosome index are 0.500-0.599 = Metacentric, 0.600-0.699 = Submetacentric, 0.700-0.899 = Subtelocentric, 0.900-1.000 = Acocetric) and standard deviations (SD) of RL and CI from metaphase chromosomes followed the methods of Saensouk P and Saensouk S (2020, 2021b). Saensouk and Saensouk (2021a, b)

RESULTS AND DISCUSSION

The chromosome numbers from the root tips of *B. rotunda*, *C. aeruginosa*, *E. elatior*, *K. elegans*, *K. pulchra*, *K. rotunda* and *Z. montanum* from Bueng Kan Province, northeastern Thailand were summarized in Table 1. In addition, karyology (fundamental number (NF), karyotype formula), ideogram, and location in this study and those studied previously of five species in this study were presented in Table 1. Moreover, the chromosome size, the relative length (RL), and the centromeric indexes (CI) were reported in Tables 2-6. The result of this study revealed that the chromosome numbers of all species were found to be 2n = 20-68, consistent with previous studies by Sirisawad et al. (2003), Eksomtramage et al. (2002) (white form inflorescence), Khamtang et al. (2014), Nopporncharoenkul et al. (2017), Saenprom et al. (2018), Mood et al. (2020), Saensouk and Saensouk (2004, 2021b, 2022).

The somatic chromosome number of *B. rotunda* was presented to be 32 (Figures 1.A and 1.B) differs from previous reports due to the effects of environmental factors, i.e. Mood et al. (2020) (2n = 24) and Saensouk and Saensouk (2022) (2n = 38). The NF was found to be 64, different from Saensouk and Saensouk (2022), who reported NF = 76. The karyotype formula of this species was a karyotype 30m+2sm (Figure 1.C and Table 2) differs from previous reports due to the effects of location, environmental factors, Saensouk and Saensouk (2022) (18m+14sm+6st). Moreover, the short arm length (Ls), the long arm length (Ll), the total chromosome length (Lt) ranged, the relative length (RL), and the centromeric indexes (CI) of karyotype structure in this study also differ from Saensouk and Saensouk (2022) (Table 2 and Figure 1.C). The ideogram was created based on the lengths of the

chromosome arms and presented the point of the centromere (Figure 1.D). The ideogram of *B. rotunda* was reported for the first time (Table 1). The symmetrical karyotype in this study differs from Saensouk and Saensouk (2022), who recorded an asymmetrical karyotype of this species due to the effects of environmental factors (Table 1).

The chromosome number of *C. aeruginosa* was found to be 66 (Figure 2.A). The somatic chromosome numbers of this study differ from Sirisawad et al. (2003) (2n = 63) due to the effects of environmental factors such as location of the plant, soil, water, air etc. The first report of NF was presented to be 132. The first report of the karyotype formula of *C. aeruginosa* was 62m+4sm (Figures 1.B and 1.C, Tables 1 and 3). The short arm length, the long arm length, the total chromosome length, the relative length and the centromeric indexes of karyotype structure in this study were reported for the first time (Table 3 and Figures 2.B 2.C). The ideogram was created based on the lengths of the chromosome arms and presented the point of the centromere (Figure 2.C). The symmetrical karyotype of *C. aeruginosa* was also reported for the first time (Tables 1 and 3).

Table 1. Chromosome number, karyology, and ideogram of seven species in the family Zingiberaceae from Beung Kan Province, Thailand, investigated in this study compared to previous studies

Species	Chromosome numbers (2n)	NF	Karyotype formula	Ideogram	Symmetrical karyotype	Location	Previous study
B. rotunda	32*	64	30m+2sm	✓*	Symmetry	Thailand	*Present study*
	24	-	-	-	-	Malaysia, Indonesia	Mood et al. (2020)
	38	76	18m+14sm+6st		Asymmetry	Thailand	Saensouk and Saensouk (2022)
C. aerugenosa	66	132*	62m+4sm*	✓*	Symmetry	Thailand	*Present study*
	63	-	-	-	-	Thailand	Sirisawad et al. (2003)
E. elatior (red form inflorescence)	68	136*	26m+30sm+12st*	✓*	Asymmetry	Thailand	*Present study*
	48	-	-	-	-	Thailand	Eksomtramage et al. (2002) (white form inflorescence)
K. elegans	22	44*	12m+8sm+2st*	✓*	Asymmetry	Thailand	*Present study*
	22	-	-	-	-	Thailand	Nopporncharoenkul et al. (2017)
	22	-	-	-	-		Eksomtramage et al. (2002)
K. pulchra	20	40*	18m+2sm*	✓*	Symmetry	Thailand	*Present study*
	22	-	-	-	-	Thailand	Nopporncharoenkul et al. (2017)
K. rotunda	22	44*	10m+12sm	✓*	Symmetry	Thailand	*Present study*
	22	-	-	-	-	Thailand	Khamtang et al. (2014)
	22	-	-	-	-	Thailand	Nopporncharoenkul et al. (2017)
	22	-	14m+16sm	-	-	Thailand	Saenprom et al. (2018)
	22	-	12m+10sm	-	-	Thailand	Saensouk and Saensouk (2021b)
Z. montanum	22	44*	2a+18m+2sm*	✓*	Asymmetry	Thailand	*Present study*
	22	-	-	-	-	Thailand	Saensouk and Saensouk (2004)

