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Karyotype analysis of *Eleutherine bulbosa* (Iridaceae) from Kalimantan, Indonesia

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Abstract. *Habibah NA, Tunjung WAS, Indrowati M, Liana A. 2025. Karyotype analysis of* Eleutherine bulbosa *(Iridaceae) from Kalimantan, Indonesia. Biodiversitas 26: 2486-2494. Eleutherine bulbosa* or Dayak onion belongs to the family Iridaceae and is widely distributed in Indonesia. However, research on *E. bulbosa*, especially for the cytogenetic aspect is poorly reported. This study aimed to analyze karyotype of *E. bulbosa* chromosomes collected from Kalimantan, Indonesia. Chromosome observation was carried out using a modified squash method. The results showed that *E. bulbosa* has basic chromosome number of 2n: 12. Although it has the same number of chromosomes, each sample shows a different chromosome formula. Those from East Kalimantan and Central Kalimantan have a chromosome formula of 2n=2x=12m. Meanwhile, samples from South Kalimantan have a chromosome formula of 2n=2x=10m+2sm. In comparison, those from North Kalimantan and West Kalimantan have a chromosome formula of 2n=2x=6m+2t+4sm and of 2n=2x=8m+2sm+2t, respectively. *Eleutherine bulbosa* was also found to have a bimodal karyotype. The most recent finding of this study is the discovery of sub-metacentric and telocentric chromosome morphologies, which have never been seen in previous studies.

Keywords: Dayak union, *Eleutherine bulbosa*, Kalimantan, karyotype, squash method

INTRODUCTION

The genus *Eleutherine* is a member of the family Iridaceae, notable for its bulbous plants. According to POWO (2025), the genus consists of three accepted species and is native from Mexico to tropical America. *Eleutherine bulbosa* (Mill.) Urb. is from the Caribbean to South Tropical America (POWO 2025) and is introduced in Indonesia. In the country, *E. bulbosa* is mainly found in the forests of Kalimantan, especially among the Dayak community, where it is locally called *Bawang Dayak* or Dayak onion (Herman et al. 2024). This species has been reported from several regions in Indonesia, including Palu (Central Sulawesi) (Bahtiar and Annisa 2018), Bogor (West Java), Purbalingga (Central Java), and Malang (East Java) (Febriani 2019) indicating its widespread occurrence across the Indonesian archipelago. Nevertheless, the highest level of biodiversity for this species has been documented in the Kalimantan region (Herman et al. 2024), suggesting that this area serves as a significant center for its distribution and may hold key insights into its evolutionary development.

Eleutherine bulbosa bulbs are generally elongated and can vary in size, influenced by environmental factors and cultivation practices (Wiendi et al. 2021). The leaves are linear to lanceolate, growing in a rosette formation, and can reach lengths of up to 50 cm. These leaves are characterized by a glossy green surface and a prominent midrib, which aids in water retention and photosynthesis (Muthia et al. 2021; Wiendi et al. 2021). The inflorescence of *E. bulbosa* is cymose, consisting of multiple intra-inflorescences, each containing 3-4 florets. The flowers are typically white to pale purple, with a distinct structure that attracts various pollinators, including bees and butterflies (Wiendi et al. 2021).

Bulb of *E. bulbosa* contains various bioactive compounds, contributing to its medicinal properties, which include antioxidant, analgesic, antibacterial, anti-inflammatory, and anti-diabetic effects (Hafizh et al. 2021; Susilawati et al. 2022; Herman et al. 2024; Nisa et al. 2024). Dayak onion has been propagated through the callus culture method to increase the production of its bioactive components (Habibah et al. 2023). The callus culture is a practical method for the production of secondary metabolites (Habibah et al. 2024). Although *E. bulbosa* is widely known as a traditional medicinal plant, information on its cytogenetic aspects, including karyotype and genetic variation, is poorly reported. On the other hand, vegetative characters need support from cytological data, because they are plastic and strongly influenced by the environment (Liana et al. 2017).

Karyotype analysis in *E. bulbosa* has previously been conducted by Alves et al. (2011), who identified metacentric and acrocentric chromosome forms, resulting in the chromosome formula 2n=11m+1a. Báez et al. (2019) also reported a bimodal karyotype in *E. bulbosa*, indicating the presence of two distinct chromosome size types. Feitoza and Guerra (2011) further demonstrated variation

in chromosome distribution and histone modifications in *E. bulbosa*, which may influence gene expression and genome stability. Despite these findings, specific studies on the karyotype of *E. bulbosa* from Kalimantan remain limited. Existing data, such as that from Mursyidin et al. (2013), focus only on populations in southern Kalimantan. Therefore, this study aims to analyze the karyotype and chromosomal variation of *E. bulbosa* collected from various regions of Kalimantan, Indonesia, to contribute to a more comprehensive understanding of its cytogenetic diversity within local populations.

