Some Observations on the Cytology in *Ochna* sp (Ochnaceae)

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Ochna is a species of medicinal interest which produces apocarpous fruit. The number of fruits is the same as the fertilized carpels (Joly 1977). The presence of abortive and imperfectly-formed fruits suggests that some factor is compromising the fertilization process. The regularity of the meiotic process is an essential condition for the gamete viability. Since it has been shown in *Aptenia cordifolia* (Pagliarini 1990a) and in *Thunbergia mysorensis* (Pagliarini 1990b) that abnormalities in meiosis endangered pollen grains, *Ochna*'s microsporogenesis was analysed to see whether cytological factors might be involved in the production of abortive and imperfectly-formed fruits.

Materials and methods

Floral buds in their best phase for meiotic study were selected from specimens extant in the Medicinal Plants Garden of the Universidade Estadual de Maringá and fixed in Carnoy (3 alcohol: 1 acetic acid) for 24 hr. Afterwards they were transferred to a solution of alcohol 70% and conserved at 5°C. Staining of microsporocyte was made with propionic carmine 1%. All meiotic phases as from diakinesis were analysed. Abnormalities that could compromise the meiotic product were taken into account. The frequency of developed, imperfectly-formed and abortive fruits was evaluated too.

Results

Fig. 1 shows some *Ochna* fruits. From left to right an increasing number of developed fruits can be observed. The second unit shows a developed fruit and a smaller imperfectly-formed one. As the maximum number of fruits observed is six, the missing fruits are imperfectly-formed or abortive. Table 1 shows a high frequency of abortive and imperfectly-formed fruits in 100 analysed units.

Table 2 presents the results obtained through analysis of microsporogenesis in *Ochna* where 1887 microsporocytes and 785 tetrads of microspores were analysed. It was shown that the frequency of the irregularities was extremely low in meiosis I and II. However it was high in tetrads of microspores. The irregularities observed in meiosis I and II consisted in the occurrence of irregular segregation of chromosomes in metaphases and anaphases. In tetrads of microspores, irregularities consisted in the presence of one or various microcytes with different size (Figs. 2a-2c). As a consequence, microspores and pollen grains of different size were observed (Figs. 2d-2e). Smaller pollen grains always proved to be sterile. Converging spindles in meiosis II were observed in many microsporocytes (Fig. 2f). As a results, telophases II with only three nuclei were observed (Fig. 2g), leading to the formation of triads at the end of meiosis (Fig. 2h).

Another specific phenomenon, observed in many microsporocytes of *Ochna*, was the persistence of nucleolus or the presence of small spherical structures, similar to nucleoli, spread

throughout the cytoplasm during meiosis. These structures were strongly stained by propionic carmine (Fig. 2i). In microsporocytes where these structures were extant, the reorganization of nucleolus in telophase was not observed.

Fig. 1. Aspects of the *Ochna* fruit.

Fig. 2. Some aspects of meiosis in *Ochna*. a) Tetrads of microspores with one microcyte. b) Tetrads of microspores with various microcytes. c) Normal and abnormal tetrads of microspores. d) Microspores of one PMC showing different sizes and abnormal number. e) Pollen grains of different sizes, the smallest is sterile. f) Microsporocytes in metaphase II with convergent spindle. g) Microsporocyte in telophase II with only three nuclei. h) Triad of microspores. i) Microsporocyte at the end of anaphase I showing innumerable globules similar to nucleoli throughout the cytoplasm.

Table 1. Number of developed (D), imperfectly formed (IF) and abortive fruits (A) in 100 analysed fruits

Class of fruits	Number of fruits
3D+3IF+0A	4
3D+0IF+3A	4
2D+1IF+3A	4
5D+0IF+1A	20
1D+4IF+0A	4
1D+0IF+3A	4
4D+1IF+1A	4
2D+4IF+0A	4
1D+5IF+0A	4
1D+2IF+3A	8
4D+2IF+0A	16
4D+0IF+2A	8
3D+2IF+1A	4
4D+1IF+1A	8
3D+1IF+2A	4

Table 2. Percentage of abnormal microsporocytes and number of cells analysed in each meiotic phase

