

## Cytomixis during microsporogenesis in the diploid and tetraploid cytotypes of *Withania somnifera* (L.) Dunal, 1852 (Solanaceae)

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**Abstract.** *Withania somnifera* (L.) Dunal, 1852, popularly known as “Ashwagandha” shows considerable morphogenetic diversity in Indian populations. Both the diploid (n=12) and tetraploid (n=24) cytotypes presently reported in the populations from Punjab, Rajasthan and Himachal Pradesh show the phenomenon of cytomixis which is a first record for the species. The inter PMC (Pollen mother cell) transfer of chromatin material in the diploid cytotype is present in 40.76% PMCs involving 2-6 PMCs. However, among tetraploids frequency of cytomixis is much less (15.80-24.32%) and involves only 2-3 PMCs. Chromatin transfer is noticed during the first and second meiotic division in the diploid as compared with the tetraploid where it is observed only during the first meiotic division. The percentage of meiotic abnormalities such as chromosome stickiness, lagging of chromatin material during anaphases and telophases is also higher in the diploid compared with the tetraploid. The microsporogenesis is also abnormal in the diploid resulting into the formation of polyads and tetrads with micronuclei whereas it is normal in the tetraploid. Cytomixis also seems to have had a greater effect on the apparent pollen fertility in the diploid compared with the tetraploid. Furthermore, cytomixis have also resulted into pollen grains of three different sizes in the diploid and only of two sizes in the tetraploid.

**Key words:** cytomixis, cytomictic channels, chromosome stickiness, micronuclei, polyads, pollen malformation, meiotic disturbances, *Withania somnifera* (L.) Dunal, 1852.

### INTRODUCTION

*Withania somnifera* (L.) Dunal, 1852 (Solanaceae) popularly known as Indian “Ginseng” or “Ashwagandha” is the most widely used herb in Ayurvedic and indigenous medical system for over 3,000 years. The species is widely distributed in Africa, the Mediterranean and the Indian sub-continent. In India it grows well in the drier parts of tropical and subtropical regions of Punjab, Haryana, Uttar Pradesh, Uttarakhand, Bihar, Jharkhand, Rajasthan, Madhya Pradesh, Maharashtra, and

some parts of Himachal Pradesh and Jammu and Kashmir ascending up to 1,650m in the Himalayas (Kapoor, 2005). Five morphological forms are known in India as documented by Atal and Schwarting (1962). The species which has been studied quite extensively from India viz. Delhi (Mohan Ram, Kamini, 1964), Punjab (Bir et al., 1978; Bir, Sidhu, 1979, 1980; Bir, Neelam, 1984) and Jammu and Kashmir (Koul et al., 1976), West Bengal (Bhaduri, 1933; Datta et al., 2005; Iqbal, Datta, 2007) and Tamil Nadu (Madhavian,



1968) depict intraspecific diploid ( $2n=24$ ), tetraploid ( $2n=48$ ) and hexaploid ( $2n=72$ ) cytotypes. Keeping in view the existence of cytomorphological diversity and medicinal value of species the present study material has been collected from different localities falling in the states of Himachal Pradesh, Punjab and Rajasthan. The emphasis of the present studies has been to locate the different cytotypes in these states and to prepare the cytogeographical maps. The aim has also been to study the comparative effects of chromatin transfer on meiotic course, pollen fertility and pollen size in the diploid and tetraploid cytotypes.

### MATERIAL AND METHODS

Materials for cytological studies was collected from four different places in the states of Punjab, Rajasthan and Himachal Pradesh. (Table 1). Floral buds of suitable sizes were fixed in Carnoy's fixative for 24 hrs and preserved in 70% alcohol at 4°C. For meiotic studies, anthers were squashed in 1% acetocarmine. A number of slides were carefully examined for chromosome counts in each population and abnormalities recorded. Pollen fertility was estimated using glycerol-acetocarmine mixture (1:1). Well filled pollen grains with stained nuclei were taken as fertile while shrivelled and unstained pollen were counted as sterile. Photomicrographs of chromosome counts were made from freshly prepared as well as from permanent slides using Leica Qwin Digital Imaging System.

### RESULTS

Detailed cytological investigations carried out in four different populations of *W. somnifera* reveal the existence of diploid ( $2n=24$ ) and tetraploid cytotypes ( $2n=48$ ). The plants studied from Jaisalmer area in Rajasthan are diploid and show the presence of 12II at meta-

**Table 1.** Locality with altitude, accession number, ploidy level and meiotic chromosome number of diploid and tetraploid plants of *Withania somnifera*.