Note**:** *the first time report, m: metacentric chromosome, sm: submetacentric chromosome, a: acrocentric chromosome, st: subtelocentric chromosome, −: not available

Table 2. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *B. rotunda* (2n = 32)

Chromosome pair	Ls (µm) ± SD	Ll (µm) ± SD	LT (µm) ±SD	RL (%)	CI	Chromosome type
1	1.11±0.08	1.19±0.04	2.30±0.04	8.80±0.08	0.52±0.04	Metacentric
2	1.03±0.20	1.15±0.06	2.17±0.04	8.33±0.02	0.53±0.02	Metacentric
3	0.93±0.18	1.17±0.08	2.10±0.06	8.04±0.04	0.56±0.01	Metacentric
4	0.79±0.14	1.14±0.14	1.93±0.02	7.40±0.04	0.59±0.04	Metacentric
5	0.88±0.12	1.00±0.10	1.89±0.06	7.24±0.02	0.53±0.06	Metacentric
6	0.80±0.14	1.06±0.08	1.86±0.08	7.15±0.01	0.57±0.01	Metacentric
7	0.84±0.24	0.94±0.04	1.78±0.10	6.82±0.02	0.53±0.04	Metacentric
8	0.76±0.16	0.86±0.14	1.61±0.06	6.19±0.04	0.53±0.08	Metacentric
9	0.72±0.18	0.85±0.18	1.58±0.04	6.05±0.08	0.54±0.02	Metacentric
10	0.76±0.20	0.81±0.04	1.57±0.08	6.00±0.02	0.52±0.04	Metacentric
11	0.66±0.04	0.76±0.08	1.42±0.02	5.44±0.02	0.53±0.04	Metacentric
12	0.65±0.18	0.74±0.14	1.38±0.04	5.29±0.04	0.53±0.06	Metacentric
13	0.58±0.10	0.69±0.18	1.27±0.05	4.86±0.08	0.54±0.02	Metacentric
14	0.53±0.04	0.59±0.14	1.11±0.04	4.26±0.04	0.53±0.01	Metacentric
15	0.48±0.08	0.59±0.14	1.06±0.04	4.08±0.04	0.55±0.04	Metacentric
16	0.41±0.18	0.65±0.18	1.06±0.04	4.06±0.02	0.61±0.02	Submetacentric

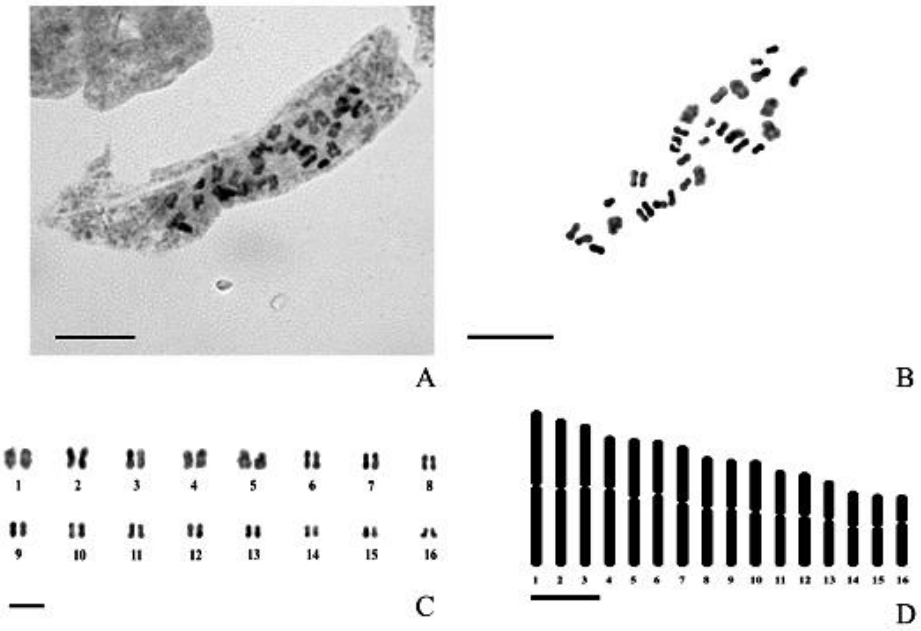

Figure 1. The chromosome of *B. rotunda* A. Photo of somatic metaphase chromosome number showing 2n = 32, B. Drawing of somatic metaphase chromosome number showing 2n = 32, C. Karyotype showing 30m+2sm, D. Ideogram, scale bars = 5 μm

Table 3. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *C. aeruginosa* (2n = 66)

