Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: Ramanpreet, R. Chand Gupta (2019) Meiotic studies in genus *Withania* Pauquy, from Indian Thar Desert. *Caryologia* 72(1): 15-21. doi: 10.13128/cayologia-247

Received: 19th July 2018

Accepted: 20th December 2018

Published: 10th May 2019

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

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

Ramanpreet*, Raghbir Chand Gupta

Department of Botany Punjabi University, Patiala, 147002, Punjab, India
* Corresponding author: ramanbrar247@gmail.com

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 subtropical 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 2*n*=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 (2*n*=24), tetraploid cytotype (2*n*=48) and hexaploid cytotypes (2*n*=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 (2*n*=12, 18, 24, 36, 48, 72) with predominance of 2*n*=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.

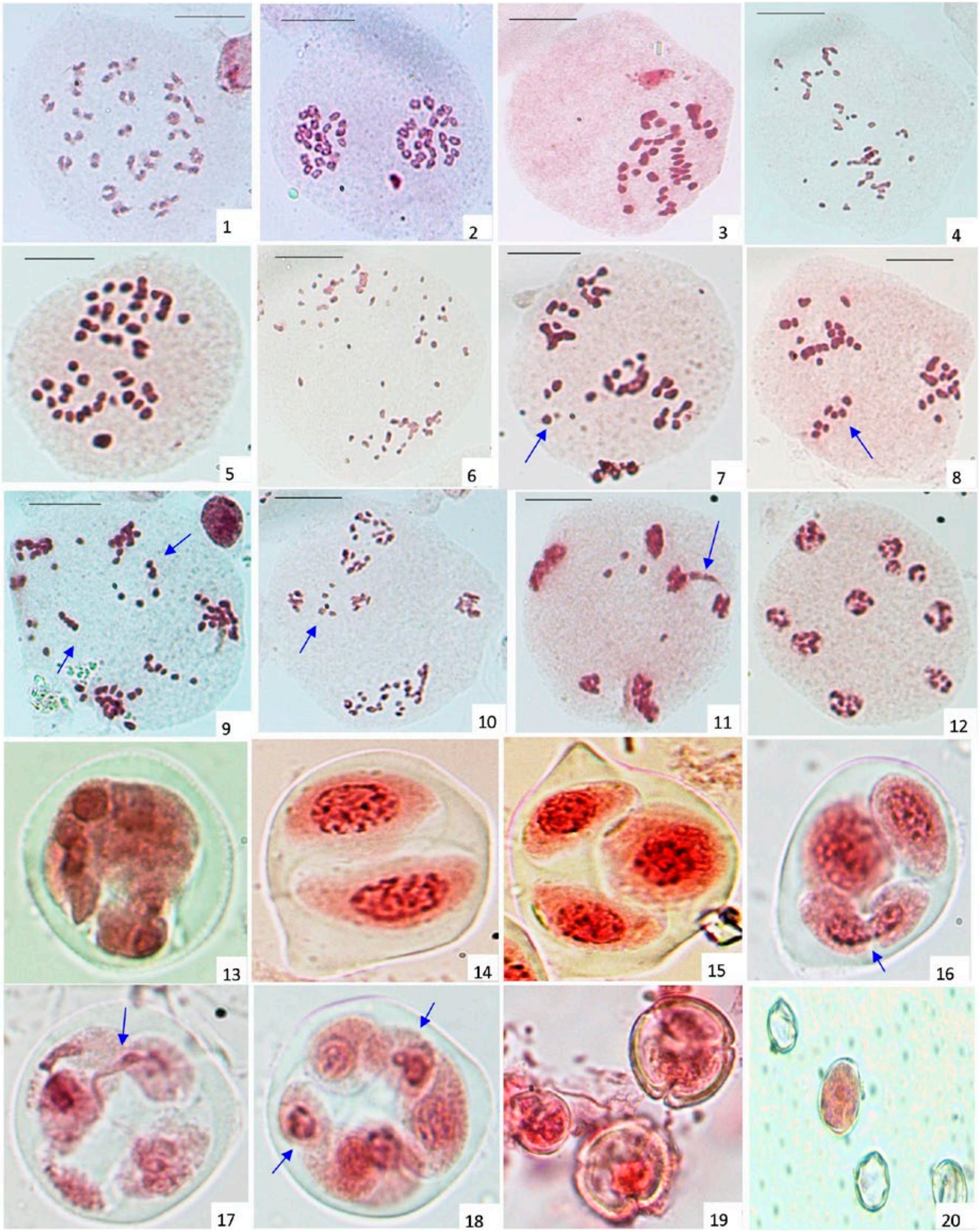
MATERIALS AND METHODS

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% 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. Pollen grain size was measured using an occulometer. 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 of Herbaria 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/teolophase-I and anaphase-II/telophase-II. Chromosomes also lack the ability of congregation at a single pole and



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.

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.

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, 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 homeologous chromosomes (Morrison and Rajhathy 1960, Gottschlk 1978). On the other hand in *W. coagulans* the meiosis is highly 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 enviornmental) 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). Spindle irregularities are generally divided into 4 categories-multipolar, monopolar, radial and apolar. Multipolar spindles are those in which 3 or more poles are formed, in monopolar only one spindle formation takes place, in radial spindles ends are located at the periphery and near the equator (Shamina *et al.* 2000a) and apolar spindles have randomly oriented set of fibers (Shamina *et al.* 2000b, Seriukova *et al.* 2003).

