



Original Article

## Karyotype of some Thai Hypoxidaceae species

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### Abstract

Somatic chromosome numbers and karyotypes of nine species in three genera of Thai Hypoxidaceae were determined in root tips. All species are diploid with  $2n = 18$  and asymmetrical karyotypes including metacentric (m) submetacentric (sm) subtelocentric (st) and also telocentric (t) in some species. Satellite chromosomes (SAT) were observed in all species studied except in *C. latifolia*. The karyotypes of *Curculigo ensifolia*  $4m+8sm+6st(2SAT)$ , *C. latifolia*  $2m+14sm+2st$ , *C. megacarpa*  $2m+12sm+4st(2SAT)$ , *C. villosa*  $4m+10sm(2SAT)+4st$ , *Hypoxis aurea*  $2m+8sm+8st(2SAT)$ , *Molineria capitulata*  $2m+10sm+2st(2SAT)+4t$ , *M. trichocarpa*  $2m+10sm+6st(2SAT)$ , *M. gracilis* and *M. latifolia*  $4m+8sm+4st(2SAT)+2t$  are reported for the first time. The chromosome relative length of *Curculigo*, *Hypoxis* and *Molineria* range between 3.44-9.55, 4.03-8.12 and 3.64-8.97, respectively.

**Keywords:** karyotype, Thai Hypoxidaceae, *Curculigo*, *Hypoxis*, *Molineria*

### 1. Introduction

Hypoxidaceae is a small family of herbaceous perennial monocotyledons with approximately 200 species in 9 genera that are mainly found in the Southern hemisphere of the Old World and in North America (Govaerts as cited in Sánchez-Ken, 2010). In Thailand, three genera with ten species have been reported (Purintavaragul, 2006). Several species are often utilized as food, traditional medicines, and ornamental plants (Prajapati, 2003; Venkumar and Latha, 2002)

Cytological knowledge of the Hypoxidaceae is poor, as thirty one species in only three genera have been studied for their chromosome numbers; all *Curculigo* Gaertn. spp., have  $2n = 18$  ( $x = 9$ ), *Hypoxis* L.,  $2n = 14-200$  ( $x = 7, 8, 9, 11, 19$ ) and *Rhodohypoxis* Nel,  $2n = 12$  ( $x = 6$ ) (Darlington and Wylie, 1955; Soontornchainaksaeng, 2005; Kocyan, 2007; Nordal *et al.*, 1985; Singh, 2009). Karyotypic studies have also been reported for *H. decumbens* L. (Naranjo, 1975) and four

species of *Curculigo* (Sheriff, 1946; Yongping *et al.*, 1989). The objective of this study was to analyse the karyotype characteristics of some Thai Hypoxidaceae in order to support species identification and their relationships.

### 2. Materials and Methods

Living specimens were collected from the southern part of Thailand and cultivated in the greenhouse of the Department of Biology, Faculty of Science. Voucher specimens were deposited at the PSU herbarium, Prince of Songkla University, Thailand.

#### 2.1 Mitotic analysis

Actively growing root tips were pretreated in saturated paradichlorobenzens (PDB) solution for 5 hours at  $12^{\circ}\text{C}$  and fixed in a mixed solution of ethanol; acetic acid (3:1 v/v) for 24 hours at  $12^{\circ}\text{C}$ . The root tips were hydrolyzed with 1 N HCl for 6 minutes at  $60^{\circ}\text{C}$  and stained in carbol fuchsin for 4 hours. Tissue slides were prepared using the squash technique. The well spread metaphase plates of ten cells in each species were photographed using an Olympus

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compound microscope with a PMC35 DX Olympus camera.

### 2.2 Karyotype analysis

The enlarged photographs of well spread metaphase chromosomes from ten cells were measured for each species. The percentage of relative length (RL % = chromosome length x 100 / total chromosome lengths) and the arm ratio (long arm length / short arm length) with their respective standard errors (SE) were calculated for each chromosome. Chromosome types as metacentric (m: 1-1.7), submetacentric (sm: 1.7-3.0), subtelocentric (st: 3.0-7.0) and telocentric (t: >7.0) were determined based on the arm ratio following Levan *et al.* (1964). The karyotype was constructed based on the mean arm ratio and arranged in descending relative lengths.

