horticulturae

MDPI

Article

Cytogenetic Study of Five Varieties of *Callisia repens* (Jacq.) L. (Commelinaceae) from Laos

Anousone Sengthong [1,2], Surapon Saensouk [1,2,*], Piyaporn Saensouk [2,3] and Phetlasy Souladeth [4]

[1] Walai Rukhavej Botanical Research Institute, Mahasarakham University, Kantarawichai District, Maha Sarakham 44150, Thailand; a.sengthong@nuol.edu.la
[2] Diversity of Family Zingibeaceae and Vascular Plant of Its Applications Research Unit, Mahasarakham University, Kantarawichai District, Maha Sarakham 44150, Thailand; pcornukaempferia@yahoo.com
[3] Department of Biology, Faculty of Science, Mahasarakham University, Kantarawichai District, Maha Sarakham 44150, Thailand
[4] Faculty of Forest Science, National University of Laos, Vientiane 7322, Laos; p.souladeth@nuol.edu.la
* Correspondence: surapon.s@msu.ac.th

Abstract: Cytogenetical studies were carried out on five varieties of *Callisia repens*, i.e., turtle vine, green, pink lady, gold, and Bianca. The morphological characteristics of all five varieties differed in leaf shape and color of the plant. All five varieties have the same chromosome number, 2n = 12, and the fundamental number (NF) = 24. The number of metacentric (m), submetacentric (sm), and subtelocentric (st) chromosomes was related to the discrepancies between the various karyotypes that were found. The formula for each of the karyotypes was 2m + 2sm + 8st (turtle vine), 2m + 10st (green), 8m + 4st 14 (pink lady), 2m + 4sm + 6st (gold), and 2m + 2sm + 8st (Bianca). Therefore, all five strains had asymmetrical karyotypes. The chromosome number of *C. repens* has been reported previously, but this is the first report of karyotype variation among the varieties. Furthermore, principal component analysis (PCA) of the karyotype formula was able to distinguish *C. repens* 'pink lady', *C. repens* 'green', and *C. repens* 'gold', but it was unable to differentiate between *C. repens* 'Bianca' and *C. repens* 'turtle vine'. Additionally, PCA conducted on the centromeric index (CI) and the leaf colors of the five varieties of *C. repens* successfully separated all of them. Therefore, the prominent morphological traits and karyotype information of the five varieties of *C. repens* from Laos can be used to distinguish between them.

Keywords: *Callisia*; chromosome count; commelinaceae; karyotype; Laos

Citation: Sengthong, A.; Saensouk, S.; Saensouk, P.; Souladeth, P. Cytogenetic Study of Five Varieties of *Callisia repens* (Jacq.) L. (Commelinaceae) from Laos. *Horticulturae* **2023**, *9*, 1050. https://doi.org/10.3390/horticulturae9091050

Academic Editor: Roberto Berni

Received: 27 June 2023
Revised: 6 September 2023
Accepted: 9 September 2023
Published: 19 September 2023

1. Introduction

The family Commelinaceae contains monocot plants known as the dayflower family or spiderwort family comprising 41 genera and over 731 species that are widely distributed, mainly in tropical and subtropical areas with considerable diversity extending into northern temperate regions of the world [1]. The family contains annual or perennial herbs, which are generally terrestrial and somewhat fleshy or succulent, perennial with rhizomes or stolons; stems clearly divided into nodes and internodes, simple or branched, sometimes rooting from the stem; roots fibrous, fine, or tuberous [2–6]; some species are used as food (including as edible plants and ingredients) [7–9], medicine [7,9–16], animal feed [7], and as ornamentals [7,9,15].

The genus *Callisia* Loefl. includes 41 accepted species of perennial plants with their native range in tropical and subtropical America [1,17]. Some species are used as ornamentals and have been introduced to many countries, i.e., Azores, Bangladesh, southeast China, Hawaii, Louisiana, Morocco, Norfolk Is., Taiwan, Thailand, and Laos. *Callisia repens* (Jacq.) L. is a well-known tiny succulent creeping plant that has the common name turtle vine. It is distributed throughout Central and South America [1,2], but has become naturalized

in various other parts of the world due to its attractive foliage and ease of cultivation. Furthermore, the species is usually used as an ornamental plant, as medicine [15], and has environmental uses, such as a green roof cooling of canopies and soil temperatures [18]. Additionally, *C. repens* has single leaves with variation in them. The colors of the upper and lower surfaces of the leaves is different, with the top of the leaves being green and the bottom purple. The color on the underside is purple on all parts of the stem. When the stems stretch out to touch the soil, roots can form, and it is currently a popular ornamental plant. Therefore, *C. repens* is a popular ornamental plant in Thailand [7,8] and Laos.

