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ORIGINAL ARTICLE

Endomitosis in tapetal cells of some Cymbidiums (Orchidaceae)

Santosh Kumar Sharma · Suman Kumaria · Pramod Tandon · Satyawada Rama Rao

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Abstract True endomitosis in the anther tapetum of the three *Cymbidium* species viz. *C. aloifolium, C. devonianum* and *C. tigrinum* is described. In these Cymbidiums, most tapetal cells go through endomitosis instead of either normal mitosis or so called "inhibited" mitosis. The nuclear membrane does not disappear, but during metaphase the chromosomes are considerably condensed far more than in normal mitosis, and nucleolus persists throughout the endomitotic cycle. Endomitosis may not be unusual to the tapetal cells of these peculiar Cymbidiums but may have a wider application and explain many of the cytological phenomena occurring in the tapetal cells of other plants. Further, physical localization of 45S rRNA genes during endomitotic divisions may help to confirm their atypical activities as well as provide insight into the course and degree of tapetum polyploidization. Analysis of DNA-methylation levels is also recommended for understanding the role of nucleolus for spindle formation during endomitosis.

Keywords *Cymbidium* · Tapetal cells · Endangered species · Endo-metaphase · Bi-nucleate

S. K. Sharma · S. R. Rao (✉)
Department of Biotechnology and Bioinformatics,
North-Eastern Hill University,
Shillong, Meghalaya, India
e-mail: srrao22@yahoo.com

S. R. Rao
e-mail: srrao@mail.com

S. Kumaria · P. Tandon
Centre for Advanced Studies in Botany,
North-Eastern Hill University,
Shillong, Meghalaya, India

Introduction

Since 1940s two contrary opinions have existed concerning the occurrence of true endomitosis in tapetal cells. According to first opinion [5, 6, 25], typical endomitoses take place in the tapetum of a great number of plants. The opposite opinion is that the stages resembling endomitosis are, in reality, phases of the "inhibited" mitosis [9, 24]. This process was first claimed to occur in the tomato [4] and subsequently in *Antirrhinum* [13], and was termed as "endomitosis" by these workers. During "inhibited mitosis" the cell goes through endomitosis up to endo-metaphase, after which the nuclear membrane dissolves and the chromosomes form a meta phase plate. They are then assumed to divide within the plane of the metaphase plate to form one restitution nucleus. The beginning of the "inhibited" mitosis would thus correspond to endomitosis, the end, at least to a certain extent, to C-mitosis.

Owing to very less blooming, different flowering periods, environment dependent flowering, insect dependent pollination, less number of flowers and more importantly rare and/or endangered status make orchids not so suitable material for male meiotic studies. In line with our earlier studies on natural genetic variation at cytogenetic and molecular level [17, 18] in Cymbidiums from north-east India, male meiotic studies were also envisaged. Interestingly, during the efforts made for meiotic studies in anthers of some Cymbidiums viz. *C. aloifolium, C. devonianum* and *C. tigrinum*, tapetal cell mitosis was observed and found to be more remarkable and informative. The present study summarizes that, at least in Cymbidiums, most tapetal cells go an endomitosis instead of either normal mitosis or through so called "inhibited" mitosis. In this type of division there is a reduplication of the chromosomes, but no spindle is formed and there is no anaphase movement of the chromosomes. Throughout

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the whole process the nuclear membrane remains intact. The resulting nucleus and cell have twice the original number of chromosomes. This study adds one more example of occurrence of endomitoses of tapetal cells in plant species, probably for the first time in orchids especially in most economically and horticulturally valued Cymbidiums.

Material and methods

Plant samples of three *Cymbidium* species viz. *C. aloifolium, C. devonianum* and *C. tigrinum* were collected from Meghalaya, Sikkim and Arunachal Pradesh, respectively. The collected plants were potted at the experimental garden located at North-Eastern Hill University, Shillong (Meghalaya). Flower buds of appropriate size from the potted plants were collected during April-May (*C. alofolium*), and June-July (*C. devonianum* and *C. tigrinum*) and fixed in freshly prepared Carnoy's fluid supplemented with a drop of ferric chloride solution for a minimum of 24 h at room temperature and subsequently stored in 70% ethanol at 10°C. Anthers were squashed in 2% aceto-carmine. For detailed analysis, more than 50 tapetal cells per species were studied which clearly revealed the somatic chromosome complements without any ambiguity. Few cells were also analyzed at anaphase for the segregation pattern of the chromosomes during endomitosis. Micro-photographs were taken using *Jenoptik* CCD camera (Germany) attached to *Labomed* LX-400 fluorescence microscope.