Phase	% of abnormal microsporocytes	Number of analysed cells
Diakinesis	0.00	170
Metaphase I	0.98	507
Anaphase I	0.47	419
Telophase I	1.06	187
Metaphase II	3.74	214
Anaphase II	1.31	306
Telophase II	0.00	84
Tetrads of microspores	16, 69	789

Discussion

The occurrence of regular meiosis as an essential condition to the formation of functional gametes has been shown in innumerable species (Powers 1932, La Fleur and Jalal 1972, Dewald and Jalal 1974, Del Duca 1976, Falcão *et al.* 1981, Moraes-Fernandes 1982). In these studies it was shown that a decline in seed production was correlated to meiotic irregularities. However, in *Ochna* the high frequency of imperfectly-formed and abortive fruits cannot be attributed

exclusively to the presence of irregularities during the meiotic process since the latter was practically normal (Table 2). Irregularities in meiotic process generally culminate in the formation of tetrads with microcytes or in the presence of micronuclei in microspores. Pollen grains thus produced are generally sterile. The influence of irregularities upon the production of abnormal tetrads and sterility of pollen has been clearly shown in *Aptenia cordifolia* (Pagliarini 1990a) and *Thunbergia mysorensis* (Pagliarini 1990b). However, in *Ochna* the occurrence of tetrads of microspores with microcytes (16.69%) was much higher than expected in view of the abnormalities observed during meiosis (Table 2). Thus, meiotic abnormalities cannot solely be responsible for the high frequency of irregular tetrads in *Ochna*. The presence of imperfectly-formed fruits suggests that fertilization should have occurred through a gamete with a imbalanced number of chromosomes where, despite the fertilization that has taken place, the seed development did not proceed in regular form. The presence of microcytes in tetrads is a factor that leads to the formation of unbalanced gametes.

Another factor that could have contributed to the formation of abnormal pollen grains in *Ochna* was the occurrence of irregular cytokinesis. *Ochna*, as a dicotyledonous species, presents cytokinesis of the simultaneous type. In various microsporocytes in metaphase II convergence of two spindles towards a simple pole was observed (Fig. 2f). This led to the formation of microsporocytes in telophase II with only three nuclei (Fig. 2g), thus, there occurs the formation of a nucleus of restitution. This nucleus produces gamete 2n. Restitution nuclei with different forms of origin were widely described in *Solanum tuberosum* and were considered responsible for the evolution of the species (Mok and Peloquin 1975, Mok *et al.* 1975, Inawaga and Peloquin 1979, 1982, Ramanna 1979, 1982).

Although the nucleolus has not been extensively studied during meiosis in plants, it is known that its behaviour is similar to that described for mitosis. During prophase it generally appears as a spherical corpuscule associated with one or more chromosomes. At the end of the prophase it disappears and begins to reorganize itself in telophase (Darlington 1965, John and Lewis 1965). In *Ochna*, however, not all microsporocytes showed this behaviour with regards the nucleolus. In many cells the nucleolus did not suffer any disorganization at the end of the prophase, in others, observations showed that there was a disintegration into the small nucleoli that spread through the cytoplasm during meiosis. Corpuscles similar to nucleoli, spread in the nucleoplasm or in the cytoplasm during meiosis, have been described in some plant species (Johansen and Flint 1959, Khidir 1969, Walters 1970). In lower plants these structures have been called micronucleoli or globuli (Giménez-Martin *et al.* 1977). The importance and origin of these structures are still unknown. Nevertheless, Giménez-Martin *et al.* (1977) suggest that they may represent products of nucleolar material disintegration or may be structures newly synthesized during prophase. This latter process of formation has been described in *Lilium* (William *et al.* 1973). However, in *Ochna* its origin seems to be linked to the disintegration of the nucleolus, since these structures were observed after prophase and such cells did not reorganize the nucleolus in telophase.

Summary

Ochna's meiosis has been analysed in search of factors that could cause the abortion of fruits. Results showed low frequency of meiotic irregularities but high frequency of tetrads with a variable number of microcytes, besides converging spindles in meiosis II leading up to the formation of triads. Abnormal behaviour of the nucleolus was also observed in many microsporocytes. These factors may explain, at least in part, the high frequency of abortion in *Ochna*'s fruit.

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