Tapetal Cell Nuclear Characteristics of Some Kuwaiti Plants

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Accepted May 15, 1996

Within anthers of flowering plants, there is a layer of highly specialized and delicate cells just outside the sporogenous cells, the tapetum, which is important to the normal process of meiosis and pollen grain development. Tapetal cells nourish the pollen mother cells until later stages of pollen formation, after which they degenerate to form precursors of pollenkit and outer wall layer or exine coating (Esau 1977, Ciampolini *et al.* 1993, Hesse 1993). The timing of tapetal degeneration has been found to influence the process of pollen embryogenesis in higher plants (Liang *et al.* 1980). As a whole tapetal cells perform 14 different functions (Pacini and Franchi 1993) and could be considered as a type of gland cells capable of synthesizing and releasing a number of diverse enzymes, carbohydrates and secondary substances. Such secondary products show constitutive periods of increase and decrease of accumulation and/or release (Rowley *et al.* 1992, Fitzgerald *et al.* 1993, Rowley and Walles 1993, Hess and Hesse 1993). Many studies have demonstrated the importance of tapetum for reproductive life of plant in the sense that any failure in their function results in pollen grain malfunction and sterility (Horner and Rogers 1974, Bino 1985, Albertini *et al.* 1987, Mariani *et al.* 1990, Muhanna *et al.* 1991, Gourret *et al.* 1992).

In a premeiotic phase, tapetal cells are uninucleate, and with advancement of meiotic prophase, they become bi- and tetranucleate showing an increase in their nuclear size (Carlson and Stuart 1936).

Incidence of endomitosis and absence of cytokinesis in tapetum tissue has been documented in members of many plant families and could be considered as characteristic features of that tissue. These and other aspects of tapetum have been dealt with in a comprehensive review of Chapman (1987). Most light microscopic data about tapetal nuclei was published in the sixties and seventies. However no micrographs, showing stages of nuclear division of tapetal cells, are so far discernible in the literature. It is manifested that compilation of information about tapetum was highly desirable to understand plant breeding and genetics. The present study focuses on observations and characteristics of tapetal cells in four plant species collected during a study aimed at genotoxic impact of oil pollution on Kuwaiti flora (Malallah *et al.* 1996).

Materials and methods

Compositae plant species *Launaea mucronata* ($2n=16$), *Picris babylonica* ($2n=10$), *Senecio glaucus* ($2n=20$), and *Sonchus oleraceus* ($2n=32$) were selected because of their well defined tapetal cells and for their copious cytological observations. These plant species were collected during 1992–1993, from areas around the blazing oil wells of Burgan and other areas away from oil fields like Khaldiya, Rumaithiya, Andulas and Qortuba.

Flower buds, at different developmental stages, were collected from at least 10 specimens of every species. The samples were fixed in Carnoy's fluid (absolute ethanol : glacial acetic acid, 3 : 1 v/v) and stored in a refrigerator until future use. For preparing microscopic slides, the fixed buds were washed with distilled water, macerated in a mixture of absolute ethanol :

sulphuric acid (1 : 1 v/v) for 5–10 min and stained with acetic orcein. Preparations were made permanent by using dry ice. Cells from at least 10–20 flower buds were analyzed. Analyses of tapetal cells included recognition of different stages of nuclear division and the variation of the number and size of nuclei per tapetal cell in addition to the recognition of any mitotic abnormalities. Microphotographs at 100 $\times$ magnification were taken, using an Olympus microscope.

Results

In the four investigated species, all tapetal cells showed nuclear division without cytokine-

Table 1. Number and percentage of cells showing detected categories of tapetal cells with variable number of nuclei

Species	No. of nuclei/cell	No. of detected cells	% of each category	Inter-phase	Pro-phase	Meta-phase	Ana-telo-phase	Dividing nucleus %	% of abnormal cells
<i>Launaea mucronata</i> 2n=16	2	480	35.42	402	57	17	4	78	0
	3	69	5.09	46	13	10	0	23	0
	4	143	10.55	137	2	2	2	6	0
	5	10	0.74	8	0	2	0	2	0
	6	10	0.74	10	0	0	0	0	0
	7	4	0.30	4	0	0	0	0	0
	1 endopolyploidy nucleus	145	10.70	13	0	58	74	132	0
	2 endopolyploidy nucleus	494	36.46	452	25	12	5	42	0
	Total	1355							
<i>Picris babylonica</i> 2n=10	2	524	59.14	473	30	19	2	51	3.48
	3	63	7.11	51	7	3	2	12	0.94
	4	193	21.78	177	16	0	0	16	2.30
	5	7	0.79	7	0	0	0	0	0
	6	16	1.80	16	0	0	0	0	0.24
	7	5	0.56	5	0	0	0	0	0
	8	5	0.56	5	0	0	0	0	0
	1 endopolyploidy nucleus	30	3.39	0	1	15	14	30	0.94
	2 endopolyploidy nucleus	43	4.85	10	32	1	0	1	0.45
	Total	886							
<i>Senecio glaucus</i> 2n=20	2	608	84.44	600	6	2	0	8	0
	3	13	1.80	13	0	0	0	0	0
	4	12	1.66	127	0	0	0	0	0
	1 endopolyploidy nucleus	79	10.97	1	6	26	46	79	0
	2 endopolyploidy nucleus	8	1.11	8		0	0	8	0
	Total	720							
<i>Sonchus oleraceus</i> 2n=32	2	144	22.39	121	9	10	4	23	0
	3	1	0.15	1	0	0	0	0	0
	4	147	22.86	133	10	4	0	14	0
	1 endopolyploidy nucleus	65	10.11	0	0	32	33	65	0
	2 endopolyploidy nucleus	286	44.48	281	1	4	0	5	0
	Total	643							

