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Research Article

Abnormal Chromosomal Behavior in a New Diploid Cytotype of *Spigelia anthelmia* L. (*Loganiaceae*) from Aravalli Mountain Range of Rajasthan

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Abstract

Spigelia anthelmia L. is cytologically worked for the time for the area of India. The diploid chromosome count ascertained here, represents new cytotype, supplementing the earlier report of a hexaploid cytotype with 2n=32 from outside India. Of the four populations presently worked out here, population collected from Mount Abu and Jhalawar region show normal meiotic course (n=8) with normal seed set and high pollen fertility (65%-100%), whereas populations collected from Ranakpur and Udaipur region shows abnormal meiotic course with the presence of cytomixis, laggards and chromatin bridges. Further, microsporogenesis was also abnormal, resulting in the formation of monads, dyads, triads, polyads, and tetrads with micronuclei. All these abnormalities lead to low pollen fertility (45%-60%) and heterogeneous sized pollen formation was also observed.

Keywords: *Spigelia anthelmia*, Meiosis, New chromosome count, Chromatin transfer, Meiotic abnormalities, Aravalli mountain range

INTRODUCTION

Rajasthan symbolizes royalty, rich cultural heritage, safaris and sand-dunes. The name itself means the land of rajas (Kings). The state is the largest in North-Western part of India, geographically it lies between 23°3´ to 30°12´ N longitude and 69°30´ to 78°17´ S latitude with an area of 3 Km^2, 42 Km^2, 269 Km^2, out of which about 1 Km^2, 98 Km^2, 100 Km^2 is arid and the rest is semi-arid. The altitude range varies from 150 m to 1,722 m. The state is blessed with the diverse landscapes and vegetation. The state is bordered by Pakistan to the West, Gujarat to Southwest, Madhya Pradesh to Southeast, Uttar Pradesh and Haryana to Northeast and Punjab to the North (Andersson et al., 1995).

Meiosis is the cell division that reshuffles genetic information between generations. It is unique and essential part of life cycle of all sexually reproducing organisms. It involves two divisions that are linked together, one the reductional division (meiosis -I) and other the equational division (meiosis-II).

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Failure in the proper execution of chromosome segregation inevitably leads to the formation of imbalanced gametes, resulting in aneuploid and ployploid progeny or polyploidization. The pairing of homologous chromosomes during zygotene or their premature separation are the most significant events in meiosis. Meiosis is one of the most sensitive and key step in the sexual life cycle of every plant species. It involves number of complex molecular and cellular events like chromosome replication, chromosome pairing, genetic recombination, chromosome segregation and cytokinesis (Andersson et al., 1991). Meiotic events have been found to be under the control of number of genes, though it is also influenced by many external factors of environment, such as temperature, humidity and nutritional aspects. Any disturbance, alteration or mutation in genes can change the rhythm of cell cycle and affect the normal meiosis (JR et al., 1988).

In previous studies we have addressed cytological aspects of plants from hot deserts of India, focusing on male meiosis, exploring more than 400 species from Rajasthan (Baker et al., 1976). The studies reveled various irregularities during male meiosis in some dicots, in family *Zygophyllaceae*, in *Malvales*, in tribe *Paniceae* and in some other species like *Sorghum bicolor Ageratum conyzoides*, *Physalis angulate* (Bellucci et al., 2012).

The family *Loganiaceae* comprises of 30 genera and 600 species distributed to tropical, sub-tropical, temperate and warm-temperate regions of the world. Phylogenetic studies in the family have been reported using nucleoide sequence data base. The genus *Spigelia* L. has 60 species distributed to temperate and warm-temperate regions of the world. Morphologically, *Spigelia* species can identified by opposite or whorled leaves, one-sided cymose inflorescences, often brightly colored pentamerous flowers with usually funnel form or tubular corollas, articulated styles, and strongly bilobed capsules with persistent style and fruit bases (Elufioye et al., 2015). The genus is represented in India and Rajasthan by 1 species *i.e., S. anthelmia*. The plant is used by local people of Rajasthan for the treatment of various diseases. The plant is used in the treatment of headaches, heart diseases and facial pains (eyes and teeth). It has powerful action on nervous system and also helps in breathing problems (Erickson et al., 2002).