MATERIALS AND METHODS

Plant materials

Plant materials were collected from five locations in Kalimantan, Indonesia (Table 1 and Figure 1). The bulbs of *E. bulbosa* were carefully excavated, cleaned from excess soil, and individually packaged in labeled paper bags to ensure proper identification and to maintain sample integrity during transport. All samples were then shipped to Yogyakarta for further analysis. This research was conducted from July to August 2024 at the Genetics and Breeding Laboratory, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Procedures

Sample preparation

Eleutherine bulbosa bulbs were grown in a petri dish containing distilled water until the roots grew. When the secondary roots of the plant had grown, the root tips were cut with a razor blade at the end with a length of about 3-5 mm. The cutting of the root tips was done every 15 minutes from 08.00 to 11.00 a.m. (UTC+07.00). After this treatment, the root samples were fixed (placed in a tube containing 45% acetic acid fixative solution at 4°C for 15-20 minutes). After fixation, the samples were washed with distilled water until clean (using a suction pipette) and then macerated with 1 N hydrochloric acid (HCl) solution at 55°C for 8 minutes.

Table 1. Sampling location and plant status

Sampling code	Location in Indonesia	Coordinate	Altitude (m asl)	Status
KU	Bulungan, North Kalimantan	3°01'42"N; 117°17'56"E	60	Cultivated
KT	Balikpapan, East Kalimantan	1°14'06"S; 116°49'25"E	5	Cultivated
KG	Palangka Raya, Central Kalimantan	2°13'42"S; 113°53'24"E	13	Cultivated
KS	Banjarbaru, South Kalimantan	3°25'40"S; 114°42'56"E	8	Cultivated
KB	Kapuas Hulu, West Kalimantan	0°50'04"N; 112°51'56"E	202	Cultivated

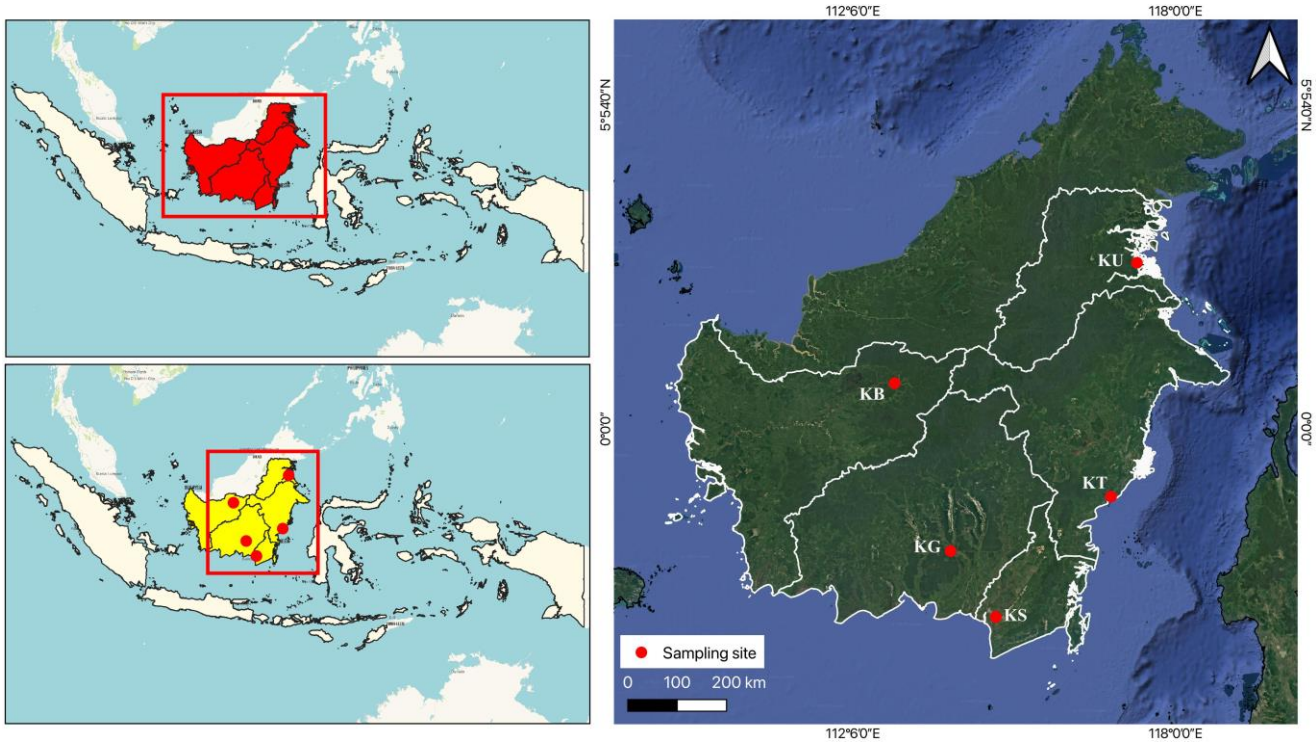

Figure 1. Map sampling sites of Dayak onion (*Eleutherine bulbosa*) in Kalimantan, Indonesia