Chromosome pair	Ls±SD (*μ*m)	Ll±SD (*μ*m)	LT±SD (*μ*m)	RL (%)	CI	Chromosome type
1	0.59±0.03	0.97±0.06	1.57±0.09	4.65±0.01	0.62±0.02	Submetacentric
2	0.55±0.03	0.76±0.04	1.31±0.08	3.88±0.01	0.58±0.03	Metacentric
3	0.57±0.03	0.72±0.04	1.30±0.07	3.85±0.02	0.56±0.01	Metacentric
4	0.56±0.03	0.68±0.04	1.24±0.07	3.68±0.05	0.55±0.02	Metacentric
5	0.56±0.03	0.67±0.04	1.23±0.07	3.66±0.04	0.54±0.01	Metacentric
6	0.61±0.04	0.62±0.04	1.23±0.07	3.66±0.02	0.50±0.03	Metacentric
7	0.52±0.03	0.70±0.04	1.22±0.07	3.62±0.01	0.57±0.02	Metacentric
8	0.57±0.03	0.59±0.03	1.17±0.07	3.46±0.01	0.51±0.01	Metacentric
9	0.51±0.03	0.65±0.04	1.16±0.07	3.43±0.02	0.56±0.04	Metacentric
10	0.55±0.03	0.58±0.03	1.13±0.07	3.35±0.01	0.52±0.01	Metacentric
11	0.45±0.03	0.65±0.04	1.11±0.06	3.29±0.01	0.59±0.03	Metacentric
12	0.47±0.03	0.60±0.03	1.07±0.06	3.18±0.02	0.56±0.01	Metacentric
13	0.46±0.03	0.60±0.03	1.06±0.06	3.15±0.04	0.56±0.01	Metacentric
14	0.44±0.03	0.60±0.03	1.05±0.06	3.10±0.05	0.58±0.05	Metacentric
15	0.50±0.03	0.55±0.03	1.05±0.06	3.12±0.01	0.52±0.03	Metacentric
16	0.43±0.02	0.58±0.03	1.00±0.06	2.98±0.01	0.57±0.02	Metacentric
17	0.46±0.03	0.54±0.03	1.00±0.06	2.97±0.02	0.54±0.01	Metacentric
18	0.46±0.03	0.51±0.03	0.97±0.06	2.88±0.01	0.52±0.01	Metacentric
19	0.43±0.03	0.52±0.03	0.96±0.06	2.84±0.01	0.55±0.05	Metacentric
20	0.45±0.03	0.50±0.03	0.95±0.05	2.81±0.02	0.53±0.02	Metacentric
21	0.40±0.02	0.54±0.03	0.94±0.05	2.78±0.04	0.57±0.01	Metacentric
22	0.43±0.02	0.51±0.03	0.94±0.05	2.78±0.04	0.54±0.02	Metacentric
23	0.43±0.02	0.50±0.03	0.93±0.05	2.76±0.01	0.54±0.02	Metacentric
24	0.41±0.02	0.51±0.03	0.92±0.05	2.73±0.02	0.56±0.01	Metacentric
25	0.44±0.03	0.44±0.03	0.88±0.05	2.62±0.01	0.50±0.03	Metacentric
26	0.39±0.02	0.49±0.03	0.88±0.05	2.62±0.01	0.56±0.02	Metacentric
27	0.40±0.02	0.42±0.02	0.82±0.05	2.43±0.01	0.51±0.03	Metacentric
28	0.41±0.02	0.41±0.02	0.82±0.05	2.43±0.01	0.50±0.03	Metacentric
29	0.40±0.02	0.42±0.02	0.82±0.05	2.44±0.02	0.51±0.02	Metacentric
30	0.39±0.02	0.41±0.02	0.80±0.05	2.37±0.01	0.51±0.02	Metacentric
31	0.35±0.02	0.42±0.02	0.77±0.04	2.29±0.02	0.55±0.01	Metacentric
32	0.34±0.02	0.42±0.02	0.76±0.04	2.25±0.01	0.55±0.01	Metacentric
33	0.26±0.01	0.40±0.02	0.66±0.04	1.95±0.01	0.61±0.01	Submetacentric

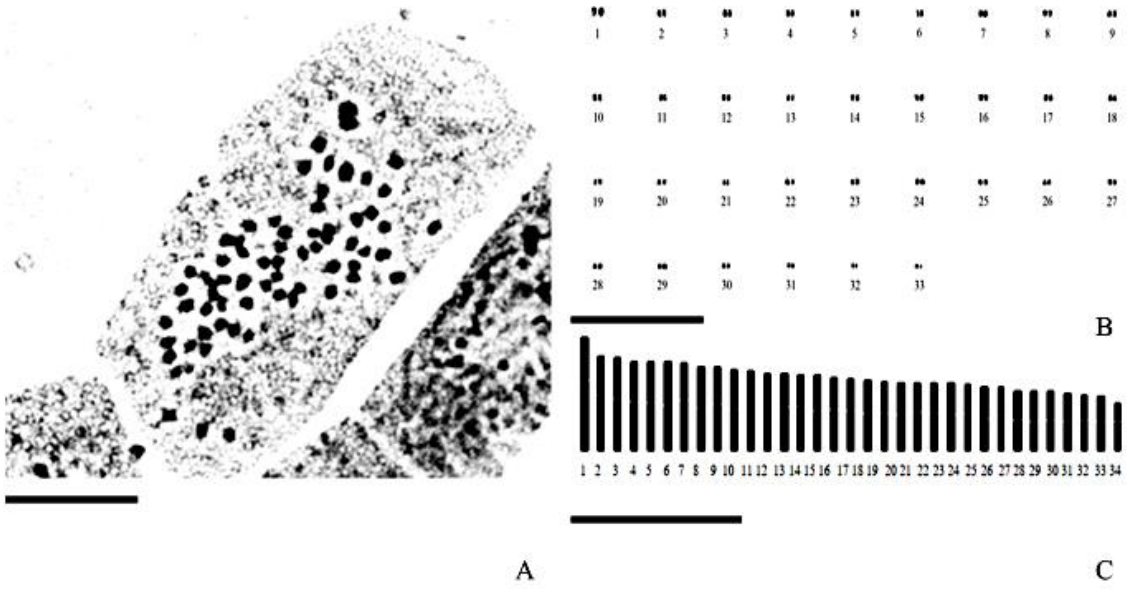

Figure 2. The chromosome of *C. aeruginosa* A. Photo of somatic metaphase chromosome number showing 2n = 66, C. Karyotype showing 62m+4sm, C. Ideogram, scale bars = 5 μm

