Journal of Cytology and Genetics 2021 VOL. **22** (NS): 1−6

RESEARCH ARTICLE

KARYOMORPHOLOGICAL STUDIES IN *HARDWICKIA BINATA* ROXB.

SHITAL DESHMUKH* AND NAMDEO GHANAWAT

Department of Botany, Yashavantrao Chavan Institute of Science, Satara 415 001, Maharashtra, India

*For correspondence. Email: coolshitaldeshmukh@gmail.com

(Received 5 March 2021, revised accepted 29 March 2021)

SUMMARY A monotypic genus *Hardwickia* belongs to the family Fabaceae. *H. binata* is a beautiful tree with huge economic potential. The present karyotypic study revealed chromosome number of 2n = 34 in *H. binata.* The chromosome length in the somatic complement ranges from 1.33 to 2.75 µm with average length being 1.93 µm and the total chromosome length of the haploid complement was 32.89 µm. All chromosomes were metacentric and karyotype is symmetric with 1A type.

Keywords: *Hardwickia binata,* Fabaceae, endemic, karyomorphology.

INTRODUCTION

The genus *Hardwickia* is monotypic (Kotresha & Seetharam 2010). *H. binata,* the sole species of the genus is distributed in India, Indonesia, Iran, Bangladesh, Myanmar, Malaysia, Afghanistan, Pakistan, Nepal, Cambodia, Brunei, Laos, Philippines, Papua New Guinea, Vietnam and Thailand. In India, it is found in the dry savannah forests of the Deccan peninsula, Central India and some parts of Bihar and Uttar Pradesh (Chaturvedi et al. 2017) and also in Nandurbar, Dhule, Jalgaon and Nashik districts in Maharashtra, Dharwad district in Karnataka, North-west Provinces and western peninsula (Cooke 1958).

It is a multipurpose tree, valuable for agro-forestry in dry regions with fodder, timber, manure, fuel wood, fibre and agricultural potentials (Korwar 1994, Kundu & Schmidt 2011). It is a medicinal plant (Ranganathan et al. 2012) categorized under the endemic biodiversity category in India (Vijaya Sankar et al. 2008) with antibacterial and antifungal (Gunaselvi et al. 2010), anticancer and antioxidant properties (Hamid et al. 2018).

A perusal of literature pertaining to cyto-logical studies in *H. binata* are very meagre and fragmentary. Cytological investigation in *H. binata* were initiated by Bir and his associates in early 1970s as a part of their programme of karyotype analyses of Indian legumes. Whereas Sareen & Kumari (1973) reported the chromo-some number of 2n = 34, for *H. binata*, Bir & Kumari (1977) and Kumari & Bir (1989) extended their study into karyomorphology of this species collected from Pachmarhi hills (Central India). Contrary to the earlier reports of 2n = 34, Watson & Dallwitz (1993) reported 2n = 68 for *H. binata* indicating tetraploid condition within a single species. However, Doyle (2012) reported 2n = 34 confirming earlier reports by Kumari & Bir (1989). From these accounts it is clear that *H. binata* exists at diploid (2n = 34) and tetraploid (2n = 68) levels and calls for extensive population studies. The present paper deals with karyomorphology of *H. binata* from Maharashtra.

MATERIAL AND METHODS

Material of *H. binata* was collected from Swatantrapur village of Sangli district, Maharashtra, India. It was authenticated and deposited (DSV 001) in the herbarium of Department of Botany, Shivaji University, Kolhapur, India. The mature, dried, viable and healthy seeds were selected and surface sterilized with 0.1% mercuric chloride for 2 min. These were thoroughly washed in distilled water, then soaked in distilled water for 12 h and placed for germination in petriplates containing moist blotting paper.

The growing root tips of 1 cm length were obtained after 2 d. These were excised and washed rigorously with distilled water and pretreated with saturated aqueous para-dichlorobenzene at 8–10°C for 4 h and again kept in room temperature for 1–2 h. These root tips were again washed in distilled water and hydrolysed in 1N HCl over a spirit lamp flame for 2 min and squashed in 2% propionic-orcein. These squash preparations were observed under light microscope to screen out desired cell plates with proper condensation and well spread chromosomes. Photomicrography of these were taken on LEICA DM 2000 fluorescence microscope with camera attached at 1000 x magnification.