While exploring cytomorphological diversity in *S. anthelmia (Loganiaceae)* from Aravalli mountain range covering Mount Abu, Jhalawar, Udaipur and Ranakpur regions, it was found that individuals with morphological variation (particularly height) were observed. It is herbaceous plant that grows up to 0.5 m tall, leaves are deeply veined ovate to lanceolate, flowers are star shaped, tubular with 5-obovate, pointed lobes, white with pink or purple stripes, fruit is 2 lobes capsule. In the present paper detailed meiotic course, microsporogenesis and pollen fertility is studied (Falistocco et al., 1995).

MATERIALS AND METHODS

Extensive field surveys have been carried out to different localities of Rajasthan. The plant specimens were identified with the help of various floras and authenticated from Botanical Survey of India, Jodhpur (Fuzinato et al., 2001). For meiotic studies, young flower buds of appropriate size were collected in freshly prepared Carnoy's fixative (6 parts ethanol: 3 parts chloroform: 1 part glacial acetic acid) for 40 h- 48 h, from the plants during the peak flowering time. Plant material was transferred to 70% ethyl alcohol and stored in refrigerator at 4°C until use. Meiotic studies were carried out by crushing anthers to prepare a smear of pollen mother cells (PMCs) in 1% aceto-carmine (Gadella, 1962). The chromosome counts and meiotic abnormalities were confirmed by observing appropriate number of PMCs. Pollen fertility was observed by heating the pollen grains in 50% glycerol-aceto carmine (1:1) solution. The photomicrographs of the PMCs and pollen grains were taken from the temporary slides by using Nikon 80i digital imaging system. Voucher specimens are deposited in Herbarium, Department of Botany, Punjabi University, Patiala (PUP) with accession numbers 60829, 60830, 59920 and 59901 (Gates, 1911).

RESULTS

The genus is cytologically worked out for the first time from India (Figures 1 and 2). The present study deals with detailed meiotic course of 4 individuals of *S. anthelmia* collected from Aravalli mountain range of Rajasthan. The species is found near wet and shady places. All the individuals collected from Mount Abu and Jhalawar regions showed the presence of 8 bivalents at diakinesis and metaphase-I, equal distribution of 8:8 chromosomes at anaphase-I and normal meiosis with high pollen fertility (Malallah et al., 2003). The individuals collected from Mount Abu showed morphological variation in the form of plant height but both the morpho-types showed same chromosome count. However, 2 individuals collected from Udaipur and Ranakpur region respectively, showed abnormal meiosis cytomixis involving transfer of chromatin material among 2 cells-4 cells in 16.84% PMCs at anaphases I/II and telophases I/II. Chromatin transfer occurs through narrow or broad cytoplasmic channels and presently is more frequently observed in second meiotic division (Bradshaw et al., 1984).

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Further, the presence of chromatin stickiness, laggards at anaphase-I and telophase-I were observed (Cai et al., 2001). Chromatin bridges were also observed at anaphase-I. Unequal distribution of chromosome towards their respective poles is observed in some PMCs (Gottschalk, 1970). The formation of multipolarity *i.e.,* formation of more than four poles is observed. All these meiotic abnormalities lead to irregular microsporogenesis. Laggards were not included in the daughter nuclei and gives rise to micronuclei (Gupta et al., 2016).

Failure of first meiotic division was frequently observed which led to the formation of restitution nuclei (Gupta et al., 2016). Analysis of 393 sporads revealed that 3.89% were dyads, 29.88% were dyads with micronuclei, 14.29% triads without micronuclei, 28.71% were triads with micronuclei and 15.29% were polyads. Besides, inter-microsporal (between the microspores of different sporads) transfer of chromatin material was also observed. The abnormal meiotic course resulted into low pollen fertility (45%-60%) (Haroun et al., 2004).

