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Cytomorphological Study in Three Species of the Genus *Chlorophytum* Ker Gawl. (Liliaceae)

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ABSTRACT: A detailed Karyotypic and meiotic study in three species of *Chlorophytum* is presented and its significance is discussed in the light of the modern concepts. *Chlorophytum elatum* R. Br. is tetraploid with *2n=28* chromosomes, *C. heynei* Baker. and *C. laxum* Baker. are diploids with *2n=14* or 16 chromosomes, in x=7 and 8 basic number lines and *C. elatum* shows asymmetrical karyotype while *C. heynei* and *C. laxum* show symmetrical karyotypes. Karyomorphological studies reveal some minor differences between the three species, such as absolute chromosomal length and relative length which clearly indicate a common origin and close relationship of these three species. However, populations of *Chlorophytum elatum*, *C. heynei* and *C. laxum* vary slightly in their intraspecific cytomorphological characteristics which may be the basis of ecotypic differentiation. The behaviour of chromosomes during meiosis in all the 3 species is found to be fairly normal. Nevertheless, in *C. elatum* quadrivalent association of chromosomes, laggards and bridges are of common occurrence. The presence of B-chromosome in *C. elatum* is recorded for the first time.

KEY WORDS: **Asphodeleae, Cytology, *Chlorophytum elatum*, *Chlorophytum heynei*, *Chlorophytum laxum*, Liliaceae.**

INTRODUCTION

Chlorophytum means the "green plant" in the Greek language. The genus *Chlorophytum* Ker Gawl. belongs to the tribe Anthericinae of the sub-family Asphodeleae of Liliaceae (Engler and Prantle, 1930). The species of *Chlorophytum* are widely known for their economic, ornamental and medicinal values (Tarafder, 1983; Wolverton *et al.*, 1984). It comprises about 326 species distributed in tropical and sub-tropical regions of the world (Willis, 1973). Most of the species are reported from Africa its possible center of origin (Patil *et al.* 1987). From India, Hooker (1892) had recorded twelve species to which one more has been added by Santapau and Fernandes (1955).

Although there are 326 species in the genus *chlorophytum* cytology of only twenty nine species is so far known (Federov, 1969; Goldblatt, 1984). Notable Chromosome reports on the Indian species are those by Sheriff (1957), Sheriff and Chennaveeraiah (1975), Naik (1977), Naik and Nirgude (1984) and Pagliarini *et al.* (1993). Among the species of *Chlorophytum, C. elatum* has been investigated cytologically by several workers, both in India and elsewhere. Several cultivar varieties exist in this species, differing principally in the pattern of foliage and colour of the scape. Surprisingly all these cultivar varieties thus for reported have found to posses the same chromosome number *2n=28*. Matsura and Sato (1935), Yamazaki (1936), Sato (1942), Sharma and Chatterjee (1958), and Muniyamma and Shiva Kameshwari (1996) have described the Karyotypes in different cultivar varieties and have also discussed their evolutionary significance. The present work deals with the Karyomorphological and meiotic studies on *C. elatum, C. heynei and C. laxum*.

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MATERIALS AND METHODS

Live plants of *C. elatum, C. heynei* and *C. laxum* were collected from Lalbagh Garden, Bangalore and Indra Vihar Garden, Mysore. All the plants were raised under uniform conditions in the Botanical garden of the department. Voucher specimens of these collections have been deposited in the herbarium of the department of Botany, Manasagangotri, University of Mysore, Mysore.

Somatic chromosomes were studied in the root tips excised from the potted plants. Root tips were pretreated with 0.01% 8-hydroxyquinoline at 4℃ and fixed in 1:3 acetic alcohol solution for 12-24 hours. They were hydrolysed in 1N HCI at 60℃ for 8 minutes, stained with haematoxylin and propionic acid.

For morphological classification of somatic chromosomes the nomenclature proposed by Levan *et al.*, (1964) was followed. Symmetry and asymmetry have been determined according to the system described by Stebbins (1958). Meiotic studies were made from aceto-carmine squashes of pollen mother cells.

RESULTS

The data on karyomorphological studies of *C. elatum, C. heynei* and *C. laxum* and the salient features are described below.

