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ORIGINAL ARTICLE

Cytopalynological studies in *Zanonia indica* (Cucurbitaceae), a monotypic genus

M. M. Lekhak[1] · P. B. Yadav[1] · U. A. Attar[2] · K. S. Rajput[3] · S. G. Ghane[2]

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Abstract
This paper presents first cytogenetical analysis of a monotypic genus, *Zanonia indica* (Cucurbitaceae). The diploid chromosome number was $2n=2x=30$. Male meiosis was normal and revealed 15 bivalents at diakinesis. Karyotype was symmetrical (Stebbins's 4a category). All chromosomes possessed median centromere. Chromosome length ranged from 1.10 ± 0.14 µm to 1.98 ± 0.33 µm. Mean chromosome length (MCL) was 1.47 ± 0.24 µm. Pollen grains were tricolporate and prolate with P/E ratio 1.49.

Keywords Cucurbits · Chromosomes · Karyotype · Pollen · SEM

Introduction

Cucurbitaceae Juss., commonly known as gourd family are an economically important plant group. Cucurbits, the members of the family are widely cultivated for their fruits such as cucumber (*Cucumis sativus* L.), melon (*C. melo* L.), water melon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) etc. Cucurbitaceae have been divided into 15 tribes and comprise 2600 species and 109 or 110 genera [8]. In India, a total of 94 species have been recognized across 31 genera [5].

Tribe Zanonieae Benth. & Hook.f comprises four genera, viz. *Gerrardanthus* Harv. ex Benth. & Hook.f., *Siolmatra* Baill., *Xerosicyos* Humbert and *Zanonia* L. Of these four, only *Zanonia* is reported from India. *Zanonia* is a monotypic dioecious woody climber. The only species *Zanonia indica* L. is distributed from India, throughout Southeast Asia to New Guinea [8]. In India, the species has been recorded from Andaman and Nicobar Islands, Assam, Goa, Karnataka, Kerala, Maharashtra, Meghalaya, Sikkim, Tamil Nadu and West Bengal [5]. As chromosome data are not available for the entire tribe Zanonieae, present study is focused on cytogenetical investigation of *Z. indica*. Further, karyotype details and palynology of the species are discussed in relation to the existing information on the allied taxa.

Materials and methods

Mitotic chromosome preparations were made from root-tips obtained from seeds collected from Alevoor, Udupi district, Karnataka. Root-tips were pre-treated with saturated para-dichlorobenzene (pDB) at 8–10 °C for 4 h and were washed in double distilled water and hydrolysed at 60 °C in 1 N HCl for 10 min. Further, the hydrolysed root-tips were washed with distilled water and squashed in 2% propionic-orcein. Chromosome pairs were identified and arranged on the basis of their length. For meiotic studies, flower buds were fixed in Carnoy's fluid (3:1 ethanol and acetic acid) and smears of floral buds were stained using 2% propionic-orcein. Suitable somatic and meiotic plates from fresh preparations were photographed using LEICA DM 2000 fluorescence microscope with attached camera at ×1000 magnification. Twenty plates with well stained and separated somatic chromosome were selected for karyotype analysis. The nomenclature used for describing karyotype composition followed Levan et al. [3]. The degree of karyotype asymmetry was determined using the categories of Stebbins [11]. Karyotype morphometric characters were

✉ M. M. Lekhak
mml_botany@unishivaji.ac.in

[1] Angiosperm Taxonomy Laboratory, Department of Botany, Shivaji University, Kolhapur, Maharashtra 416 004, India

[2] Plant Physiology Laboratory, Department of Botany, Shivaji University, Kolhapur, Maharashtra 416 004, India

[3] Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat 390002, India

evaluated by calculating haploid complement length together with A_1 (intrachromosomal asymmetry index) and A_2 (interchromosomal asymmetry index) [6].