Table 4. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *E. elatior* (2n = 68)

Chromosome pair	Ls±SD (*μ*m)	Ll±SD (*μ*m)	LT±SD (*μ*m)	RL (%)	CI	Chromosome type
1	4.50±0.20	7.92±0.35	12.42±0.56	5.56±0.01	0.64±0.01	Submetacentric
2	4.56±0.21	6.00±0.27	10.56±0.48	4.73±0.01	0.57±0.01	Metacentric
3	3.43±0.17	7.00±0.31	10.43±0.47	4.67±0.02	0.67±0.02	Submetacentric
4	1.55±0.10	8.72±0.36	10.28±0.46	4.60±0.01	0.85±0.01	Subtalocentric
5	4.05±0.18	5.36±0.25	9.41±0.42	4.22±0.05	0.57±0.01	Metacentric
6	2.26±0.12	6.73±0.29	8.99±0.41	4.03±0.03	0.75±0.03	Subtalocentric
7	2.92±0.15	6.03±0.26	8.95±0.41	4.01±0.01	0.67±0.02	Submetacentric
8	3.48±0.16	5.14±0.24	8.62±0.39	3.86±0.01	0.60±0.02	Submetacentric
9	2.15±0.12	6.03±0.26	8.18±0.38	3.66±0.02	0.74±0.02	Subtalocentric
10	2.99±0.14	4.83±0.22	7.82±0.36	3.50±0.03	0.62±0.03	Submetacentric
11	1.73±0.10	5.90±0.25	7.63±0.35	3.42±0.04	0.77±0.01	Subtalocentric
12	2.83±0.13	4.76±0.22	7.59±0.35	3.40±0.01	0.63±0.02	Submetacentric
13	2.57±0.12	5.00±0.23	7.57±0.35	3.39±0.02	0.66±0.03	Submetacentric
14	3.22±0.15	4.31±0.19	7.53±0.35	3.37±0.03	0.57±0.03	Metacentric
15	2.07±0.11	5.09±0.22	7.17±0.33	3.21±0.02	0.71±0.02	Subtalocentric
16	1.91±0.10	4.30±0.19	6.21±0.30	2.78±0.01	0.69±0.05	Submetacentric
17	3.16±0.15	3.68±0.17	6.84±0.32	3.06±0.03	0.54±0.05	Metacentric
18	1.97±0.11	4.49±0.20	6.46±0.30	2.89±0.01	0.69±0.01	Submetacentric
19	2.00±0.11	4.26±0.19	6.26±0.30	2.81±0.02	0.68±0.01	Submetacentric
20	2.71±0.13	3.48±0.16	6.19±0.29	2.77±0.05	0.56±0.02	Metacentric
21	2.77±0.13	3.39±0.16	6.15±0.29	2.76±0.05	0.55±0.01	Metacentric
22	1.56±0.09	4.00±0.18	5.56±0.27	2.49±0.02	0.72±0.05	Subtalocentric
23	1.82±0.10	3.03±0.15	4.85±0.24	2.17±0.01	0.63±0.02	Submetacentric
24	1.65±0.09	3.17±0.15	4.81±0.24	2.16±0.01	0.66±0.03	Submetacentric
25	2.02±0.11	2.79±0.14	4.81±0.24	2.15±0.02	0.58±0.01	Metacentric
26	1.71±0.08	3.07±0.16	4.78±0.24	2.14±0.03	0.64±0.01	Submetacentric
27	1.97±0.09	2.56±0.14	4.53±0.23	2.03±0.01	0.56±0.02	Metacentric
28	1.36±0.08	2.74±0.13	4.10±0.21	1.84±0.02	0.67±0.02	Submetacentric
29	1.50±0.07	2.49±0.14	3.99±0.21	1.79±0.05	0.62±0.02	Submetacentric
30	1.57±0.08	1.94±0.10	3.50±0.19	1.57±0.05	0.55±0.01	Metacentric
31	1.47±0.08	1.92±0.11	3.39±0.18	1.52±0.01	0.57±0.02	Metacentric
32	1.27±0.07	1.74±0.09	3.01±0.16	1.35±0.03	0.58±0.05	Metacentric
33	1.24±0.06	1.24±0.07	2.38±0.13	1.06±0.03	0.52±0.02	Metacentric
34	1.15±0.06	1.15±0.06	2.29±0.12	1.03±0.01	0.50±0.01	Metacentric