Abnormalities like cytomixis, chromosome stickiness, unoriented bivalents, laggards, bridges which ultimately lead to abnormal microsporogenisis with the production of dyads, triads, polyads, tetrads with micronuclei, and sterile and fertile pollen grains.

ACKNOWLEDGMENTS

This study was funded by Department of Biotechnology (DBT), New Delhi, DBT-IPLS Project with reference number BT/PR 4548/NF/22/146/2012. The authors are also thankful to Head, Department of Botany, Punjabi University, Patiala, for all the necessary laboratory facilities.

REFERENCES

Ahmad, Q. N., Britten, E. J. and Byth, D. E. 1984. Effect of interacting genetic factors and temperature on meiosis and fertility in soybean×*Glycine soja* hybrids. Can. J. Genet. Cytol. **26**: 50-56.

Al-Turki, T. A., Filfilan, S. A. and Mehmood, S. F. 2000. A cytological study of flowering plants from Saudi Arabia. Willdenowia **30**: 339-358.

Anesini, C. and Perez, C. 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol. **39**: 119-128.

Bajpai, A. and Singh, A. K. 2006. Meiotic behavior of *Carica papaya* L. spontaneous chromosome instability and elimination in important cvs in North Indian conditions. Cytologia **71**: 131-136.

Baquar, S. R. 1967. Cytomorphological studies in the family Solanaceae from West Pakistan. Genetica **38**: 388-397.

Baum, M. E., Lagudah, E. S. and Appels, R. 1992. Wide crosses in cereals. Annu. Rev. Plant Physiol. Plant Mol. Biol. **43**:117-143.

Bedini, G., Garbari, F. and Peruzzi, L. 2012. Does chromosome number count? Mapping karyological knowledge on Italian flora in a phylogenetic framework. J. Syst. Evol. **298**: 739–750.

Belling, J. 1921. On counting chromosomes in pollen mother cells. American Naturalist. **55**: 573-574.

Bhaduri, P. N. 1933. Chromosome numbers of some Solanaceous plants of Bengal. J. Ind. Bot. Sci. **12**: 56-64.

Bhandari, M. M. 1978. *Flora of Indian desert*. Pp. **471**. Scientific Publishers. Jodhpur.

Bir, S. S. and Sidhu, M. 1979. Cytological observations in weed flora of orchards of Patiala district, Punjab. Recent Res. Plant Sci. **7**: 261-271.

Bir, S. S. and Sidhu, M. 1980. Cyto-palynological studies on weed flora of cultivable lands of Patiala district (Punjab). J. Palynol. **16**: 85-105.

Bir, S. S., Kumari, S., Shoree, S. P. and Saggoo, M. I. S. 1978. Cytological studies in certain Bicarpellatae from North and Central India. J. Cytol. Genet. **13**: 99-106.