sis. They were characterized by the presence of two or more nuclei embedded in the cytoplasm with no separation through a cell wall formation.

Table 1 shows that there was a variation in the number of nuclei per tapetal cell among the studied species. The four plant species were divided into two groups depending on the number of nuclei in each group of plants. The first group included *S. glaucus* and *S. oleraceus*. It showed cells with up to 4 nuclei while the second group represented by *L. mucronata* and *P. babylonica* showed up to 8 nuclei in their tapetal cells. Plate 1 shows micrographs of the number of nuclei per tapetal cell of the two groups. The percentage of tapetal cells with 5 to 8 nuclei was 1.78 and 3.71 in each group, respectively. A tapetal cell with more than 8 nuclei was observed only in two cases (Plate 1, Fig. 8).

It is conspicuous from table 1 that tapetal cells with two and four nuclei occurred frequently in all the four plant species. The percentage of such cells was appreciably higher in *P. babylonica* and *S. glaucus* (80.92 and 86.10%, respectively) than that of *L. mucronata* and *S. oleraceus* (45.97 and 45.25%, respectively). *S. glaucus* showed the highest percentage (84.44%) of binucleated cells and the lowest (1.66%) of tetranucleated cells. This trend was opposite in *S. oleraceus* which showed 22.39% bi- and 22.86% tetranucleated tapetal cells. The sizes of nuclei within the multinucleated tapetal cell were not necessarily similar (Plate 1).

Nuclei of tapetal cells showed the typical phases of normal mitosis, predominantly in the interphase stage, but also in the form of dividing cells undergoing prophase, metaphase and ana-telophases. Cells showing two and less frequently 3 or 4 of such stages were also detected (Plate 2). Movement of chromosomes to anaphase poles demonstrated the existence of functional spindle apparatus (Plate 2, Figs. 3, 6). In each of the four species investigated, some tapetal cells were found to have one or two nuclei showing endopolyploidy demonstrated by an increase in multiples of their diploid chromosome numbers without cytokinesis. The frequency of tapetal cells with one endopolyploidized nucleus ranged from 3.39% in *P. babylonica* to about 11% in the other three plant species (Table 1). It was remarkable that nearly all those

Plate 1. Tapetal cells with different number of nuclei.

Plate 2. Tapetal cells showing mitotic division with typical mitotic phases.

cells were seen dividing showing either metaphase or ana-telophase stages (Table 1, Plate 3, Figs. 2, 5, 6). These cells showed multiples of well defined metaphase chromosomes (about $4n$ and rarely more) aligned at the equatorial plate as well as multiples of separated anaphasic chromosomes moving to the opposite poles. Some of these cells showed a separation difficulty with varying number of chromatid bridges. For cells with two endopolyploid nuclei, the frequency varied from as low as 1.11% in *S. glaucus* to 36.46% and 44.48% in *L. mucronata* and *S. oleraceus*, respectively. Additionally a low frequency of other chromosomal aberrations, mainly in the form of excluded micronuclei, lagging chromosomes, and anaphasic bridges was observed in *P. babylonica*.

Discussion

In all the four plant species, a minimum number of two nuclei was found in their tapetal cells indicating that a first obligatory acytokinetic mitosis must have taken place. In both *S. glaucus* and *S. oleraceus* a maximum number of four rounded equal-sized nuclei were observed in their tapetum thus indicating the incidence of a second mitotic division in a part of the binucleated cells. Typical mitotic phases, have been detected in this second division pointing out the presence of two spindle apparatus. On the other hand, in both species *L. mucronata* and

Plate 3. Tapetal cells of the studied species showing the behaviour of the nuclei during the developmental stages.

P. babylonica, a third cycle of mitotic division proceeded in some tetranucleated cells to form the observed low percentage of octanucleated tapetal cells. Similar findings have been documented by Turala-Szybowska (1993) in the tapetum of tetraploid plants of *Ranunculus fluitans*.