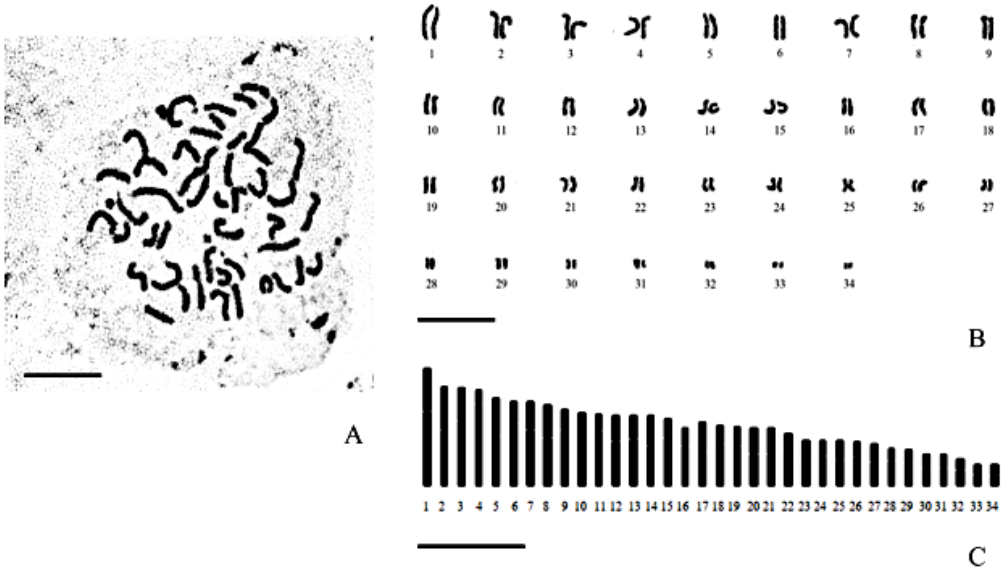

Figure 3. The chromosome of *E. elatior* A. Photo of somatic metaphase chromosome number showing 2n = 66, C. Karyotype showing 62m+4sm, C. Ideogram, scale bars = 5 µm

The somatic chromosome number of *E. elatior* (red form inflorescence) was found to be 68 (Figure 3.A). The somatic chromosome numbers of this study differ from Eksomtramage et al. 2002 (white form inflorescence) (2n = 48) due to the morphological character of inflorescence and the effects of environmental factors such as location of the plant, soil, water, air etc. The NF was the first reported to be 136. The karyotype formula of *E. elatior* (red form inflorescence) was an asymmetrical, karyotype 26m+30sm+12st (Figures 3.B, 3.C and Table 4). The short arm length, the long arm length, the total chromosome length, the relative length, and the centromeric indexes (Table 4 and Figures 3.B, 3C) were provided. The ideogram of this species was the first created based on the lengths of the chromosome arms and presented the point of the centromere (Figure 3.C). Therefore, the karyotype formula, the relative length, and the centromeric indexes and the symmetrical, karyotype of *E. elatior* (red form inflorescence) were reported for the first time (Tables 1, 4).

The somatic chromosome number of *K. elegans* occurred to be 22 (Figure 4.A). The somatic chromosome numbers of this study are consistent with previous studies by Eksomtramage et al. (2002) and Nopporncharoenkul et al. (2017). The NF was the first reported to be 44. The karyotype formula of *K. elegans* was the first reported as an asymmetrical, karyotype 12m+8sm+2st (Figures 4.B, 4.C and Tables 1, 5). The short arm length, the long arm length, the total chromosome length, the relative length of the karyotype, and the centromeric indexes were the first provided (Table 5 and Figures 4.B, 4.C). The ideogram was first created (Figure 4.C and Tables 1, 5).

Table 5. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *K. elegans* (2n = 22)

Chromosome pair	Ls±SD (*µ*m)	Ll±SD (*µ*m)	LT±SD (*µ*m)	RL (%)	CI	Chromosome type
1	1.69±0.10	3.77±0.85	5.47±0.95	12.48±0.01	0.69±0.05	Submetacentric
2	2.01±0.09	2.93±0.16	4.93±0.26	11.26±0.03	0.59±0.02	Metacentric
3	2.11±0.10	2.52±0.15	4.64±0.24	10.59±0.01	0.54±0.01	Metacentric
4	1.96±0.10	2.05±0.13	4.01±0.23	9.16±0.05	0.51±0.01	Metacentric
5	1.22±0.08	2.73±0.13	3.95±0.21	9.02±0.02	0.69±0.02	Submetacentric
6	1.06±0.08	2.76±0.13	3.82±0.21	8.72±0.01	0.72±0.03	Subtalocentric
7	1.41±0.08	2.27±0.13	3.68±0.21	8.40±0.01	0.62±0.01	Submetacentric
8	1.22±0.07	2.29±0.11	3.51±0.18	8.01±0.02	0.65±0.02	Metacentric
9	1.51±0.08	1.96±0.10	3.47±0.18	7.92±0.05	0.56±0.05	Metacentric
10	1.46±0.07	1.89±0.10	3.35±0.17	7.64±0.01	0.56±0.01	Metacentric
11	1.09±0.06	1.89±0.09	2.98±0.16	6.80±0.01	0.63±0.02	Submetacentric