C. elatum R. Br.: Mitotic studies in root tip cells showed *2n=28* (Fig. 1a). The Karyotype consists of 1 pair of 'M', 6 pairs of 'm', 6 pairs of 'sm' and 1 pair of 'st' type chromosomes. The chromosome length ranges from 3.32 to 6.00 μm and the absolute chromosomal length is 61.25 μm. The idiogram is given in figure 1b. Thus this taxon has karyotype formula *2n=28*, n=14, M_1 +m_6+sm_6+st_1. The karyotype is of asymmetrical type.

C. heynei Baker.: Mitotic studies revealed the presence of *2n=14* (Fig. 1c). The karyotype consist of 2 type of chromosomes. Chromosome length ranges from 4.66 to 7.99 μm. and the absolute chromosomal length is 43.59 μm. The idiogram is given in Fig 1d and the karyotype formula is *2n=14*, n=7=M_6+sm_1. Occasionally 12 chromosomes have also been recorded.

C. *laxum* Baker.: Mitotic studies revealed the presence of *2n=16* (Fig. 1e). The karyotype consist of two type of chromosomes. The chromosome length ranges from 5.33 to 8.00 μm and the computed absolute length is 57.29 μm. The idiogram is given in Fig1f. This taxon has a karyotype formula *2n=16*, n=8=M_2+m_6.

Meiosis

C. elatum: Meiosis in this taxon, appears to be regular in a majority of pollen mother cells observed. Prophase I was normally distinct (Fig. 2a). Diakinesis revealed the presence of 14 bivalents in nearly about 80% of cells (Fig. 2b). In about 10% of the cells tetravalents were constantly present in diakinesis and metaphase-I (Fig. 2c). About 9% of the cells show precocious movement of chromosomes. Anaphase-I shows normal segregation of chromosomes in 60% of cells observed and lagging chromosomes in 40% of the cells (Figs. 2d-f). Meiosis second leads to the formation of isobilateral tetrads (Fig. 2g). 78% fertility has been recorded (Fig. 2h). The presence of B-chromosome is recorded for the first time in root tip cells (Fig. 2i).