### 3. Results and Discussion

Chromosome numbers in metaphase cell of *Curculigo ensifolia* R.Br., *C. latifolia* Dryand. ex W.T. Aiton, *C. megacarpa* Ridl., *C. villosa* Wall. ex Merr., *Hypoxis aurea* Lour., *Molineria capitulata* (Lour.) Herb., *M. trichocarpa* (Wight) N.P. Balakr., *M. gracilis* Kurz and *M. latifolia* (Dryand. ex W.T. Aiton) Kurz are diploid of  $2n = 18$  (Figure 1-2A) including 9 pairs of homologous chromosomes (Table 1, Figure 1-2B) as asymmetrical karyotype. These 3 genera studied have basic chromosome number of  $x=9$ . Moreover satellites were found on the short arm of the subtelocentric chromosome of various chromosome pairs in 7 species except in *C. villosa*, in which the satellite was found on the short arm of submetacentric chromosome. *Curculigo latifolia* is the only species without satellite chromosome. The karyotype formula, satellite positions (SAT) and relative length with SE are shown in Table 2.

#### 3.1 Curculigo

Four species studied of *Curculigo* have  $2n=18$  ( $x=9$ ) with asymmetrical karyotype including metacentric, sub-

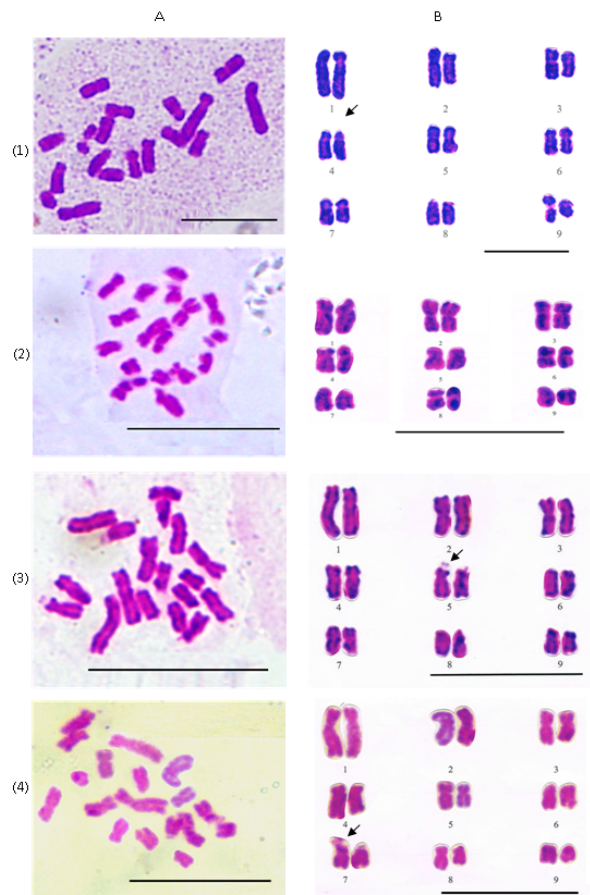


Figure 1. Somatic metaphase chromosomes ( $2n = 18$ ) (scale bar = 10  $\mu$ m) (A) and karyotype (B) (arrow = satellite)  
 1) *Curculigo ensifolia*      2) *C. latifolia*  
 3) *C. megacarpa*          4) *C. villosa*

metacentric and subtelocentric chromosomes. The chromosome numbers ( $2n = 18$ ,  $x = 9$ ) agree with those of previous studies (Darlington and Wylie, 1955; Yongping *et al.*, 1989; Soontornchainaksaeng, 2005; Kocyan, 2007). However, the karyotypes are different from those of *C. crassifolia*, *C. capitulata* and *C. sinensis* where subtelocentric chromo-

Table 1. Karyotype details of somatic chromosomes of nine Thai Hypoxidaceae species