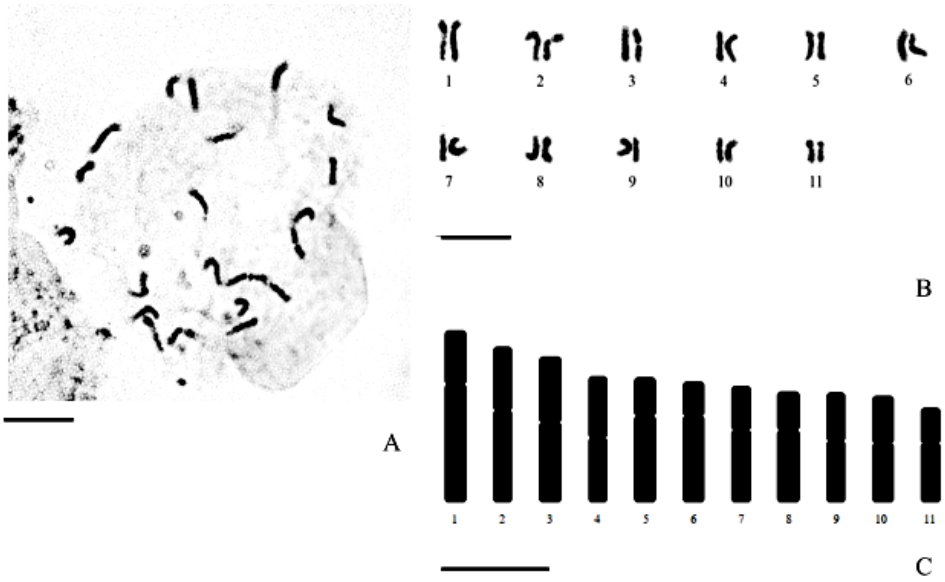

Figure 4. The chromosome of *K. elegans* A. Photo of somatic metaphase chromosome number showing 2n = 44, C. Karyotype showing 12m+8sm+2st, C. Ideogram, scale bars = 5 µm

Table 6. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *K. pulchra* (2n = 20)

Chromosome pair	Ls±SD (*µ*m)	Ll±SD (*µ*m)	LT±SD (*µ*m)	RL (%)	CI	Chromosome type
1	1.88±0.07	2.44±0.14	4.32±0.21	12.30±0.01	0.57±0.05	Metacentric
2	1.88±0.07	2.19±0.13	4.07±0.19	11.60±0.05	0.54±0.05	Metacentric
3	1.84±0.07	2.15±0.12	3.99±0.18	11.37±0.03	0.54±0.03	Metacentric
4	1.15±0.06	2.56±0.10	3.71±0.16	10.56±0.01	0.69±0.01	Submetacentric
5	1.52±0.07	1.99±0.10	3.51±0.16	10.00±0.01	0.57±0.01	Metacentric
6	1.62±0.06	1.82±0.10	3.45±0.16	9.82±0.03	0.53±0.03	Metacentric
7	1.44±0.06	1.88±0.09	3.32±0.14	9.44±0.05	0.57±0.05	Metacentric
8	1.39±0.05	1.89±0.09	3.28±0.14	9.33±0.01	0.58±0.01	Metacentric
9	1.41±0.05	1.48±0.08	2.89±0.13	8.23±0.03	0.51±0.05	Metacentric
10	1.08±0.04	1.50±0.06	2.58±0.10	7.36±0.01	0.58±0.01	Metacentric

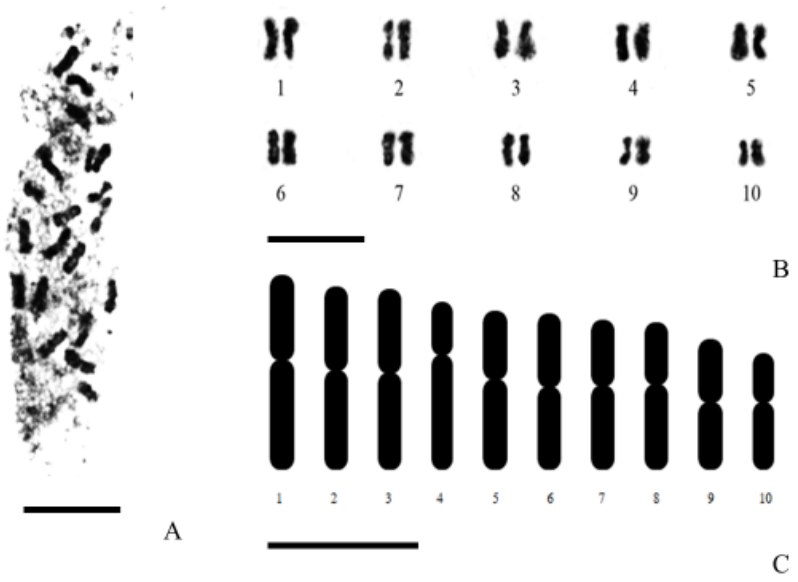

Figure 5. The chromosome of *K. pulchra.* A. Photo of somatic metaphase chromosome number showing 2n = 20, C. Karyotype showing 18m+2sm, C. Ideogram, scale bars = 5 µm

The somatic chromosome number of *K. pulchra* occurred to be 20 (Figure 5.A). The somatic chromosome numbers of this study differ from previous studies by Nopporncharoenkul et al. (2017), who reported chromosome numbers of *K. pulchra* to be 2n = 22 due to the effects of environmental factors such as location of the plant, soil, water, air etc. The NF was the first studied to be 40. The karyotype formula of *K. pulchra* was a symmetrical, karyotype 18m+2sm (Figures 5.B, 5.C and Table 6). The short arm length (Ls) ranged from 1.08±0.04 to 1.88±0.07 μm, the long arm length, the total chromosome length, the relative length of the karyotype, the centromeric indexes were provided (Figures 5.B, 5.C and Tables 1, 6). The ideogram was created based on the lengths of the chromosome arms and presented the point of the centromere (Figure 5.C). Moreover, the karyotype formula, the relative length of the karyotype, the centromeric indexes, the ideogram and the symmetrical karyotype of *K. pulchra* were reported for the first time (Tables 1, 6).