Pollen viability was assessed with freshly prepared 1% aceto-carmine. Pollens grains, fixed in glacial acetic acid were acetolysed (in freshly prepared 9:1 acetic anhydride: concentrated sulphuric acid) following the technique of Erdtman [1]. Pollen study was also carried out using electron microscopy. Pollen grains were mounted on a double sided sticky carbon tape bound to an aluminium stub. Pollen grains were then coated with gold/palladium for 75 s on a Quorum SC7620 sputter coater and examined using a TESCAN VEGA3 scanning electron microscope (SEM) at 5 kV. The pollen grain measurements were made from semi-permanent preparation of acetolysed pollen grains mounted in glycerine jelly. Measurements of total twenty pollen grains were taken and expressed as mean ± standard deviation (SD). The values of P (polar axis length) and E (equatorial diameter) were calculated to find out P/E ratio.

Results

The chromosome number of *Zanonia indica* was $2n = 2x = 30$. The karyotype morphology of this taxon, consisting of 30 m chromosomes, is reported for the first time (Fig. 1a). The ratio of the longest to the shortest chromosome was 1.80. Mean chromosome length (MCL) was recorded 1.47 ± 0.24 µm with chromosome length ranging from 1.10 ± 0.14 µm to 1.98 ± 0.33 µm. Total haploid chromosome length (THL) was 22.12 ± 0.24 µm. Karyotype was symmetrical and occupied Stebbins's 4a category (Fig. 1b).

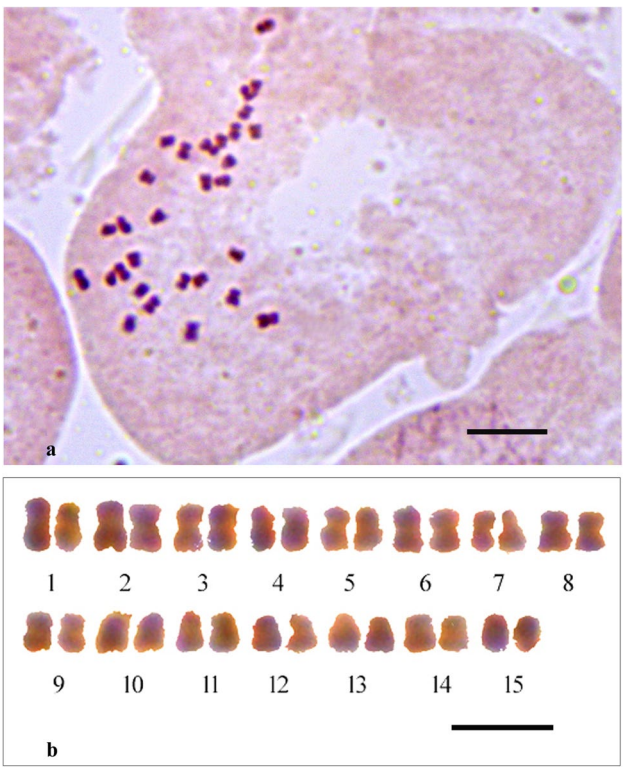

Fig. 1 **a** Mitotic metaphase chromosomes of *Zanonia indica* ($2n = 30$), **b** Karyogram. *Scale bars* = 5 µm

Meiotic behavior was found to be normal. Meiotic course has been depicted in Fig. 2. A total of 315 pollen mother cells (PMCs) were analysed. At diakinesis, PMCs showed 15 bivalents ($n = 15$). Different stages such as diplotene, diakinesis, metaphase-I, II, anaphase-I, II and telophase-I, II were also observed. Four bivalents were found associated with the nucleolus (Fig. 2a). Microspore tetrads were tetrahedral. Multivalent associations were observed in very few PMCs (2.86%).

A total of 412 pollen grains were observed for viability. More than 99% pollens were found to be viable. Pollen grains were usually monad, isopolar, 24–36 × 16–24 µm, prolate with P/E ratio 1.49, tricolporate; colpi with granular membrane, endoaperture circular and thick walled. Exine was thick, subtectate, striate with subparallel muri (Fig. 3).