The samples were then washed again with distilled water until clean and then stained with 1% aceto-orcein (SIGMA) for approximately 120 minutes. After staining, the sample was placed in an object glass and the tip of the root sample was cut (characterized by a darker color). The aceto-orcein solution still attached to the edge of the sample was absorbed with a paper towel. Next, the root sample was dabbed with glycerin, covered with a cover glass, and then squashed with the base of a wooden brush while tapping in such a way that the roots were squashed. Then the preparation was covered with a glass cover and fixed with nail varnish (cutex). In order to identify the preparations, they were labeled with the time and date of preparation (Mursyidin et al. 2013).

Mitotic observation

The stages of mitotic division in *E. bulbosa* roots were then observed under an Optilab microscope with immersion oil to increase the refractive index and documented with a digital camera (Olympus U-TV0.5XC-3). The cells used for cytological characterization were those in prometaphase stage with an intact state, evenly distributed and not overlapping. Cells in interphase, prophase, metaphase, anaphase, and telophase were used to study the cell cycle. The results of prometaphase cell documentation were used for cytological characterization analysis and karyogram preparation (Mursyidin et al. 2013; Yuniastuti et al. 2023).

Data analysis

Data was analyzed descriptively from selected chromosome images. The image data were then interpreted by several applications based on the observed variables. Chromosomes were measured using raster images. Microscopic images of chromosomes were redrawn in Corel Draw to better visualize the shape of the chromosomes. Chromosome size was determined by adding the sum of the long and short arms (q+p), where q was the long arm and p was the short arm. Chromosome shape was determined based on the ratio of chromosome arms (AR) and Centromere Index (CI). Identification of chromosome shape was calculated from AR and CI according to the method developed by Levan et al. (1964) (Table 2). The karyotype arrangement of Dayak onion bulb plants was expressed in the form of karyograms and idiograms. The preparation of karyotype and idiogram was done using CorelDraw Graphic Suite X3 program application. Karyograms were arranged based on the order of chromosomes from the longest to the shortest, while idiograms were arranged by connecting pairs of chromosomes according to the length and shape of the whole chromosome.

Table 2. Chromosome shape based on Arm Ratio (AR) and Centromere Index (CI)

Chromosome shape	Symbol	AR	CI
Metacentric	m	1.00-1.67	37.50-50.00
Submetacentric	sm	1.68-3.00	25.50-37.49
Acrocentric	a	3.01-7.00	12.5-25.49
Telocentric	t	>7.00	0-12.49

Asymmetry chromosomes are calculated using the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2) formula described by Zarco (1986) as follows:

$$A1 = 1 - \frac{\sum_{i=1}^{n} \frac{bi}{Bi}}{n}$$

Where, bi is average short arm of each homologous chromosome pair, Bi is average of the long arms of each homologous chromosome pair, and n is number of homologous chromosome pairs.

Asymmetric index between chromosomes is calculated as follows:

$$A2 = \frac{SD}{\bar{X}}$$

Where, SD is standard deviation of chromosome lengths within a karyotype and $\bar{X}$ is average length of chromosomes in a karyotype.

RESULTS AND DISCUSSION

Chromosome number

Chromosome number is one of the important genetic information needed in breeding programs (Yuniastuti et al. 2023). Observation of the number of chromosomes in plants can be performed readily in the prometaphase of cell division. This is obtained from image data which is then redrawn with Corel draw to get a clearer profile of chromosome number. The results showed several phases of cell division of *E. bulbosa* are observed completely, from the beginning of cell division (prophase) to the end of cell division (telophase). Chromosomes in prometaphase stage are shown in Figure 2. *Eleutherine bulbosa* from Kalimantan experienced cell division between 09:00 and 11:00 a.m. (UTC+07.00). In complete cell division, the phases that occur are prophase, prometaphase, metaphase, anaphase, and telophase. More specifically, prometaphase occurs optimally at 10:00 a.m. (UTC+07.00). Mursyidin et al. (2013) get the optimum cell division of *E. bulbosa* at 10:30 a.m. (UTC+08.00). Based on our observation, the best range for collecting root tips in *E. bulbosa* at 09.00 to 11.00 a.m. The timing of mitotic activity in plants varies among species. For instance, Sangur et al. (2021) reported that the optimal time for collecting root tips in *Cajanus cajan* is at 08:00 a.m. Meanwhile, Syakhril et al. (2019) found that *Ricinus communis* exhibits active mitotic cell division within a broader time window, between 08:00 and 12:00 a.m. These findings indicate that the optimal time for root tip sampling should be adjusted according to the species-specific mitotic rhythm.