The somatic chromosome number of *K. rotunda* was 22 (Figures 6.A, 6.B). The somatic chromosome numbers of this study are consistent with previous studies by Khamtang et al. (2014), Nopporncharoenkul et al. (2017), Saenprom et al. (2018) and Saensouk and Saensouk (2021b). The NF was the first presented to be 44. The karyotype formula of *K. rotunda* was a symmetrical, karyotype 10m+12sm (Figures 6.C, 6.D and Table 7). The karyotype formula of this study differs from previous studies by Saenprom et al. (2018) (14m+16sm) and Saensouk and Saensouk (2021b) (12m+10sm) due to the effects of environmental factors such as location of the plant, soil, water, air etc. The short arm length, the long arm length, the total chromosome length, relative length, centromeric indexes of karyotype were reported (Table 7 and Figures 6.C, 6.D). The ideogram was first created based on the lengths of the chromosome arms and presented the point of the centromere (Figure 6.C). Moreover, the relative length, centromeric indexes and symmetrical karyotype of *K. rotunda* were reported for the first time (Tables 1, 7).

Table 7. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *K. rotunda* (2n = 22)

Chromosome pair	Ls (μm) ± SD	Ll (μm) ± SD	LT (μm) ±SD	RL (%)	CI	Chromosome type
1	0.74±0.08	1.15±0.04	1.88±0.04	12.10±0.08	0.61±0.04	Submetacentric
2	0.68±0.18	1.17±0.08	1.85±0.02	11.86±0.04	0.63±0.02	Submetacentric
3	0.62±0.12	0.93±0.10	1.55±0.06	9.97±0.01	0.60±0.04	Submetacentric
4	0.64±0.20	0.88±0.14	1.53±0.08	9.79±0.02	0.58±0.04	Metacentric
5	0.62±0.14	0.89±0.06	1.51±0.04	9.68±0.02	0.59±0.06	Metacentric
6	0.48±0.14	0.90±0.08	1.38±0.06	8.84±0.04	0.65±0.04	Submetacentric
7	0.51±0.24	0.85±0.18	1.36±0.04	8.71±0.08	0.62±0.08	Submetacentric
8	0.48±0.18	0.78±0.04	1.25±0.08	8.05±0.02	0.62±0.04	Submetacentric
9	0.54±0.20	0.69±0.14	1.23±0.02	7.88±0.02	0.56±0.06	Metacentric
10	0.54±0.16	0.57±0.04	1.11±0.10	7.10±0.04	0.51±0.04	Metacentric
11	0.44±0.04	0.49±0.08	0.94±0.06	6.01±0.02	0.53±0.08	Metacentric

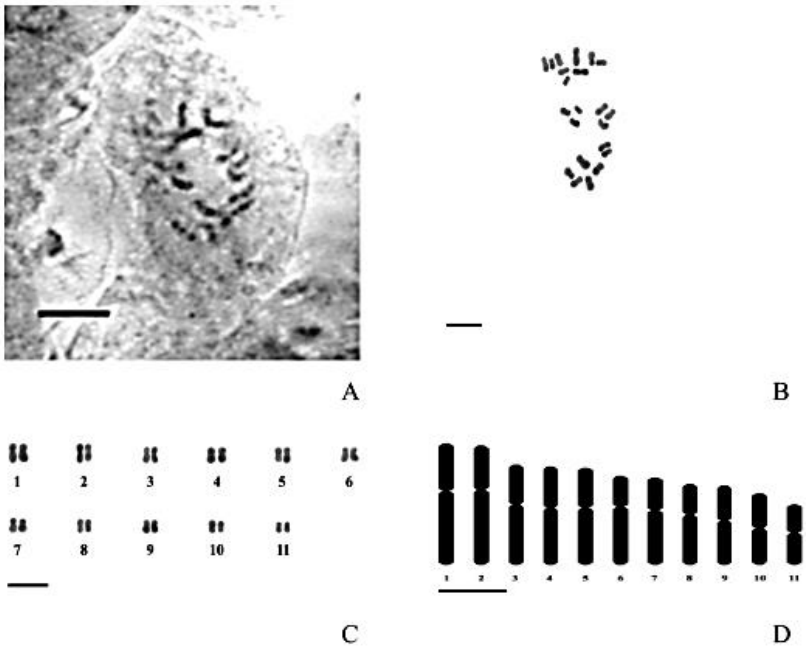

Figure 6. The chromosome of *K. rotunda* A. Photo of somatic metaphase chromosome number showing 2n = 22, B. Drawing of somatic metaphase chromosome number showing 2n = 22, C. Karyotype showing 10m+12sm, D. Ideogram, scale bars = 5 μm

Table 8. Mean length of short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT), relative length (RL), centromeric index (CI), standard deviation (SD) of RL and CI from 20 metaphases, and chromosome size of *Z. montanum* (2n = 22)