Results

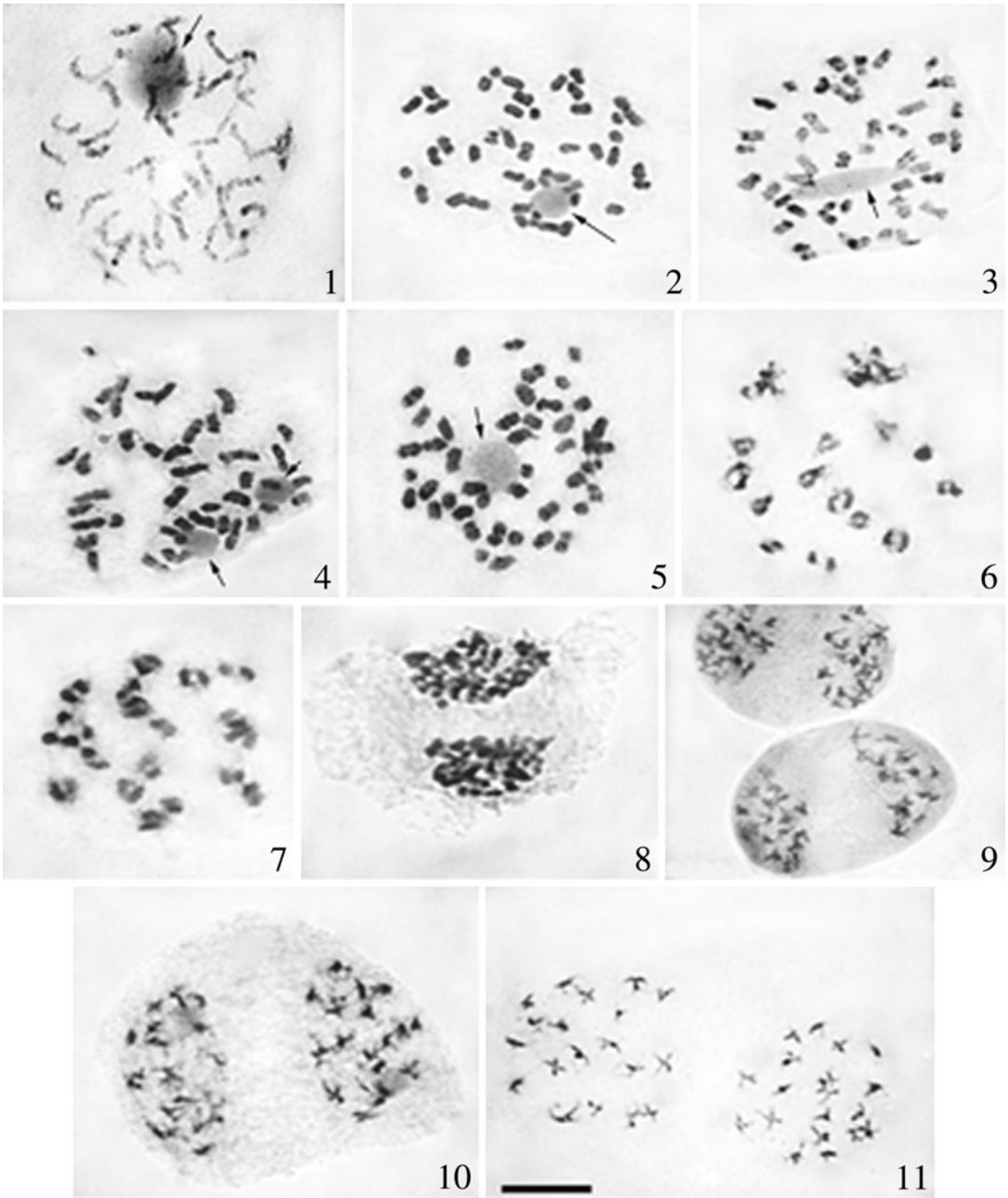
A minimum of 50 cells for each of the species viz. *C. aloifolium*, *C. devonianum* and *C. tigrinum* were analyzed for endomitosis in anther's tapetal cells. All the three Cymbidiums presently investigated had shown the occurrence of 2n=40 chromosomes (Figs. 1–5) in anther tapetal cells which could be clearly resolved into 20 pairs of homologous chromosomes. Interestingly, some of the cells revealed the presence of 20 paired homologues, an event that is otherwise scarce in somatic tissues (Figs. 6–7). The chromosomes were condensed and exhibited more or less similar morphology in terms of total length and centromeric position. The presence of a decondensed, round to oval shape nucleolus in most of the tapetal cells was the hallmark feature of endomitosis in all the Cymbidiums presently investigated (Figs. 1–3, 5). Some of the cells showed two nucleoli at a time (Fig. 4), however, the numbers of such cells are negligible. The nuclear membrane does not disappear, but during metaphase the chromosomes are remarkably condensed, often considerably more than in normal mitosis. Some of the cells in all the Cymbidiums analyzed were at endo-anaphase stages (Figs. 8–9) whereas a few cells exhibited chromosome stickiness (Fig. 8). In *C. aloifoium*, most of the cells were at pro-metaphase stage with less condensation of chromosomes (Fig. 1), whereas some of the cells revealed highly condensed metaphases (Figs. 2, 5). At the time of the last pre-meiotic mitosis in the PMCs, each tapetal cell has one diploid nucleus. Cells with one nucleus most probably come about through restitution in the first mitosis, which ordinarily results in the formation of binucleate cells. This nucleus divides, but the cell does not, resulting in a cell with two diploid nuclei (Figs. 10–11). There is no spindle and the chromosomes do not congress upon an equatorial plate. After attaining their maximum contraction, the spindle attachment regions divide and the chromosomes separate slightly.