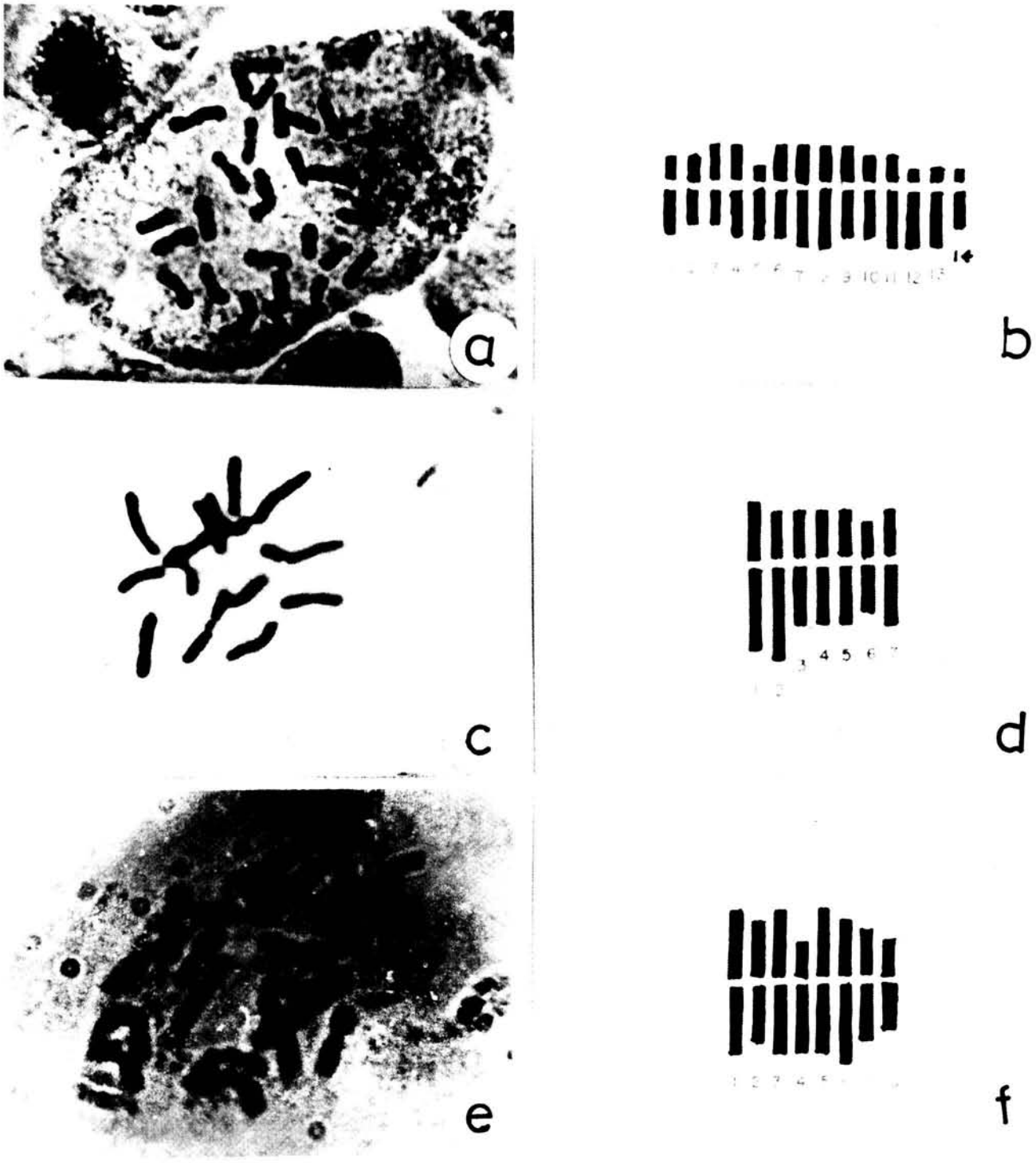

Fig. 1. Karyotype of three species of *Chlorophytum* Ker Gawl. a: *C. elatum*, *2n=28*. x 6000. b: *C. elatum*, Idiogram. x 6000. c: *C. heynei*, *2n=14*. x 6000. d: *C. heynei*, Idiogram. x 6000. e: *C. laxum*, *2n=16*. x 6000. f: *C. laxum*, Idiogram. x 6000.

