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SPINDLE ANOMALIES IN MICROSPOROGENESIS IN A POPULATION OF *WITHANIA SOMNIFERA* (L.) DUNAL FROM KARACHI

Viqar Anjum Saeed, S. Afaq Husain and S. Shahid Husain

PCSIR Laboratories, Off University Road, Karachi-39

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A population of *Withania somnifera* (L.) Dunal from Karachi, was investigated, primarily for its cytological behaviour. The observations revealed various types of anomalies during the course of microsporogensis. At metaphase-I, all chromosomes were not arranged on metaphase plate, some were found lying away and bridges, univalents and laggards were recorded in many pollen mother cells. Multipolar and supernumerary spindles at 2nd meiotic divisions were of frequent occurrence resulting in 5-8 nucleate telophase-II and subsequently in abortive pollens.

Key words: Withania, Microsporogensis, Spindle anomalies.

INTRODUCTION

Withania somnifera Dun. belongs to family solanaceae and is distributed in drier subtropical regions of India and Pakistan. Bahaduri [1] and Miege [2] reported 2n = 48, whereas Gottschalk [3], Baquar *et. al.* [4] and Surriaya and Ali [5] n = 24. The present authors have investigated cytological behaviour or *Withania somnifera* and have reported spindle anomalies for the first time. Spontaneous multipolar spindles have been reported in *Melandrium* by Heitz [6], in *Zea* by Beadle and MaClintock [7] in hybrids of *Bromus* by Walters [8,9] as well as in human cancer cells by Therman and Temonin [10]. Multipolar spindles have also been induced by various physical and chemical means by Heplar and Jackson [11], Coss *et. al.* [12] and Ceoloni etc. [13].

MATERIALS AND METHODS

Suitable young buds were fixed in carnoy solution (ethanol-chloroform-acetic acid 6: 3: 1) for meiotic studies and stored in 70% alcohol. Microsporogensis was studied by staining and squashing the pollen mother cells (PMC's) in acetocarmine for temporary preparations. *Camera lucida* drawings were made at a magnification of approximately 2000x. Pollen fertility was studied by staining pollen grains with acetocarmine and recording stained-rounded and unstained-shrunken pollens.

RESULTS

During cytological studies, various types of meiotic anomalies were observed. Strikingly, the spindle fibres were lightly stained with acetocarmine which made the present study of spindle activity easier (Fig. 1). Normal metaphase - I was observed in few cells with 24 bivalents (Fig. 2), whereas in others 1 to 4 chromosomes or bivalents were found lying away from the metaphase plate (Fig. 3).

Bridges and laggards were of common occurrence, 1-3 bridges were observed at anaphase-I (Fig. 4) and 1-4 laggards at telophase-I (Fig. 5, 6). Small chromatin bodies were also present besides lagging chromosomes in some PMC's (Fig. 6). It was observed that the two nuclei at times behave asynchronously in second miotic division, cells have been found with metaphase-II and anaphase-II stages simultaneously (Fig. 8). Laggards were also fairly common at anaphase-II as seen in Fig. 8 and 9. A chromatin body,

Fig. 1. Metaphase-I plate with spindle fibres clearly visible.

Fig. 2. Normal metaphase-I with 24 bivalents.

Fig. 3. Metaphase-I showing 4 bivalents lying away from the plate.

Fig. 4. Anaphase-I with 3 bridges and a laggard.

Fig. 5, 6. Telophase-I showing lagging chromosomes and a small chromatin body.

probably a multivalent has been observed lying away from the spindle at metaphase-II and anaphase-II (Fig. 7, 9). Spindle anomalies were frequently noted at second miotic division, branched, bifurcated (Fig. 10) and tripolar spindles were recorded (Fig. 11). In certain cells three separate spindles or two tripolar spindles were observed (Fig. 12, 13). Chromatin mass was found to be persistent in some cells upto telophase-II (Fig. 14, 15), though normal telophase-II and tetrads were also observed in very small numbers (Fig. 18). PMC's having 4-9 nuclei were common resulting in abortive pollens (Fig. 16, 17). The percentage of abortive pollens was found to be 99.5.

