Cytological Investigations on the Endosperm in *Zephyranthes citrina* Herb.

Radha Soman Bobby*, Joshua Stephen and Narayanan Omanakumari

Department of Botany, University of Kerala, Kariavattom, Trivandrum.-695581, India

Received March 12, 2003; accepted July 11, 2003

Summary A detailed investigation on the endosperm in *Zephyranthes citrina* Herb. was undertaken to understand its mode of development, cytological constitution and its failure in some fruits. The endosperm was found to be basically triploid ($3x=72$). Upon increasing maturity of the endosperm, cells showing higher ploidy levels, aneuploidy, abnormal mitosis and structural aberrations of chromosomes began to appear. Stickiness of chromosomes and mitotic arrest at various stages of the cell cycle were of common occurrence. Various types of spindle abnormalities such as tropokinesis, split spindle, somatic segregation of chromosomes, multipolar mitosis, atypical distribution of chromosomes at anaphase, lagging of chromosomes as well as nuclear polymorphism and the occurrence of micronuclei were the other salient features.

Key words *Zephyranthes*, Endosperm, Mitotic arrest, Stickiness.

Zephyranthes is one of the evolutionarily dynamic genera of the family Amaryllidaceae. Taxa belonging to 8 species and 65 cultivars of the genus are commonly grown for their showy flowers in Indian gardens. *Zephyranthes citrina* Herb. is a perennial bulbous herb. During the autumn it gives out solitary bright yellow flowers with long slender pedicels arising from the underground bulb which also produces thin long slender leaves. Whereas the cytology of the endosperm in *Zephyranthes rosea* and *Z. ajax* has been well worked out by Kapoor (1963) and Stephen (1973) that of *Z. citrina* had not been investigated so far. Moreover although fruits are formed most of these fall away prematurely. Hence a detailed cytological investigation on the endosperm of *Z. citrina* was undertaken to assess its basic ploidy status and cytological features and also to investigate into the premature fruit fall.

Materials and methods

The endosperm material for the present work was collected from the developing fruits of *Zephyranthes citrina* Herb. grown in the botanic garden of the Department of Botany, University of Kerala, Kariavattom. The material was fixed in 3 : 1 : 1 mixture of absolute ethyl alcohol, glacial acetic acid and chloroform in the noon hours. Fixative was changed several times and acetocarmine squash preparations were made and these were examined under Leitz Laborlux research microscope. Interesting cytological features were photographed.

Mitotic indices and percentage of aberrations were calculated from these squash preparations. Nuclear diameters of endosperm cells with varying maturity were measured using previously calibrated ocular micrometer. Developmental stages of the endosperm were followed by the microscopic examination of thin hand sections, double stained with haematoxylin and fast green. The somatic chromosome number of the plant was ascertained from acetocarmine squashes of root tip cells. Percentage of pollen sterility was determined by the criterion of shriveled morphology and non stainability with acetocarmine. Percentage of seed sterility was also determined by the dissection of

* Corresponding author, e-mail: BOBBYRS@hotmail.com

mature fruits.

Observations

Hand sections of the fertilized ovules of *Zephyranthes citrina* Herb. at various stages of seed development showed that the endosperm follows nuclear type of development. Wall formation was found to be initiated within 10 d after pollination, starting from the periphery all around making it completely cellular by 13 d.

Acetocarmine squashes of the root tip cells of *Z. citrina* showed $2n=48$ chromosomes. Detailed cytological examination of the endosperm showed that it is basically triploid ($3x=72$) in chromosome constitution (Fig. 1), although cells showing variations in chromosome number were observed such as tetraploid, $4x=96$ (Fig. 2), pentaploid, $5x=120$ (Fig. 3) and even higher levels of polyploids (Fig. 4) in course of maturation of the endosperm.

Somatic segregation of chromosomes was often seen in the endosperm of *Z. citrina*. Stickiness of chromosomes was another salient feature and was found from prophase onwards. Several prophase cells showed highly elongated chromosomes with varying levels of stickiness giving the appearance of somatic synapsis (Fig. 5). Prometaphase and metaphase spreads of chromosomes showing somatic segregation were observed often and in some of these, those chromosomes which had not been held together by stickiness were found to move to opposite direction altogether giving a false anaphase like appearance (Fig. 6).