Chromosome pair	<i>C. ensifolia</i>	<i>C. latifolia</i>	<i>C. megacarpa</i>	<i>C. villosa</i>	<i>H. aurea</i>	<i>M. capitulata</i>	<i>M. trichocarpa</i>	<i>M. gracilis</i>	<i>M. latifolia</i>
1	st	sm	sm	sm	st*	sm	sm	sm	sm
2	sm	m	m	m	st	sm	sm	sm	sm
3	sm	sm	sm	sm	st	sm	sm	sm	sm
4	st*	sm	sm	st	sm	sm	st	st	st
5	m	sm	st*	sm	sm	st*	st*	st*	st*
6	sm	sm	sm	st	st	t	sm	m	t
7	sm	st	sm	sm*	sm	sm	st	sm	m
8	st	sm	st	sm	m	t	sm	t	sm
9	m	sm	sm	m	sm	m	m	m	m

\* = satellite chromosome

m = metacentric    sm = submetacentric    st = subtelocentric    t = telocentric

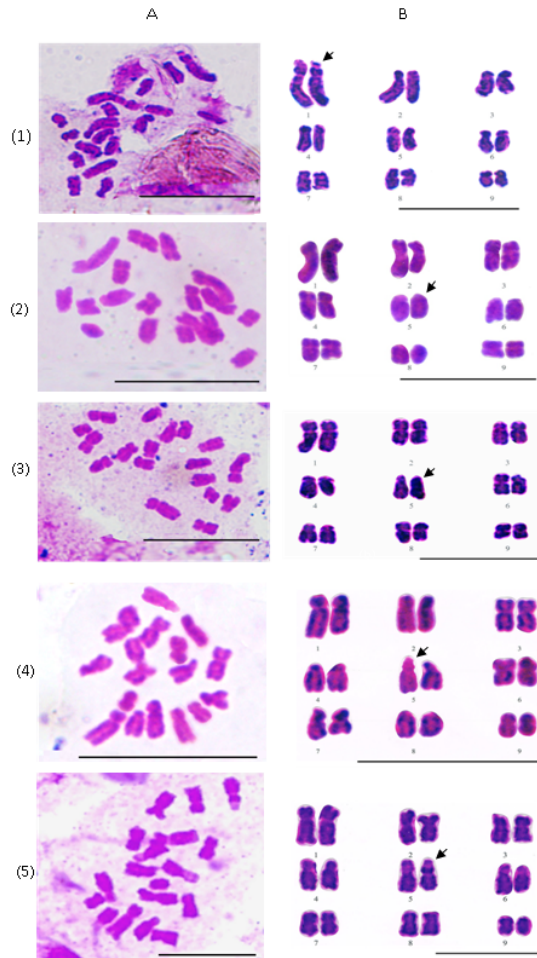


Figure 2. Somatic metaphase chromosomes ( $2n = 18$ ) (scale bar =  $10 \mu\text{m}$ ) (A) and karyotype (B) (arrow = satellite)  
 1) *Hypoxis aurea*                      2) *Molineria capitulata*  
 3) *M. trichocarpa*                    4) *M. gracilis*  
 5) *M. latifolia*

some were not found (Yongping *et al.*, 1989). Karyotypes of *C. ensifolia*, *C. megacarpa* and *C. villosa* with satellite on the short arm of the 4<sup>th</sup>, 5<sup>th</sup> and 7<sup>th</sup> chromosome pairs are reported for the first time. *Curculigo latifolia* is a distinct species without satellite chromosome. The chromosome relative lengths (RL%) in *C. ensifolia*, *C. latifolia*, *C. megacarpa* and *C. villosa* range between 3.84-9.55, 3.52-8.80, 3.54-9.15 and 3.44-8.86, respectively (Table 2).

### 3.2 *Hypoxis*

The results from this study indicate that *H. aurea* has  $2n = 18$  ( $x = 9$ ) with 2 metacentric, 8 submetacentric and 8 subtelocentric chromosomes. Satellites were found on the short arm of the 1<sup>st</sup> chromosome pair. A previous chromosome count (Kim *et al.*, 2008) from Jeju island, Korea, showed  $2n = 54$  for this species. This variation in chromosome number ( $2n = 18, 54$ ) is similar to that in other species of the genus, for example *H. angustifolia* has  $2n = 14$  and  $2n = 28$  (Nordal *et al.*, 1985). These ploidy levels indicate and support the presence of polyploidy in the genus. For chromosome length, RL% of *H. aurea* ranges from 4.03-8.12 (Table 2). The chromosome pair 1 of *H. aurea* (8.12) is the shortest one compared to all species studied.