S. No.	Locality and a.s.l	Accession numbers (PUN)	Meiotic chromosome number (n)	Ploidy level
1	<b>Rajasthan:</b> Jaisalmer, 225 m	49322 49323	12	2x
2	<b>Punjab:</b> Patiala, 250 m	49328 49329	24	4x
3	<b>Punjab:</b> Kapurthala, 235 m	49326 49327	24	4x
4	<b>Himachal Pradesh:</b> Kullu, 1230 m	49324 49325	24	4x

phase I (MI) (Fig. 1). The individuals worked out from Patiala and Kapurthala districts of Punjab and Kullu district of Himachal Pradesh are tetraploid showing 24II at MI (Fig. 12). The meiotic course in the tetraploid population from Kullu is normal with regular microsporeogenesis and high pollen fertility (97.81%). On the other hand, the rest of the tetraploid and the diploid individuals show abnormal meiotic course due to inter transfer of chromatin material at different stages of meiosis. The data on cytomixis, meiotic course, pollen fertility and pollen size for diploid and tetraploid cytotypes are provided in Table 2. The detailed observations under each cytotype are given separately.

A. Diploid: The phenomenon of cytomixis involving inter PMC transfer of chromatin material through cytomictic channels involving 2-6 PMCs is present in 40.76% PMCs (Fig. 4). The cytomictic channels in majority of the cases are narrow (Fig. 3). However, in some cases these channels are much broader (Fig. 2). The phenomenon of chromatin transfer occurs

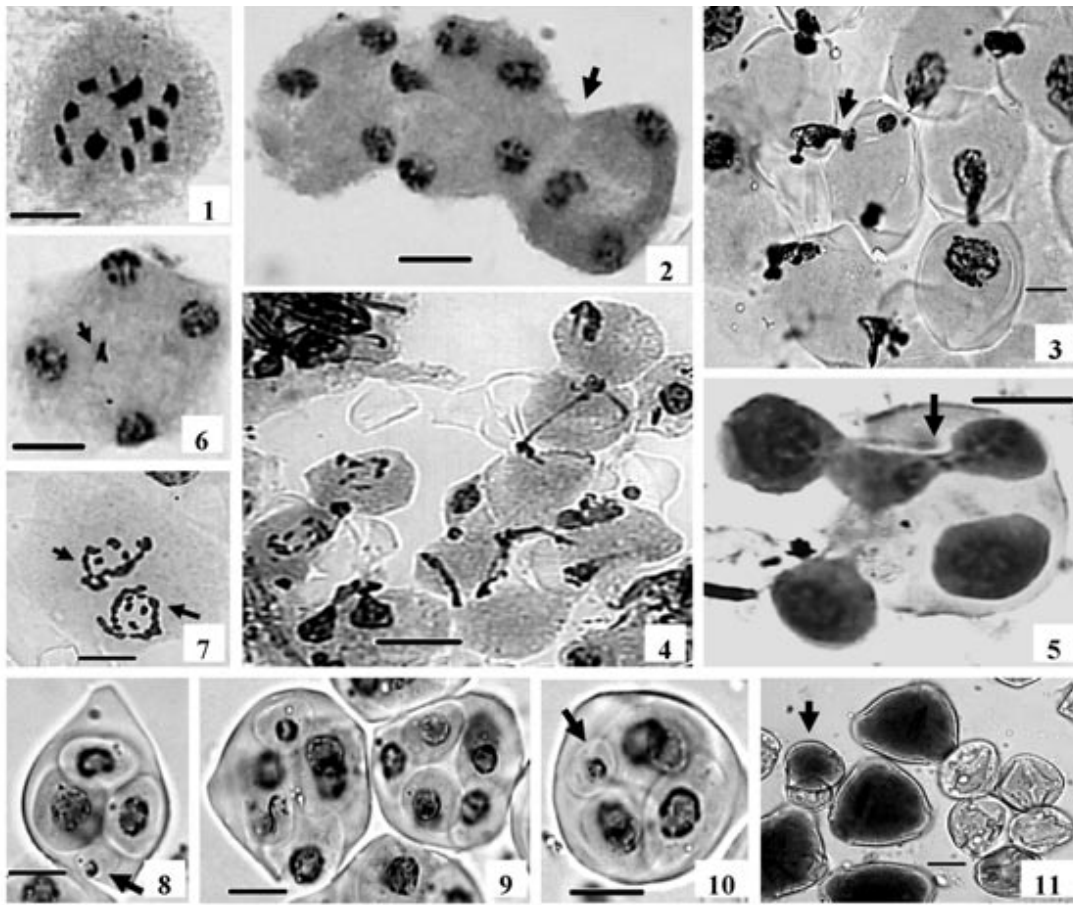
**Table 2.** Cytomixis, meiotic course and pollen fertility and pollen size in the diploid and tetraploid cytotypes of *Withania somnifera*. PMC's = pollen mother cells; EPI = Early Prophase-I; AI = anaphase I; TI = telophase I; AII = anaphase II; TII = telophase II; TS = Tetrad Stage; WM = with micronuclei; WMT = without micronuclei; CS=Chromosome stickiness; RF=Relative frequency (observed number of different sized pollen grains/total number of fertile pollen grains).