Clumping of chromosomes at metaphase was a consistent feature of the endosperm cells and it gave rise to figures resembling ball metaphase, exploded metaphase (Fig. 7), arch metaphase (Fig. 8), star metaphase and “Y”-shaped (tripolar) metaphase (Fig. 9). Rarely cells showing only small undersized chromosomes with varying degrees of stickiness were observed (Fig. 10). These chromosomes were nearly five times lower than that of normal chromosome. Stickiness was found to be caused by a process by which the chromosomes swelled up and gave out some sticky substance which caused the chromosomes to clump together (Fig. 11). In some highly polyploid cells, the chromosomes were found to be clumped together due to acute stickiness along with certain intact chromosomes and fragments which had not fused with the other chromosomes (Fig. 12). Some achromatic materials faintly stainable with acetocarmine could be visualized between the fusing chromosomes. Often the arrest of the cell cycle at metaphase coupled with amalgamation of chromosomes resulted in highly polymorphic nuclei with occasional projecting chromosomes and fragments as in Fig. 13. Normal metaphases were rare in mature endosperm cells.

Majority of anaphases encountered in the endosperm cells during this study were abnormal. Occasionally disturbed asynchronous anaphases with lagging chromosomes were observed (Fig. 14). Double star anaphases were encountered both in triploid and higher ploid cells (Fig. 15). In general, most of the aberrant anaphases showed chromosome stickiness and sticky bridges (Fig. 16). Anaphasic arrest amidst acute stickiness and sticky bridges were also observed (Fig. 17). Anaphases characterized by precociously moving chromosomes with split spindle showing tendency for separation into separate multiple chromosome groups could also be found (Figs. 18, 19). Cells with 3 groups of chromosome clumps were often observed presumably due to tripolar spindle activity (Fig. 20). Rarely, tetra polar anaphases were observed (Fig. 21). Arrested anaphases were also found to occur along with persistent sticky bridges (Figs. 22, 23).

A characteristic feature of the mature endosperm was the presence of polymorphic nuclei which assumed irregular shapes due to budding of micronuclei (Fig. 24). In certain cells of the mature endosperm, chromatinic or nuclear extrusions through cytoplasmic blebbing were observed (Fig. 25).

Micrometer readings of endosperm nuclei at varying stages of maturity showed that the average diameter of nuclei dropped considerably although anisonucleosis was more prevalent in the

Figs. 1–14. Acetocarmine squash preparations of the endosperm cells from *Zephyranthes citrina* Herb. All Figs. $\times 700$ except 7 and 8 ($\times 1050$). 1) a cell from 5 day old endosperm showing triploid ($3x=72$) chromosomes, 2) a cell from 7 day old endosperm showing tetraploid ($4x=96$) chromosomes, 3) pentaploid ($5x=120$) number of chromosomes in another endosperm cell, 4) a highly polyploid cell from the maturing endosperm, 5) highly extended and thin prophase chromosomes apparently giving the appearance of somatic synapsis due to stickiness, 6) somatic segregation of chromosomes mimicking anaphase. Here the chromosomes rather than the daughter chromosomes segregate, 7) exploded metaphase amidst stickiness, 8) arch metaphase coupled with acute stickiness, 9) Y-shaped tripolar metaphase, 10) a metaphase spread of undersized chromosomes with varying degrees of stickiness, 11) chromosome inflammation and the initiation of stickiness at metaphase in a polyploid cell, 12) acute chromosome stickiness causing amalgamation of most of the chromosomes, 13) acute stickiness causing the amalgamation of chromosomes and mitotic arrest at metaphase, 14) asynchronous anaphase with lagging chromosomes in a polyploid cell.

more mature stages (Table 1). There were also wide variations in the number and size of nucleoli per endosperm nuclei. But these had no correlation with ploidy level.

With the progression of the age of the endosperm an increased mitotic activity up to 7 d along with percentage of aberrations was observed. Up to 10 d the mitotic activity was observed to be almost at the same rate while percentage of aberrations was increasing. Later, towards the end of 15 d the mitotic activity was found to be decreasing and the percentage of aberrations was also found to

Figs. 15–25. Acetocarmine squash preparations of the endosperm cells from *Zephyranthes citrina* Herb. (contd). All Figs. $\times 700$ except 24 and 25 ($\times 1050$). 15) double star anaphase, 16) anaphase with sticky bridges, 17) anaphase arrest with multiple sticky bridges, acute stickiness, 18) anaphase with sticky bridges, split spindle and precociously moving chromosomes, 19) multiple sticky bridges, split spindle and acute stickiness causing mitotic arrest at anaphase, 20) tripolar anaphase in a highly polyploid cell, 21) tetrapolar anaphase also in a polyploid cell, 22) a pair of persistent bridges in between 2 dissimilar sticky masses of amalgamated chromosomes, 23) a Figure formed by the arrest of sticky bridges, 24) budding of micronuclei from a large polymorphic nucleus, 25) nuclear and chromatin extrusion through cytoplasmic buds.

