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Male Meiotic and Taxonomical Studies in *Withania Somnifera* (L.) Dunal

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Abstract- Detailed male meiosis has been made in one species of genus *Withania* collected from Ujjain region of Madhya Pradesh, India. The study revealed $2n=48$ for the species. In addition to this, some cells showed the occurrence of various meiotic irregularities for example, lagging chromosomes (1.76%), chromatin bridges (1.76%) and cytomixis (0.87%). The fertility of pollen grains was also determined which was observed 97.52%. The species are widely used for various medicinal purposes by local/tribal people of the state.

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I. Introduction

The genus *Withania* belongs to the family Solanaceae. The family comprises of 100 genera and about 2500 species (Hunziker 2001, Olmstead *et al.* 2008). The genus is distributed throughout the tropical and sub-tropical regions of the world with 26 species (Ahmad 2014). *W. somnifera* is well distributed in India with and is growing well in dry parts of tropical and subtropical regions extending to the elevation of 1500 m. The plant possesses antitumor, antimicrobial and anti-inflammatory properties. Since the plant is toxic in nature so it should be used with caution (Purohit and Yyas 2004). Flowers of its species are used to treat nervous exhaustion, insomnia and impotence. The meiotic course of the species reveals the presence of $2n=48$ from West Pakistan (Baquar 1967) as well as outside India. *W. somnifera* commonly known as "Ashwangandha" or "Indian ginseng" is extensively studied from India by Bir *et al*. (1978) Bir and Sidhu (1979 and 1980) from Punjab plains, Koul *et al.* (1976) from Jammu and Kashmir, Madhavadian (1968) from Tamil Nadu, Bhaduri (1933), Datta *et al.* (2005), Iqbal and Datta (2007) from West Bengal. It is also extensively worked out from Pakistan by Baquar (1967) and Khatton and Ali (1982) and from Saudi Arabia by Al-Turki *et al*. (2000). Earlier meiotic studies reveals the presence of intraspecific diploid cytotype ($2n=24$), tetraploid cytotype ($2n=48$) and hexaploid cytotypes ($2n=72$). The karyotype analysis of the species shows seven groups of the chromosomes with occurrence of metacentric and sub-metacentric types (Samaddar *et al.* 2012). The species also shows polysomatomy ($2n=12$, 18, 24, 36, 48, 72) with predominance of $2n=48$. Present research work is undertaken by keeping in view the existence of cytological diversity.

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II. Materials and Methods

a) Collection of plants materials

Species was collected from Ujjain city especially in the premises of Vikram University Ujjain. The photographs of the collected plants species were preferably taken from their natural habitats. The photography was done during the complete flowering seasons. For identification of plants species, different floras were consulted during the present study such as Flora of Madhya Pradesh Western Part by Singh (2012), various internet sources. The plant is an important medicinal plant of the Indian subcontinent. In additions to this, the plant specimens were taken to some taxonomists for further documentation. The identified plants specimens have been reposted in School of Studies in Botany, Vikram University, Ujjain (India).

b) Fixation and preservation of plant materials

Floral buds were fixed in freshly prepared Carnoy's fixative (6 parts of absolute alcohol: 3 parts of chloroform: 1 part of glacial acetic acid) for 24-48 hours. Afterwards, these were transferred to 70% ethyl alcohol and stored in refrigerator at 4°C until use. For chromosomal preparations, anthers were crushed and tapped to prepare a smear of pollen mother cells (PMCs) in 1% acetocarmine (Belling 1921). A number of PMCs were observed and chromosome counts were confirmed. In case of species with meiotic abnormalities, large numbers of PMCs are observed to confirm frequency of various abnormalities. Pollen fertility was observed by mounting the pollen grains in 50% glycerol-aceto carmine (1:1) solution (Marks 1954). Pollen grains with stained nuclei were taken as fertile and viable, whereas, unstained pollen grains marked as sterile ones.

c) Photomicrography

Photomicrographs were taken from freshly prepared temporary slides. The photographs were taken with the aid of the microscope Camera (Digital Eyepiece) on a field of 40% objective lens and 10X eyepiece of Olympus microscope.

III. Results

a) Taxonomical and morphological studies

Kingdom: Plantae (Plants);
Sub-kingdom: Tracheobionta (Vascular plants);
Super division: Spermatophyta (Seeds plants);
Division: Angiosperma
Class: Dicotyledons
Order: Tubiflorae
Family: Solanaceae
Genus: *Withania*
Species: *Somnifera Dunal*

The plant is usually erect, green, branched/unbranched herb, up to 1.25 m in height. The aerial part, especially the stem, leaves and calyx are sparsely covered with fine hairy tomentum. Branches are round; leaves simple, petiolated, ovate, entire, shiny, smooth and opposite; flowers inconspicuous, greenish or yellow, in axillary umbellate cymes, bisexual; fruit is berry in a persistent calyx; seeds are small, flat, yellow, reniform, very light. In this species, the period of flowering and fruiting occurred during the months from August to October. The plant has tap root system having 15-25 cm in length and light yellow in colour. The branches are erect and are of about 60-120 cm in length. It requires dry weather conditions for development of better root quality and alkaloid content.