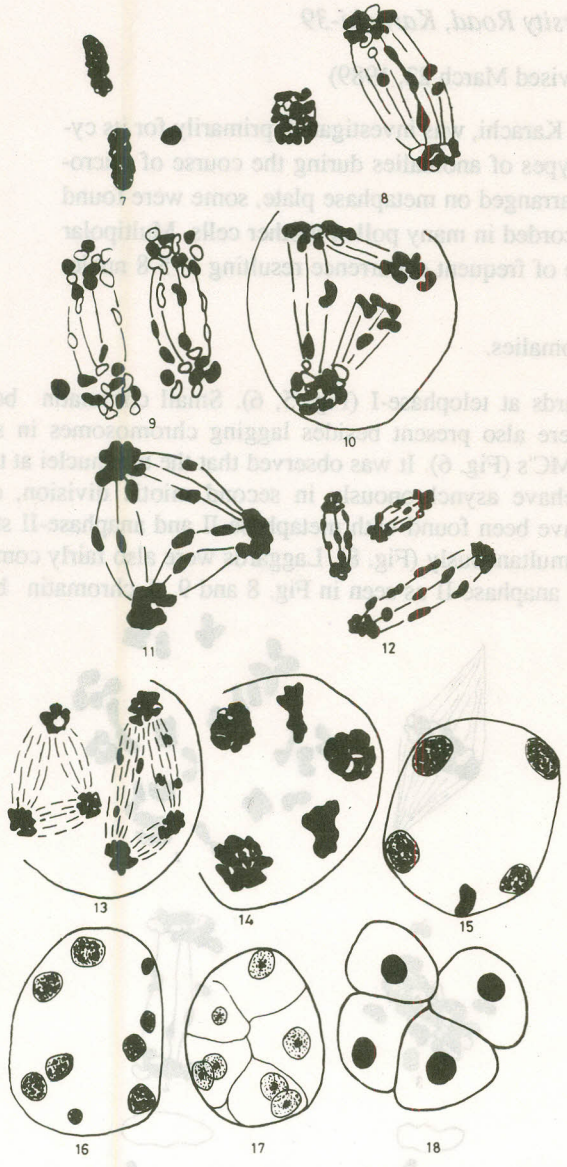

Fig. 7, 9. Metaphase-II and anaphase-II with a chromatine body lying by.

Fig. 8. Asynchronous behaviour within the cell at 2nd miotic division with metaphase-II and anaphase-II stages simultaneously.

Fig. 10, 11. 2nd miotic division with branched, bifurcated and tripolar spindle.

Fig. 12. Anaphase-II showing 3 separate spindles and 2 tripolar spindles.

Fig. 14, 15. Telophase-II with chromatin mass.

Fig. 16. Pollen mother cell with 9 nuclei.

Fig. 17. A Polyad. Fig. 18. A normal tetrad.

DISCUSSION

Microsporogensis was studied in 68 plants of *W. somnifera* Dun. from eastern part of Karachi (PCSIR Labs. Campus, Karachi), all exhibiting anomalies at different stages of miosis. A total of 2070 PMC's were studied, out of which 24 cells only were found with normal metaphase-I or anaphase-I, others exhibited anomalies such as asynaptic behaviour of few chromosomes, laggards, bridges, univalents, and chromatin mass at different stages were observed. Chromatin was found to be persistent upto telophase-II and multi-nucleate cells were common. Most prominent of all the irregularities were supernumerary, multipolar, branched and bifurcated spindles resulting in multinucleate cells. These anomalies are reported for the first time in *W. somnifera* by present authors. These anomalies may indicate some hybrid factor but morphologically it was not indicated. Previously, multipolar spindles have been induced in plants and animals by various physical and chemical treatments.

In nature, spontaneous multipolar and supernumerary spindles have been reported in *Melendrium* [6] Zea [7] Triton [14] Sterile bull [15] human cancer cells [10] interracial hybrids of *Drosophila* [16] and in interspecific hybrids of *Bromus* [8, 9].

Swanson and Nelson [17] have suggested that in plants multipolar spindles arise due to asynchronous division of chromosomes and spindle organizers. Walters [9], has suggested that there exists a relationship between an altered rate of meiosis and the spindle irregularities resulting in multipolar spindle and multinucleate cells. Multipolar spindles cannot always be attributed to asynchronous behaviour of chromosomes, as in all the taxa where asynchrony of chromosomes in meiosis is recorded, it is not always preceded by multipolar spindles [19].

Schrader [19], Thermen and Timonen [10] and Kundsen [15] have suggested that in animal cells multipolar spindles arise due to disturbances in the division cycle of centrioles. This has been further attributed to the change in relative duration of prophase and metaphase [10].

It has been established that the organization of spindle depends on the capacity of microtubules to polymerize and depolymerize in an orderly and controlled manner [20] and microtubule organizing centres (MTOCS) control the initiation, orientation, directionality and patterning of microtubule polymerization, the assembly of microtubule is under spatial and tamporal control [21]. In several systems microtubule orientation, directionality and patterning turned out

to be markedly disturbed following IPC (antimitotic herbicide) treatment. Hepler and Jackson [11] have shown in *Heamnthus katrini* endosperm cells that multipolar spindles are formed following treatment of IPC. This was also confirmed by Coss and Heaps [22] in *Oedogonium* a green alga. They further suggested that the multipolar spindles resulted following fragmentation of spindle organizing centres.

The studies, leading to the effect of antimitotic drugs has led to the understanding of the role of MTOCS and microtubule in spindle organization. The IPC appears to have a direct effect on spindle organizing centres but still its actural mechanism is not known. However, the information relating to spontaneous supernumerary and multipolar spindle formation in nature is fragmentary and hence more comprehensive investigations are needed for the understanding of exact mechanism as to how spindle disturbances and misalignment occur in nature resulting in multipolar spindles which can be induced in laboratory on treatment with IPC/hydrostatic pressure. Such abnormal behaviour of spindle may be due to a gene as suggested by Cocloni [13] or due to some biophysical factors in microenvironment of anthers or PMCs which disturbs the usual pattern of spindle organizing centres which subsequently initiate formation of multipolar/or supernumerary spindle.

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