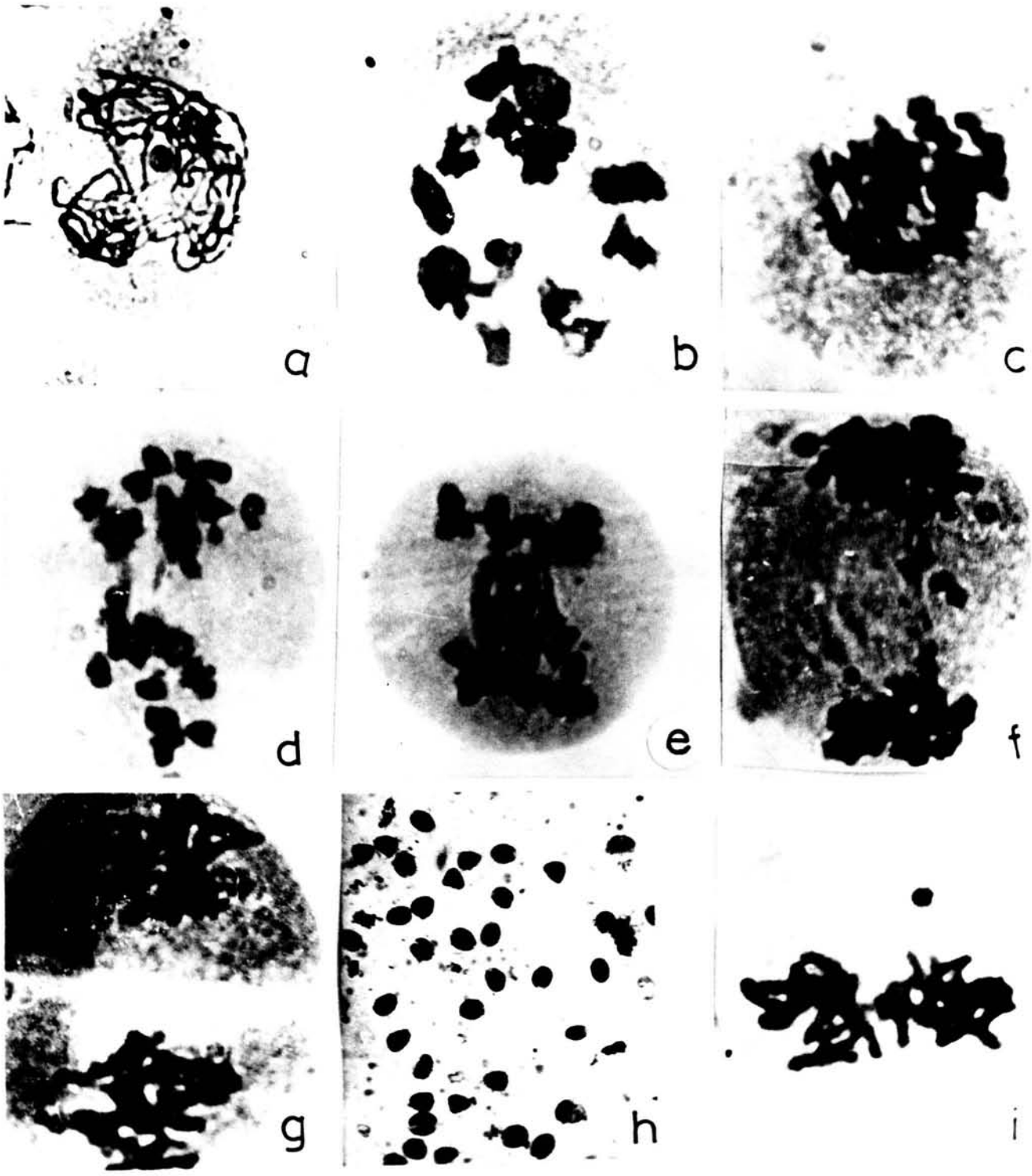

Fig. 2. Meiosis in *C. elatum* R. Br. a: Prophase-I. x 600. b: Diakinesis. x 600. c: Metaphase-I. x 600. d-f: Anaphase-I showing chromatin bridge and lagging chromosomes. x 600. g: Metaphase-II. x 600. h: 78% Pollen fertility. x 600. i: Root tip cell showing B-chromosome. x 600.

C. heynei: Meiosis appears to be regular in a majority of pollen mother cells that were investigated. Prophase-I was normally distinct by the presence of one nucleolous (Fig. 3a). Seven bivalents were counted at diakinesis (Figs. 3b & c). Two nucleoli during diakinesis were noticed in about 20% of cells (Fig. 3d). First and second divisions also appeared normal (Fig. 3e-h) and tetrads are of isobilateral type (Fig. 3i). 89% of pollen fertility was observed.

C. laxum: At the pachytene stage, chromosome pairing was complete and normal (Fig. 4a). Diakinesis showed consistently 8 bivalents in about 80% of Pollen mother cells (Figs. 4b-e). During metaphase -I, 8-bivalents were seen arranged on the equatorial plate (Fig. 4f). Anaphase- I and Telophase- I show normal segregation of chromosomes (Figs. 4g & h). Meiosis second leads to the formation of isobilateral tetrads of microspores (Fig. 4i). About 85% of Pollen was found to be fertile.

DISCUSSION

Karyomorphological studies made on three species of *Chlorophtum C. elatum, C. heynei & C. laxum* revealed *2n=28, 14* and *16* chromosomes respectively. Most of the reports on *C. elatum* reveal that it is a tetraploid with *2n=28* chromosomes (Matsura and Sato, 1935; Sheriff, 1967; Koul and Gohil, 1973; Muniyamma and Shiva Kameshwari, 1996). The chromosome counts *2n=28* made for *C. elatum* in the populations of all cultivar varieties are remarkably uniform. The present documentation of chromosome numbers for the four populations of this species confirms the earlier reports. In the genus *Chlorophytum*, although it was widely investigated cytologically by the earlier workers, (Sheriff, 1957), Sheriff and Chennaveeraiah (1975), Naik and Nirgude (1984) and Pagliarini *et al.* (1993) the occurance of B-chromosomes was not observed. However the presence of such chromosomes in the other genera of the family was known. In *Scilla autumnalis* (Battaglia, 1964 and Ruiz Regon *et al.* 1980) and in *urginea indica* (Sen, 1974; Shiva Kameshwari and Muniyamma, 1992) B-Chromosomes have been reported. The present work, records for the first time, the presence of B-chromosome in Mitotic cells of *C. elatum.*