Table 1. Size variation of the endosperm nuclei in *Zephyranthes citrina* Herb. in relation to seed maturity

Age of the endosperm (d)	Total number of nuclei measured	Range in diameter of nuclei (μm)	Average diameter of nuclei (μm)
5	1000	5.00–10.00	6.80 ± 0.92
6	1000	3.75–7.50	5.90 ± 0.63
7	1000	3.75–7.50	5.88 ± 0.83
8	1000	3.75–7.50	5.85 ± 0.97
9	1000	3.75–7.50	5.55 ± 0.83
10	1000	3.75–6.25	5.30 ± 0.71
11	1000	3.75–6.25	4.88 ± 0.78
12	1000	2.50–7.50	4.80 ± 1.14
13	1000	2.50–7.50	4.68 ± 0.89
14	1000	2.50–6.25	3.73 ± 0.68
15	1000	2.50–5.00	3.48 ± 0.54

Table 2. Mitotic index and percentage of aberration in *Zephyranthes citrina* Herb. in relation to seed maturity

Age of the endosperm (d)	Mitotic index (Average)	Percentage of aberration (Average)
5	9.88	11.83
6	7.59	8.16
7	17.79	17.59
8	14.94	14.95
9	11.29	12.45
10	16.34	15.70
11	5.50	9.13
12	6.21	7.07
13	6.39	7.29
14	4.10	4.93
15	0.97	1.23

be decreasing (Table 2). There was a negative correlation coefficient of -0.65 existing between the mitotic index and increase in the age of the endosperm and a negative correlation of -0.70 existing between the percentage of aberrations and increase in the age of endosperm, where as a positive correlation coefficient of $+0.97$ was found to be existing between the mitotic index and percentage of aberrations.

The percentage of morphologically 'good' and stainable pollen grains (fertile) was as high as 96. Only a few of the pollen grains were deformed and sterile. However seed setting was highly variable ranging between 14.29–96.55%.

Discussion

The above observations indicate that the endosperm is basically triploid ($3x=72$) in *Zephyranthes citrina* Herb. although it becomes highly mixoploid in course of maturation. Its development follows the nuclear type. These findings are in conformity with the reports on the endosperm in other species of *Zephyranthes* (Tandon and Kapoor 1962, Kapoor 1963, Stephen 1973). The present observations also have revealed that although the endosperm nuclei are almost of uniform size undergoing normal mitosis in young fruits, anisonucleosis and aberrant mitosis occur in the course of seed development. Although a number of highly polyploid nuclei appear sporadically in the endosperm tissue, the general trend is the diminution in the average size of nuclei during maturation as revealed from micrometer readings. The occurrence of highly polyploid nuclei in the endosperm does not appear to be due to exact doubling of the triploid number of chromosome, rather it appears to be due to the preferential replication of one or two sets of chromosomes resulting in tetraploid, pentaploid chromosome numbers. Such situations have already been reported in addition to exact doubling of the basic triploid set of chromosomes in the endosperm of at least a few plants (Kapoor 1963, Stephen 1974, 1977).

The hypotriploid or even hypodiploid conditions presently observed could have resulted from unequal distribution of chromosomes in otherwise bipolar anaphases, abnormal distribution of chromosomes through multipolar anaphases or even through irregular somatic segregation of chromosomes. Similar observations have already been made in the endosperm cells of *Zephyranthes ajax* (Stephen 1973). It was Geitler (1948) who first reported the decrease of nuclear volume during endosperm maturation in *Gagea lutea* and he assumed it to be due to either dehydration or depolyploidization. In *Z. citrina* presently investigated it was not due to dehydration but due to the decrease in the number of chromosomes through mechanisms cited above. The formation of micronuclei either by the lagging chromosomes, or by the scattered chromosomes as in exploded metaphase or even through budding of nuclei appears to be another mechanism which lowers the mean volume of endosperm nuclei upon maturity. The occurrence of highly undersized chromosomes as in Fig. 10 may be yet another mechanism which brings about nuclear size diminution. Busch (1974) considers that undersized chromosomes arise when chromosomes replicate in the absence of DNA replication.