Discussion

Considerable cytogenetical data, particularly, in the form of chromosome count exist for Cucurbitaceae. Nevertheless, three tribes, namely Indofevilleeae H. Schaef. & S.S. Renner, Triceratieae A. Rich. and Zanonieae Benth. & Hook.f. have not been studied from cytogenetical point of view [8]. A perusal of the available literature revealed that gametophytic chromosome number (n) can range from $n = 7$–44 [8]. Tribe Sicyoeae Schrad. exhibits maximum diversity in chromosome numbers as gametophytic number can be 11, 12, 13, 14, 15, 16 and 44. Gomphogyneae Benth. & Hook.f. ($n = 11$, 14 and 16), Thlandiantheae H. Schaef. & S.S. Renner ($n = 9$ and 16), Momordiceae H. Schaef. & S.S. Renner ($n = 11$ or 14), Schizopeponeae C. Jefferey ($n = 10$ and 11), Coniandreae Endl. ($n = 12$, 13 and 14) and Benincaseae Ser. ($n = 7$ or 12) show two or more gametic numbers [8]. However, Actinostemmateae H. Schaef. & S.S. Renner ($n = 8$), Siraitieae H. Schaef. & S.S. Renner ($n = 14$), Joliffieae Schrad. ($n = 12$), Bryonieae Dumort. ($n = 10$) and Cucurbiteae ($n = 20$) exhibit a single gametic number [8].

A symmetrical type of karyotype was noted in *Z. indica* as all the chromosomes had median centromere. Chromosomes were usually small (1.10–1.98 µm) and hence THL value was quite low (22.12 ± 0.24 µm). Karyotype data are not available for taxa related to *Zanonia,* however, small chromosomes with median and submedian region centromeres have been reported in many cucurbits. For instance, Singh and Roy [10] investigated five different species of *Cucumis* L. and five varieties of *C. melo* and found that chromosomes were mostly submedian and their length varied from 1.22 to 2.94 µm. The THL value