Cells with three unequal nuclei (Plate 1, Fig. 2) could be attributed either to inhibition of one of the two spindles or to the fusion of two adjacent chromosomal groups to form a $4n$ nucleus besides the two normal $2n$ nuclei [Diagram 1(3)]. Such fusion could be expected if two spindles were arranged linearly within the same cell furnishing the opportunity to the two middle telophases to fuse together. The fusion would also be facilitated by the presence of chromatid bridges in anaphase impeding normal separation of the resultant 2 daughter nuclei. If such unequal nuclei underwent another acytokinetic division, the involved cell would contain six unequal nuclei such as those detected in both *L. mucronata* and *P. babylonica* [Plate 1, Figs. 5, 6, Diagram 1(23)]. Endomitotic division, accompanied by inhibition of spindle formation in a binucleated cell could lead to the formation of a cell with 2 endopolyploidized nuclei, $4n$ each [Diagram 1(4)]. Existence of tapetal cells with a wide variation in the number and size of their nuclei could be explained on the basis of spindle absence, formation of chromatid bridges and/or fusion of adjacent chromosomal groups. Diagram (1) depicts possible explanation for the origin of some tapetum cell categories presenting different number and sizes of nuclei. Similar conclusions have been reported in a study with tapetal cells of other species (Battaglia 1949).

The occurrence of a single or more than one of these events would determine number and size of nuclei in tapetal cells. If a single polyploidized nucleus has been formed, it undergoes

a next cycle of typical mitosis ending up with two daughter nuclei with the same ploidy level [Diagram 1(11,12)]. These newly formed polyploid nuclei would usually show no tendency of further divisions since the major part of these nuclei were inhibited at interphase stage. However, in a part of these cells endomitosis could take place giving rise to enlarged endopolyploids, depending upon the frequency of occurred endomitotic cycles. Tapetal cells with polyploid level of $8n$ or $16n$ or even more, originate in this way and have been recorded in numerous cases (Chapman 1987). Determination of the exact chromosome numbers in endopolyploid nuclei proved to be difficult.

Acytokinetic mitosis as well as endomitosis are associated with no increase in the number of tapetal cells since cell division does not take place due to lack of cell wall formation (Rowley 1993). It seems that an increase in the ploidy level and chromosomal material of tapetal cell by endomitosis compensate for the increase of number of those cells.

Incidence of polyploidy for all tapetal cells is known to be correlated to its role as secretory cells (D'Amato 1984). Similarly, nutritive tissues such as endosperm are shown to be characterized by the same mode of development (Annie and Nair 1992). Supportive evidence for the occurrence of high levels of endoploidy has been given by Chatterji and Jayaramu (1981) in four safflower cultivars as well as by Turala-Szybowska (1993) in diploid cytotypes of *Ranunculus fluitans*. Endomitosis and nuclear fusion have been suggested to occur during anther culture experiments of some *Brassica* species leading to the presence of spontaneous diploid regenerations in addition to the expected haploids (Keller *et al.* 1975, 1983). Data obtained in the present studies point out the importance of tapetal endopolyploidy in the four examined plant species for a normal development of their anthers and microsporocytes. Abnormalities in tapetum tissue either structural or in its proper degeneration time are found to be associated with male sterility in many plants (Bhandari 1984, Kaul 1988). This fact stresses the importance of cytological examination of tapetal cells in studies aiming to follow-up any possible effects of environmental pollutants on the normal development and fertility of plants selected as indicators. Present results showed a few abnormalities in one species (*P. babylonica*) indicating insufficiency either in the level of pollution or in the sensitivity of tapetal cells.

Summary

A detailed cytological study of tapetum tissue accompanied by micrographic confirmation has been performed in a high number of cells in the four plant species, *Launaea mucronata*, *Picris babylonica*, *Senecio glaucus* and *Sonchus oleraceus*. While in these species, all tapetal cells were binucleated as a result of an obligatory first acytokinetic mitosis, in two species this was followed by a second division forming tetranucleated cells and in the other two species a further third division followed to form octanucleated cells. Existence of variable percentages of tapetal cells with other numbers of nuclei has been documented and interpreted. Similarly endopolyploidy phenomena was found to be of general occurrence in all four species. Its possible origin and significance for the developing microspores has been stipulated. Diagrammatic illustrations are insinuated to elucidate the possible origin of nuclei having variable ploidy levels and sizes. These illustrations have been based mainly on fusion of neighboring chromosomal groups, an inhibition of spindle formation or incidence of persisting chromatid bridges which could prevent proper separation of chromatids.

Acknowledgement

This work was supported by a research grant No. SOO54, from Research Management

Unit, Kuwait University, for which the authors are thankful. The authors are also thankful to Prof. M. Hesse (University of Vienna), Dr. D. Brigg (University of Cambridge) for their valuable consultations and to Prof. M. W. Miller (University of Rochester, NY) for his very helpful criticism of the first draft of the paper which helped us to rewrite it in the present form. Taxonomic identification was kindly provided by Prof. L. Boulos and statistical treatment of original data was kindly attempted by Dr. M. Ghitany (University of Kuwait).

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