Karyotype analysis was carried out on the basis of 10 cell plates. All chromosomes in the somatic complement have median centromeres. On the basis of their lengths they were categorized into long and short types. The former ranging in length from 2.75 to 2.03 µm and those measuring 1.33 to 1.71 µm are considered short types. First seven pairs (I to VII) come under long category and the rest of the chromosomes (pairs VIII to XVII) of the complement are treated as short types. The assessment of long and short arms were used for estimation of total length (c), difference between long and short arms (d), arm ratio (r), centromeric index (i) and relative length (RL%) whereas total form percentage (TF%) was calculated by using formula given by Huziwara (1962) while gradient index (GI) and symmetry index (SI) were calculated as formulae given by Pritchard (1967). For calculation of karyotype asymmetry viz. intrachromosomal asymmetry (A1), interchromosomal asymmetry (A2) and asymmetry index (AI) were used (Zarco 1986). The nomenclature of chromosomes according to their difference between long and short arms (d), arm ratios (r) and centromeric index (i) was followed as suggested by Levan et al. (1964). For the comparison and analysis of the karyotype, the chromosomes of *H. binata* could be divided on the basis of chromosomes length and centromeric position. The karyotype asymmetry was determined as per Stebbins (1971).

OBSERVATIONS

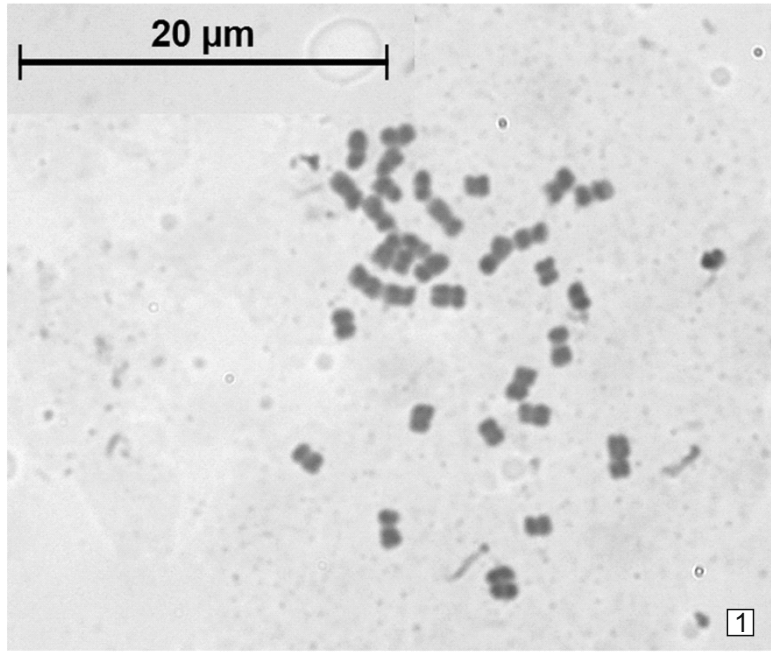
The details of karyomorphology are presented in Table 1 and Figs 1–3.

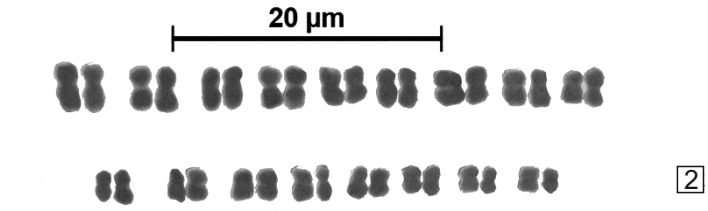
The somatic complement in *H. binata* consists of 34 metacentric chromosomes (Figs 1–3). There are 7 pairs of long chromosomes (chromosome pairs I to VII) and 10 pairs of short chromosomes (chromosome pairs VIII to XVII). The individual chromosome length ranges from 1.33 µm to 2.75 µm with an average length of 1.93 µm and the total chromosome length of the haploid complement (THCL) was 32.89 µm. The difference between long and short arms of chromosomes was found to vary from 0.07 µm to 0.45 µm and average ratio of shortest to longest chromosome was 0.83 µm as well as arm ratios range from 1.1 µm to 1.39 µm. The centromeric index varies from 41.82 to 47.52 with an average