### 3.3 *Molineria*

Chromosome numbers in the metaphase cell of *M. capitulata*, *M. trichocarpa*, *M. gracilis* and *M. latifolia* are diploid of  $2n = 18$  ( $x=9$ ) with metacentric, submetacentric, subtelocentric and telocentric chromosomes. Satellites were found on the short arm of subtelocentric chromosome pair 5 in all species studied. The karyotype formulae of *M. gracilis* and *M. latifolia* are similar (Table 2) but karyotypic details are different such as the 6<sup>th</sup> pair of *M. gracilis* are metacentric

Table 2. Karyotype formula (KF), satellite position (SAT), range of relative length (RL%, Range) of *Curculigo*, *Hypoxis* and *Molineria* species

Genus / species	KF	SAT(pair no.)	Range (RL % $\pm$ SE)	Voucher number
<i>Curculigo</i>				
<i>C. ensifolia</i>	4m+8sm+6st (2SAT)	4s*	3.84 $\pm$ 0.12–9.55 $\pm$ 0.05	M.01 (PSU)
<i>C. latifolia</i>	2m+14sm+2st	-	3.52 $\pm$ 0.16–8.80 $\pm$ 0.26	M.03 (PSU)
<i>C. megacarpa</i>	2m+12sm+4st(2SAT)	5s*	3.54 $\pm$ 0.11–9.15 $\pm$ 0.28	M.04 (PSU)
<i>C. villosa</i>	4m+10sm(2SAT)+4st	7s*	3.44 $\pm$ 0.15–8.86 $\pm$ 0.33	M.05 (PSU)
<i>Hypoxis</i>				
<i>H. aurea</i>	2m+8sm+8st(2SAT)	1s*	4.03 $\pm$ 0.41–8.12 $\pm$ 0.28	M.06 (PSU)
<i>Molineria</i>				
<i>M. capitulata</i>	2m+10sm+2st(2SAT)+4t	5s*	3.70 $\pm$ 0.16–8.69 $\pm$ 0.36	M.07 (PSU)
<i>M. trichocarpa</i>	2m+10sm+6st(2SAT)	5s*	4.08 $\pm$ 0.14–8.16 $\pm$ 0.20	M.09 (PSU)
<i>M. gracilis</i>	4m+8sm+4st(2SAT)+2t	5s*	3.67 $\pm$ 0.16–8.97 $\pm$ 0.49	M.10 (PSU)
<i>M. latifolia</i>	4m+8sm+4st(2SAT)+2t	5s*	3.64 $\pm$ 0.10–8.92 $\pm$ 0.33	M.12 (PSU)

s\* = short arm

chromosomes while those of *M. latifolia* are telocentric chromosomes (Table 1).

The ranges of RL% in *M. capitulata* (3.70-8.69), *M. gracilis* (3.67-8.97) and *M. latifolia* (3.64-8.92) are almost equal, supporting their inclusion in the same genus. The range of three *Molineria* species is slightly different from four species of *Curculigo*, while the range of *M. trichocarpa* (4.08-8.16) is nearly equal to the range of *H. aurea* (4.03-8.12). According to the relative length, presumably *Curculigo* is more closely related to *Molineria* than to *Hypoxis*.

In this study, we found that the karyotype of *Curculigo* is similar to *Molineria*, i.e. chromosome pairs 1 and 3 of three *Curculigo* species and four *Molineria* species have the same submetacentric chromosome. Besides, satellites on the subtelocentric chromosomes were also found in the four species of *Molineria* and the two species of *Curculigo* (Table 1). Thus, the karyotype analysis suggest a close relationship between the genera *Curculigo* and *Molineria*.

In conclusion, nine species studied of Hypoxidaceae are diploid of  $2n = 18$  ( $x=9$ ) with different karyotypes. The karyotypes of these species are reported for the first time.

#### Acknowledgement

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