Based on the observations of chromosome behavior in the prometaphase, it is known that *E. bulbosa* has 6 pairs of chromosomes or a total of 12 chromosomes (2n: 12). This finding is in line with Goldblatt (1982) in Peru and Alves et al. (2011) in Brazil, which showed that *E. bulbosa* has a diploid chromosome number of 2n: 12. The same results have also been reported by Mursyidin et al. (2013) in *E.*

americana, which is a synonym of *E. bulbosa*. In addition, *E. latifolia* of the same genus is known to have the same number of chromosomes (Báez et al. 2019). However, the number of chromosomes in members of the family Iridaceae varies, for example, the number of chromosomes in *Cipura paludosa* is 2n: 14, *Neumerica candida* is 2n: 18, and *Trimezia connata* is 2n: 82 (Alves et al. 2011).

Chromosome size

The calculation will produce short arm, long arm, total chromosome length, long arm, and short Arm Ratio (AR), and Centromere Index (CI). The values of AR and CI are used to determine the chromosome shape (Table 2). The size and shape of *E. bulbosa* in this study are presented in Tables 3 to 7. The total chromosome length varies from 81.53 (KB) to 123.53 (KT). The shortest average of total chromosome length is 6.79 (KB) and the longest is 10.46 (KT). This value is higher than the average chromosome length studied by Alves et al. (2011) and (Mursyidin et al. 2013) with an average chromosome length of 2.62 and 2.08, respectively (Table 8). The average of AR ranged from 1.09 (KB) to 1.26 (KU). This value is lower than the results of Mursyidin's et al. (2013) study, which obtained an average of AR 0.31. Meanwhile, the average value of CI ranged from 34.35 (KU) to 47.27 (KT), the range of average CI is in accordance with the research of Alves et al. (2011) and Mursyidin et al. (2013) who got CI values of 41.92 and 45.86, respectively. Complete data are presented in Table 8. AR and CI values are used to determine the shape of the chromosome. The variation in chromosome size was caused by mitotic division factors. In different cells, there can be differences in chromosomes due to the level of chromosome condensation (Yuniastuti et al. 2023).

The longest and shortest absolute chromosome length ratio (R) was also measured. The R value shows the variation in chromosome size. The larger of R value, the greater of variation in chromosome size. In addition, the difference in R values between plants can be used to show differences in chromosomal characteristics or genetic variation in the plants studied by Aristya et al. (2018). In this study, the R value ranged from 2.13 (KG) to 3.65 (KB). This means that the KB sample has the most varied chromosome size. However, the variation in chromosome size in Alves et al. (2011) and Mursyidin et al. (2013) study was greater than in this study (Table 8). The R value is more fixed than the chromosome size. Moreover, Aristya et al. (2018) explained that various treatments in the implementation of chromosome preparation tend to cause changes in chromosome size. This condition makes it difficult to parameterize the chromosome size, the calculation of R value is expected to help to make chromosome comparisons between plants.

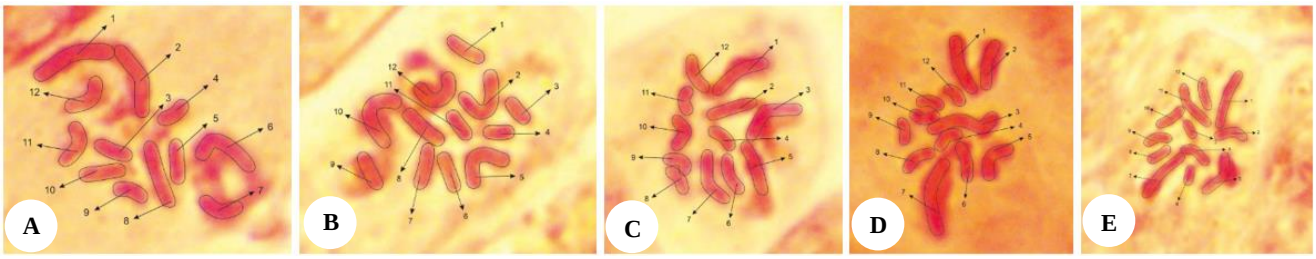

Figure 2. Chromosomes in prometaphase stage. A. KU, B. KT, C. KG, D. KS, E. KB. 100x magnification

Table 3. Chromosome size and shape of *Eleutherine bulbosa* from North Kalimantan (KU), Indonesia