Discussion

True endomitosis, as defined above, was first described in the heteropteran insect by Geitler [8]. Thereafter, it has been found in many insects, as well as in other animal groups [9, 10]. It is also found in malignant tumors, such as mouse ascites tumors [12, 15, 23], and in human cancer [22], Tschermak-Woess [24] denied the occurrence of endomitosis in animal tumor cells. In plants, endomitosis seems to have been described for the first time in the tapetal cells of spinach by Witkus [25]. Various stages of tapetal cells have been interpreted as phases of endomitosis in several classical reviews by early workers [5, 6].

The present observation did reveal the presence of endometaphase followed by nuclear membrane brake-up and finally formation of metaphase plates by somatic chromosomes. The observations also indicate that at least in Cymbidiums, most tapetal cells go through an endomitosis instead of either normal mitosis or through "inhibited" mitosis which is contrary to the observation of Mechelke [13] and did show sign of true endo-mitosis. Mechelke [13] stressed in his study that the "inhibited" mitoses can only be studied in heavily squashed cells which draws favor from this study and is the hallmark feature. Such scrutiny also support the view of Oksala and Therman [16] that "inhibited" mitoses could take place in plant species, however, the documentation [4, 13] is not sufficient to establish the existence of this process.

The association (pairing) of homologous chromosomes in tapetal cells seems to be unique since the pairing of homologous chromosomes has not been observed in other somatic, dividing tissues, except for the tapetum and PMCs at the early premeiotic stage and prophase I [1]. Extrapolating these results, it can be speculated that other factors may