The present study of *C. heynei and C. laxum* having 2n=14 and 16 chromosomes respectively confirms the previous reports by Sheriff and Chennaveeraiah (1972) and Mathew and Thomas (1974). In *C. laxum,* diploids (*2n=16*) and a rare tetraploid (*2n=32*) have been recorded by Sheriff and Nagraj (1976) and Patil *et al.* (1988). Thus the cytological studies of different populations, have revealed that Indian populations of *C. laxum* are characterised by having 16 chromosomes. The diploidy being found predominantly, in most of the populations while the tetraploids are of rare occurrence. Cytological data on different species of *Chlorophytum* in addition to the present study, clearly show 7 and 8 basic euploid series in the genus. It is also interesting to note that all populations of *C. elatum* have tetraploidy while the populations of *C. heynei* and *C. laxum* are diploids. Such a phenomenon where in different ploidy exist in different species of the same genus is not very uncommon in the Liliaceae. In *Urginea polyphylla* Shiva Kameshwari and Muniyamma (1999), *U. Coromandeliana*, Naik (1973) *Scilla indica* Sheriff and Rao (1981) *C. laxum* Sheriff and Nagaraj (1976). In the above taxa although diploids are common aneuploidy is widely reported.

Karyomorphological studies made in the three species of *Chlorophytum* reveal only some minor differences between the three species which clearly indicates close relationship. Karyotype is symmetrical in *C. heynei* and *C. laxum* where as, asymmetrical in *C. elatum.* A

