Indian J. Genet., 76(1): 111-115 (2016)
DOI: 10.5958/0975-6906.2016.00017.1

Short Communication

Meiotic chromosomal studies in family Zygophyllaceae R. Br. from Rajasthan

Raghbir Chand Gupta, Kuljit Kaur* and Vijay Singh

Department of Botany, Punjabi University, Patiala 147 002

(Received: June 2015; Revised: October 2015; Accepted: November 2015)

Abstract

Present paper deals with the cytomorphological study of 4 genera and 5 species belonging to family Zygophyllaceae collected from various localities of Rajasthan. Three cytomorphovariants of *Tribulus terrestris* have been observed with 2n=24, 36 and 48. Peganum harmala with 2n=12 (based on x=6) is reported for the first time from world level, earlier the species is based on x=12. The presence of B-chromosomes also makes the first report of the species from world level. *Fagonia cretica* (2n=22), *Tribulus alatus* (2n=24) and *Zygophyllum simplex* (2n=16) have been cytologically worked out for the first time from India.

Key words: Zygophyllaceae; Rajasthan; meiosis; base number; cytotype

The family Zygophyllaceae comprises of 27 genera and 285 species subdivided into 5 subfamilies (Sheahan and Chase 2000). From India, 8 genera and 18 species are known. The family is widely distributed, mainly growing in arid and semiarid areas in the tropics and sub-tropics. It is considered to be monophyletic based on morphological and DNA characters after a few genera are segregated as distinct families (Judd et al. 1999).

To study the genetic diversity at intra-and interspecific levels, present meiotic studies on the members of family Zygophyllaceae have been undertaken from various localities of Rajasthan.

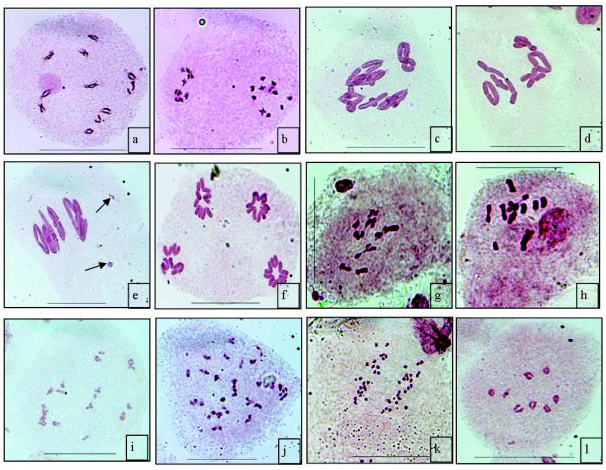
For meiotic study, floral buds of appropriate size were collected and fixed in Carnoy's fixative for 24 hrs and preserved in 70% alcohol at 4^{o}C. Meiotic studies of the appropriate sized anthers have been made by squashing in 1% acetocarmine. Pollen fertility was estimated by mounting mature pollen grains in glycero-acetocarmine (1:1). Well-filled pollen grains with stained nuclei were counted as fertile, while shrivelled and unstained pollen grains as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using a Nikon 80i Eclipse Digital Imaging System. Voucher specimens are deposited in the Herbarium of the Department of Botany, Punjabi University, Patiala, India (PUN).

Detailed data regarding the different accessions, locality with altitude, flowering and fruiting period, chromosome number, ploidy level, meiotic course, pollen fertility and previous reports have been given in Table 1. Meiotic study of *Tribulus alatus* depicts the presence of 2n=24 (Fig. 1g) which is in conformity with the previous reports from outside India (Table 1) and the species is first time cytologically worked out from India. Three populations of *Tribulus terrestris* collected from different localities shows intraspecific cytotypes with 2n=24, 36 and 48 (Figs. 1h-k).

Three populations of *Fagonia cretica* show the diploid cytotype with 2n=22 (Figs. 1a-b), which is reported for the first time from India. Earlier the species is known to have 2n=18, 20 and 22 from outside India (Baquar 1967) and 2n=20 from India (Table 1).