Cytotype and population	Cytomixis			Meiotic course		Microsporogenesis				Pollen		
	% PMC's involved	No. of PMC's involved	Meiotic Stage/s	PMCs with laggards at AI/TI, AII/TII (%)	PMCs with CS (%)	Polyads (%)		Tetrads (%)		Fertility (%)	Size (µm)	RF
						WM	WMT	WM	WMT			
<b>A. Diploid</b> Jaisalmer	40.76	2-6	EPI, MI & TS	24.25	27.16	2.02	17.64	14.84	65.50	77.99	22.44×19.34 21.66×15.08 18.52×15.38	51.77 28.88 19.35
<b>B. Tetraploid</b> I. Patiala	24.32	2-3	MI, AI & TI	2.00	16.91	0.00	0.00	0.00	0.00	95.99	26.69×23.99 20.68×17.63	60.74 39.26
II. Kapurthala	15.80	2-3	EPI & MI	0.00	9.05	0.00	0.00	0.00	0.00	93.27	23.80×23.64 17.74×17.18	93.13 6.87
III. Kullu	0.00	0.00	—	0.00	0.00	0.00	0.00	0.00	0.00	97.81	19.18×18.84	100

from early prophase stages (Fig. 3) and lasts up to the tetrad stage (Fig. 5). However, the frequency of PMCs involved in cytomixis during meiosis-I is much higher compared to those during meiosis-II. Consequent to cytomixis, the PMCs also show chromosome stickiness (Fig. 7) and lagging of chromatin material during anaphases and telophases (Fig. 6). These meiotic disturbances lead to abnormal microsporogenesis resulting into polyads (19.66%) and tetrads with micronuclei (14.84%) (Figs 8-10). Owing to these abnormalities, the diploid individuals show a considerable amount of pollen malformation (22.01%) and heterogeneous sized pollen grains (Fig. 11).

**B. Tetraploid:** Out of the three tetraploid populations, individuals from Kullu district depict perfectly normal meiotic behaviour with regular 24II formation at MI and their normal segregation at anaphase I (AI). The microsporogenesis is also regular resulting into high pollen fertility (98%). In the rest of two populations, occurrence of cytomixis has

been observed with the involvement of 15.80-24.32% PMCs in transfer of chromatin material. Compared with diploid plants, the frequency of PMCs involved in chromatin transfer is low and only 2-3 PMCs are involved (Fig. 13). Furthermore, chromatin transfer which occurs only through narrow cytomictic channels was observed only during meiosis-I. The chromatin transfer in these individuals is either partial or complete resulting into hypo- and hyperploid (Fig. 15) and enucleated PMCs (Fig. 14). It is quite possible that meiocytes with no chromatin are lost during the further meiotic course, whereas those with unusual chromatin content resulted into abnormal microspore development and formation of more or less viable gametes with an unbalanced chromosome number. The formation of giant or double sized microspores as a consequence of chromatin transfer is also resulted in some cases. Simultaneous transfer of chromatin from two different PMCs to single PMC is also observed (Fig. 17). The PMCs with laggards at telo-