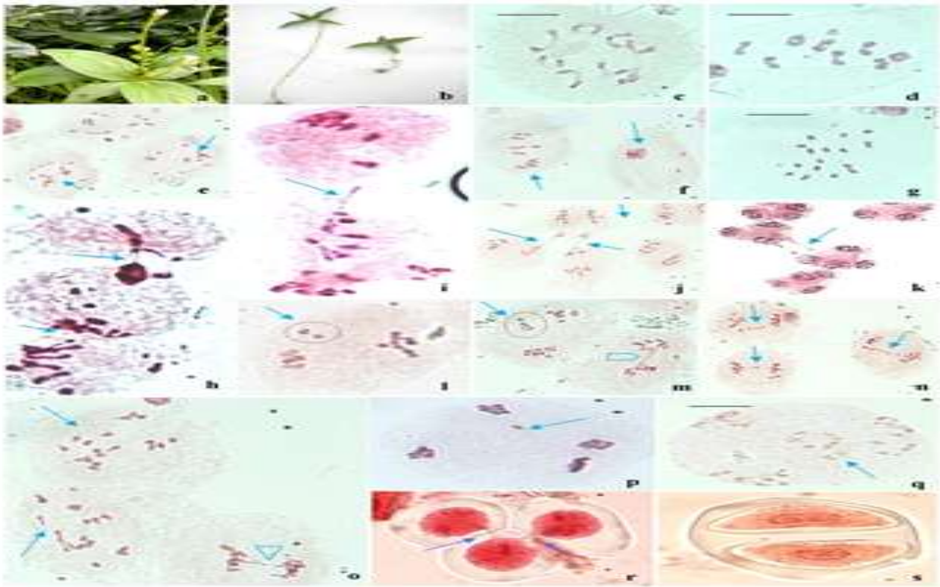

Figure 1. a) Spigelia anthelmia, b) Morphological variation in two populations, c) Pollen Mother Cells, PMCs, at diakinesis showing 8 bivalents, d) 8 bivalents at M-I, e) Random dispersa, chromosomes in cytoplasm at M-I, f) Chromosome stickiness, g) Equal distribution of 8:8 chromosomes at A-I, h,i) PMCs involved in chromatin transfer at M-I, j) Group of 4 PMCs showing chromatin transfer at A-I, K) PMCs involved in chromatin transfer at T-II, l) PMC with laggards at A-I, m) PMCs with laggards (arrow) and chromatin bridges (arrow head), n) PMCs showing chromatin bridge at A-I, o) PMCs showing unequal distribution of chromosomes (arrow) and chromatin bridges (arrow head), p) Laggards at T-II, q) Multipolarity, r) Cytoplasmic channels among microspores of monads, s) Dyad. Scale Bar=10µm.

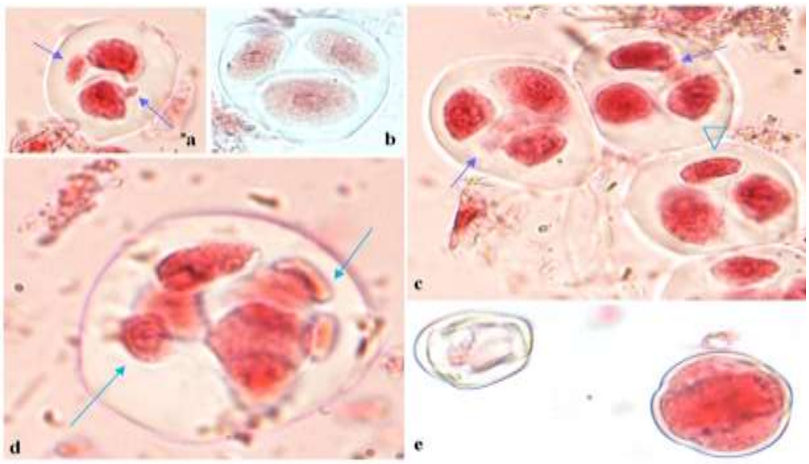

Figure 2. a) Dyad with two micronuclei, b) Triad, c) Triad with 2-3 micronuclei (arrow) dyad with micronuclei (arrow head), d) Polyad, e) Sterile and fertile pollen grains. Scale Bar=10.