Two populations of *Peganum harmala* depict the

*Corresponding author's e-mail: kuljitkajla88@gmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com



Figs. 1. a-l: a-b. *Fagonia cretica* (2n=22) a. PMC at Diakinesis showing 9_{II} b. PMC at M-II showing 9:9 distribution of chromosomes c-f. *Peganum harmala* (2n=12) c-d. PMC at M-I showing 6_{II} with ring and rod shaped bivalents e. PMC at M-I showing 6_{II} and 2-B chromosomes f. PMC at A-II showing 6:6 distribution of chromosomes at four poles g. *Tribulus alatus* (2n=24) PMC at M-I showing 12_{II} h-k. *Tribulus terrestris* (2n=24, 36, 48) h. PMC at Diakinesis showing 12_{II} i. PMC at M-I showing 16_{II} j. PMC at M-I showing 24_{II} k. PMC at A-I showing 24:24 distribution of chromosomes at A-I l. *Zygophyllum simplex* (2n=16) PMC at M-I showing 8_{II}. (*Scale* 10µm)

presence of 2n=12+0-2B chromosomes (Figs. 1c-f) which is new chromosomal report at world level. Earlier the species is known to have 2n=22 and 24 (Ma et al. 1984, 1990) from outside India while 2n= 24 from India (Singh 1984). One population of *Zygophyllum simplex* shows the chromosomal count of 2n=16 (Fig. 1l) which is cytologically worked out from India for the first time.

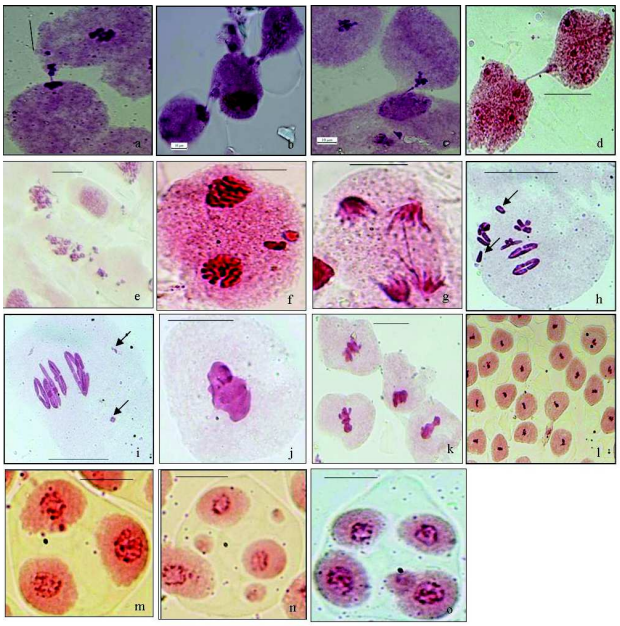
Meiotic abnormalities have been observed in some of the populations of three species as *Tribulus terrestris, T. alatus* and *Peganum harmala*. Chromatin transfer/ Cytomixis is quite common in *Tribulus terrestris* and *Tribulus alatus* (Figs. 2a-d). 0-2 B-chromosomes are observed for the first time in some PMCs of *Peganum harmala* (Fig. 2e), but it does not show much effect on its pollen fertility. Early and late disjunction of bivalents have been noticed in *Peganum harmala* (Fig. 2h). Laggards and chromatin bridge have been observed in case of *Tribulus terrestris* (2n=24) (Figs. 2f-g) in high frequency which ultimately results in the formation of triads and tetrads with micronuclei. Chromatin stickiness involving few bivalents or whole complement is observed in case of *Fagonia cretica* and *Peganum harmala* (Figs. 2i-j). All these meiotic abnormalities ultimately lead to the reduction of pollen fertility.

Chromosome numbers

Tribulus L.: Out of 25 taxonomically known species, 8 species (32%)/16 cytotypes are cytologically worked out whose chromosome number varies from 2n=12-48. The genus is dibasic (x=6, 10). From India, five species are taxonomically known, of which 3 species (60%)/ 7 cytotypes are cytologically worked out.

Fagonia L.: Out of 34 taxonomically known species, 14 species (41.17%)/20 cytotypes are cytologically worked out. The genus is polybasic depending on the base number x=5, 9 and 11. From India, 4 species are taxonomically known and 3 species (75%)/ 4 cytotypes are cytologically worked out.

Peganum L.: Out of six taxonomically known species, 2 species (33%)/3 cytotypes are cytologically worked out depending on base numbers x=12, 11. According to the present chromosomal report of 2n=12, new base number x=6 is proposed for the first time. From India, 1 species (*Peganum harmala*) is cytologically worked out.