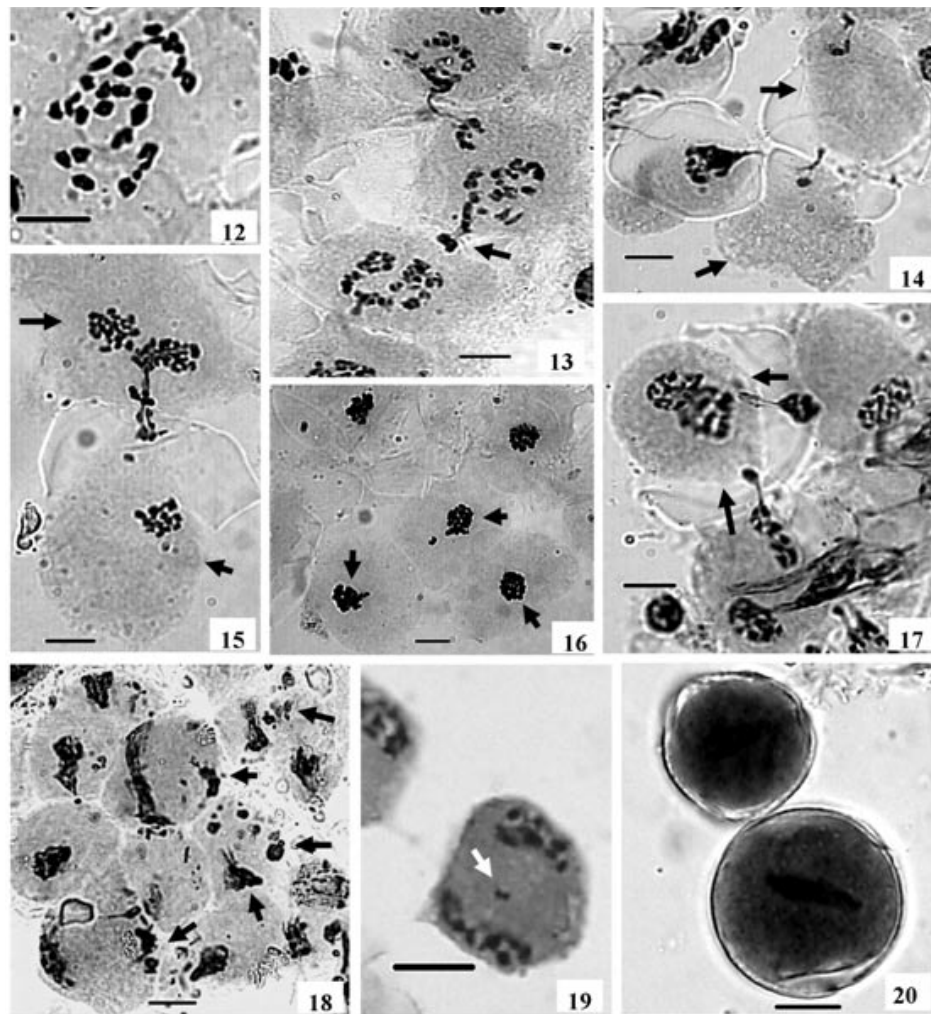


**Figs 1-11.** Diploid cytotype of *Withania somnifera*. **1** - PMC with 12II at MI. **2** - PMCs showing broad cytomictic channels (arrowed). **3** - chromatin transfer in early prophase through narrow cytomictic channels (arrowed). **4** - a group of PMCs involved in chromatin transfer. **5** - microspores in a polyad involved in chromatin transfer (arrowed). **6** - laggard at TII (arrowed). **7** - chromosome stickiness at AI (arrowed). **8** - tetrad with micronuclei (arrowed). **9** - polyads. **10** - polyad with micronuclei (arrowed). **11** - unequal sized apparently fertile (arrowed), and sterile pollens grains. Bar = 10  $\mu$ m.

## DISCUSSION

phase I (TI) (Fig. 19), chromosome stickiness at MI (Fig. 16), AI, and TI, and disintegration of chromatin into cytoplasm (Fig. 18) were observed only in a few PMCs. Because of the very low frequency of PMCs with cytomixis, the tetraploid plants show less meiotic abnormalities and consequently high pollen fertility (93.27-95.99%). But, the formation of two different sized pollen grains could be the products of hypo- and hyperploid PMCs (Fig. 20).