Previous cytogenetic studies on *C. repens* have reported chromosome numbers 2n = 12 [15,19–21], n = 6 [21], NF = 24 [15,21], and a karyotype formula of 2m + 2sm + 6st + 2t [21], 4sm + 8st [15]. Thus, *C. repens* exhibits variations in its karyotype formula; however, there is no mention of variations based on the morphology of *C. repens*. In previous studies of *C. repens*, researchers mainly focused on presenting the chromosome number. Only two research papers [15,21] have provided karyotype formula and their results were different from each other. Therefore, the experimental setup of this focuses on the differences in the morphology of *C. repens* and whether these differences correlate with its karyotype. To address this question, we divided *C. repens* into five varieties (including green, pink lady, Bianca, gold, and turtle vine from Vientiane province, Laos) and conducted a comprehensive study of their karyotypes. So, this study aims to obtain cytological information, including the chromosome number, NF, and karyotype, in five varieties of *C. repens* with leaf variations from Laos. These data contribute to identifying differences among the varieties and support further studies on *C. repens* in other fields such as plant breeding.

2. Materials and Methods

2.1. Sample Collection

Experimental plant material from five varieties of *C. repens*, namely green (collected from Vientiane province, coll. no. A. Sengthong 402), pink lady (collected from Vientiane province, coll. no. A. Sengthong 403), Bianca (collected from Vientiane province, coll. no. A. Sengthong 404), gold (collected from Vientiane province, coll. no. A. Sengthong 405), and turtle vine (collected from Vientiane province, coll. no. A. Sengthong 406), was collected from Laos (Table 1) and voucher specimens were kept in the Faculty of Forest Science Herbarium (FOF), National University of Laos, and the Mahasarakham University Herbarium, Thailand. All fresh cultivars were grown in a nursery at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Maha Sarakham Province, Thailand.

Table 1. Information about five *Callisia repens* species and their corresponding references, including chromosomal numbers, NF = 24, and karyotype formula.

Species	2*n*	*n*	Karyotype Formula	Origin	Previous Research
C. repens	12	-	-	Brazil	[19]
	12	-	-	Brazil	[20]
	12	6	2m + 2sm + 6st + 2t	Argentina	[21]
	12	-	4sm + 8st	Thailand (Maha Sarakham province)	[15]
C. repens 'turtle vine'	12	-	2m + 2sm + 8st	Laos (province of Vientiane)	Present research
C. repens 'green'	12	-	2m + 10st	Laos (province of Vientiane)	Present research
C. repens 'pink lady'	12	-	8m + 4st	Laos (province of Vientiane)	Present research
C. repens 'gold'	12	-	2m + 4sm + 6st	Laos (province of Vientiane)	Present research
C. repens 'Bianca'	12	-	2m + 2sm + 8st	Laos (province of Vientiane)	Present research

2.2. Mitotic and Karyotype Analyses

For the purpose of studying somatic chromosomes, the root tips (about 1 cm long) were taken from living specimens. These root tips had a paradichlorobenzene (PDB) pretreatment for 6 h at 4 °C, fixed in ethanol–acetic acid 3:1 (*v*/*v*) for 30 min at room temperature,

and after that stored at 4 °C or immediately used. All root tips were cleaned with distilled water, hydrolyzed in 1N HCl for 5 min at 60 °C, and then cleaned once more with distilled water. All root tips were dyed and squashed in 2% aceto-orcein. The chromosomes were observed by using a light microscope, Zeiss: Axiostar plus, to take the micrographs. Adobe Photoshop CS3 Extended was used to quantify the chromosome length and to rearrange the chromosomes. The study of chromosome count followed, [10–12]. The chromosomal morphology nomenclature was developed using the techniques described in [5]. Therefore, the chromosomes of all varieties were classified to be metacentric (m), submetacentric (sm), subtelocentric (st), or acrocentric (a). For each variety, 20 metaphase cells were used to determine the chromosomal count, chromosome structure, and karyotype. For the karyotyping, the parameters for the average length of Ls (the short arm), average length of Ll (the long arm), LT of each chromosome (total length), average relative length (RL = LT/∑LT, formula for relative length), and chromosome index (CI = Ll/LT, formulae for chromosome index are 0.500–0.599 (metacentric), 0.600–0.699 (submetacentric), 0.700–0.899 (subtelocentric), and 0.900–1.000 (acrocentric)) [15,16,22–25].

2.3. Statistical Analysis

The means of five varieties of *C. repens*, including length of short arm chromosome, long arm chromosome, total arm chromosome, relative length, and centromeric index, for all variables plus one standard deviation (SD), were used to represent all results, and they were calculated based on the methods of [15,16,22–25], and principal component analysis (PCA) [26] was performed using SPSS version 29.

3. Results

C. repens 'turtle vine' has ovate and small leaves. The color of the leaves on the top is green. While the color of the leaves on the bottom is purple (Figure 1a). *C. repens* 'green' has large ovate leaves, which are larger than in the other variants. The color of the leaves is a green color on both sides (Figure 1b). *C. repens* 'pink lady' has ovate and small leaves. Leaves are elongated with different colors from green to pink and cream stripes, and the underside of the leaf is magenta (Figure 1c). *C. repens* 'gold' has ovate and small leaves. The leaves are golden green. The bottom of the leaf is magenta (Figure 1d). *C. repens* 'Bianca' is characterized by small and oval shaped leaves, which are either pure green, spotted, or pink color on both surfaces (Figure 1e).