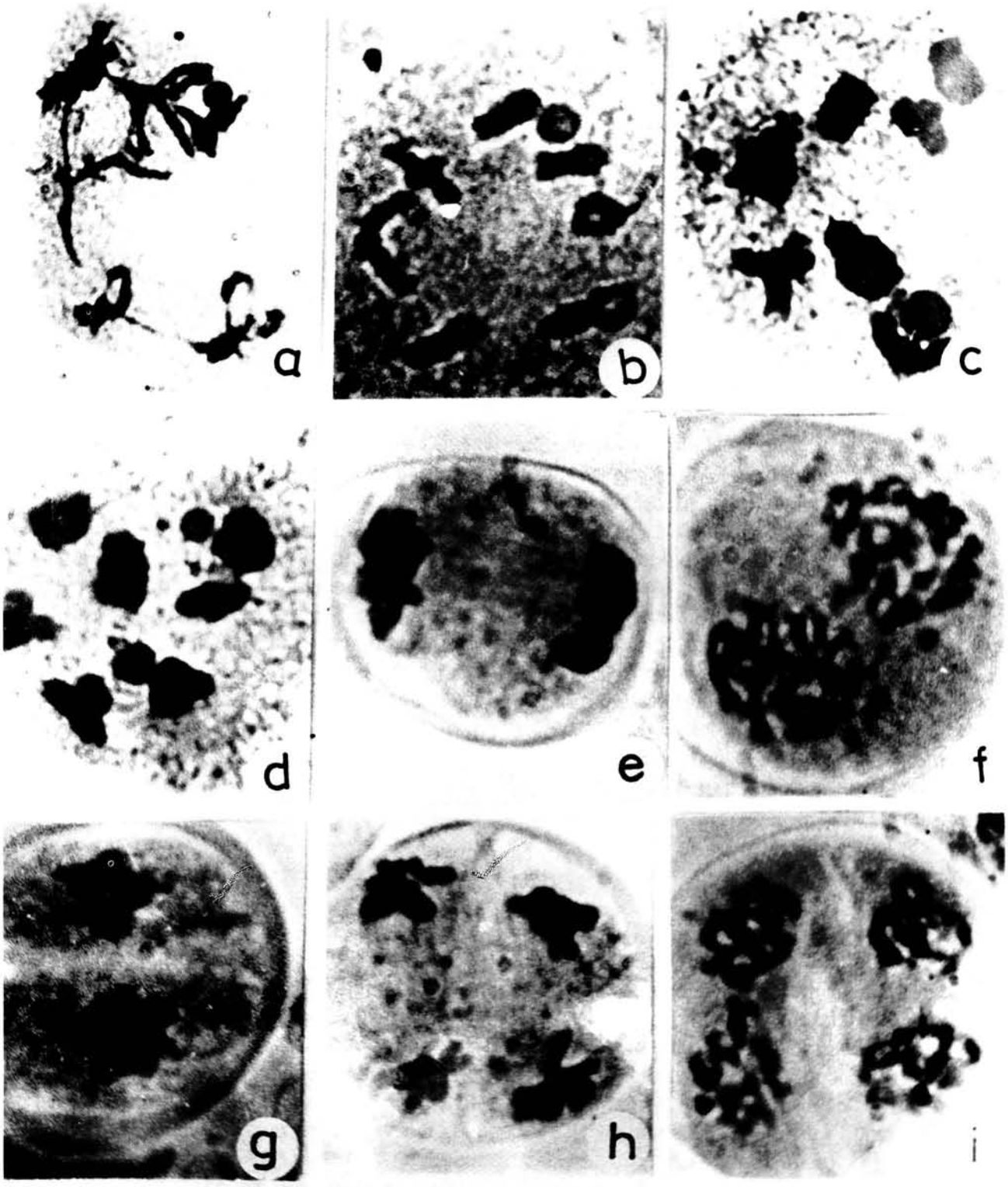

Fig. 3. Meiosis in *C. heynei* Baker. a: Prophase-I. x 600. b & c: Diakinesis. x 600. d: Diakinesis showing 2 nucleoli. x 600. e & f: Telophase-I. x 600. g & h: 2nd Metaphase and Anaphase. x 600. i: Isobilateral tetrad. x 600.