Male meiotic studies In this plant species, meiotic characterization of anthers revealed regular 24 bivalents ($2n = 48$) in cells at diakinesis as well as metaphase I. At anaphase I, the chromosomes successively demonstrated normal segregation of 24:24 chromosomes towards opposite poles (Figs. A-I). In addition to this, some cells showed the occurrence of various meiotic irregularities (Figs. A-I; Table-1-3), for example, lagging chromosomes (1.76%), chromatin bridges (1.76%) and cytomixis (0.87%). The incidence of chromosome association in the form of ring and rod bivalents per PMC ranged from 13 to 22 (mean = 17.2) and 2 to 11 (mean = 6.8), respectively. The frequency of chiasma per cells, on an average, was observed 45.8 (SD = ±3.25) in which 41.20 chiasma (SD = ±3.12) were found terminalized and 4.60 (SD = ±0.80) were identified as untereminalized. As a result, the terminalization coefficient was calculated as 0.90 (Table-8). The fertility of pollen grains was also determined which was observed 97.52%.

IV. Discussion and Conclusions

Meiosis is most sensitive stage in the life cycle for all sexual species and has direct relevance to natural selection; it leads to the formation of gametes, contributes to genome stability and generates genetic diversity. The process of meiosis depends upon interrelated events of homologous chromosome recognition, intimate association, synapsis and recombination (Hamant *et al.* 2006, de Muyt *et al.* 2009). In plants, it is affected by various genetic and environmental factors (Ahmad *et al.* 1984, Viccini and Carvalho, 2002, Sun *et al*. 2004, Bajpai and Singh 2006, Rezaei *et al*. 2010). There are various meiotic abnormalities which hinder the path of normal meiosis and are the cause of changes in the morphology and genetic constitutions of the plant. The evolution of vascular plants is dependent upon the variation in chromosome numbers which may be caused due to genomic mutations especially polyploidy (auto or allopolyploidy) (Soltis *et al.* 2009, Bedini *et al.* 2012). There are number of research papers on the phenomena of polyploidy, emphasizing its origin, impact and role in speciation (Stebbins 1985, Ramsey and Schemske 1998, Otto and Whitton 2000, Cifuentes *et al.* 2010, Jiao *et al.* 2011). The autotetraploids are generally characterized by the presence of quadrivalents due to homology of 4 sets of chromosomes, whereas, in allopolyploids there is normal pairing because of existence of two separate sets of chromosomes. On the other hand in segmental allotetraploids due to the partial homology of two genomes there is low frequency of quadrivalent formation. In the present study *W. somnifera* shows normal bivalent formation in all the PMCs, without any quadrivalent formation which indicates its allotetraploid behavior. However, the absence of quadrivalents does not confirm that it is an allotetraploid because there are many artificially produced autotetraploids where there is only bivalent formation because the formation of quadrivalents depends upon many other factors such as localization of chiasmata, small size of chromosomes, and presence of some suppressor genes etc., which does not allow the pairing between the homologous chromosomes (Morrison and Rajhathy 1960, Gottschalk 1978). On the other hand in *W. somnifera* the meiosis is abnormal with the presence of spindle abnormalities which indicates the absence of multivalents and also indicates that it might be hybrid or more probably due the presence of specific genes which interfere in the pairing and functioning of spindle (Baum *et al.*1992, Risso-Pascotto *et al.* 2003; Kumar and Singhal 2008; Singhal and Kaur 2009). The basic function of the spindle is to attach at kinetochore and separate the chromosome or chromatids at anaphases (Wadsworth *et al*. 2011), these attach to the centromeres (Qu and Vorsa 1999) and rearrange the chromosomes on the equatorial plate and bring them together at metaphase-I (Qu and Vorsa 1999). But, if due to some factors (genetic or environmental) the spindle activity fails then chromosomes are unable to line up in the equator and then separate at Anaphases of the meiosis, which leads to abnormal meiotic course. Earlier, a number of plants have been reported with abnormalities like irregular spindle activity, cytomixis and chromatin stickiness

leading to abnormal microsporogenesis (Baum *et al*. 1992, Caetano-Pereira and Pagliarinini 2001, Kumar and Singhal 2008, Rai and Kumar 2010, Singhal and Kaur 2009). Abnormalities like lagging chromosomes, chromatin bridges and cytomixis which ultimately lead to abnormal microsporogenesis with the production of dyads, triads, polyads, tetrads with micronuclei, and sterile and fertile pollen grains.