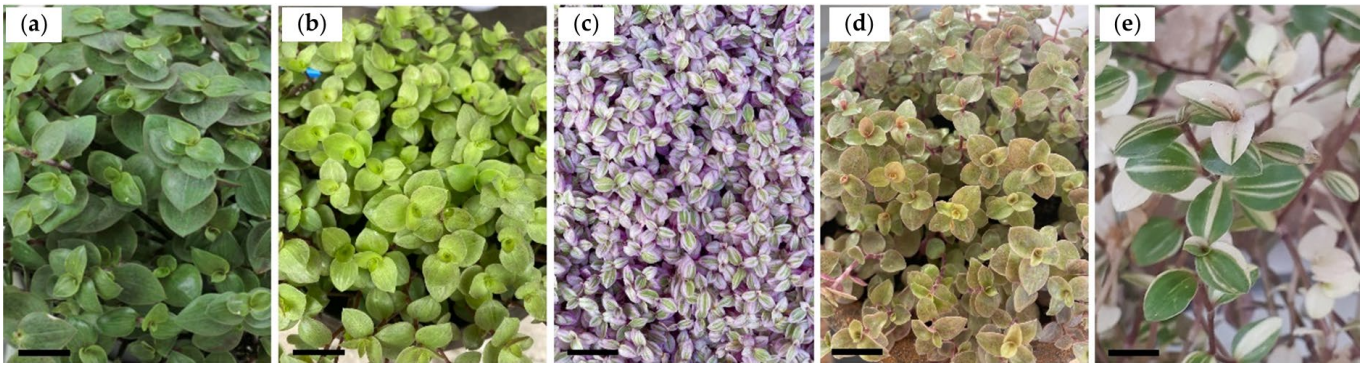

Figure 1. Five varieties of *Callisia repens* from Vientiane province, Laos: (**a**) turtle vine, (**b**) green, (**c**) pink lady, (**d**) gold, and (**e**) Bianca (scale bars = 1 cm).

The diploid chromosomes of the five varieties of *C. repens* in this study were observed as having 12 chromosomes (Figure 2, Table 1). The characteristics of the karyotypes of the five varieties of *C. repens* are provided in Figure 3 and Table 1.

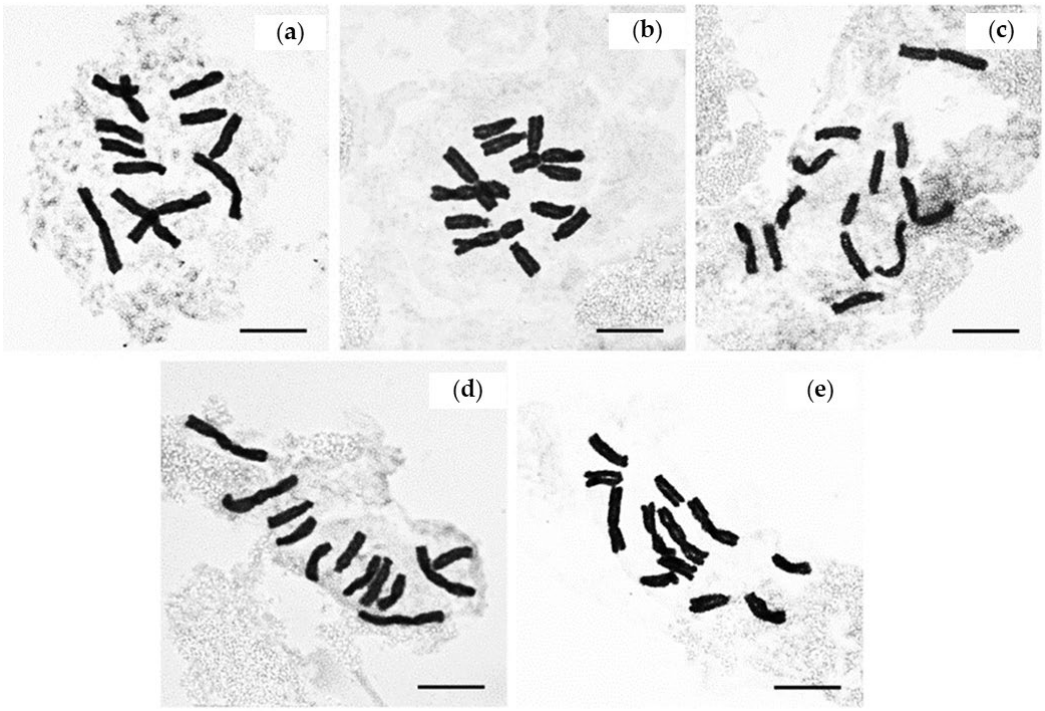

Figure 2. Microphotographs of somatic metaphase of *Callisia repens* (five varieties) 2n = 12. Scale bars = 10 μm. (**a**) turtle vine, (**b**) green, (**c**) pink lady, (**d**) gold, and (**e**) Bianca.