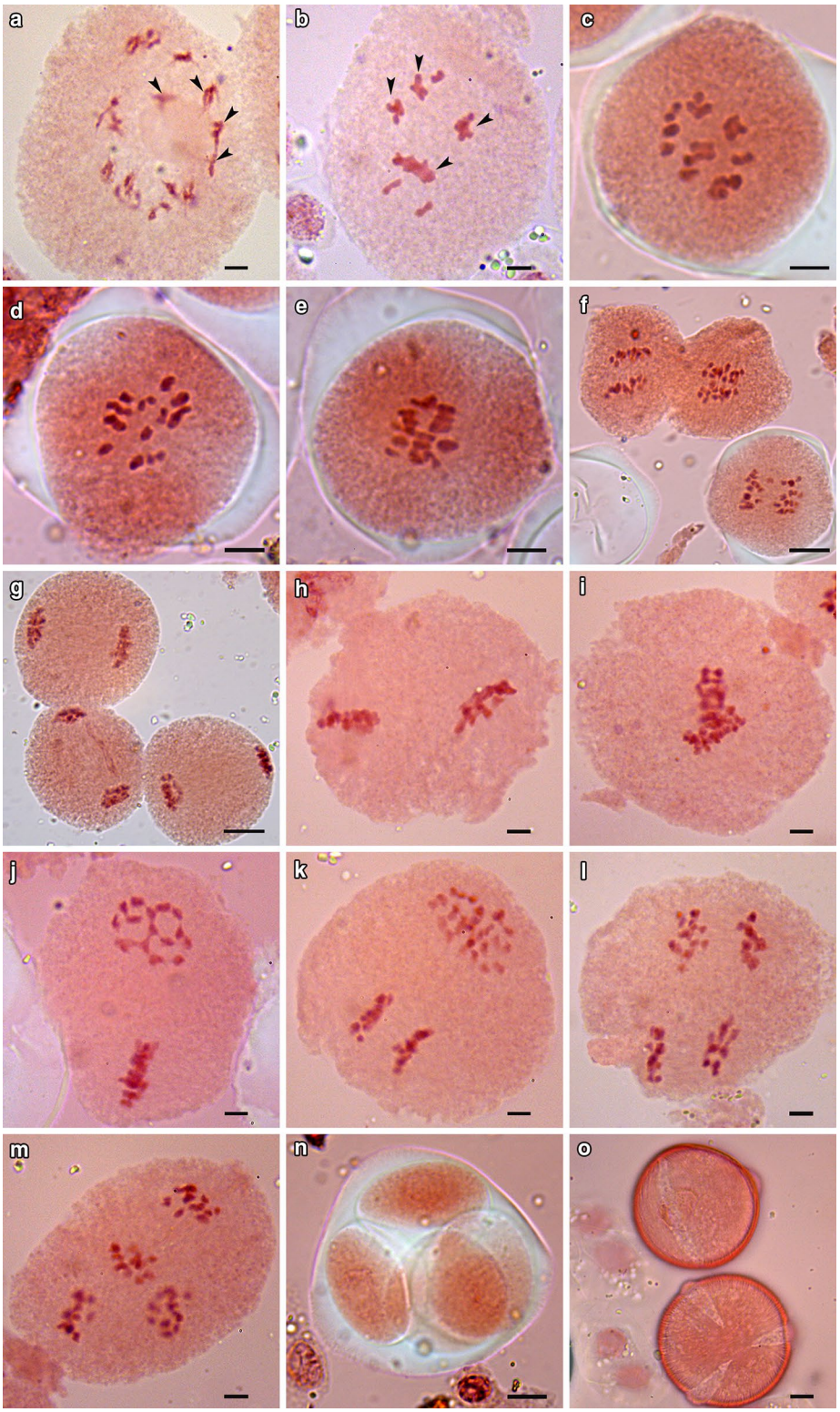

Fig. 2 Male meiosis in *Zanonia indica*. **a** PMC at diplotene (arrowheads depict four bivalents associated with nucleolus), **b** PMC at diakinesis (arrowheads depict chromosomal associations, 4II + 3IV + 1V), **c**, **d** PMCs at diakinesis showing $n = 15$ bivalents, **e** PMC at metaphase-I, **f** PMCs at anaphase-I, **g** PMCs at telophase-I, **h** PMC at metaphase-II, **i** PMC at metaphase-II, disorientation of spindle, **j**, **k** PMCs at meta-anaphase-II, **l** PMC at telophase-II, **m** PMC at telophase-II, disorientation of the spindle, **n** microspore tetrad, **o** pollens. *Scale bars* = 5 µm

