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Cytological studies in Four Endemic Genera of Apiaceae from India

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ABSTRACT: The family Apiaceae comprises 428 genera worldwide, of which 68 are represented in India. *Karnataka* P.K. Mukh. & Constance, *Pinda* P.K. Mukh. & Constance, *Polyzygus* Dalzell, *Sivadasania* N. Mohanan & Pimenov and *Vanasushava* P.K. Mukh. & Constance are endemic genera to the country. The present communication is an attempt to provide chromosome counts and basic karyomorphology of *Karnataka*, *Pinda*, *Polyzygus* and *Vanasushava*. Somatic chromosome counts $2n = 22$ and meiotic chromosome counts $n = 11$ are reported for the first time in *Karnataka* and *Pinda* while in *Polyzygus* $2n = 36$ and in *Vanasushava* $2n = 44$ are reported as new cytotypes in present investigation. Karyomophologically *Pinda* and *Vanasushava* showed affinities with *Heracleum* L. while *Polyzygus* showed advanced karyotype symmetry.

KEY WORDS: Apiaceae, Chromosome counts, Endemic genera, India, Karyomorphology.

INTRODUCTION

The family Apiaceae has a cosmopolitan distribution with about 3500 species and 428 genera worldwide (Mabberley, 2008). It comprises 240 species in India belonging to 68 genera (Mukherjee and Constance, 1993). *Buplerum* L., *Heracleum* L. and *Pimpinella* L. are the most speciose genera in India consist about 20 species each (Mukherjee and Constance, 1993). *Anethum* L., *Berula* W.D.J. Koch, *Karnataka* P.K. Mukh. & Constance, *Kedarnatha* P.K. Mukh. & Constance, *Pinda* P.K. Mukh. & Constance, *Pleurospermopsis* C. Norman *Polyzygus* Dalzell, *Tordyliopsis* DC., *Turgenia* Hoffm., *Sivadasania* N. Mohanan & Pimenov and *Vanasushava* P.K. Mukh. & Constance are monotypic genera reported from India (Mukherjee and Constance, 1993; Mohanan and Pimenov, 2007). Among these *Karnataka*, *Pinda*, *Polyzygus*, *Sivadasania* and *Vanasushava* are endemic to the Western Ghats of India (Irwin and Narasimhan, 2011; Mohanan and Pimenov, 2007).

Genus *Karnataka* is restricted to the Western Ghats of Karnataka state while, *Polyzygus* is found growing throughout the Western Ghats of Maharashtra, Karnataka and Kerala (Irwin and Narasimhan, 2011) and is reported to be of rare in occurrence (Nayar, 1996).

Pinda concanensis (Dalzell) P. K. Mukh. & Constance and *Vanasushava pedata* (Wight) P.K. Mukh. & Constance were initially treated under the genus *Heracleum*; however of based on morphological characters, Mukherjee and Constance transferred *H. concanense* Dalzell into *Pinda* (Mukherjee and Constance, 1974) and *H. pedatum* Wight into *Vanasushava* (Mukherjee and Constance, 1986a). Both *Pinda* and *Vanasushava* are of rare in occurrence (Nayar, 1996; Mukherjee, 1988). Mohanan and Pimenov (2007) raised *Peucedanum josephianum* Wadhwa & H.J. Chowdhery into a separate monotypic genus *Sivadasania* on the basis of subterete to slightly laterally compressed fruit and equally keeled mericarp ribs.

There are different advance cytological characters have been reported in different members of Apiaceae from India viz., highly asymmetric karyotypes and aneuploidy in 21 species from South India (Subramanian, 1986), highly advance karyotype and autopolyploidy in the genus *Polyzygus* (Janardhanan and Thoppil, 2003), aneuploidy, tetraploidy and advanced karyotype in eight species of the genus *Heracleum* from South India (Subramanian, 1986; Deng et al., 2009; Yu et al., 2011) and many meiotic abnormalities in 17 species from Western Himalayas (Kumar et al., 2014).

Many common morphological characters which have been used for identification of many Apiaceae members are confusing, viz., seasonal tuberous habit in the generas *Karnataka*, *Pinda* and *Polyzygus* (Mukherjee and Constance, 1986a; 1986b). As karyomorphological evidences are unique enough at generic level the cytological work would be helpful to dissect the intricate interrelationships between different Apiaceae members. In this article, we reported the karyomorphology of four monotypic genera from Western Ghats.