Chromosome pair	Ls (µm) ± SD	Ll (µm) ± SD	LT (µm) ±SD	RL (%)	CI	Chromosome type
1	1.77±0.10	2.55±0.04	4.33±0.02	14.55±0.02	0.59±0.02	Metacentric
2	1.66±0.08	2.22±0.06	3.88±0.04	13.04±0.01	0.57±0.02	Metacentric
3	0.98±0.06	2.38±0.08	3.36±0.06	11.28±0.02	0.71±0.04	Acrocentric
4	1.24±0.06	1.86±0.10	3.10±0.08	10.43±0.04	0.60±0.06	Submetacentric
5	1.15±0.08	1.39±0.04	2.54±0.08	8.55±0.02	0.55±0.02	Metacentric
6	1.24±0.05	1.29±0.02	2.53±0.04	8.49±0.01	0.51±0.02	Metacentric
7	1.20±0.04	1.24±0.06	2.43±0.02	8.18±0.01	0.51±0.02	Metacentric
8	1.11±0.05	1.20±0.08	2.31±0.06	7.75±0.02	0.52±0.02	Metacentric
9	0.86±0.08	1.05±0.04	1.90±0.04	6.40±0.01	0.55±0.06	Metacentric
10	0.78±0.06	1.01±0.02	1.78±0.04	5.99±0.04	0.57±0.02	Metacentric
11	0.70±0.02	0.88±0.02	1.58±0.04	5.33±0.02	0.56±0.01	Metacentric

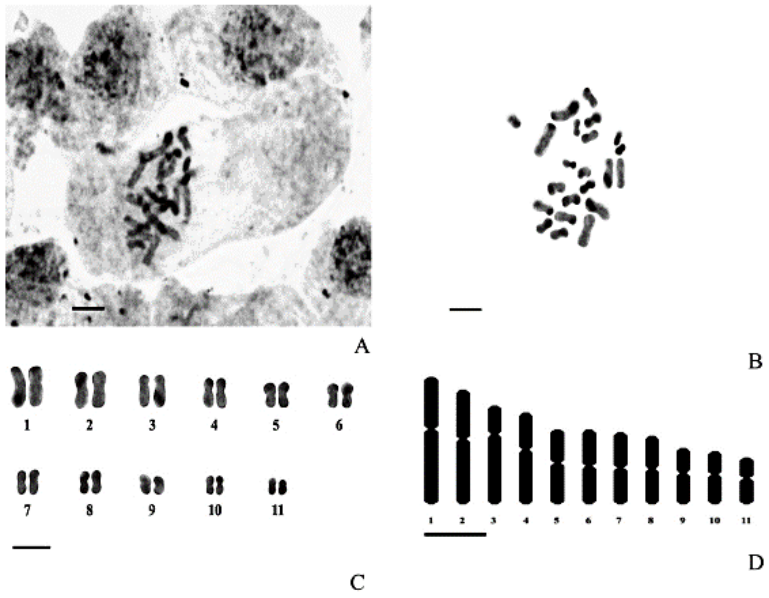

Figure 7. The chromosome of *Z. montanum* A. Photo of somatic metaphase chromosome number showing 2n = 22, B. Drawing of somatic metaphase chromosome number showing 2n = 22, C. Karyotype showing 2a+18m+2sm, D. Ideogram, scale bars = 5 µm

The somatic chromosome number of *Z. montanum* occurred to be 22 (Figures 7.A, 7.B). The somatic chromosome numbers of this study are consistent with previous studies by Saensouk and Saensouk (2004). The NF was the first presented to be 44. The karyotype formula of *Z. montanum* was a symmetrical, karyotype 2a+18m+2sm (Figures 7.C, 7.D and Table 8). Therefore, the karyotype formula and symmetrical karyotype of *K. elegans* were reported for the first time. The short arm length, the long arm length, the total chromosome length, and relative length, centromeric indexes of this karyotype were reported for the first time (Table 8 and Figures 7.C, 7.D). The ideogram was the first created based on the lengths of the chromosome arms and presented the point of the centromere (Figure 7.C and Tables 1, 8).

The cytogenetic study of family Zingiberaceae was few studied, especially karyotype and ideogram because the somatic chromosome number of this family was difficult to find clearly metaphase stage, consistent with Saensouk and Saensouk (2004). Furthermore, Stebbins (1971) suggested the karyotype of organisms involves chromosome size and chromosome types. These cytological analysis data would be a help in the classification of species, but further reports are warranted. Moreover, Chaiyasut (1989) reported a study on karyotypes of plants that can help identify the cause of the morphological changes that occurred from the environment, such as location of the plant, soil, water, air and etc. or from a change involving chromosomes, either structural or numerical. It is also used as evidence in the study of evolutionary relationships.

In conclusion, the chromosome number, karyotype, NF, symmetrical karyotype, the relative length, centromeric indexes, and ideogram of *B. rotunda*, *C. aeruginosa*, *E. elatior* (red form inflorescence), *K. elegans*, *K. pulchra*, *K. rotunda* and *Z. montanum* from Bueng Kan Province, northeastern Thailand was studied. The chromosome numbers of all species were found between 2n = 20 (*K. pulchra*)-68 (*E. elatior*). The NF had 40 (*K. pulchra*)-136 (*E. elatior*). The symmetrical karyotype formula of this study found a symmetrical karyotype (four species) and an asymmetrical karyotype (three species). The ideogram of seven species in this study was reported for the first time. The NF of six species was reported for the first time. The karyotype formula of five species, namely *C. aeruginosa*,

E. elatior (red form inflorescence), *K. elegans*, *K. pulchra*, and *Z. montanum* was reported for the first time. Whereas, the karyotype formula of two species (*B. rotunda* and *K. rotunda*) differs from the previous study. Therefore, the cytogenetic data in this study can be used for identification.

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