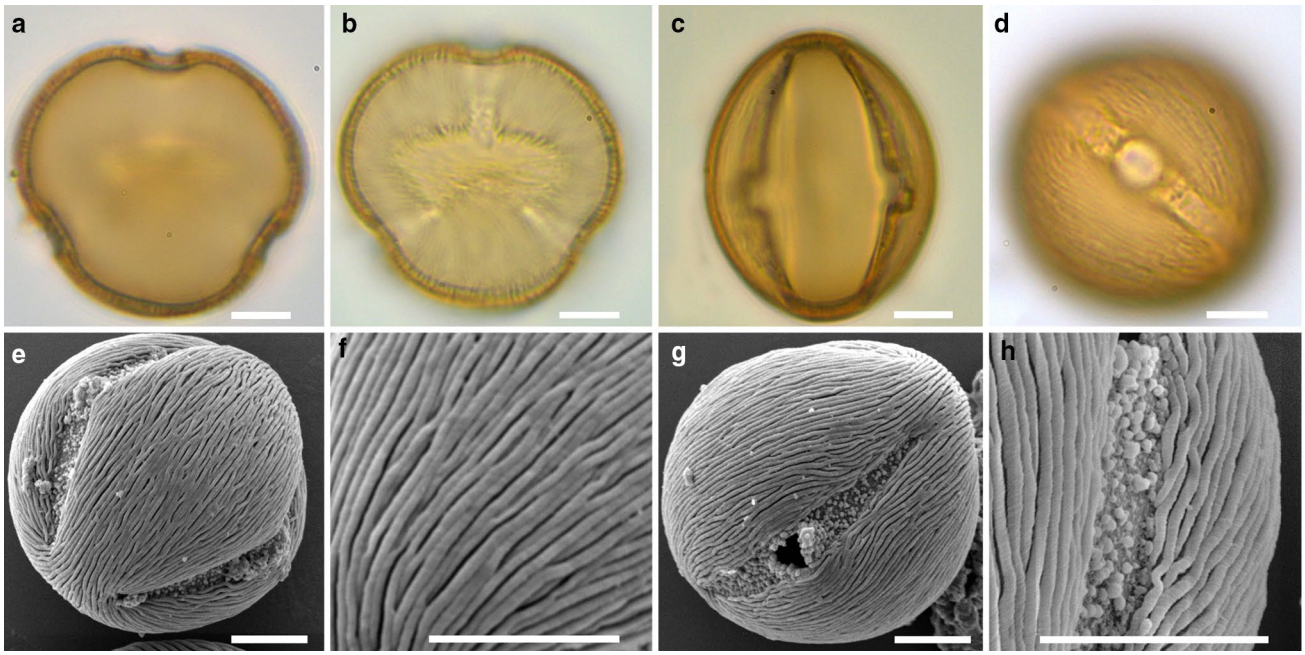

Fig. 3 Pollen grains of *Zanonia indica*. **a**, **b** Polar view of pollen (LM) (×1000), **c** Equatorial view of pollen (LM) (×1000), **d** Equatorial view showing colporate pollen (LM) (×1000), **e** Equatorial view of pollen (SEM) (×7640), **f** Enlarged view of pollen surface (SEM) (×20,000), **g** Pollen grain shows colpi with pore (SEM) (×8220), **h** Enlarged view of granular membranous surface of colpi (SEM) (×25,000). *Scale bars* = 5 µm

was found to be the lowest (30.80 µm) in *C. callosus* (Rottler) Cogn. and highest (41.64 µm) in *C. melo* var. *agrestis* Naudin. Range of chromosome length in *Melothria assamica* Chakrav. (0.80–2.40 µm) and *Mukia maderaspatana* (L.) M. Roem. (1.36–2.14 µm) [9] also support the fact that the chromosomes in Cucurbitaceae are usually small in size. Surprisingly, *Gomphogyne cissiformis* Griff. (Gomphogyneae) has been reported to have large chromosomes [7, 12]. However, karyotypic features of this species were not discussed by the authors. Thakur and Sinha [12] observed male meiosis in the species. They had found that 12% of the PMCs exhibit multivalent formation (8 quadrivalents) whereas 22% of the cells showed regular bivalent formation. It was suggested that taxon is of autoploid origin. Multivalent fomation in *Z. indica* was very low (2.86%). As abundant seed set was observed in the species, multivalent formation seems to be environment induced rather than a consequence of autoploidy. Chromosomal studies have also been conducted on *Gynostemma* Blume (Gomphogyneae). Eight species and one variety were studied [2]. Chromosome numbers $2n = 22$, 33, 44, 66 and 88 were reported but karyotype features not detailed. Authors concluded that the genus has the base number $x = 11$.

The present investigation revealed that Zanonineae may have a base number of $x = 15$. However, more cytogenetical data from other members (*Gerrardanthus, Siolmatra* and *Xerosicyos*) are still awaited to ascertain chromosome diversity and evolution within the tribe. Chromosomal information from members of related tribes such as Gomphogyneae and Indofevilleeae may also provide essential information to ascertain the primary or original base number (x) of the family. Furthermore, two Indian taxa, viz. *Indofevillea khasiana* Chatterjee and *Neoalsomitra clavigera* (Wall.) Hutch. belonging to Indofevilleeae and Gomphogyneae, respectively are woody climbers and hence it would be worthwhile to investigate their karyotype features vis-a-vis *Z. indica.*

Pollens in Zanonineae are monad, tricolporate with striate exine ornamentation mostly with long subparallel muri [13]. van der Ham et al. [13] studied the pollens of five genera of Zanonineae, viz. *Gerrardanthus, Siolmatra, Xerosicyos, Zanonia* and *Zygosicyos* Humbert (now merged into *Xerosicyos*). The studies revealed that pollen grains are mostly striate except *Gerrardanthus* (which has microreticulate pollen). The size of the pollen grain (length of the polar axis) ranged from 22 to 52 µm. The smallest being the pollens of *Siolmatra* and *Xerosicyos* and the largest of *Gerrardanthus.* For *Z. indica* the length of the polar axis ranged from 24 to 40 µm. Our values of 24–36 µm for the length of the polar axis are very much in sync with the earlier findings [13]. In the present investigation, we also found that pollens of *Zanonia indica* are monad, tricolporate with striate exine ornamentation and with long subparallel muri. Pollen type similar to *Z. indica* have also been reported for the genus *Sicydium* Schltdl. (now placed in tribe Triceratieae) [4] and

in tribes Actinostemmateae, Fevilleeae and Gomphogyneae of previously recognized subfamily Fevilleioideae [13].

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