Caetano-Pereira, C. M. and Pagliarini, M. S. 2001. A new meiotic abnormality in *Zea mays* multiple spindles associated with abnormal cytokinesis in both divisions. Genome **44**: 865-871.

Cifuentes, M., Grandont, L., Moore, G., Chèvre, A. M. and Jenczewski, E. 2010. Genetic regulation of meiosis in polyploidy species: new insights into an old question. New Phytol. **186**: 29-36.

Datta, A. K., Mukherjee, M. and Iqbal, M. 2005. Persistent cytomixis in *Ocimum basilicum* L. (Lamiaceae) and *Withania somnifera* (L.) Dun. (Solanaceae). Cytologia **70**: 309-313.

de Muyt, A. D., Mercier, R., Mezard, C. and Grelon, M. 2009. Meiotic recombination and crossovers in plants. Genome dynamics **5**: 14-25.

Dymock, W., Waden, C. J. H. and Hopper, D. 1972. Pharmacographia Indica: Institute of health and TB Research, Karachi, pp. 306.

Golubovskaya, I. N. and Distanova, E. E. 1986. Mapping mei gene ms43 by B-A translocation stocks. Genetica **22**: 1173-1180.

Gottschalk, W. 1978. Open problems in polyploidy research. Nucleus 21: 99-112.

Hamant, O., Ma, H. and Cande, W. Z. 2006. Genetics of meiotic prophase I in plants. Anu. Rev. Plant Biol. **57**: 267-302.

Hunziker, A. T. 2001. Genera Solanacearum: the genera of the Solanaceae illustrated, arranged according to a new system; Gantner Verlag: Ruggell, Liechtenstein.

Iqbal, M. and Datta, A. K. 2007. Cytogenetic studies in *Withania somnifera* (L.) Dun. (Solanaceae). Cytologia **72**: 43-47.

Jiao, Y., Wickett, N. J., Ayyampalayam, S., Chanderbali, A. S., Lena. L., Ralph, P. E., Tomsho, L. P., Hu, Y., Liang, H., Soltis, P. S., Soltis, D. E., Clifton, S. W., Schlarbaum, S. E., Schuster, S. C., Ma, H., Leebens-Mack, J. and de Pamphilis, C. W. 2011. Ancestral polyploidy in seed plants and angiosperms. Nature **473**: 97-102.

Khatoon, S. and Ali, S. I. 1982. Chromosome numbers of some plants of Pakistan. Pak. J. Bot. **14**: 117-129.

Koul, A. K., Wakhlu, A. K. and Karihaloo, J. L. 1976. Chromosome numbers of some flowering plants of Jammu (Western Himalayas) II. Chr. Inform. Ser. **20**: 32-33.

Krishnamurthi, A. 1969. The Wealth of India, Vol VIII, Publication Information Directorate, SIR, New Delhi. pp. 582.

Kumar, P. and Singhal, V. K. 2008. Cytology of *Caltha palustris* L. (Ranunculaceae) from cold regions of Western Himalayas. Cytologia **73**:137-143.

Madhavadian, P. 1968. Chromosome numbers in South Indian Solanaceae. Caryologia **21**: 343-347.

Marks, G. E. 1954. An acetocarmine glycerol jelly for use in pollen fertility counts. Stain Tech. **29**: 277.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science **264**: 421-423.

Mishra, J., Dash, A. K., Mishra, S. N. and Gupta, A. K. 2013. *Withania Coagulans* in treatment of diabetics and some other diseases: A review. Res. J. Pharm. Bio. Chem. Sci. **4**: 1251-1258.

Ahmad, M. B 2014. Characterization of Genetic Variability in *Withania somnifera* L Dunal using Biochemical and Molecular Markers. http://hdl.handle.net/10603/23450.

Morrison, J. W. and Rajhathy, T. 1960. Frequency of quadrivalents in autotetraploid plants. Nature **187**: 528-530.

Naz, S., Masud, T. and Nawaz, M. A. 2009. Characterization of milk coagulating properties from the extract of *Withania coagulans*. Int. J. Diary Tec. **62**: 315-320.

Olmstead, R. G., Bohs, L., Migid, H. A., Santiago-Valentin, E., Garcia1, V. F. and Collier, S. M. 2008. A molecular phylogeny of the Solanaceae. Taxon **57**: 1159-1181.