Figs. 2a-j: a-e. PMCs showing chromatin transfer/ cytomixis f. PMC showing laggards at T-I g. PMC showing chromatin bridge at A-II h-i. PMCs showing 2B-chromosomes (arrowed) j-l. PMCs showing chromatin stickiness at M-I m. Triad n . Triad with micronuclei o. Tetrad with micronucleus. (*Scale* 10µm)

Table 1. Data showing accession numbers, locality with altitude, flowering and fruiting period, chromosome number (2n), ploidy level, pollen fertility and previous reports of various taxa belonging to family Zygophyllaceae from various localities of Rajasthan.

S.No.	Taxon		Accession number (PUN)	Locality with altitude (mts)	Flowering and fruiting period	Chromosome number (2n)	Ploidy	Pollen fertility	Previous reports
1.	*Fagonia cretica* L.	P-I	59432	Massuria Hill Garden, Jodhpur (350)	September-February	22*	2x	99	2n=18: Baquar 1967; Diosdado et al. 1993; Negodi 1939; Ruiz de Clavijo Jimenez et al. 1988 2n=20: Baquar 1967; Bhansali & Bhandari 1974
		P-II	59433	On the way to Hemawas Dam, Pali (237)		22*	2x	100	2n=22: Baquar et al. 1965; Baquar 1967
		P-III	59434	Circuit House, Pali (230)		22*	2x	98	
2.	*Peganum harmala* L.	P-I	59435	Khema ka kuan, Jodhpur (300)	August-November	12+0-2B**	2x	89	2n=22: Ma et al. 1984; Negodi 1937 2n=24: Amin 1973; Aryavand 1975; Baquar et al 1965; Diaz Lifante 1991a; Hilu 1979; Humphries
		P-II	59436	Pali (230)		12**	2x	92	1978; Kaul & Wakhlu 1976; Lorenzo-Andreu 1951; Ma et al. 1990; Magulaev 1979a; Ruiz De Clavijo 1991; Singh 1984; Warburg 1938
3.	*Tribulus terrestris* L.	P-I	59429	On the way to Hemawas Dam, Pali (237)	August - December	24	4x	76	2n=12 Baquar et al. 1965 2n=24 Sugiura 1940b,d; Heiser & Whitaker 1948; Bhansali 1980; Pastor et al. 1988; Khatoon 1991;
		P-II	59430	On the way to Circuit House, Pali (230)		36	6x	92	Khatoon & Ali 1993; Baltisberger 2006 2n=24, 36, 48, 30 Morrison & Scott 1996; Yang et al. 1996
		P-III	59431	Botanical Survey of India, Jodhpur (282)		48	8x	98	2n=36 Baxap&eBa, AcTauoBa 1968; Huang & Hsieh 1994; Mesicek & Sojak 1995; 2n=36, 48 Husain 1986 2n=36, 48 Sidhu 1979; Sanjappa 1979; Hilu 1979; Bir & Sidhu 1980; Hilu 1981 2n=32, 36, 48 Koul et al. 1976; Sarkar et al. 1977 a, Rostovtseva 1977; Kumar 1978 2n=24, 48, 72, 96 Suguira 1940 b,d; Whitaker 1948; Shetty 1961; Schnack & Covas 1947; Baquar et al. 1965; Malik 1966b 2n=32 Sarkar et al. 1977 2n=36 Shetty 1961; Zakharyeva & Astanova 1968; Ramachandran & Kuriachan 1970; Rashid 1974; Hilu 1979 2n=48 Schnack & Covas 1947; Fotedar & Roy 1969b; Sanjappa 1979; Singh 1984
4.	*Tribulus alatus* Delile		59428	Hemawas Dam, Pali (237)	August-October	24*	4x	98	2n=24 Hilu 1979, 1981
5.	*Zygophyllum simplex* L.		59437	Kaali Sindh Dam, Jhalawar (331)	October-December	16*	2x	100	2n=16: Khatoon & Ali 1982, 1993

*First cytotype report from India; **First cytotype report from wold level; PUN= Punjabi University, Patiala.
The reports & authors names in bold represents the work done from India

Table 2. Data on cytomixis and meiotic course in the studied accessions of family Zygophyllaceae from different areas

Accession no.	Cytomixis			Meiotic course		
	PMCs involved (%)	No. of PMCs involved	PMCs with chromatin stickiness at M-I (%)	PMCs with B-chromosomes (%)	PMCs with bridge (at A-I/II, T-I/II) (%)	PMCs with laggards (at A-I/ T-I/II) (%) II,
59432	—	—	15.3 (15/98)	—	—	—
59435	7.6 (7/92)	2-3	17.30 (9/52)	9.5 (9/94)	—	—
59428	19.16 (23/120)	2-4	—	—	—	—
59429	12.69 (16/126)	2-6	—	—	15.2 (19/125)	17.5 (21/120)