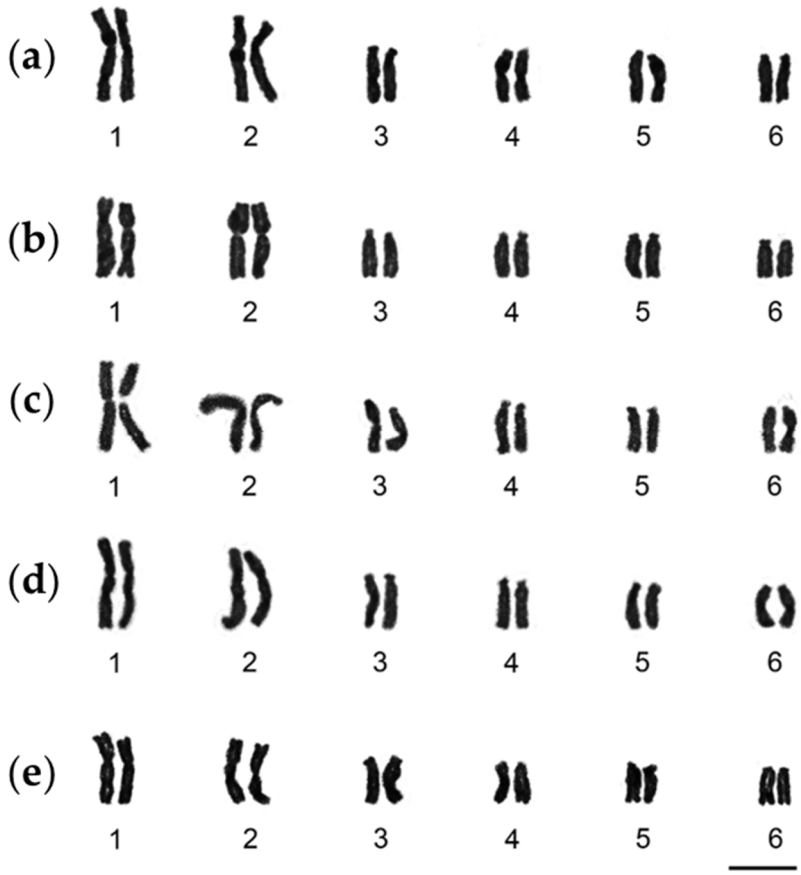

Figure 3. Karyotypes of five varieties of *Callisia repens*. Scale bars = 10 μm. (**a**) turtle vine, (**b**) green, (**c**) pink lady, (**d**) gold, and (**e**) Bianca.

The somatic chromosome number of turtle vine was 2n = 12 and NF = 24 (Figure 2a). The karyotype formula was 2m + 2sm + 8st. The arm ratio caused the karyotype to be asymmetrical. The measured lengths of the short and long arms ranged from 0.89 ± 0.01 to 5.79 ± 0.02 µm, 4.38 ± 0.02 to 8.43 ± 0.03 µm, respectively, while the total arm length ranged from 7.28 ± 0.02 to 14.21 ± 0.05 µm, the RLs ranged from 12.49 ± 0.01 to 24.38 ± 0.01, and the CIs from 0.55 ± 0.01 to 0.88 ± 0.01, as shown in Table 2 and Figure 3a.

Table 2. Mean length of chromosome pairs 1–6 (short arm chromosome (Ls), long arm chromosome (Ll), total arm chromosome (LT)), relative length (RL), and centromeric index (CI) of five varieties 2n = 12, obtained from 10 metaphase plates.