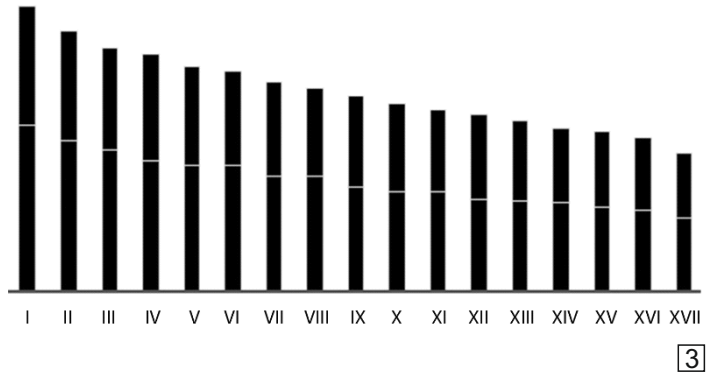
TABLE 1: Karyotype analysis of *H. binata.*

Chrom. pair	L. arm (l) (µm)	S. arm (s) (µm)	Total length (c = l + s) (µm)	d (l–s) (µm)	r (l/s) (µm)	i (s/c × 100)	RL (%)	Centromere position
I	1.6 ± 0.29	1.15 ± 0.16	2.75 ± 0.41	0.45	1.39	41.82	8.36	m
II	1.45 ± 0.36	1.06 ± 0.14	2.51 ± 0.44	0.39	1.37	42.23	7.63	m
III	1.36 ± 0.22	0.98 ± 0.16	2.34 ± 0.33	0.38	1.39	41.88	7.11	m
IV	1.25 ± 0.16	1.03 ± 0.12	2.28 ± 0.24	0.22	1.21	45.18	6.93	m
V	1.21 ± 0.19	0.95 ± 0.12	2.17 ± 0.25	0.26	1.27	43.78	6.6	m
VI	1.21 ± 0.19	0.91 ± 0.09	2.12 ± 0.23	0.3	1.33	42.92	6.45	m
VII	1.1 ± 0.11	0.92 ± 0.09	2.03 ± 0.19	0.18	1.2	45.32	6.17	m
VIII	1.1 ± 0.16	0.86 ± 0.06	1.97 ± 0.2	0.24	1.28	43.65	5.99	m
IX	1 ± 0.12	0.89 ± 0.12	1.89 ± 0.22	0.11	1.12	47.09	5.75	m
X	0.96 ± 0.06	0.85 ± 0.11	1.81 ± 0.16	0.11	1.13	46.96	5.5	m
XI	0.96 ± 0.09	0.79 ± 0.08	1.75 ± 0.14	0.17	1.22	45.14	5.32	m
X11	0.89 ± 0.1	0.81 ± 0.06	1.71 ± 0.14	0.08	1.1	47.37	5.2	m
XIII	0.87 ± 0.11	0.78 ± 0.08	1.65 ± 0.17	0.09	1.12	47.27	5.02	m
XIV	0.85 ± 0.09	0.72 ± 0.08	1.57 ± 0.16	0.13	1.18	45.86	4.77	m
XV	0.81 ± 0.08	0.73 ± 0.08	1.54 ± 0.14	0.08	1.11	47.4	4.68	m
XVI	0.78 ± 0.08	0.7 ± 0.11	1.47 ± 0.17	0.08	1.11	47.52	4.47	m
XVII	0.7 ± 0.09	0.63 ± 0.06	1.33 ± 0.14	0.07	1.11	47.37	4.04	m

m, metacentric; ±, standard deviation.







Figs 1–3 : *H. binata.* 1. Somatic chromosomes. 2. Karyogram. 3. Idiogram.

of 45.22. The average relative length of the longest chromosome was 8.36% while shortest chromosome was 4.04%. The total form per cent (TF) was 44.88 and gradient index (GI) was 48.36%. All chromosomes in the complement possess median centromeres. The karyotype formula was K = 34m. The analysis of karyotype symmetry index (SI %) was 81.55 while karyotype asymmetry index showed 0.17 and 0.2 for intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) respectively.

DISCUSSION

The present report of 2n = 34 in *H. binata* is in conformity with the earlier findings of Sareen & Kumari (1973), Bir & Kumari (1977), Kumari & Bir (1989) and Doyle (2012). While investigating a population from Pachmarhi hills with 2n = 34, 12 metacentric, 20 submetacentric and secondary constrictions in 2 chromosomes at the complement were reported (Kumari & Bir 1989). In the present study, no secondary constrictions were observed and all chromosomes were metacentric. According to Kumari & Bir (1989) the karyotype is categorized under 2B; however, in the present study, it conforms to 1A type. However, in respect of other karyological features the present findings are more or less in agreement with those of Kumari & Bir (1989).

