THAI FOR. BULL. (BOT.) 38: 90–94. 2010.

Karyotypic studies in four species of *Gynura* in Tribe Senecioneae (Asteraceae)

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ABSTRACT. Karyotypes of four *Gynura* species were investigated from root tips in mitotic metaphase. All species have the same chromosome numbers (2n = 2x = 20). Chromosome designations revealed that these species have the same symmetrical karyotype, consisting of metacentric (m) and submetacentric (sm) chromosomes. However, the differences in karyotype formulas of the four species were elucidated as follows: 7m + 3sm in two varieties of *G. pseudochina* DC., 8m + 2sm in *G. procumbens* Merr. and *G. calciphila* Kerr and 9m + 1sm in *Gynura* sp. These results show relationships among these four species.

KEY WORDS: karyotype, *Gynura pseudochina*, *G. procumbens*, *G. calciphila*, *Gynura* sp.

INTRODUCTION

Gynura Cass. is a herbaceous plant belonging to tribe Senecioneae in the Asteraceae. About twenty species of *Gynura* are distributed in warm regions of Asia, Africa and Australia (Hooker, 1882). Five species, *G. cusimbua* (D.Don) S.Moore, *G. integrifolia* Gagnep., *G. procumbens* (Lour.) Merr. and *G. pseudochina* (L.) DC. var. *hispida* Thwaites and var. *pseudochina*, are found in Thailand (Smitinand, 2001). *G. procumbens* and *G. pseudochina* are used for medicinal purposes (Jeamamornrat, 1990). *Gynura crepidoides* Benth. is a synonym of *Crassocephalum crepidoides* (Benth.) S.Moore and is weedy plant (Forest Herbarium, 2001).

Many species of plants contain constant karyotypes. Among herbaceous genera, which have medium to large sized chromosomes, the great majority possess variation between species, with related species having similar karyotypes (Stebbins,1971; Bai & Kuriachan,1997) and the varieties of a species having same karyotype (Sarbhoy,1980).

The chromosome numbers of many species of *Gynura* have been analyzed previously. Most *Gynura* taxa have a somatic chromosome number of 2n = 20 (x = 10) and a few have 2n = 34 and 60 (Darlington & Wylie, 1955; Jose & Mathew,1990; Tzanoudakis & Kypriotakis, 1987). For the Thai species, Soontornchainaksaeng (2005) reported chromosome numbers in *Gynura pseudochina*, *G. procumbens* and *Gynura* sp. (2n = 20) but the karyotype was not reported.

In this study, karyotypes of *G. pseudochina*, var. *hispida* and var. *pseudochina*, *G. procumbens*, *G. calciphila* and *Gynura* sp. are reported and compared to investigate how they might support species identification and their relationships.

MATERIALS AND METHODS

The plants were collected from provinces of southern Thailand. Five samples of each species were cultivated in the nursery of Department of Biology, Prince of Songkla University, Songkhla Province, Thailand. Voucher specimens were deposited in PSU herbarium, Department of Biology. Somatic chromosomes were prepared from their active root tips. They were pretreated with saturated solution of α - Bromonaphthalene for 24 h at 10°C. The material was fixed in the mixture of 95 % ethanol and glacial acetic acid (3 : 1) for 24 h at 10°C and then preserved in 70% ethanol at 10°C. Samples were hydrolyzed in 1 N HCl for 4 min. at 60°C. They were rinsed in tap water, stained and squashed in Carbol Fuchsin. The selected metaphase plates of ten cells in each species were photographed on a Nikon OPTIPHOT-2 light microscope at 100x magnification with a

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Nikon FX series model UFX-DX II camera. The length of diploid chromosomes were measured from enlarged photographs of ten metaphase cells and the percentage of relative length (RL%) and arm ratio calculated according to Levan et al. (1964). Each chromosome pair number, mean RL% and mean arm ratio were analysed.

The chromosomes were classified according to their arm ratio i.e. metacentric (1.00–1.70), submetacentric (1.70–3.00) acrocentric (3.00–7.00) and telocentric (∞) (Levan et al.,1964). The paired homologous chromosomes were arrayed in descending order of length.

Comparison of mean RL% of haploid chromosome (Haplotype Karyotype Length, HKL) and mean arm ratio among these four species were tested by analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using the program SPSS.