Variety	Mean Length of Chromosome Pairs 1–6, RL, and CI					Chromosome Type
	Ls ± SD (µm)	Ll ± SD (µm)	LT ± SD (µm)	RL (%)	CI	
Turtle vine	5.79 ± 0.02	8.43 ± 0.03	14.21 ± 0.05	24.38 ± 0.01	0.59 ± 0.02	Metacentric
	4.59 ± 0.01	8.34 ± 0.03	12.94 ± 0.04	22.19 ± 0.01	0.64 ± 0.02	Submetacentric
	1.02 ± 0.01	7.31 ± 0.03	8.33 ± 0.03	14.28 ± 0.01	0.88 ± 0.01	Subtelocentric
	1.09 ± 0.02	6.90 ± 0.02	7.99 ± 0.03	13.70 ± 0.02	0.86 ± 0.01	Subtelocentric
	0.92 ± 0.02	6.64 ± 0.03	7.56 ± 0.04	12.96 ± 0.01	0.88 ± 0.02	Subtelocentric
	0.89 ± 0.01	6.39 ± 0.02	7.28 ± 0.02	12.49 ± 0.01	0.88 ± 0.01	Subtelocentric
Green	3.42 ± 0.02	8.09 ± 0.03	11.51 ± 0.05	23.45 ± 0.01	0.70 ± 0.01	Subtelocentric
	4.65 ± 0.01	6.75 ± 0.03	11.40 ± 0.04	23.22 ± 0.01	0.59 ± 0.01	Metacentric
	0.92 ± 0.02	6.15 ± 0.02	7.07 ± 0.03	14.39 ± 0.02	0.87 ± 0.01	Subtelocentric
	0.92 ± 0.01	5.81 ± 0.02	6.73 ± 0.02	13.70 ± 0.02	0.86 ± 0.01	Subtelocentric
	0.72 ± 0.01	5.96 ± 0.02	6.69 ± 0.02	13.62 ± 0.01	0.89 ± 0.01	Subtelocentric
	0.83 ± 0.02	4.86 ± 0.02	5.70 ± 0.02	11.60 ± 0.01	0.85 ± 0.02	Subtelocentric
Pink lady	6.02 ± 0.02	8.18 ± 0.03	14.20 ± 0.05	24.58 ± 0.02	0.58 ± 0.02	Metacentric
	5.81 ± 0.01	6.85 ± 0.03	12.66 ± 0.04	22.13 ± 0.02	0.54 ± 0.02	Metacentric
	4.08 ± 0.02	4.16 ± 0.02	8.23 ± 0.03	14.38 ± 0.01	0.51 ± 0.03	Metacentric
	0.97 ± 0.01	6.82 ± 0.02	7.80 ± 0.03	13.63 ± 0.01	0.88 ± 0.01	Subtelocentric
	0.89 ± 0.02	6.35 ± 0.02	7.24 ± 0.02	12.66 ± 0.02	0.88 ± 0.01	Subtelocentric
	3.19 ± 0.01	3.90 ± 0.02	7.08 ± 0.02	12.38 ± 0.01	0.55 ± 0.01	Metacentric
Gold	5.34 ± 0.02	8.22 ± 0.03	13.56 ± 0.05	24.40 ± 0.01	0.61 ± 0.01	Submetacentric
	5.06 ± 0.01	7.56 ± 0.03	12.62 ± 0.04	22.71 ± 0.02	0.60 ± 0.02	Submetacentric
	1.08 ± 0.01	7.10 ± 0.02	8.17 ± 0.03	14.71 ± 0.02	0.87 ± 0.01	Subtelocentric
	0.90 ± 0.02	6.61 ± 0.02	7.52 ± 0.03	13.53 ± 0.02	0.88 ± 0.01	Subtelocentric
	1.45 ± 0.01	5.63 ± 0.02	7.08 ± 0.02	12.74 ± 0.01	0.81 ± 0.02	Subtelocentric
	2.81 ± 0.01	3.82 ± 0.02	6.63 ± 0.02	11.92 ± 0.01	0.58 ± 0.02	Metacentric
Bianca	4.15 ± 0.02	6.29 ± 0.03	10.44 ± 0.04	23.29 ± 0.01	0.60 ± 0.01	Submetacentric
	4.13 ± 0.01	5.54 ± 0.03	9.67 ± 0.04	21.56 ± 0.01	0.57 ± 0.01	Metacentric
	0.92 ± 0.01	6.03 ± 0.02	6.95 ± 0.03	15.49 ± 0.02	0.87 ± 0.01	Subtelocentric
	1.68 ± 0.01	4.41 ± 0.02	6.09 ± 0.02	13.59 ± 0.02	0.72 ± 0.02	Subtelocentric
	0.97 ± 0.02	5.05 ± 0.02	6.02 ± 0.02	13.43 ± 0.01	0.84 ± 0.02	Subtelocentric
	0.47 ± 0.02	5.21 ± 0.02	5.68 ± 0.02	12.65 ± 0.01	0.92 ± 0.02	Subtelocentric

The somatic chromosome number of green was 2n = 12 and NF = 24 (Figure 2b). 2m + 10st represented the karyotype formula. The arm ratio caused the karyotype to be asymmetrical. The total arm length varied between 5.70 ± 0.02 and 11.51 ± 0.05 µm, the long arm length between 4.86 ± 0.02 and 8.09 ± 0.03 µm, and the short arm length between 0.72 ± 0.01 and 4.65 ± 0.01 µm. RLs varied from 11.60 ± 0.01 to 23.45 ± 0.01 and CIs varied from 0.59 ± 0.01 to 0.89 ± 0.01 (Table 2, Figure 3b).

Pink lady's somatic chromosomal count was 2n = 12 and NF = 24 (Figure 2c). It was 8m + 4st in the karyotype formula. Because of the arm ratio, the karyotype was asymmetrical. The total arm length varied between 7.08 ± 0.02 and 14.20 ± 0.05 µm, the long arm length varied between 3.90 ± 0.02 and 8.18 ± 0.03 µm, and the short arm length varied between 0.89 ± 0.02 and 6.02 ± 0.02 µm. RLs ranged from 12.38 ± 0.01 to 24.82 ± 0.02 and CIs were between 0.54 ± 0.02 and 0.88 ± 0.01 (Table 2, Figure 3c).

Gold had somatic chromosomes 2n = 12 and NF = 24 (Figure 2d). It was 2m + 4sm + 6st in the karyotype formula. The arm ratio caused the karyotype to be asymmetrical. The total arm length varied between 6.63 ± 0.02 and 13.56 ± 0.05 µm, the long arm length varied between 3.65 ± 0.02 and 8.22 ± 0.03 µm, and the short arm length varied between 0.90 ± 0.02 and 5.34 ± 0.02 µm. RLs ranged from 11.92 ± 0.01 to 24.40 ± 0.01 and CIs from 0.55 ± 0.02 to 0.88 ± 0.01 (see Table 2 and Figure 3d).