The present observations also point out that there is blockage to the sub stages of the M-phase of mitosis in the endosperm cells of *Z. citrina*. Chromosome stickiness appears to be the most important mechanism which brings about mitotic arrest in this material. Varying grades of stickiness starting from the stickiness of a few chromosomes up to acute stickiness resulting in the amalgamation of all the chromosomes into a deeply pycnotic chromatin mass could be visualized presently. Sticky bridges at anaphase and telophase stages were also observed. In most of these cases the sticky bridges were multiple and these persisted even after telophase. Acute stickiness caused the arrest of mitosis mostly at late prophase and metaphase stages. A few anaphase arrests were also encountered. It was also possible to trace the origin of stickiness during this study. At first the affected chromosomes undergo swelling or inflammation and it is followed by the extrusion of some

less stained material which serves as a glue to cause adhesion of chromosomes. The molecular mechanism of chromosome stickiness is a matter of controversy. Stephen (1979) has proposed that it is caused by the stripping of protein covering of the DNA in chromosomes and that the sticky material is chromosomal matrix rather than DNA. Gaulden (1987) states that chromosome stickiness results from changes in specific nonhistone proteins (topoisomerase II and the peripheral proteins) that are integral components of chromosomes, necessary for the disjunction of daughter chromosomes. The present observations are in conformity with the findings of the above 2 authors. Acute stickiness leading to mitotic breakdown has been reported in the endosperm materials of *Zephyranthes ajax* and Triticale (Stephen 1973).

Apart from chromosome stickiness, inhibition and disturbances to the functioning of the spindle mechanism also contribute to mitotic arrest as evidenced from star metaphases, ball metaphases, exploded metaphases, multipolar mitosis, tropokinesis, split spindles *etc.*, presently observed. Also chromosome aberrations occasionally encountered may also contribute to mitotic arrest. Such mechanisms have already been described by other authors in different endosperm materials (Kapoor 1963, Abraham *et al.* 1965, 1966, Stephen 1973, 1974, 1979). These abnormalities were originally described by Eigsti and Dustin Jr. (1955) in colchicine treated cells.

Nuclear polymorphisms presently observed may be explained to be due to endopolyploidy, aneuploidy, atypical distribution of chromosomes through multipolar mitosis, mitotic arrests and stickiness resulting in the amalgamation of chromosomes after the chromosomes had undergone condensation, lagging chromosomes forming micronuclei, budding from large nuclei and persistent bridges, all of which have been encountered during this investigation on *Z. citrina* endosperm.

The wide variations in the percentage of seed fertility (ranging from 14.29 to 96.55) suggest that the cytology of the endosperm may vary from seed to seed. Only those seeds with less cytological abnormalities mature where as those with serious cytological abnormalities undergo endosperm breakdown leading to seed abortion even though the pollen fertility was found to be as high as 96%.

References

- Abraham, A., Ninan, C. A. and Gopinath, P. 1965. Cytology of development of abnormal endosperm in Phillipine makapuno coconuts. *Caryologia* **18**: 395–408.
- , — and — 1966. Cytology of the endosperm in some varieties of coconut. *Indian J. Genet.* **26A**: 234–246.
- Busch, H. (ed.) 1974. *The Cell Nucleus*. Vol. 11. Academic Press. New York.
- Eigsti, O. J. and Dustin Jr., P. 1955. Colchicine “in Agriculture, Medicine, Biology and Chemistry.” The Iowa State College Press, Ames, Iowa, U.S.A.
- Gaulden, M. E. 1987. Hypothesis: Some mutagens directly alter specific chromosomal proteins (DNA topoisomerase II and peripheral proteins) to produce chromosome stickiness, which causes chromosome aberrations. *Mutagenesis*. **2**: 357–365.
- Geitler, L. 1948. Notizen zur endomitotischen Polyploidisierung in Trichozyten und Elaiosomen sowie über Kernstrukturen bei *Gagea lutea*. *Chromosoma* **3**: 271–281.
- Kapoor, B. M. 1963. Cytology of Endosperm of Some Angiosperms. Ph.D. thesis. University of Delhi, India.
- Stephen, J. 1973. Cytology of Endosperm in Angiosperms. Ph.D. thesis. University of Kerala, Trivandrum.
- 1974. Cytological investigations on the endosperm of *Borassus flabellifer* Linn. *Cytologia* **39**: 195–207.
- 1977. Cytological analysis of the endosperm failure in a hybrid clone of *Tradescantia*. *Cytologia* **42**: 137–145.
- 1979. Cytological causes of spontaneous fruit abortion in *Haemanthus katherinae* Baker. *Cytologia* **44**: 805–812.
- Tandon, S. L. and Kapoor, B. M. 1962. Contributions to the cytology of endosperm in some angiosperms I. *Zephyranthes ajax* Sprenger. *Caryologia* **15**: 21–41.