Meiotic studies in genus *Withania* Pauquy, from Indian Thar Desert

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**Abstract.** Detailed male meiosis has been made in two species of genus *Withania* collected from desert regions of Rajasthan, India. The study revealed $2n=48$ for both the species. The meiotic analysis of *W. somnifera* is reported to be normal whereas, *W. coagulans* showed abnormal meiosis with the presence of abnormalities like spindle irregularities, chromatin transfer, laggards and irregular microsporogensis with the presence of monads, dyads, triads, polyads and tetrads with micronuclei which further lowered the pollen fertility and giving rise to varying size pollen grains. Both the species are widely used for various medicinal purposes by local/tribal people of the state.

**Keywords.** *Withania*, Medicinal plant, Abnormal meiotic behavior, Indian Thar desert.

**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 sub-tropical regions extending to the elevation of 1500 m. But *W. coagulans* is rare and endangered plant species found only in few localities in Rajasthan. Both the species are well distributed in East of Mediterranean regions extending to South Asia.

Regarding the medicinal properties of the species, a composite Ayurvedic medicine “Liv 52” commonly used as intestinal infections is a hepatoprotective herbal preparation and contains extracts from *W. coagulans* and *W. somnifera* (Mishra *et al.* 2013). *W. coagulans* is commonly known as “Indian cheese maker” since fruits and leaves of the plants are used as coagulant, as it contain an enzyme called Withanin, having milk coagulating activity (Naz *et al.* 2010). In some parts of India and Pakistan, both the species are used as blood purifier, for cleaning teeth and plant smoke is inhaled for relief in toothache (Dymock *et al.* 1972, Krishnamurthi 1969). 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 “Ashwagandha” 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 polysomy (2n=12, 18, 24, 36, 48, 72) with predominance of 2n=48.Whereas cytologically, *W. coagulans* is not as much explored. There is no cytological report of it from the studied area. Present research work is undertaken by keeping in view the existence of cytological diversity.

**MATERIALS AND METHODS**

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% aceto-carmine (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. Pollen grain size was measured using an oculometer. The photomicrographs of the PMCs and pollen grains were taken from the temporary slides by using, Nikon 80i digital imaging system.

The species are collected from different localities of district Churu and Jodhpur. *W. somnifera* is more frequent in its appearance as compared to *W. coagulans*, which is a rare medicinal plant and is collected only from single locality of the studied area. The plant specimens were identified with the help of various floras such as Flora of North East Rajasthan (Sharma and Tiagi 1979), Flora of the Indian Desert (Bhandari 1978), Flora of Rajasthan (Shetty and Singh 1993) and by comparing the plant specimens with the samples lying in Herbarium of Department of Botany, Punjabi University, Patiala (PUP) and Botanical Survey of India, Jodhpur (BSI). The identified voucher specimens of plants have been deposited in the Herbarium, Department of Botany, Punjabi University Patiala (PUN), India.

**RESULTS**

Detailed cytological investigation is carried out in two species of genus *Withania* i.e., *W. somnifera* and *W. coagulans*. Meiotic studies of *W. somnifera* reveals the presence of 24 bivalents at metaphase-I (Fig. 1). PMCs also show the presence of equal segregation of 24:24 chromosomes at anaphase-I (Fig. 2). The meiotic course is normal with high pollen fertility (97.66%). The meiosis of *W. coagulans* depicts the presence 24 bivalents at metaphase-I (Fig. 3). These chromosomes are unable to segregate on the spindle and are scattered all over the cytoplasm (Fig. 4). A very few the cells also shows equal segregation of 24:24 chromosomes at anaphase-I (Fig. 5). The meiotic course reveals spindle irregularities in the PMCs as the chromosomes are unable to move towards the poles (Figs. 6, 7) and several chromosomes remain in the cytoplasm to the form of laggards (Fig. 8) at anaphase-I. The spindle abnormalities (Figs. 9, 10) and laggard formation is also observed at anaphase-II stages of meiosis (Fig. 10). Further the formation of bridges (Fig. 11) is also observed at telophase-II. All these abnormalities lead to the formation of multipolarity (Fig. 12) as chromosomes are not able to move towards their respective poles and remain in the cytoplasm. Microsporogenesis is highly abnormal with the presence of monad (Fig. 13), dyad (Fig. 14) and triad (Fig. 15). Chromatin transfer within tetrads (Fig. 16), polyad (Fig. 17) and polyads with micronuclei (Fig. 18) is also observed. Pollen grains with unequal size (Fig. 19) and fertile and sterile pollen are also observed (Fig. 20) which leads to low pollen fertility. The majority of the PMCs depicted abnormal spindle formation which resulted in irregular arrangement of bivalents at the spindle plate during metaphase-I and segregation of chromosomes during anaphase-I/telophase-I and anaphase-II/telophase-II. Chromosomes also lack the ability of congregation at a single pole and...
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Figs. 1-20. *Withania somnifera* 1) PMC at metaphase-I showing 24 bivalents; 2) PMC at anaphase-I showing 24:24 chromosomes; *W. coagulans* 3-4) PMC at metaphase-I showing 24 bivalents; 5) PMC at anaphase-I showing 24:24 chromosomes; 6-7) PMC showing spindle irregularities with scattered chromosomes (scattered chromosomes arrowed); 8) PMC at anaphase-I showing laggards (laggards arrowed); 9, 10) PMCs at anaphase-II showing spindle irregularities in form of laggards (laggards arrowed); 11) PMC at telophase-II showing laggards and bridges (bridge arrowed); 12) PMC showing multipolarity; 13) Monad; 14) Dyad; 15) Triad; 16) Chromatin transfer within tetrad (chromatin transfer arrowed); 17) Chromatin transfer within polyad (chromatin transfer arrowed); 18) Polyad with micronuclei (micronuclei arrowed); 19) Unequal sized pollen grains; 20) Sterile and fertile pollen grains. Scale bar=10µm.
remained scattered in the cytoplasm or in small groups. Chromosomes in these PMCs failed to reach their respective poles and constitute micronuclei during late telophase stages and sporad formation. Irregular spindles in these plants are also depicted in the meiocytes, which showed multipolar presence of chromosomes.

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, synopsis 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 allo-tetraploids 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 homeologous chromosomes (Morrison and Rajhathy 1960, Gottschlk 1978). On the other hand in W. coagulans the meiosis is highly abnor-

Map A) Map showing the geographical location of Rajasthan in India, B) Map depicting various localities in Rajasthan, C) Map depicting the boundary of Thar Desert and the amount of rainfall received by the State.
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