Table 1 Mean of relative length (RL%) from ten metaphase chromosomes cells between *G. pseudochina* var. *hispida* and var. *pseudochina*, *G. procumbens*, *G. calciphila* and *Gynura* sp.

Chromosome pair number	RL% of haploid chromosome				
	G. pseudochina var. *hispida*	*G. pseudochina* var. *pseudochina*	*G. procumbens*	*G. calciphila*	*Gynura* sp.
1	6.19	6.00	6.06	6.15	6.14
2	5.73	5.69	5.61	5.45	6.04
3	5.42	5.43	5.37	5.36	5.49
4	5.23	5.18	5.20	5.28	5.36
5	5.02	5.07	5.01	5.13	5.09
6	4.82	4.89	4.93	4.98	4.81
7	4.73	4.73	4.79	4.92	4.72
8	4.53	4.55	4.50	4.75	4.42
9	4.32	4.45	4.43	4.35	4.17
10	3.99	4.00	4.05	4.06	3.77

Table 2 Mean of arm ratio from ten metaphase chromosomes cells and Karyotype formula between *Gynura pseudochina* var. *hispida* and var. *pseudochina*, *G. procumbens*, *G. calciphila* and *Gynura* sp.

Chromosome pair number	arm ratio				
	G. pseudochina var. *hispida*	*G. pseudochina* var. *pseudochina*	*G. procumbens*	*G. calciphila*	*Gynura* sp.
1	1.11b	1.14 b	1.09 b	1.10 b	1.46 a
2	1.15 a	1.13 a	1.10 a b	1.07 b	1.05 b
3	1.18c	1.14 cd	1.26b	1.70a	1.09 d
4	1.73 a	1.69 a	1.11 c	1.50 b	1.46 b
5	1.14 b	1.09 bc	1.74 a	1.15 b	1.05 c
6	1.20 b	1.20 b	1.24 b	1.24 b	1.45 a
7	1.24 b	1.25 b	1.24 b	1.37 a	1.08 c
8	1.35 b	1.34 b	1.26 b	1.76 a	1.13 c
9	1.76 a	1.77 a	1.74 a	1.13 c	1.44 b
10	1.88 ab	1.96 a	1.17 d	1.60 c	1.76 b
Karyotype formula	7m+3sm*	7m+3sm*	8m+2sm*	8m+2sm*	9m+1sm*
Locality	Songkhla	Songkhla	Songkhla	Nakhon Si Thammarat	Phatthalung