MATERIALS AND METHODS

The plant materials of all four genera were collected from the Western Ghats of India. The voucher specimens are deposited in the Herbarium, Department of Botany, Shivaji University, Kolhapur (SUK). The tuberous roots were grown in earthen pots in the departmental botanical garden. Mitosis was studied from healthy root tips. The root tips of 6–10 mm length were

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Table 1. Different karyomorphological parameters of ***Karnataka, Pinda, Polyzygus*** and ***Vanasushava***

Sr. no.	Parameters	*Karnataka*	*Pinda*	*Polyzygus*	*Vanasushava*
1.	TF%	33.23	36.92	13	35.97
2.	SI	49.77	58.52	14.94	56.17
3.	GI	65.35	61.87	36.32	51.49
4.	TCL%	7.27–11.12	7.15–11.56	3.71–10.2	3.1–5.3
5.	CV_{CL}	12.02	14.41	40.28	15
6.	CV_{CI}	13.54	20.12	177.05	5.12
7.	AI	1.63	2.9	71.32	0.77
8.	A1	0.41	0.49	0.094	0.52
9.	A2	0.12	0.14	0.40	0.15
10.	TCLH	22.84	22.24	19.7	39.64
11.	Classification as per Stebbin (1971)	3A	2A	4B	2A
12.	Karyotype formula	6m+16sm	10m+8sm+4st	8sm+2st+26t	8m+36sm

TF%: Total form percent, **SI:** Symmetric Index, **GI:** Gradient Index, **TCL%:** Total haploid chromosome length percent, **CV_{CL}:** Coefficient of variation (CV) of the centromeric index, **CV_{CL}:** Coefficient of variation (CV) of chromosome lengths, **AI:** Asymmetry index, **A1:** Intrachromosomal asymmetry index, **A2:** Interchromosomal asymmetry index, **TCLH:** Total chromosome length of haploid compliment.

pretreated with saturated Para-dichlorobenzene solution for 4 hours at 9 ± 3^0 C. The root tips were squashed in 2% propionic orcein. The well-spread somatic plates were photographed by LEICA EC3 camera under LEICA DM 2000 fluorescent microscope at 1000x magnification. Ten well-separated somatic chromosome plates of each species were selected for karyotype analysis. Types of chromosomes were evaluated by implement the method of Levan et al. (1964). A1 and A2 indices (Romero, 1986), CV_{CL}, CV_{CI} and AI indices (Paszko 2006) as well as the categories of Stebbins (1971) were used for determine degree of karyotype asymmetry.

For meiotic studies young anthers were fixed in Cornoy's fluid in morning time (7.30 am to 8.00 am) and fixed anthers were squashed in 2% propionic-orcein results were obtained from mounted slides.

RESULTS

Chromosome counts of *Pinda* ($2n = 22$ and $n = 11$) and *Karnataka* ($2n = 22$ and $n = 11$) are reported here for the first time while, chromosome counts of *Vanasushava* ($2n = 44$) and *Polyzygus* ($2n = 36$) are reported as new cytotypes in the present study.

The diploid and haploid chromosome numbers of *Karnataka* were determined as $2n = 22$ and $n = 11$, respectively (Fig. 1 E, I). Chromosome size ranged from 1.66 to 2.54 µm. Total chromosome lengths of haploid compliment (TCLH) were found to be 22.84. The arm ratio ranged from 1.59 to 2.69. Median (m) and sub-median (sm) centromeric chromosomes were found with a karyotype formula K: 6m+16sm (Fig. 1 K for karyogram).

Pinda showed $2n = 22$ and $n = 11$(Fig. 1 F, J). Chromosome size ranged from 1.59 to 2.57 µm. Total chromosome lengths of haploid compliment (TCLH) were found to be 22.24. The arm ratio ranged from 1.18 to 2.98. Median (m), sub-median (sm) and sub-terminal (st) centromeric chromosomes were found with a karyotype formula K: 10m+8sm+4st (Fig. 1L for karyogram).

Polyzygus showed $2n = 36$ (Fig. 1G). Chromosome size ranged from 0.73 to 2.01 µm. Total chromosome lengths of haploid compliment (TCLH) were found to be 19.7. The arm ratio ranged from 1.67 to ∞. Sub-median (sm), sub-terminal (st) and terminal (t) centromeric chromosomes were found with a karyotype formula K: 8sm+2st+26t (Fig. 1M for karyogram).