Bianca's somatic chromosomal count was 2n = 12 and NF = 24 (Figure 2e). It was 2m + 2sm + 8st in the karyotype formula. The arm ratio caused the karyotype to be asymmetrical. The total arm length varied between 5.68 ± 0.02 and 10.44 ± 0.04 µm, the short arm length between 0.47 ± 0.02 and 4.15 ± 0.02 µm, and the long arm length between 4.41 ± 0.02 and 6.29 ± 0.03 µm. RLs were 12.65 ± 0.01 to 23.29 ± 0.01 and CIs were 0.57 ± 0.01 to 0.87 ± 0.01 (Table 2, Figure 3e).

The sizes and karyotypes of the five varieties look similar to each other, but they differed slightly. Therefore, we used PCA to help classify the five of them. The results of the PCA are shown below.

- PCA score plot for karyotype formula

The PCA score plot for five varieties of *C. repens*, based on their karyotype formula, revealed that component 1 accounted for 66.57% of the total variance, while component 2 accounted for 31.34%. Pink lady and green were in the same stage, while Bianca, turtle vine, and gold were in the same stage. This indicates that the karyotype formulae of pink lady and green had the same chromosome types but a different number of each chromosome type. Additionally, Bianca and turtle vine had the same chromosome types and numbers, indicating that they could not be separated (Figure 4a). Therefore, when only using the karyotype formula, it is not sufficient to separate all five varieties of *C. repens*. It is necessary to include other information, such as CI and leaf color, to distinguish between different variations.

- PCA score plot for CI

The PCA score plot for the five varieties of *C. repens* based on CI showed that component 1 accounted for 58.66% of the total variance, while component 2 accounted for 37.23%. Bianca was in a different stage compared to the other variations, as indicated by its wider range of CI closely related to component 1. On the other hand, the CIs of green and turtle vine were similar to each other and different from the other CI variations (Figure 4b). This result aligns with the findings of the karyotype formula, indicating that the CIs of Bianca and turtle vine can be used to separate them into different stages, although they remain closely related.

- PCA score plot for leaf color

The PCA score plot for the five varieties of *C. repens* based on leaf color successfully separated all variations. Component 1 accounted for 72.25% of the total variance, while component 2 accounted for 22.66%. Green, Bianca, turtle vine, and gold were in the same stage, whereas only pink lady was in a different stage, exhibiting distinct leaf color compared to the other variations (Figure 4c). In conclusion, the karyotype formula alone is insufficient to separate the five varieties of *C. repens*. However, when combined with CI and leaf color, it becomes possible to differentiate between the variations successfully.

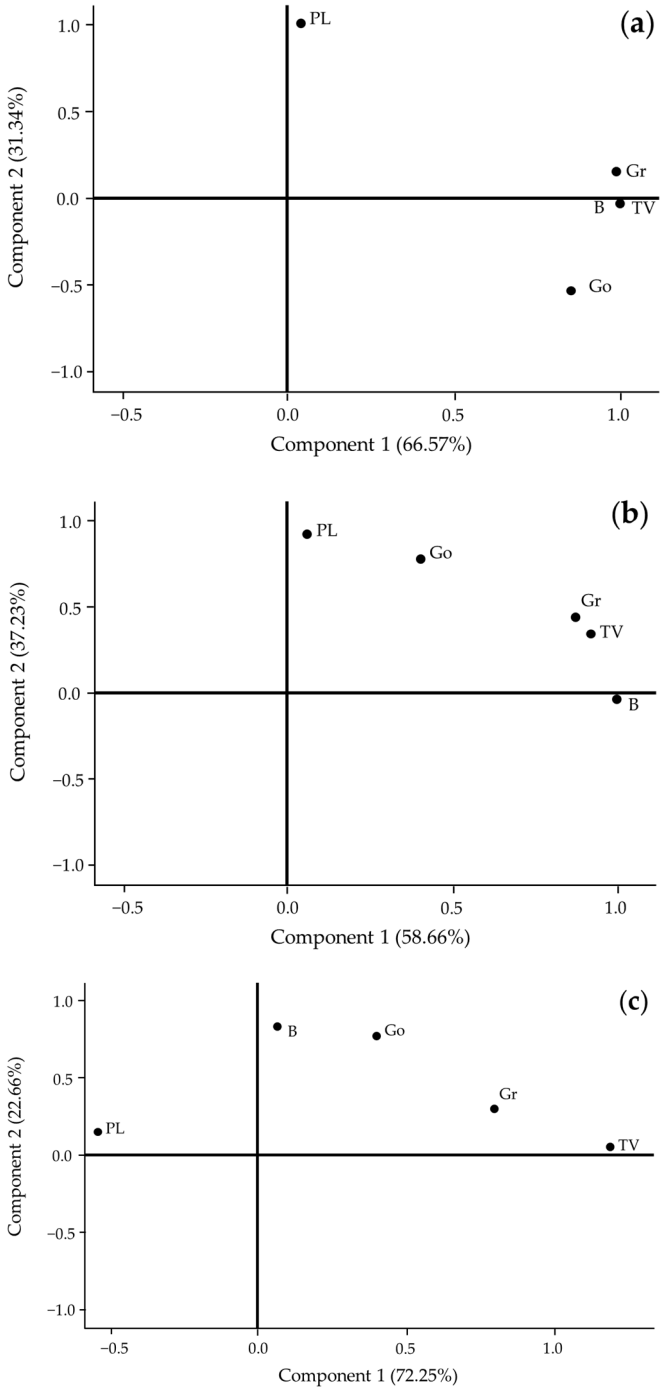

Figure 4. PCA score plots for five varieties of *Callisia repens* (**a**) based on karyotype formula, (**b**) based on CI, and (**c**) based on leaf color (TV: turtle vine; Gr: green; PL: pink lady; Go: gold; B: Bianca).