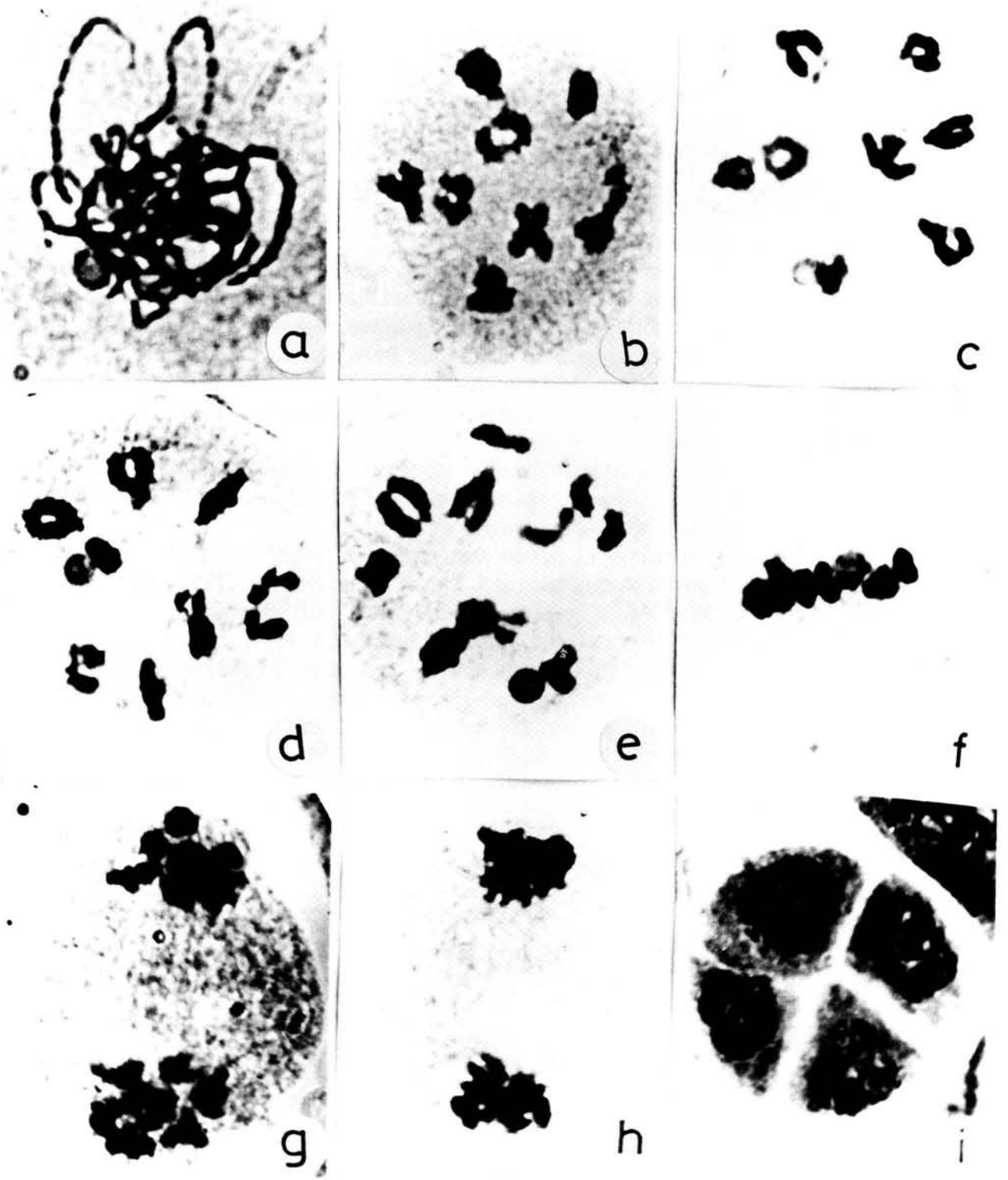

Fig. 4. Meiosis in *C.laxum* Baker. a: Prophase-I. x 600. b-e: Diakinesis. x 600. f: Metaphase-I. x 600. g & h: Anaphase-I and Telophase-I. x 600. i: Isobilateral tetrad. x 600.

distinctive feature is the presence of two relatively shorter chromosomes with sub-telocentric primary constriction in *C. elatum* of the present study under 7-basic series and the significant absence of these in the other two species. The karyotypes under 8-basic series i.e. of *C.laxum* are more symmetrical and the chromosomes are relatively longer when compared to those in the 7-basic series i. e., *C. elatum* and *C. heynei*. Basic chromosomes X=7 line showed more asymmetrical karyotypes than X=8 which is indicative of an association between decreasing basic number and increasing karyotype specialization in the genus *Chlorophytum* Vijayavalli and Mathew (1990). Therefore the evolutionary advancement, in the species of the genus *Chlorophytum* appears to be more towards the decreasing basic number and the increasing specialisation on the Karyotype. However, as the genus has 326 species more work is needed to predict the trends in evolutionary specialization.

In the three species of the present study, chromosome behaviour during meiosis is found to be fairly normal. Nevertheless, in *C. elatum*, a tetraploid (2n=28), quadrivalent association of chromosomes, chromosome bridges and irregular spindles were of common occurrence. Sometimes the chromosomes are irregularly distributed from pole to pole on the anaphase spindles, such abnormalities have also been reported by Naik, (1977), Pagliarini *et al.* (1993). Sheriff (1957) in a cultivar variety of *C.elatum* has observed spontaneous chromosome inversions.

The diploid *C. heynei* shows normal meiosis. However, in about 20% of the cells, 2 nucleoli (one small and one large) were observed in early stages. Upcott (1939) had also noted such nucleoli in triploid tulips and in grasses.

Normal meiosis was noticed in *C. laxum* that results in fertile pollen. According to Pagliarini *et al.* (1993) and Muniyamma and Shiva Kameshwari (1996), the meiotic abnormalities such as tetravalents, precocious movement of chromosomes, lagging chromosomes and chromatin bridges are found frequently in most of the species of *Chlorophytum*. In fact the genus *Chlorophytum* offers a good material for cytogeographical and biosystematic studies. Although the present investigation confirms the earlier cytological findings, Several significant new findings are reported here. These include the presence of B-Chromosomes in *C. elatum* and presence of two nucleoli in 20% of cells in *C. heynei* and 95% pollen viability in *C. laxum*.

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三種吊蘭屬(百合科)之細胞形態學的研究

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摘　　要

本文詳細報導三種吊蘭屬的核型及體細胞分裂的研究，且藉現代學理以討論其意義。彈絲吊蘭是四倍體，染色體為 2n=28。赫氏吊蘭及小花吊蘭是雙倍體，染色體兼具 2n=14 及 16，而基數為 7 及 8。彈絲吊蘭表現不對稱核型，而赫氏吊蘭及小花吊蘭則表現對稱核型。三種間之核型形態稍異，例如染色體絕對及相對長度明示此三種為同緣及親緣極相近。但此三種之族群內之細胞型特徵均稍異而顯示具有生態型分化現象。細胞分裂中之染色體行為在三種均為正常。但彈絲吊蘭常見染色體為四價，遲滯及橋之現象。B－染色體出現於彈絲吊蘭為首次之報告。

關鍵詞：異蕊草族、細胞學、彈絲吊蘭、赫氏吊蘭、小花吊蘭、百合科。

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