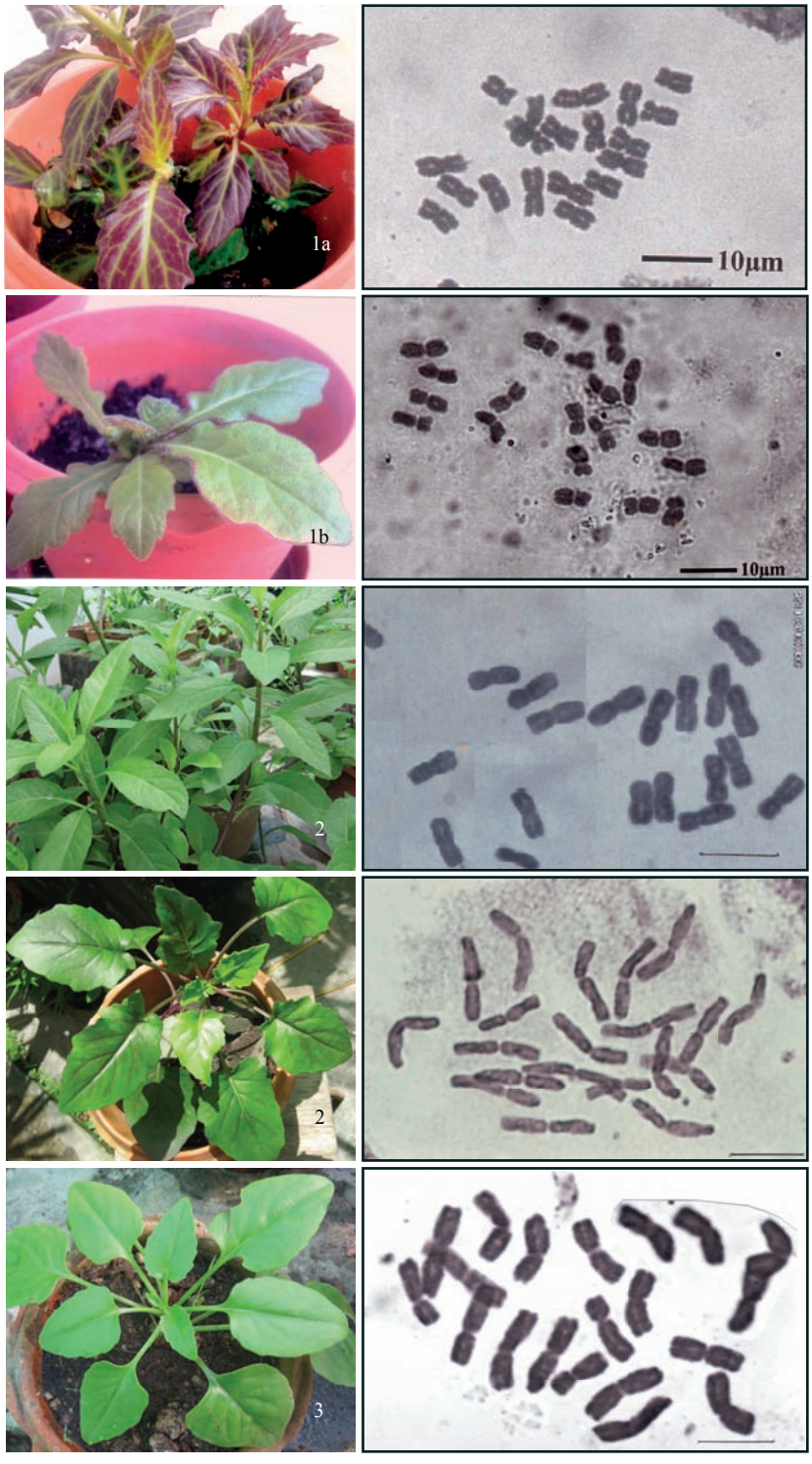

Figure 1. Habit (left) and Metaphase chromosomes (right) of the four *Gynura* species;

1a *Gynura pseudochina* var. *hispida*, 2n = 20 (scale bar = 10µm)

1b *G. pseudochina* var. *pseudochina*, 2n = 20

2 *G. procumbens*, 2n = 20

3 *G. calciphila*, 2n = 20

4 *Gynura* sp., 2n = 20

RESULTS AND DISCUSSION

The somatic chromosome numbers of *Gynura pseudochina*, *G. procumbens*, *G. calciphila* and *Gynura* sp. are the same, 2n = 20 (Fig. 1). The results are consistent with the reported data for *G. pseudochina* and *G. procumbens* (Soontornchainaksaeng, 2005). The genus *Gynura* has a basic number of x = 10 (Darlington & Wylie, 1955) and Jeamamornrat (1990) reported that *G. procumbens* showed 10 bivalents at the first metaphase in meiosis. Thus it can be concluded that four species of *Gynura* are diploid (2n = 2x = 20).

The results of the karyotype analysis are presented in Tables 1 and 2 and Fig. 2. It was found that the average RL% of the longest haploid chromosome in *G. pseudochina* var. *hispida*, var. *pseudochina*, *G. procumbens*, *G. calciphila* and *Gynura* sp. was 6.19, 6.00, 6.06, 6.15 and 6.14 and the average RL% of shortest haploid chromosome in each plants was 3.99, 4.00, 4.05, 4.06 and 3.77, respectively. The chromosome complements of all four *Gynura* species consisted of two chromosome types: metacentric chromosomes and submetacentric chromosomes.

In this study, *Gynura pseudochina* var. *hispida* and var. *pseudochina* were shown to have the same symmetrical karyotype, consisting of 14 metacentric chromosomes and 6 submetacentric chromosomes. In addition to similarity in the karyotype, RL% and arm ratio values of each chromosome pair were not significantly different ($p < 0.05$) between the two varieties, confirming that they belong to the same species. Two varieties of the same species possessing the same karyotype was in agreement with the findings of Sarbhoy (1980) on *Phaseolus vulgaris* var. *climbing* and var. *dwarf*.