Vanasushava showed $2n = 44$ (Fig. 1H.). Chromosome size ranged between 2.35–1.21 µm. Total chromosome lengths of haploid compliment (TCLH) were found to be 39.64. The arm ratio ranged from 1.51 to 1.97. Median (m) and sub-median (sm) centromeric chromosomes were found with a karyotype formula K: 8m+36sm (Fig. 1N for karyogram).

Karyotypic parameters of these genera are summarized in Table no. 1

DISCUSSION

Janardhanan and Thoppil (2003) reported somatic counts $2n = 56$ and Krishnappa and Basappa (1988) $2n = 22$ in *Polyzygus*; however, we found somatic counts $2n = 36$ during present investigation in the genus. Earlier report (Janardhanan and Thoppil, 2003) showed Range of Chromosome Length (RCL) = 1.8–3.6 µm as against 0.73–2.01 µm, Total Forma percentage (TF%) 31 as against 13 and dominance of sub-median centromeric chromosomes as against terminal centromeric chromosomes observed in the present investigation. The reasons for these differences are unknown but may be due to use of image with overlapped chromosomes for image analysis system by earlier workers.

Subramanian (1986) reported somatic counts $2n = 46$ in *Vanasushava* as against $2n = 44$, Range of size of Chromosome (RCL) = 2–5 µm as against 1.21–2.35 µm, Total Chromosome Length (TCL) = 140.6 µm as against 45.68 µm and average chromosome length = 3.06 µm as against 2.07 µm in present investigation. He

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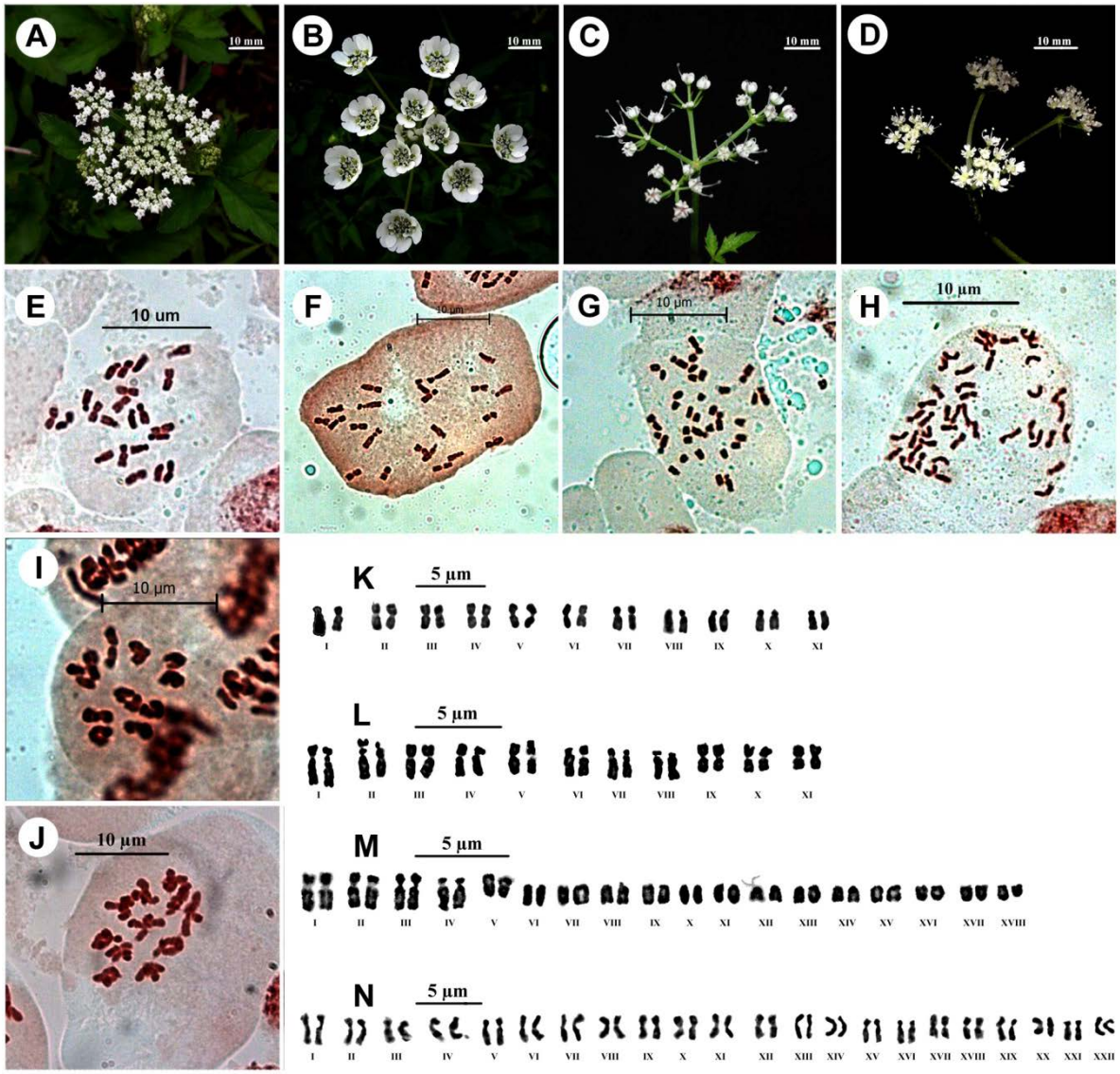