In the present study, all chromosomes in *H. binata* are metacentric as reported in *Flemingia nilgheriensis* (Lekhak et al. 2011*), Nesphostylis bracteata* (Bagane et al. 2014)*, Nogra dalzellii* (Gavade et al. 2015) and *Mucuna* species (Gaikwad et al. 2017). In higher plants, karyotype evolution pattern is generally from symmetry to asymmetry Stebbins (1971). According to Stebbins (1950) the higher percentage of metacentric chromosomes indicate primitiveness of a species. Thus, the *H. binata* represent a primitive pattern of karyotype in the scale of evolution.

ACKNOWLEDGEMENTS

Authors are thankful to the Head, Department of Botany, Shivaji University, Kolhapur; Principal as well as Head, Department of Botany, Yashavantrao Chavan Institute of Science, Satara for providing laboratory facilities. The authors are also indebted to Dr. M. M. Lekhak and Dr. N. B. Gaikwad for continuous help and encouragement. Thanks are also due to Miss. Pradnya Yadav, Mr. Suraj Patil, Mr. Pradeep Deshmukh and Miss. Rupali Chougule for necessary help and cooperation.

REFERENCES

BAGANE S K, KATTEE A V, SARDESAI M M, LEKHAK M M & YADAV S R 2014 Mitotic chromosome studies in *Nesphostylis bracteata* an endemic legume from Western Ghats *Cytologia* **79** 59–62

BIR S S & KUMARI S 1977 Evolutionary status of leguminosae from Pachmarhi Central India *Nucleus* **20** 94–98

CHATURVEDI O P, HANDA A K, UTHAPPA A R, SRIDHAR K B, NARESH KUMAR, CHAVAN S B & RIZVI J 2017 *Promising agroforestry tree species in India* Central Agroforestry Research Institute (CAFRI) Jhansi South Asia Regional Programme based in Delhi India of the World Agroforestry Research Centre (ICRAF) pp 116–122

COOKE T 1958 *The Flora of the Presidency of Bombay* **vol I** Botanical Survey of India Calcutta

DOYLE J J 2012 Polyploidy in legumes *In* Pamela S S & Douglas E S (eds) *Polyploidy and genome evolution* Springer New York pp 147–180

GAIKWAD S V, GURAV R V & YADAV S R 2017 Karyotype studies in *Mucuna macrocarpa* Wall and *Mucuna sanjappae* Aitawade et Yadav (Fabaceae) from India *Chrom Bot* **12** 52–55

GAVADE S K, LEKHAK M M & YADAV S R 2015 Taxonomy and karyology of *Nogra dalzellii* (Baker) Merr (Leguminosae: Papilionoideae) a little-known Indian legume *Webbia J Pl Taxon Geogr* **70** 1–6

GUNASELVI G, KULASEKAREN V & GOPAL V 2010 Antibacterial and antifungal activity of various leaves extracts of *Hardwickia binata* Roxb (Caesalpiniaceae) *Int J Pharm Tech Res* **2** 2183–2187

HAMID S Y, ELEGAMI A E, KOKO W S, ABDELWAHAB S I & BOSTMAN A 2018 Anticancer and antioxidant activity of five Sudanese medicinal plants from the family Fabaceae *J Fac Sci* **5** 153–175

HUZIWARA Y 1962 Karyotype analysis in some genera of compositae VIII Further studies on the chromosomes of *Aster Am J Bot* **49** 116–119

KORWAR G R 1994 *Hardwickia binata*- a promising MPTS for agroforestry in dry land areas *In* Haregde N G & Daniel J N (eds) *Multipurpose tree species for agro forestry in India* BAIF Development Research Foundation Pune pp 72–75

KOTRESHA K & SEETHARAM Y N 2010 Biosystematics of *Bauhinia hookeri* Muell and *Hardwickia binata* Roxb *Ind Forester* **136** 821–826

KUMARI S & BIR S S 1989 Karyomorphological evolution in Caesalpiniaceae *J Cytol Genet* **24** 149–163

KUNDU M & SCHMIDT L H 2011 *Hardwickia binata* Roxb *Seed Leaflet* No 152 Forest and Landscape Denmark University of Copenhagen