Otto, S. P. and Whitton, J. 2000. Polyploid incidence and evolution. Ann. Rev. Genet. **34**: 401-437.

Purohit, S. S. and Vyas, S. P. 2004. Medicinal Plant cultivation: A Scientific Approach. Publisher Agrobios: Jodhpur. pp 547.

Qu, L. and Vorsa, N. 1999. Desynapsis and spindle abnormalities leading to 2*n* pollen formation in *Vaccinium darrowi*. Genome **42**: 35-40.

Rai, P. K. and Kumar, G. 2010. The genotoxic potential of two heavy metals in inbred lines of maize (*Zea mays* L.). Turk. J. Bot. **34**: 39-46.

Ramsey, J. and Schemske, D. W. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Ann. Rev. Ecol. Syst. **29**: 467-501.

Rezaei, M., Arzani, A. and Syed-Tabatabaei, B. E. 2010. Meiotic behavior of tetraploid wheats (*Triticum turgidum* L.) and their synthetic hexaploid wheat derivates influenced by meiotic restitution and heat stress. J. Genet. **89**: 401-407.

Risso-Pascotto, C., Pagliarini, M. S. and Valle, C. D. 2003. A mutation in the spindle checkpoint arresting meiosis II in *Brachiaria ruziziensis*. Genome 724-728.

Samaddar, T., Nath, S., Halder, M., Sil, B., Roychowdhury, D., Sen, S. and Jha, S. 2012. Karyotype analysis of three important traditional Indian medicinal plants, *Bacopa monnieri*, *Tylophora indica* and *Withania somnifera*. Nucleus **55**: 17-20.

Seriukova, E. G., Dorogova, N. V., Zharkov, N. V. and Shamina, N. V. 2003. Aberrations in prometaphase leading to the formation of restitution nuclei in cereal wide hybrids. Tsitologiya **45**: 244-248.

Shamina, N. V., Dorogova, N. V. and Perelman, P. L. 2000a. Radial spindle and the phenotype of maize meiotic mutant, dv. Cell Bio. Int. **24**: 729-736.

Shamina, N. V., Dorogova, N. V. and Perelman, P. L. 2000b. Disruption of male meiosis in *Pisum sativum* L. induced by mutation ms3. Tsitologiya **42**: 404-411.

Sharma, S. and Tiagi, B. 1979. *Flora of North East Rajasthan*. Kalyani Publishers. New Delhi.

Shetty, B. V. and Singh, V. 1993. *Flora of Rajasthan*, Vol. I, II, III. Botanical Survey of India, Calcutta.

Singhal, V. K. and Kaur, D. 2009. Spontaneous occurrence of meiotic spindle abnormalities in 'Long Headed Poppy' (*Papaver dubium* L.) from Indian cold regions. Cytologia **74**: 385-389.

Soltis, D. E., Albert, V. A., Leebens-Mack, J., Bell, C. D., Paterson, A., Zheng, C., Sankoff, D., dePamphilis, C. W., Wall, P. K. and Soltis, P. S. 2009. Polyploidy and angiosperm diversification. Am. J. Bot. **96**: 336-348.

Staiger, C. J. and Cande, W. Z. 1990. Microtubule distribution in dv, a maize meiotic mutant defective in the prophase to metaphase transition. Dev. Biol. **138**: 213-242.

Stebbins, G. L. 1938. Cytological characteristics associated with the different growth habits in the dicotyledons. Am. J. Bot. **25**: 189-198.

Stebbins, G. L. 1985. Polyploidy, hybridization, and the invasion of new habitats. Ann. Missouri Bot. Gard. **72**: 824-832.

Sun, K., Hunt, K. and Hauser, B. A. 2004. Ovule abortion in *Arabidopsis* triggered by stress. Plant Physiol. **135**: 2358-2367.

Viccini, L. F. and Carvalho, C. R. 2002. Meiotic chromosomal variation resulting from irradiation of pollen in maize. J. Appl. Genet. **43**: 463-469.

Wadsworth, P., Lee, W. L., Murata, T. and Baskin, T. I. 2011. Variations on theme: spindle assembly in diverse cells. Protoplasma **248**: 439-446.