4. Discussion

This study revealed that the chromosome number in all varieties of *C. repens* was 2n = 12, which is consistent with previous reports [1,4,6,8] (Figure 2, Table 1). The NF of all varieties was determined to be 24, which aligns with previous findings [4,8] (Table 1). Notably, all varieties of *C. repens* exhibited an asymmetrical karyotype, as they comprised not only metacentric and submetacentric chromosomes, but also other types, which is in line with previous studies [4,8] (Table 1). The karyotype characteristics of the five varieties in this study differed from those reported in [4,8] (Table 1). In fact, the karyotypes of the five varieties of *C. repens* (Figure 3, Tables 1 and 2) displayed variations in terms of chromosome

length, arm length, total arm length, and karyotype asymmetry. The chromosomal features allowed for the identification of the five varieties of *C. repens*, as they differed from one another in terms of short arms, long arms, relative lengths (RL), and centromeric index (CI). Therefore, the karyotype data, along with dominant morphological characteristics, can be utilized to differentiate between the five varieties of *C. repens* from Laos.

Among the five varieties of *C. repens*, four exhibited different karyotype formulas. Specifically, pink lady showed a karyotype formula of 8m + 4st, green had 2m + 10st, *C. repens* 'gold' displayed 2m + 4sm + 6st, while both Bianca and turtle vine had a karyotype formula of 2m + 2sm + 8st. However, it is noteworthy that Bianca and turtle vine shared the same karyotype formula, indicating that the karyotype formula alone cannot be used to distinguish between these two varieties. In fact, the CI can differentiate between the variations. Although the CI values appear to be in the same direction, Bianca does not fit within the accepted range. Furthermore, the leaf color of *C. repens* also serves as a distinguishing factor among the varieties. The leaf color can differentiate between them, as they may appear similar, but pink lady is not considered to be part of the same varieties. Following the results in this study, it is possible to classify all five varieties of *C. repens* by using the PCA of the karyotype formula, CI, and leaf color.

5. Conclusions

This study focused on five varieties of *C. repens*, which included turtle vine, green, pink lady, gold, and Bianca. All of these varieties share the same chromosome number, 2n = 12, and NF = 24. The karyotype formulae for turtle vine and Bianca are 2m + 2sm + 8st, for green it is 2m + 10st, for pink lady it is 8m + 4st, and for gold it is 2m + 4sm + 6st. The data regarding chromosome length, arm length, total arm length, and karyotype asymmetry represents the first report of these findings for *C. repens* in Laos. The sizes of the chromosomes are quite similar among all the varieties, but PCA can be used to classify them. This is the first published cytogenetic study in Laos. It provides valuable data and information for researchers interested in studying this field. This is because there are many varieties of *Callisia repens* that have different external characteristics. The research results suggest that plant species can be classified by studying their chromosomes. Therefore, this serves as fundamental information that can be further explored in the field of genetic editing. This exploration can potentially enhance the beauty and economic value of these plants in the future.

Author Contributions: Methodology, A.S. and S.S.; data curation, A.S.; writing—original draft preparation, A.S. and P.S. (Piyaporn Saensouk); writing—review and editing, A.S., P.S. (Piyaporn Saensouk) and P.S. (Phetlasy Souladeth); supervision, S.S., P.S. (Piyaporn Saensouk) and P.S. (Phetlasy Souladeth). All authors have read and agreed to the published version of the manuscript.

Funding: The financial support for this research project came from Mahasarakham University's Postmaster Fellowship.

Data Availability Statement: All data produced and examined are available in this article.

Acknowledgments: This research was financially supported by Mahasarakham University (Postmaster Fellowship). We are grateful to the Walai Rukhavej Botanical Research Institute, Mahasarakham University, Thailand, for their facilities during this study. Thanks to Jolyon Dodgson for language editing and suggestions to improve the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. POWO. Plant of the World Online, Facilitated by the Royal Botanic Gardens, Kew. Available online: http://www.plantsoftheworldonline.org/ (accessed on 12 June 2023).
2. Cronquist, A. *An Integrated System of Classification of the Flowering Plants*; Columbia University Press: New York, UY, USA, 1981.
3. Faden, R.B. The morphology and taxonomy of Aneilema R. Brown (Commelinaceae). In *Smithsonian Contributions to Botany*; Smithsonian Institution Press: Washington, DC, USA, 1991.