Figs. 1–11 Stages of endomitosis in three *Cymbidium* species. **1** Pro-endometaphase (2n=40); **2** Endometaphase (2n=40) in *C. aloifolium;* **3**–**4** Endometaphase (2n=40) in *C. tigrinum*; **5** Endometaphase (2n=40) in *C. devonianum;* **6**–**7** Pro-endoanaphases in *C. devonianum* showing paired chromosomes; **8** and **9** Endoanaphases in *C. aloifolium* and *C. tigrinum*, respectively; **10** and **11**. Cells showing two actively dividing nuclei in *C. tigrinum* and *C. aloifolium,* respectively. Arrow heads showing presence of nucleolus

also exist that correlate the pattern of divisions in tapetal cells with PMC development during prophase I of meiosis. The present outline of endomitosis in tapetal cells of Cymbidiums may be useful for such analyses. The presence of the nucleolus through whole of the endomitotic cycle is of considerable interest. Its persistence through endomitosis may suggest a connection between the nucleolus and the formation of the spindle but very little is known about mechanism of spindle formation in plants [2]. Recently, Hussain et al. [11] suggested the role of Myc-induced SUN domain–containing protein (Misu or NSun2), a nucleolar RNA methyltransferase, is required for mitotic spindle stability, which might mediate its function at the spindle by recruiting nucleolar and spindle-associated protein (NuSAP),

an essential microtubule-stabilizing and bundling protein. The analysis of DNA-methylation levels in the genome may help to interpret the spindle formation during endomitosis.

Like most of the angiosperms, tapetal cells of Cymbidiums did not undergo high levels of endo-polyploidization, however, such event may be species-specific. Although, the role of this process is not yet fully understood, it may be linked to functioning of the tapetum, increasing the copy number of genes needed for the synthesis of specific factors required by developing pollen mother cells (PMCs) and pollen grains. In present case, the absence of polyploidization during tapetal cells division might be connected to mitotic spindle disturbance which is general feature of endomitosis [7, 16]. Additionally, the lack of chromosome segregation and cytokinesis saves energy and time [14]. These factors are undoubtedly important for short-lived and specialized tissues. Stickiness and clumping of chromosomes in tapetal cells [3] were proposed to favor spindle disturbances [7], which was also observed in the present study and supported the hypothesis that the last cell cycle is restitution mitosis rather than endomitosis.

Cymbidium tigrinum and *C. aloifolium* are known for their peculiar morphological and climatic characteristics [18, 19]. *C. tigrinum* is a smallest member of the genus which does not look like a typical *Cymbidium* when it is not flowering with wide leaves and small, round, clustered pseudo bulbs. It exclusively grows in cooler and dry climatic regions where temperature rises up to 20°C. On the other hand, *C. aloifolium* is a medicinal, cultivated tropical *Cymbidium* and also a biological indicator of tropical environment [21] with very thick and rigid leaves which reminds *Aloe*. *C. devonianum* also possesses narrow, acuminate leaf margins and shorter seed type compared to other Cymbidiums. *C. tigrinum* and *C. aloifolium* also showed decondensed, dispersed, extended form of hybridization signals of rDNA as dots of fluorescence (transcriptionally active), hence demonstrated the heteromorphism in size, intensities and their appurtenance during physical localization of 45 S rDNA using fluorescent in situ hybridization [19]. It also might be associated with nucleolar RNA methyltransferase (Misu) translocates which can include 18 S ribosomal RNA [11]. Their abnormal behavior was also notified by apparent clustering during genetic variation analysis using SPAR (single primer amplification reaction) methods [18] as well as phylogenetic analysis using sequence data of nuclear ribosomal internal transcribed spacer (nrITS) region [20]. An alternative but not mutually exclusive explanation is that habitat as well as climatic conditions may simply allow a greater fixation rate of chromosomal (and other) variation occurring within small populations occupying novel ecological niches. Endomitosis may not be unusual to the tapetal cells of these peculiar Cymbidiums but may have a wider application and may explain many of the cytological phenomena occurring in the tapetal cells of other plants, which till now have been obscure. Further, molecular cytogenetic analysis with repetitive DNA (45 S rDNA) in somatic chromosomes of tapetal cells *C. aloifolium, C. devonianum* and *C. tigrinum* may confirm their atypical activities as well as provide insight to course and degree of tapetal polyploidization (if any) as demonstrated in *Arabidopsis thaliana* [26]

The present report on endomitosis in anther's tapetal cells of some Cymbidiums adds one more example of occurrence of such specialized mitotic division and provides useful insight into tapetum-meiocytes relationships.

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