The karyotypes of *G. procumbens* and *G. calciphila* are similar, distinguished by having 16 metacentric and 4 submetacentric chromosomes. However, the former species has submetacentric chromosomes in pairs 5 and 9 whereas the latter species has submetacentric chromosomes in pairs 3 and 8. For a karyotype of *Gynura* sp. with 2n = 20 having 18 metacentric and 2 submetacentric chromosomes is also reported here. The three species of *Gynura* studied have symmetrical karyotype (Stebbins, 1971).

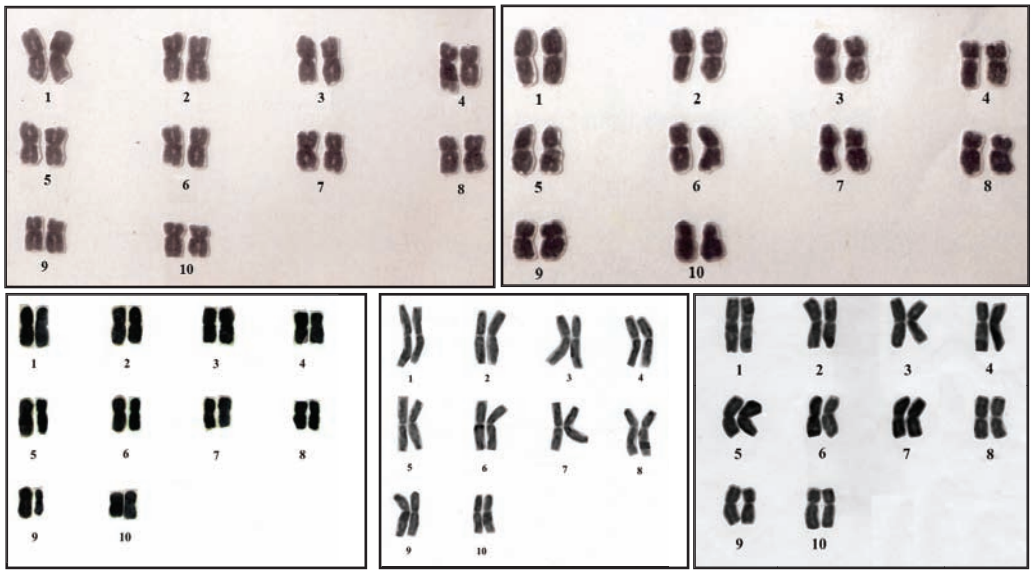

Figure 2. Karyotype of the four *Gynura* species;
1a. *Gynura pseudochina* var. *hispida* 1b. *G. pseudochina* var. *pseudochina*
2 *G. procumbens* 3 *G. calciphila* 4 *Gynura* sp.

The karyotypes of different species of a genus may either be alike or vary greatly (Stebbins, 1971) and widely different karyotypes have been found among plants with the same chromosome numbers (Frello & Heslop-Harrison, 2000). In this study, the karyotypes of the four *Gynura* species studied were different. These results correspond with previous reports (Sarbhoy,1980; Bai & Kuriachan,1997) in which different species with the same somatic number have different karyotypes.

Investigations of the karyotype of the four *Gynura* taxa show that they are diploid, since the chromosome could be matched together to form 10 homologous chromosomes. These results confirm that Thai *Gynura* has basic chromosome number x =10.

Comparison of RL% of haploid chromosome and arm ratio in each chromosome pairs in four Gynura species in Table 1 and 2 reveals that they have similarity in chromosome size but differ in the position of the centromere ($p<0.05$). These differences are usually brought about by unequal translocation and inversion (Stebbins, 1971). However, in some chromosome pairs both RL% and arm ratio between the species were not significantly different ($p<0.05$). These results suggest possible relationships between the four Gynura species.

ACKNOWLEDGEMENTS

We are grateful to Dr. Charan Leeratiwong, Department of Biology, Faculty of Science, Prince of Songkla University, Songkhla Province, Dr. Ongkarn Vanijajiva Biology Division, Faculty of Science and Technology, Phranakhon Rajabhat University, Bangkok for plant materials, Miss Jantra Sukkasem and Mr. Wachirapat Jiwanit for statistical analysis, respectively.

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