DISCUSSION

This paper presents the first cytological study of genus *Spigelia* from India. During the present study all the 4 plant populations of *S. anthelmia* collected from Aravalli mountain range of Rajasthan, showed the same meiotic chromosome count of n=8, which exhibits at the diploid level. Earlier, a tetraploid cytotype with 2n=32 (based on x=8) was already reported from outside India. The population collected from Mount Abu (even though exhibited morphological variations in the terms of plant height) and Jhalawar region, showed perfectly normal meiosis with regular 8 bivalents formation at diakinesis and metaphase-I stages of meiosis.

Equal segregation of chromosomes during anaphase-I resulted into normal tetrad formation and high pollen fertility. Morphological variations are generally seen within the species having wide range of distribution. These variations provide ways for the species to adapt and survive in different environmental conditions. Geographical changes play a significant role in causing morphological variations in the flowering plants and may also be caused due to genetic changes. Whereas plants collected from Ranakpur and Udaipur regions showed meiotic abnormalities in the form of cytomixis, laggards, chromatin bridges and abnormal sporads formation resulting into high pollen sterility. It is the phenomena of chromatin transfer from one PMC to other PMCs through cytoplasmic channels. Cytomixis was recorded for the first time by Körnicke in *Crocus sativus* and the name ‘cytomixis’ was coined by Gates. It has profound impact on the meiotic course and meiotic end products (Jiang et al., 2011).

It also affects the overall reproductive potential of the species and leads to variation in chromosome number of the gametes. There are different opinions regarding the causes of the cytomixis like pathological effects, temperature, physiological changes, fixation or mechanical injury, stress factors coupled with genetic control, direct genetic influence and pollution. Those chromosomes which fail to move towards their respective poles during anaphases and telophases of the meiosis are considered as laggards (Kaul et al., 1985). Chromatin bridges may be formed because of chiasma formation in heterozygous inversions or due to spontaneous breakage and fusion of chromosomes. Laggards may lead to the formation of micronuclei during microsprogenesis, micro pollen grains and gametes with unbalanced chromosome numbers. Various workers suggested different views regarding the formation of laggards and bridges. Pagliarini, et al. and Fuzinatto, et al. suggested that production of laggards to be under genetic control. Some researchers linked the formation of laggards and bridges to the phenomena of cytomixis. Chromatin bridges may arise due to paracentric inversions.

CONCLUSION

According to Ranjbar, et al. chromatin stickiness is also responsible for the bridge formation mainly at anaphases and telophases stages. All the abnormalities encountered during meiosis lead to abnormal microsporogenesis. In normal meiotic course tetrad formation takes place at the end of the meiosis resulting into four daughter cells. But in case of abnormal meiotic course, formation of monads, dyads, triads, polyads and tetrads with or without micronuclei are formed, leading to the formation of heterogeneous sized pollen grains and pollen sterility. If both the meiotic divisions fail, the production of monad takes place and if second division fails, then dyads formation takes place. If second division at one pole fails triads are formed and polyads formation takes due to the production of spindle abnormalities.

The micronuclei are the result of laggards which further lead to micropollen formation and chromosomally unbalanced gametes. The main reason in the production of heterogenous sized pollen grains is cytomixis but its genetic origin in some species cannot be overruled. Of the total 60 taxonomically known species, only 3 species of the genus are cytologically studied with chromosome count of n=13, 16, 24.

Earlier, 2n=32 was reported for *S. anthelmia*. The presence of intraspecific polyploidy cytotypes, combined with distinctions in morphology and meiotic course, clearly points out the fact that the species of the genus are in active process of evolution.

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CONFLICT OF INTEREST

Author declare no conflict of interest.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

No animal and/or human participation involved in the research.

INFORMED CONSENT

Author gives full consent.

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