LEKHAK M M, NANDIKAR M D & YADAV S R 2011 Karyomorphology of *Flemingia nilgheriensis* (Baker) Wight ex T Cooke: An endemic from Western Ghats *Cytologia* **76** 243–248

LEVAN A, FREDGA K & SANDBERG A A 1964 Nomenclature for centromeric position on chromosomes *Hereditas* **52** 201–220

PRITCHARD A J 1967 The somatic chromosomes of *Trifolium cherleri* L *T hirtum* All *T ligusticum* Balb and T *scabrum* L *Caryologia* **20** 323–331

RANGANATHAN R, VIJAYALAKSHMI R & PARAMESWARI P 2012 Ethnomedicinal survey of Jawadhu hills in Tamil Nadu India *Asian J Pharma Clin Res* **5** 45-49

SAREEN T S & KUMARI S 1973 IOPB chromosome number reports XLII *Taxon* **22** 647–654

STEBBINS G L 1950 *Variation and evolution in plants* Columbia University Press New York

STEBBINS G L 1971 *Chromosomal evolution in higher plants* Edward Arnold London

VIJAYA SANKAR R, RAVIKUMAR K & GORAYA G S 2008 Floristic wealth of Jawadhu hills Eastern Ghats with special emphasis on threatened plants *In* Rawat G S (ed) *Special habitats and threatened plants of India* ENVIS Bulletin and Protected Areas Wildlife vol **11** Wildlife Institute of India Dehradun India pp187–193

WATSON L & DALLWITZ M J 1993 The genera of Leguminosae- Caesalpinioideae and Swartzieae: Descriptions Identification and Information Retrieval in English and French Version 29th May 2015 Australia

ZARCO R C 1986 A new method for estimating karyotype asymmetry *Taxon* **35** 526–530

Journal of Cytology and Genetics 2021 VOL. **22** (NS): 7−12

RESEARCH ARTICLE

KARYOMORPHOLOGICAL STUDIES IN FOUR ENDEMIC SPECIES OF *CROTALARIA* (FABACEAE) FROM INDIA

K. H. ROKADE AND N. B. GAIKWAD*
Department of Botany, Shivaji University, Kolhapur 416 004, Maharashtra, India
*For correspondence. Email: nbgaikwadsuk@gmail.com

(Received 7 July 2021, revised accepted 25 July 2021)

SUMMARY The karyotype analysis was carried out in *Crotalaria clarkei, C. filipes* var. *trichophora, C. globosa* and *C. vestita* which are endemic to India. All species studied here have the diploid chromosome number of 16. In *C. globosa*, the total chromosome length (TCL) of 20.59 µm was highest with mean chromosome length (MCL) of 2.69 µm. The minimum TCL of 12.14 µm was found in *C. vestita* with mean chromosome length of 1.51 µm. Among the four species, karyotype of *C. globosa* is comparatively asymmetrical while remaining three have symmetrical karyotypes. The karyotypes in all the species conform to 1A category.

Keywords: *Crotalaria*, endemic, karyotype.

INTRODUCTION

Crotalaria comprises c. 700 species distributed worldwide (Yaradua 2018). It is highly diversified, and found in open places, plains, hilly regions, along forest margins and grasslands. In India, it is represented by 102 species, 3 subspecies, 19 varieties, and 4 formae (Ansari & Chauhan 2020). Among them, 47 species, 2 subspecies, 12 varieties and 2 formae are endemic to India. Many species of *Crotalaria* are used as important source of alkaloids, paper pulp, fibres, ornamentals and green manure.

Chromosome counts and karyotype analysis of Indian species of *Crotalaria* were carried out by many workers (Bhaumik 1975, Chennaveeraiah & Patil 1972,1973, Datta & Biswas 1963, Gupta & Gupta 1978, Mangotra & Koul 1991, Raina & Verma 1979, Verma et al. 1984). Kumar & Subramanyam (1987) enumerated chromosome numbers of 57 Indian species. *Crotalaria* is a dibasic genus with x = 7 and 8, the latter being the most frequent (Almada et al. 2006). Most species are diploids with 2n = 16, while some are with 2n = 14, 32, 48 and 64 on basic number of 7 and 8 (Ansari 2008, Boulter et al. 1970, Gupta & Gupta 1978, Koul et al. 2000, Mangotra & Koul 1991). The genus shows a distinct uniformity in chromosome size, symmetry and morphology with very similar karyotypes (Fernando et al. 2005). In *Crotalaria,* majority of chromosomes are metacentric or submetacentric (Gupta & Gupta 1978). Most of the endemic species from India are yet to be investigated for chromosome counts and karyotypic study. For understanding the cytotaxonomical relationships amongst *Crotalaria* species, karyotype studies are important. The present study was focused on chromosome count and karyotype analysis of four species of *Crotalaria* viz., *C. clarkei* Gamble*, C. filipes* var. *trichophora* (Benth. ex