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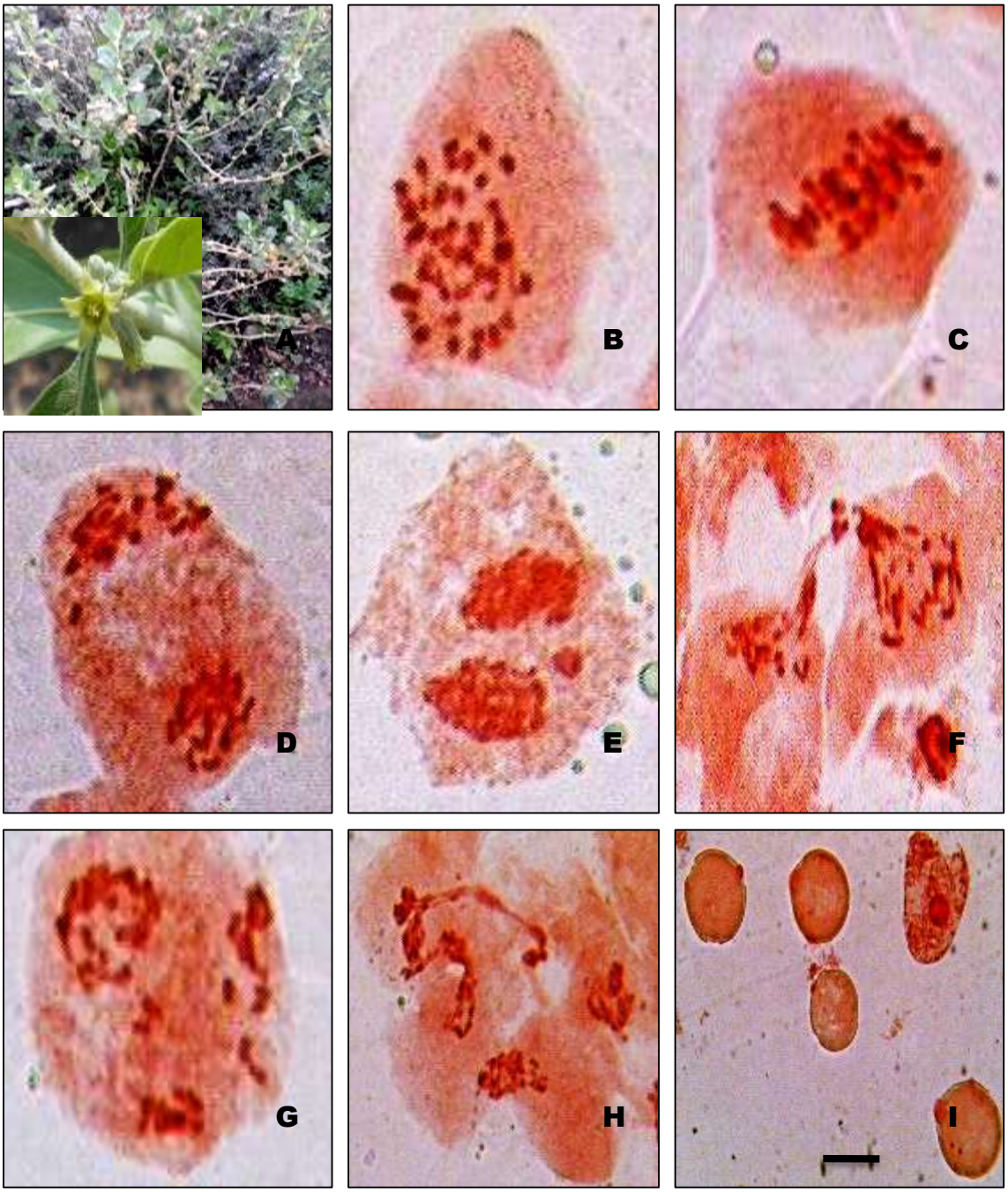

Figure (A-I): Microsporocytes showing different meiotic stages in *Withania somnifera* Dunal. A) Morphology of *Withania somnifera* Dunal. B) Diakinesis showing 24 bivalents (2*n* = 48) C) Metaphase I D) Anaphase I showing 24:24 chromosome segregation E) Laggard at telophase I F) Cytomixis G) Tripolar movement with laggards H) Bridge at telophase II I) Pollens (Scale bar = 10μm).

Table 1: Place of collection, meiotic chromosome count, ploidy level and pollen fertility in *Withania somnifera* L.

Name of taxon	Place of collection	Meiotic chromosome Number (*n*)	Ploidy level	Pollen fertility
Withania somnifera L.	VU Campus, Ujjain	*n* = 24	4*x*	97.52

Table 2: Chromosome association (Mean and range) and distribution of chromosomes at anaphase I in *Withania somnifera* L.

Species	No. of Cells analyzed	Ring bivalents		Rod bivalents		Anaphase separation
		Mean	Range	Mean	Range	
Withania somnifera	25	17.2	13-22	6.8	2-11	24:24

Table 3: Average chiasma frequency per PMC, terminalized, untereminalized and terminalization coefficient of *Withania somnifera* L.

Taxon	No. of cells analyzed	Chiasma/cell Mean ± SD	Terminalized	Untereminalized	Terminalization Coefficient
Withania somnifera	25	45.8 ± 3.25	41.20 ± 3.12	4.60 ± 0.80	0.90