Fig. 1. A–D: An inflorescence. **A:** ***Karnataka benthamii*** (C.B. Clarke) P.K. Mukh., **B:** ***Pinda concanensis*** (Dalzell) P.K. Mukh. & Constance, **C:** ***Polyzygus tuberosus*** Walp., **D:** ***Vanasushava pedata*** (Wight) P.K. Mukh. & Constance. **E–H:** Mitotic plate. **E:** ***Karnataka benthamii*** (C.B. Clarke) P.K. Mukh., **F:** ***Pinda concanensis*** (Dalzell) P.K. Mukh. & Constance, **G:** ***Polyzygus tuberosus*** Walp., **H:** ***Vanasushava pedata*** (Wight) P.K. Mukh. & Constance. **(I–J)** Meiotic counts. **I:** ***Karnataka benthamii*** (C.B. Clarke) P.K. Mukh., **J:** ***Pinda concanensis*** (Dalzell) P.K. Mukh. & Constance. (**K–N)** Ideograph. **K:** ***Karnataka benthamii*** (C.B. Clarke) P.K. Mukh., **L:** ***Pinda concanensis*** (Dalzell) P.K. Mukh. & Constance, **M:** ***Polyzygus tuberosus*** Walp., **N:** ***Vanasushava pedata*** (Wight) P.K. Mukh. & Constance. **Scalebar: A–D** = 10 mm, **E–J** = 10 µm, **K–N** = 5 µm.

reported 8 chromosomes with secondary constrictions but we failed to observe secondary constriction even in early metaphase. He reported different chromosome types viz. median (m) = 16, sub-median (sm) = 6 and sub-terminal (st) = 16 centromeric chromosomes while we observed just two types viz. median (m) = 8 and sub-median (sm) = 36 centromeric chromosomes.

Karnataka, *Pinda* and *Vanasushava* shared common basic chromosome number, x = 11. While *Polyzygus* showed x = 12. The placement of *Karnataka* in Seselinae group is justified through base chromosome number x = 11. Same base chromosome number predominantly reported in Seselinae (Wanscher, 2010).

Vanasushava and *Pinda* share common characters in irregular petals and fruit anatomy (Cauwet-Marc, 1977), leaf surface (Guyot, 1978), phytochemistry (Carbonnier and Molho, 1977) and pollen morphology (Cerceau-Larrival et al., 1977). By molecular work both genera showed affinity with the genus *Heracleum* (Logacheva et al., 2010; Downie et al., 2010). Cytologically also both genera showed common basic chromosome number (x) = 11, same category of Stabbins (1971) 2A and very less differences in Total Forma percentage (TF %), Symmetric Index (SI),

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interchromosomal asymmetry index (A2) and Coefficient of variation (CV) of the centromeric index (CV_{CI}) etc (Table 1). Thus, karyomorphology also supports their close relationships. *Vanasushava* and *Pinda* both showed similarities with the genus *Heracleum* in basic chromosome number (*x*) = 11, category of Stebbins (1971) = 2A and median (m), sub-median (sm) and sub-terminal (st) centromeric types of chromosomes (Puneet and Singhal, 2011; Deng et al., 2009; He, 1994). Karyomorphological work of *Pinda* and *Vanasushava* are supported to phylogenetic analyses of nrDNA ETS and ITS sequence data (Logacheva et al., 2010).