Table 3. Data on abnormal microsporogenesis in different species of family Zygophyllaceae from different areas

Accession no.	Dyads		Triads		Tetrads	
	WMN (%)	WM (%)	WMN (%)	WM (%)	WMN (%)	WM (%)
59435	—	—	6.09 (5/82)	2.19 (2/91)	95.32 (102/ 107)	—
59428	—	—	2.1 (2/95)	—	94.78 (109/115)	2.8 (3/107)
59429	—	2.04 (2/98)	1.6 (2/125)	3.4 (4/117)	95.04 (115/121)	5.0 (6/120)

WMN=Without micronuclei; WM=With micronuclei

Zygophyllum L.: From total 100 taxonomically known species, 25 species (16.6%)/ 28 cytotypes are cytologically worked out worldwide. The chromosome number of the genus depends on the base number x= 8, 10, 11. From India, 2 species are cytologically worked out.

The present study reveals the presence of different meiotic abnormalities in some species in the form of cytomixis, chromatin stickiness, laggards, bridges and abnormal microsporogenesis, besides B-chromosomes. B-chromosomes are dispensable supernumerary chromosomes that does not recombine with the A-chromosomes and follows its own evolutionary pathway (Jones and Houben 2003). These were first came to observe in rye and maize in 1920s (Gotoh 1924; Kuwada 1925 respecively). Previously, Jones and Rees (1982) observed the high pollen sterility with the increase in B-chromosomes numbers and have been suggested to change the morphological characteristics of the individual as well.

The occurrence of such an enormous variation of chromosome numbers and meiotic behavior in the studied species supports the need for an extensive morphological and cytological exploration at the population level on different members of Zygophyllaceae from different areas of India. As there is considerable difference in the amount and quantity of the active constituents in different cytotypes of some medicinally important plants (Berkov 2001). Therefore there is a need to evaluate the bioactive compounds in *Tribulus terrestris* on the basis of their cytotypes/morphotypes, so as to mark the best chemo-/cytotype for future use.

Acknowledgment

The authors are highly thankful to University Grant Commission(DRS-SAP programme, RGNF & BSR), New Delhi and DBT (IPLS project) for providing financial assistance. Thanks are also due to the Head of Department, Punjabi University, Patiala.

References

Baquar S. R. 1967. Intraindividual numerical chromosome instability in *Fagonia cretica* L. Genet. Iber., **19**: 99-102.

Berkov S. 2001. Size and alkaloid content of seeds in induced autotetraploids of *Datura innoxia*, *Datura stramonium* and *Hyocyamus niger*. Pharm. Biol., **39**: 329-331.

Gotoh K. 1924. Über die chromosomenzahl von *Secale cereale* L. Bot. Mag. Tokyo, **38**: 135-152.

Jones N. and Houben A. 2003. B-chromosomes in plants: escapes from the A-chromosome genome? Trends Plant Sci., **8**: 417-423.

Jones R. N. and Rees H. B. 1982. Chromosomes. Academic Press, London.

Judd W. S., Campbell C. S., Kellogg E. A. and Stevens P. F. 1999. Plant systematics. A phylogenetic approach. Sunderland, Massachusetts: Sinauer Associates, Inc. Publishers.

Kuwada Y. 1925. On the number of chromosomes in maize. Bot. Mag. Tokyo, **39**: 227-234.

Ma X. H., Ma X. Q. and Li N. 1990. Chromosome observations of some drug plants in Xinjiang. Acta Bot. Boreal. Occid. Sin., **10**: 203-210.

Ma X. H., Qin R. L. and Xing W. B. 1984. Chromosome observations of some medicinal plants in Xinjiang. Acta Phytotax. Sin., **22**: 243-249.

Rawat D., Kumar A. and Rao S. A. 2006. Meiotic chromosome studies in *Tribulus rajasthanensis*, an endemic and threatened medicinal herb of Rajasthan. Cytologia, **71**: 365-370.

Sheahan M. C. and Chase M. W. 2000. Phylogenetic relationships within Zygophyllaceae based on DNA sequences of three plastid regions, with special emphasis on Zygophylloideae. Syst. Botany, **25**: 371-384.

Singh P. 1984. In A. Love (ed.)10PB Chromosome number reports, LXXXV. Taxon, **33**: 759.