Perusal of cytological data reveals that *W. somnifera* has been investigated quite extensively from India and elsewhere. So far, the species is known to have intraspecific chromosome variations depicting diploid,  $2n=24$  (Mohan Ram, Kamini, 1964), tetraploid,  $2n=48$  (Bhaduri, 1933; Madhavadian, 1968; Koul et al., 1976; Bir et al., 1978; Bir, Sidhu, 1979, 1980; Bir, Neelam, 1984; Datta et al., 2005; Iqbal, Datta, 2007) and hexaploid,



**Figs 12-20.** Tetraploid cytotype of *Withania somnifera*. **12** - PMC with 24II at MI. **13** - three PMCs involved in chromatin transfer and showing narrow channels (arrowed). **14** - anucleated PMCs (arrowed). **15** - hypoploid (small arrow) and Hyperploid PMCs (large arrow). **16** - PMCs showing chromosome stickiness at MI (arrowed). **17** - transfer of chromatin from two different PMCs to a single PMC (arrowed). **18** - disintegrated chromatin scattered in cytoplasm (arrowed). **19** - laggard at TI (arrow). **20** - fertile pollen grains of unequal sizes. Scale Bar = 10  $\mu$ m.

$2n=72$  (Bir, Neelam, 1980, 1984) cytotypes located in the different regions of India. However, only tetraploid cytotype exists outside India (Gottschalk, 1954; Baquar, 1967; Renard et al., 1983; Slavik et al., 1993; Badr et al., 1997). The present studies reveal the existence of both diploid and tetraploid cytotypes. Both the cytotypes show the phenomenon of cytomixis during microsporogenesis which is

a first record for the species.

Cytomixis was discovered for the first time in *Crocus sativus* L. by Kornicke (1901). Since then, the phenomenon of cytomixis has been reported mainly during microsporogenesis in a wide variety of plants (Levan, 1941; Sarvella, 1958; Omara, 1976; Singhal, Gill, 1985; Bedi, 1990; Ghanima, Talaat, 2003; Singhal et al., 2007). In addition, cytomixis

has also been reported to occur in tapetal cells (Cooper, 1952), meristematic cells (Tarkowska, 1960; Bobak, 1976; Kostriyina, Soldatov, 1991), leaf epidermal and sub epidermal layers (Tarkowska, 1960), and ovary cells (Koul, 1990). It has been inferred that polyploid taxa seem to exhibit more cytomixis than their diploid counterparts as observed in "sugarbeet" by Semyarkhina and Kuptsou (1974) and "jamun" by (Singhal et al., 2007). However, cytomixis which exists in both the cytotypes of *W. somnifera* is much higher in the diploid compared to the tetraploid.

In *W. somnifera* chromatin transfer is observed to occur during the first and second meiotic divisions in the diploid, compared with the tetraploid where it exists only during the first meiotic division. The frequency and intensity of cytomixis depend on the nature of connection between adjacent cells. Presently two types of connections, narrow and broad, are observed through which chromatin transfer takes place. Similar kinds of connections have also been observed in the *Diplotaxis harra* (Forssk.) (Ghanima, Talaat, 2003). The transfer of chromatin in the diploid occurs through narrow as well as broad channels but in the tetraploid it occurs only through narrow channels. In the diploid, the number of PMCs involved in chromatin transfer ranges between 2-6 while in the tetraploid only 2-3 PMCs are involved. The percentage of meiotic abnormalities such as chromosome stickiness, lagging of chromatin material during anaphases and telophases is also higher in the diploid. The low frequency of meiotic abnormalities, normal microsporogenesis and high pollen fertility in the tetraploid individuals is directly correlated with less number of PMCs involved in chromatin transfer. On the basis of comparative studies on cytomixis in the two cytotypes of *W. somnifera* it is quite apparent that chromatin transfer in the meiocytes dur-

ing microsporogenesis is directly responsible for causing meiotic disturbances, abnormal microsporogenesis and consequently heterogeneous sized pollen grains and pollen malformation.

Although transfer of chromatin material has been reported in countless species, there are conflicting opinions and explanations regarding the causes and significance of cytomixis. Possible causes suggested earlier include the effect of fixation (Heslop-Harrison, 1966; Haroun, 1995), pathological conditions (Bobak, Herich, 1978; Morisset, 1978), physiological changes (Bell, 1964; Bahl, Tyagi 1988), chemical and herbicides (Bobak, Herich, 1978; Ajay, Sarbhoy, 1987; Haroun, 1995) and temperature (Narain, 1976). Pressure difference (Tarkowska, 1965; Morisset 1978) and clumped chromatin bridges during premeiotic anaphase (Mendes, Rijo, 1951) are the other explanations put forth by some authors. In the present case the cytomixis seems to be a natural phenomenon controlled by some genes as has been suggested earlier by other workers (Gottschalk, 1970; Bhagvandoss et al., 1973; Brown, Bertke, 1974; Singhal, Gill, 1985; Chatha, Bir, 1988; Bedi, 1990; Singhal et al., 2007).

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