The scatter diagram of A1 (intrachromosomal asymmetric index) against A2 (interchromosomal asymmetric index) asymmetry indices (Fig. 2) showed that, *Polyzygus* with the lowest A1 value, presented the small difference in the length of chromosome arms but highest A2 value, presented the large variation in length among chromosomes. However, *Vanasushava* showed highest A1 value, presented the highest difference in the length of chromosome arm while *Karnataka* showed lowest A2 value, presented lowest variation length among chromosome. The scatter diagram of CV_{CI} (coefficient of variation of centromeric index) against CV_{CL} (coefficient of variation of chromosome length) asymmetry indices (Fig. 3) also showed that, *Polyzygus* possess higher asymmetric karyotype as compare to *Karnataka*, *Pinda* and *Vanasushava*. This results also strongly supported by higher karyotype asymmetry index (AI) = 71.32 and advance type of Stabbins class, 4B showed by *Polyzygus* (Table 1) among the *Karnataka*, *Pinda* and *Vanasushava.*

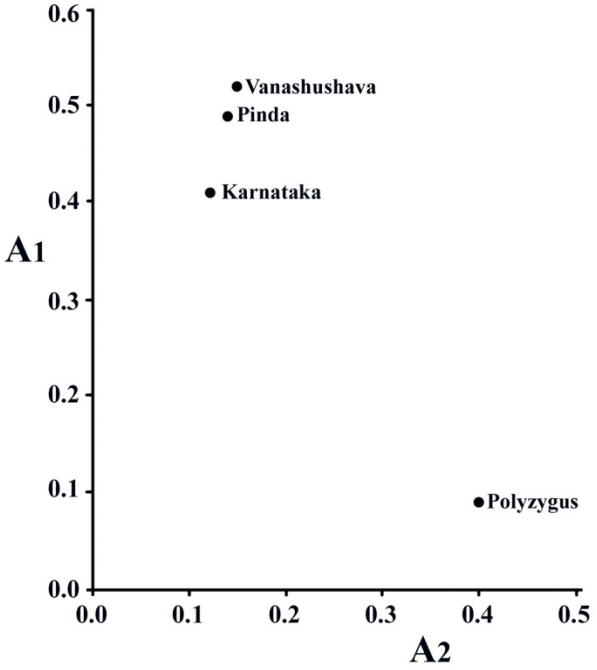


Fig. 2. Scatter diagram of intrachromosomal (A1) and interchromosomal (A2) asymmetry of ***Karnataka***, ***Pinda***, ***Polyzygus*** and ***Vanasushava***.

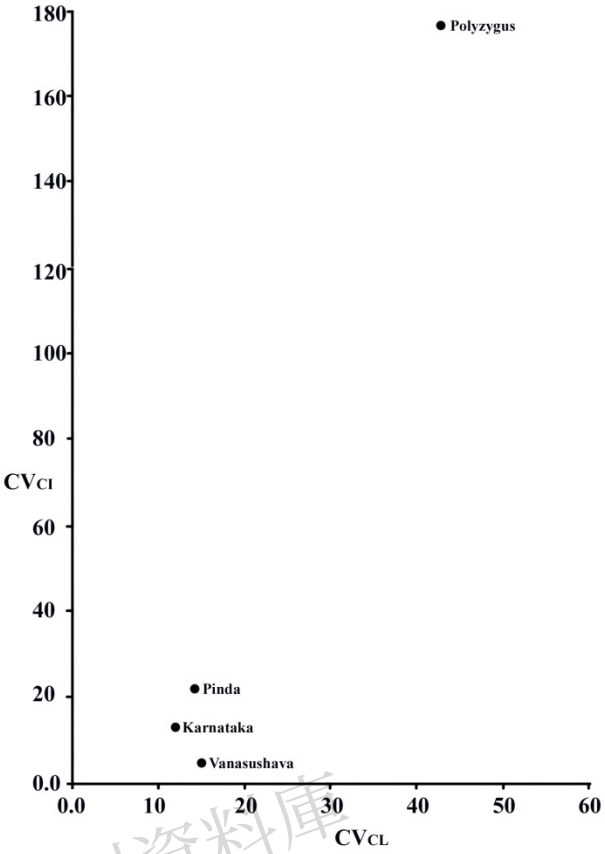


Fig. 3. Scatter diagram of the relative variation in chromosome length (CV_{CL}) and the relative variation in centromeric index (CV_{CI}) of ***Karnataka***, ***Pinda***, ***Polyzygus*** and ***Vanasushava***.

CONCLUSION

Karyomorphologically *Pinda* and *Vanasushava* are close to each other and showed affinity with the genus *Heracleum*. However, the genus *Polyzygus* is highly advanced genus among the *Karnataka*, *Pinda* and *Vanasushava.* Detail cytological work viz. basic chromosome number and different karyomorphological parameters of the Indian Apiaceae members will be focus on interrelationship with these endemic genera.

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