Chromosome	Chromosome length			AR	CI	Chromosome shape
	p	q	p+q			
1A	5.02	10.25	15.27	2.04	32.87	sm
1B	5.04	9.64	14.68	1.91	34.33	sm
2A	4.25	9.14	13.39	2.15	31.74	sm
2B	4.04	8.58	12.62	2.12	32.01	sm
3A	4.35	5.91	10.26	1.36	42.40	m
3B	4.98	5.22	10.20	1.05	48.82	m
4A	4.29	5.05	9.34	1.18	45.93	m
4B	4.40	4.42	8.82	1.00	49.89	m
5A	0.00	8.35	8.35	∞	0.00	t
5B	0.00	6.92	6.92	∞	0.00	t
6A	3.32	3.56	6.88	1.07	48.26	m
6B	2.89	3.40	6.29	1.18	45.95	m

Note: p: Short arm length, q: Long arm length, p+q: Total length of chromosome, AR: Arm Ratio, CI: Centromeric Index, m: Metacentric, sm: Submetacentric, t: Telocentric

Table 4. Chromosome size and shape of *Eleutherine bulbosa* from East Kalimantan (KT), Indonesia

Chromosome	Chromosome length			AR	CI	Chromosome shape
	p	q	p+q			
1A	6.83	9.38	16.21	1.37	42.13	m
1B	6.88	9.06	15.94	1.32	43.16	m
2A	6.98	7.24	14.22	1.04	49.09	m
2B	6.25	6.44	12.69	1.03	49.25	m
3A	4.77	5.34	10.11	1.12	47.18	m
3B	4.81	4.86	9.67	1.01	49.74	m
4A	4.07	4.7	8.77	1.15	46.41	m
4B	4.32	4.41	8.73	1.02	49.48	m
5A	4.03	4.36	8.39	1.08	48.03	m
5B	3.41	3.74	7.15	1.10	47.69	m
6A	3.38	3.74	7.12	1.11	47.47	m
6B	3.11	3.42	6.53	1.10	47.63	m

Note: p: Short arm length, q: Long arm length, p+q: Total length of chromosome, AR: Arm Ratio, CI: Centromeric Index, m: Metacentric

Table 5. Chromosome size and shape of *Eleutherine bulbosa* from Central Kalimantan (KG)

Chromosome	Chromosome length			AR	CI	Chromosome shape
	p	q	p+q			
1A	7.03	7.59	14.62	1.08	48.08	m
1B	6.35	6.58	12.93	1.04	49.11	m
2A	6.33	6.54	12.87	1.03	49.18	m
2B	4.83	6.8	11.63	1.41	41.53	m
3A	4.44	6.53	10.97	1.47	40.47	m
3B	4.55	4.63	9.18	1.02	49.56	m
4A	4.2	4.78	8.98	1.14	46.77	m
4B	4.22	4.71	8.93	1.12	47.26	m
5A	3.05	4.37	7.42	1.43	41.11	m
5B	3.37	3.74	7.11	1.11	47.40	m
6A	3.25	3.74	6.99	1.15	46.49	m
6B	3.12	3.75	6.87	1.20	45.41	m

Note: p: Short arm length, q: Long arm length, p+q: Total length of chromosome, AR: Arm Ratio, CI: Centromeric Index, m: Metacentric

Table 6. Chromosome size and shape of *Eleutherine bulbosa* from South Kalimantan (KS), Indonesia

Chromosome	Chromosome length			AR	CI	Chromosome shape
	p	q	p+q			
1A	5.89	9.87	15.76	1.68	37.37	m
1B	5.45	9.97	15.42	1.83	35.34	m
2A	6.74	6.86	13.6	1.02	49.56	m
2B	5.22	6.05	11.27	1.16	46.32	m
3A	5.22	5.55	10.77	1.06	48.47	m
3B	4.56	4.81	9.37	1.05	48.67	m
4A	3.82	4.92	8.74	1.29	43.71	m
4B	3.88	4.05	7.93	1.04	48.93	m
5A	3.89	3.92	7.81	1.01	49.81	m
5B	3.24	3.85	7.09	1.19	45.70	m
6A	3.2	3.41	6.61	1.07	48.41	m
6B	2.54	3.92	6.46	1.54	39.32	m

Note: p: Short arm length, q: Long arm length, p+q: Total length of chromosome, AR: Arm Ratio, CI: Centromeric Index, m: Metacentric

In this study, three shapes of chromosomes were found, namely metacentric (m), submetacentric (sm), and telocentric (t). In addition to size differences, this study did not find heteromorphism of homologous chromosome pairs, as found by Alves et al. (2011). Chromosomal heteromorphism is a condition in which there are homologous chromosome pairs that have different morphological shapes or sizes, as Alves et al. (2011) found that the first chromosome pair was metacentric and the second was acrocentric. In this study, each homologous chromosome had the same shape.