Baker) Cooke, *C. globosa* Wight and *C. vestita* Baker, which have hitherto remained un-investigated.

MATERIALS AND METHODS

Seeds were collected from plants grown in natural habitats from various localities of Maharashtra (Radhanagari), Karnataka (Chitradurga) and Kerala (Wayanad) states. For cytological study, seeds were treated with conc. sulphuric acid for 15–20 min followed by a thorough washing in running water and germinated on moist blotting paper. Healthy root tips were pre-treated with aqueous saturated solution of para-dichlorobenzene for 3 h at room temperature. For squash preparation, root tips were hydrolysed in 1N HCL for 10–15 min at 60°C and stained in 2% aceto-orcein. Photomicrographs were taken with a Zeiss microscope at 1000 x magnification. The karyotypic analysis was done with 10 well spread mitotic plates. Nomenclature of chromosomes was done according to Levan et al. (1964). Different methods of evaluating karyotype asymmetry such as the coefficient of variation in chromosome length $CV_{CL,}$ the coefficient of variation of the centromeric index CV_{CI}, asymmetry index AI and the total form per cent TF% were used (Paszko 2006). Stebbins' (1971) classification was employed to determine karyotype symmetries. Instead of traditional measurement of metaphase spreads, *IdeoKar* software was used for calculation of chromosomal and karyotype parameters to build ideograms (Mirzaghaderi & Marzangi 2015).

OBSERVATIONS

All 4 species have somatic chromosome number of 16.

C. clarkei

The somatic complement has 8 pairs of m-type chromosomes (Figs 1–3). The chromosome length varies from 1.44 to 2.31 µm with a longest / smallest ratio of 1.6 and total length of haploid set is 14.43 µm. (Table 1).

C. filipes var. *trichophora*

This species has somatic complement with 8 pairs of m-type chromosomes (Figs 4–6). The chromosome length varies from 1.32 to 2.47 µm with a longest/smallest ratio of 1.86. The total length of haploid set is 13.60 µm (Table 1).