4. AONA, L.Y.S. Revisão Taxonomica e Analise Cladistica do Genero Dichorisandra J.C. Mikan (Commelinaceae). Ph.D. Thesis, Universidade Estadual de Campinas, Campinas, Brazil, 2008.
5. Mabberley, D.J. *Mabberley's Plant Book*, 3rd ed.; Cambridge University Press: Cambridge, UK, 2008.
6. Angiosperm Phylogeny Group; Chase, M.W.; Christenhusz, M.J.; Fay, M.F.; Byng, J.W.; Judd, W.S.; Stevens, P.F. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* **2016**, *181*, 1–20. [CrossRef]
7. Rahman, A.H.M.M.; Sultana, Z.; Rani, R.; Islam, A.K.M.R. Taxonomic Studies of the Family Commelinaceae at Rajshahi, Bangladesh. *Int. J. Adv. Res.* **2015**, *3*, 978–989.
8. Łuczaj, Ł.; Lamxay, V.; Tongchan, K.; Xayphakatsa, K.; Phimmakong, K.; Radavanh, S.; Kanyasone, V.; Pietras, M.; Karbarz, M. Wild food plants and fungi sold in the markets of Luang Prabang, Lao PDR. *J. Ethnobiol. Ethnomed.* **2021**, *17*, 6. [CrossRef] [PubMed]
9. Santos Moraes, Q.D.; de Oliveira Pellegrini, M.O.; Alves-Araújo, A. Flora of Espírito Santo: Commelinaceae. *Rodriguésia* **2022**, *73*, e00522021. [CrossRef]
10. Long, C.L.; Li, R. Ethnobotanical studies on medicinal plants used by the red-headed yao people in Jinping, Yunnan Province, China. *J. Ethnopharmacol.* **2004**, *90*, 389–395. [CrossRef]
11. Delang, C.O. Ecological Succession of Usable Plants in an Eleven-Year Fallow Cycle in Northern Lao P.D.R. *Ethnobot. Res. Appl.* **2007**, *5*, 331–350. [CrossRef]
12. Panyaphu, K.; On, T.V.; Sirisa-ard, P.; Srisa-nga, P.; ChansaKaow, S.; Nathakarnkitkul, S. Medicinal plants of the Mien (Yao) in Northern Thailand and their potential value in the primary healthcare of postpartum women. *J. Ethnopharmacol.* **2011**, *135*, 226–237. [CrossRef]
13. Khuankaew, S.; Srithi, K.; Tiansawat, P.; Jampeetong, A.; Inta, A.; Wangpakapattanawong, P. Ethnobotanical study of medicinal plants used by Tai Yai in Northern Thailand. *J. Ethnopharmacol.* **2014**, *151*, 829–838. [CrossRef] [PubMed]
14. Xiong, Y.; Sui, X.; Ahmed, S.; Wang, Z.; Long, C. Ethnobotany and diversity of medicinal plants used by the Buyi in eastern Yunnan, China. *Plant Divers.* **2020**, *42*, 401–414. [CrossRef] [PubMed]
15. Saensouk, P.; Saensouk, S. Karyological study of four species in *Callisia* and *Cyanotis* (Commelinaceae) from Thailand. *Cytologia* **2021**, *86*, 339–343. [CrossRef]
16. Saensouk, S.; Saensouk, P. A karyological study of six Commelinaceae species from Thailand. *Cytologia* **2020**, *85*, 57–62. [CrossRef]
17. Gledhill, D. *The Names of Plants*, 4th ed.; Cambridge University Press: Cambridge, UK, 2008.
18. Cao, J.; Hu, S.; Dong, Q.; Liu, L.; Wang, Z. Green roof cooling contributed by plant species with different photosynthetic strategies. *Energy Build.* **2019**, *195*, 45–50. [CrossRef]
19. Beltrão, G.T.d.A.; Guerra, M. Citogenética de angiospermas coletadas em Pernambuco—III. *Ciênc. Cult.* **1990**, *42*, 839–845.
20. Pitrez, S.R.; Felix, L.P.; Barreto, R.; Guerra, M. Númeroscromossômicos de espécies de Commelinaceae R. Br. ocorrentes no nordeste do Brasil. *Bol. Bot. Univ. São Paulo* **2001**, *19*, 7–14.
21. Grabiele, M.; Daviña, J.R.; Honfi, A.I. Cytogenetic analyses as clarifying tools for taxonomy of the genus *Callisia* Loefl. (Commelinaceae). *Gayana Bot.* **2015**, *72*, 34–41. [CrossRef]
22. Saensouk, P.; Saensouk, S. Karyological Study of Four Genera and Seven Species (Commelinaceae) in Thailand. *Cytologia* **2022**, *87*, 41–47. [CrossRef]
23. Saensouk, P.; Saensouk, S. Cytogenetics of five species of genus *Murdannia* (Commelinaceae) from North-eastern Thailand. *Biologia* **2022**, *78*, 23–30. [CrossRef]
24. Senavongse, R.; Saensouk, S.; Saensouk, P. Comparative karyotype analysis in five morphological forms of Bon or *Colocasia esculenta* (L.) Schott (Araceae) in Thailand. *Cytologia* **2018**, *83*, 169–173. [CrossRef]
25. Senavongse, R.; Saensouk, S.; Saensouk, P. Karyological study in three native species of genus *Alocasia* (Araceae) in the northeast of Thailand. *Nucleus* **2020**, *63*, 81–85. [CrossRef]
26. Bro, R.; Smilde, A.K. Principal component analysis. *Anal. Methods* **2014**, *6*, 2812–2831. [CrossRef]