Karyotype analysis

The chromosome formula of *E. bulbosa* in the five samples studied is presented in Figure 3 and Table 8. Despite having the same number of chromosomes, the five *E. bulbosa* samples showed different shapes and sizes (Figures 3 and 4). Only KT and KG have the same chromosome formula, which is entirely metacentric. The chromosome formulas of KU, KS, and KB is different. KS has metacentric and sub-metacentric chromosome forms, while KU and KB have metacentric, sub-metacentric, and telocentric chromosomes, respectively. In this study, no acrocentric chromosome form was found as those of Alves et al. (2011) and Mursyidin et al. (2013). Their findings on chromosome formula are 2n=11m+1a (Table 8). Thus, the discovery of sub-metacentric and telocentric chromosomes is the first reported in the observation of *E. bulbosa* chromosome morphology.

Karyotype asymmetry is a good expression for the general morphology of karyotypes in plants. Changes in morphological characters of the genome are often associated with evolution in higher plants (Zarco 1986). Morphological characteristics of chromosomes can be described according to the degree of karyotype symmetry, namely intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2). A1 is used to determine the form of chromosome variation within a karyotype. A1 values range from 0 to 1. It should be noted that A1 is independent of chromosome number and size. A low A1 indicates a high proportion of metacentric chromosomes. From the value of A1, it is known that the order of samples with the lowest A1 to the highest is KT, KG, KS, KB, and KU. Thus, *E. bulbosa* from East Kalimantan has the highest proportion of metacentric chromosomes, while *E. bulbosa* from North Kalimantan has the lowest proportion of metacentric chromosomes. The interchromosomal asymmetry index (A2) is used to determine the bias (dispersion) of chromosome size in a karyotype. Low A2 values indicate low chromosome size deviations in a karyotype.

Table 7. Chromosome size and shape of *Eleutherine bulbosa* from West Kalimantan (KB), Indonesia

Chromosome	Chromosome length			AR	CI	Chromosome shape
	p	q	p+q			
1A	4.81	5.56	10.37	1.16	46.38	m
1B	5.11	5.16	10.27	1.01	49.76	m
2A	2.84	6.45	9.29	2.27	30.57	sm
2B	3.2	5.65	8.85	1.77	36.16	sm
3A	3.08	4.06	7.14	1.32	43.14	m
3B	3.36	3.42	6.78	1.02	49.56	m
4A	2.8	3.34	6.14	1.19	45.60	m
4B	3.01	3.02	6.03	1.00	49.92	m
5A	2.85	3.15	6	1.11	47.50	m
5B	2.19	2.77	4.96	1.26	44.15	m
6A	0	2.86	2.86	∞	0.00	t
6B	0	2.84	2.84	∞	0.00	t

Note: p: Short arm length, q: Long arm length, p+q: Total length of chromosome, AR: Arm Ratio, CI: Centromeric Index, m: Metacentric, t: Telocentric, sm: Submetacentric

Table 8. Karyology, ideogram, and chromosome measurement of five accession *Eleutherine bulbosa* from Kalimantan, Indonesia, investigated in this study in comparison to previous studies

Accession	Location	Karyotype Formula	Ideogram	TCL	CL	AR	CI	R	A1	A2	Reference
KU	Bulungan	6m + 2t + 4sm	√	123.02	10.25	1.26	34.35	2.43	0.39	0.30	*Present study*
KT	Balikpapan	12m	√	125.53	10.46	1.12	47.27	2.48	0.10	0.33	*Present study*
KG	Palangka Raya	12m	√	118.5	9.88	1.18	46.03	2.13	0.14	0.26	*Present study*
KS	Banjarbaru	10m + 2sm	√	120.83	10.07	1.24	45.13	2.44	0.16	0.33	*Present study*
KB	Kapuas Hulu	8m + 2sm + 2t	√	81.53	6.79	1.09	36.89	3.65	0.32	0.38	*Present study*
E. americana	South Kalimantan	11m + 1a	√	12.49	2.08	1.31	45.86	4.14	-	-	Mursyidin et al. (2013)
LPFelix, 2000	Areia Paraiba, Brazil	11m + 1a	-	31.34	2.62	-	41.92	5.00	-	0.77	Alves et al. (2011)

Note: TCL: Total Chromosome Length, CL: Mean Chromosome Length, AR: Mean Arm Ratio, CI: Mean Centromere Index, R: Proportion between the largest and smallest chromosomes, A1: Intrachromosomal asymmetry index, A2: Interchromosomal asymmetry index

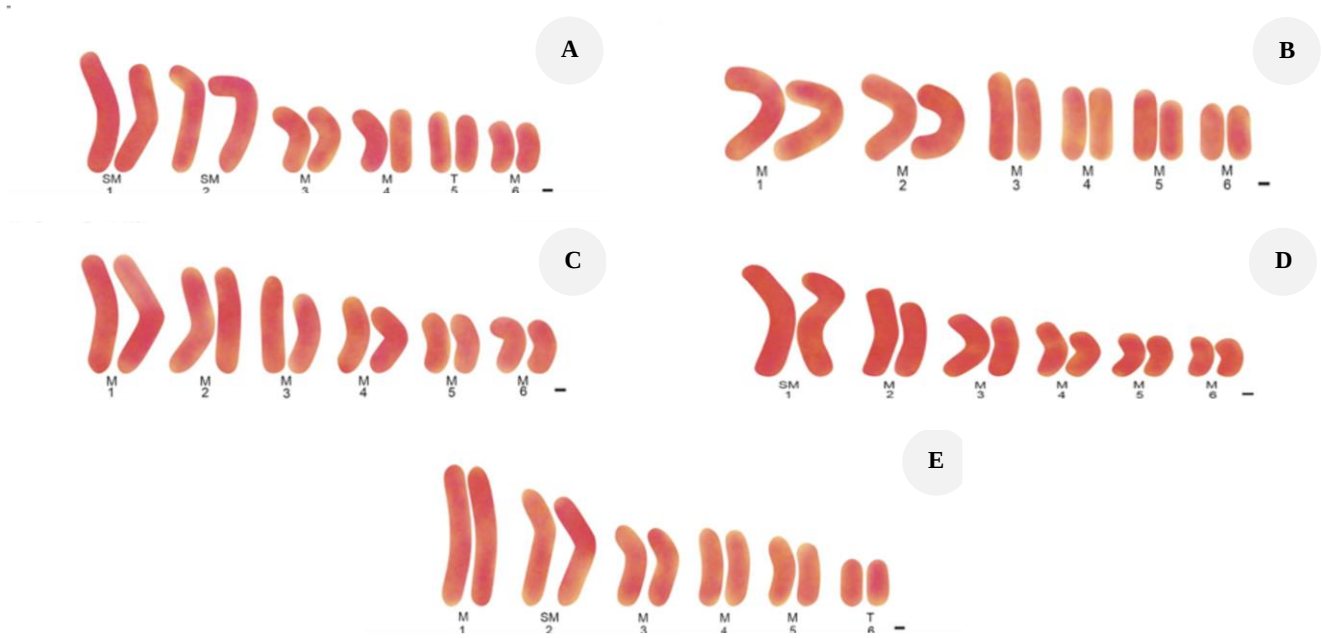

Figure 3. Karyotype of *Eleutherine bulbosa* from Kalimantan, Indonesia. A. KU, B. KT, C. KG, D. KS, E. KB (Scale bar 1 µm)

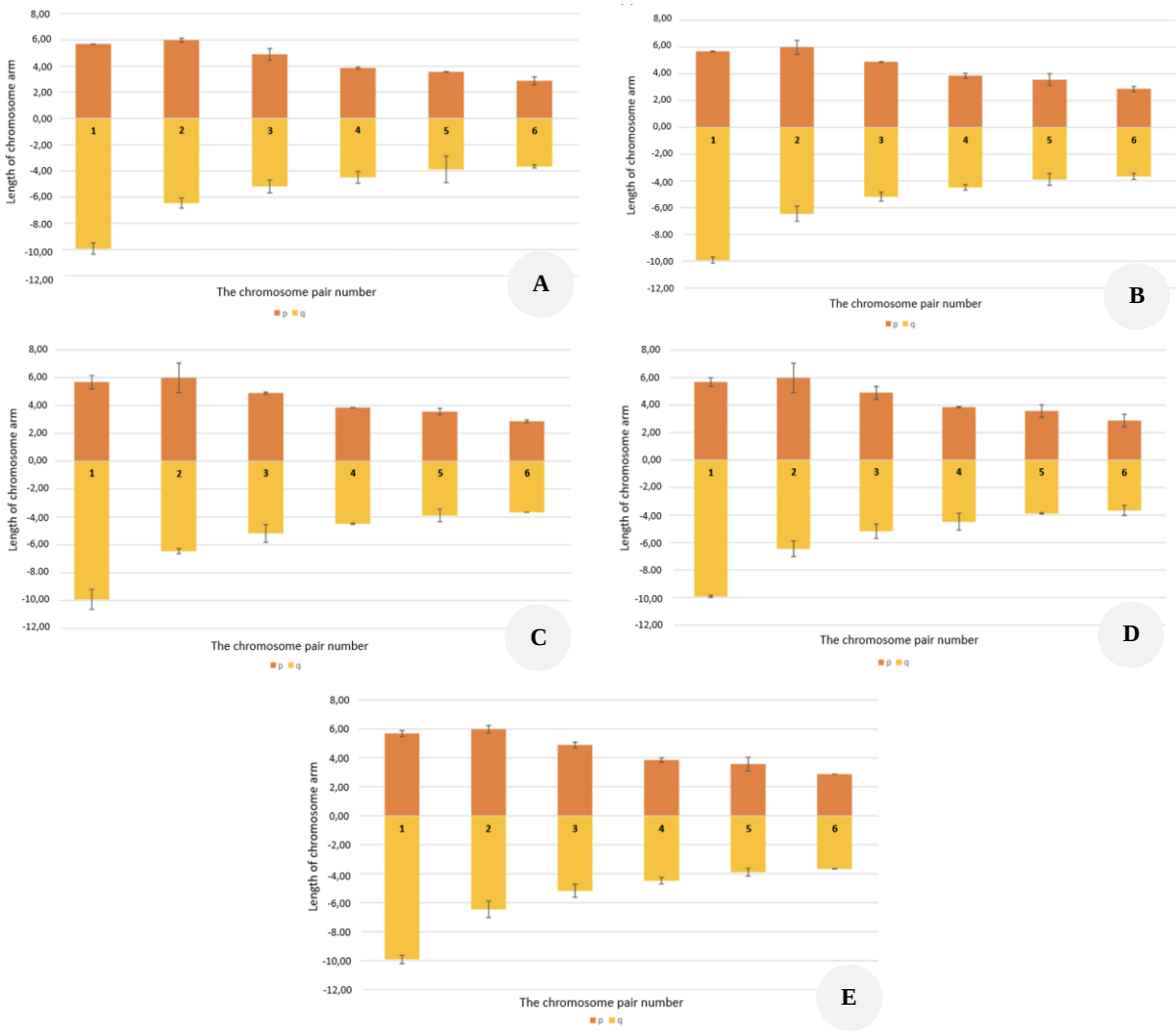

Figure 4. Ideogram of *Eleutherine bulbosa* chromosomes from Kalimantan, Indonesia. A. KU, B. KT, C. KG, D. KS, E. KB

In this study, the lowest A2 value was shown by the KG sample, while the highest value was shown by the KB sample. Thus, it is known that the KB sample has the largest chromosome size deviation. Based on the results of A1 and A2 calculations, it can be explained that chromosomes in the *E. bulbosa* karyotype are predominantly metacentric, contributing to the overall symmetry of the karyotype. This symmetry is often associated with primitive karyotypes, which can provide insight into the evolutionary lineage of the species (Alves et al. 2011). This chromosome structure reflects not only the evolutionary history of the plant, but also its adaptability to different habitats, highlighting the importance of chromosome morphology in understanding plant genetics and evolution.

Two different size classes of chromosomes (bimodal karyotype) were observed in this study. The first class had one to two pairs of large chromosomes and the second class consisted of four or five pairs of smaller chromosomes. In a bimodal karyotype, chromosomes are categorized into two distinct groups based on size: the larger ones are referred to as "macrochromosomes" and the smaller ones as "microchromosomes." These two types can be easily distinguished under a microscope due to their pronounced size differences, which serve as the key diagnostic feature of bimodal karyotypes (Báez et al. 2019; Ramos et al. 2023). There are three possible origins of the bimodal karyotype. First, these karyotypes are the results of chromosome rearrangements involving fusion-fission events, where the macrochromosomes are the product of microchromosome fusions in *Agave* (Asparagaceae) (Ramos et al. 2023), or these are the results of macrochromosome fission, as observed in some bimodal karyotypes of the tribe Lilieae (Liliaceae) (Du et al. 2017). Second, the bimodal karyotype may result from allopolyploid by hybridization of parental species with different chromosome sizes, as in *Aloe* (Asphodelaceae) (Sánchez et al. 2018). Third, differential accumulation of repetitive DNA sequences has contributed to an increase in the size of a subset of chromosomes, as occurred in *E. bulbosa* (Iridaceae) (Báez et al. 2019).

In conclusion, five samples of *E. bulbosa* collected from Kalimantan showed karyotype variation. The variation was observed in the chromosome shapes, sizes, and formulas. The number of *E. bulbosa* chromosome has 2n=2x=12 with different shapes including metasentric, submetasentric, and telosentric. Although it has the same number of chromosomes, each sample shows a different chromosome formula. Only samples from East Kalimantan and Central Kalimantan have the same chromosome formula. The size of Arm Ratio (AR), Centromere Index (CI), and the ratio of longest and shortest absolute chromosome length (R) vary between samples. A1 and A2 karyotype asymmetries also show variations with each other.

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