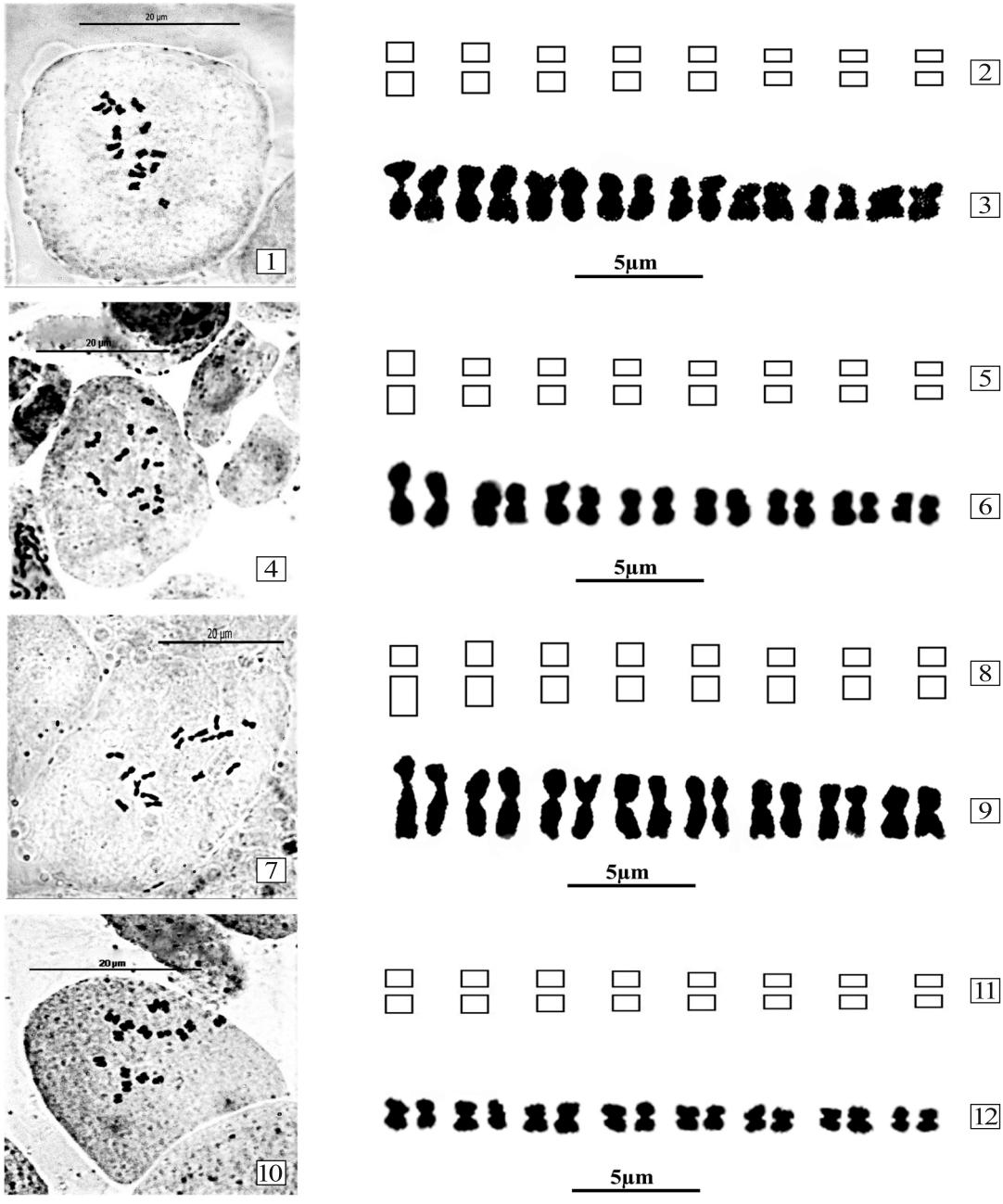
C. globosa

The diploid complement consists of 1 pair of sm-type and 7 pairs of m-type chromosomes (Figs 7–9). The chromosome length ranges from 2.19 to 3.17 µm with a longest/smallest ratio of 1.44 and the total length of haploid set is 20.59 µm. (Table 1).

C. vestita

All 16 chromosomes in the complement are of m-type (Figs 10–12). The chromosome length varies from 1.25 to 1.76 µm with a longest/smallest ratio of 1.40. The total length of haploid set is 12.14 µm (Table 1).

The longest chromosome pair of 3.17 µm was observed in *C. globosa* while shortest chromosome pair of 1.25 µm was found in *C. vestita*. *C. globosa* has the maximum mean chromosome length (MCL) of 2.69 µm and highest total chromosome length (TCL) of 20.59 µm, while minimum MCL of 1.51µm and TCL of 12.14 µm was observed in *C. vestita. C. clarkei* and *C. filipes* var. *trichophora* had intermediate values between *C. globosa* and *C.vestita.* The karyotypes of all the four species come under 1A category (Stebbins 1971). *C. filipes* var. *trichophora* has highest CV_{CL} value and lowest CV_{CL} value was found in *C. vestita*. Highest CV_{CI} and AI values are reported in *C. globosa* while



Figs 1–12: 1–3. *Crotalaria clarkei.*1. Somatic chromosomes. 2. Ideogram. 3. Karyogram. 4–6. *C. filipes* var. *trichophora.* 4. Somatic chromosomes. 5. Ideogram. 6. Karyogram. 7–9. *C. globosa.*7. Somatic chromosomes. 8. Ideogram. 9. Karyogram. 10–12. *C. vestita.* 10. Somatic chromosomes. 11. Ideogram. 12. Karyogram.

TABLE 1: Karyotype data for *C. clarkei*, *C. filipes* var. *trichophora*, *C. globosa* and *C. vestita*.

Taxon	Long arm Mean ± SD (μm)	Short arm Mean ± SD (μm)	TCL (μm)	MCL (μm)	r ± SD (μm)	R
C. clarkei	0.97 ± 0.19	0.82 ± 0.13	14.43	1.80	0.85 ± 0.04	1.60
C. filipes var. *trichophora*	0.90 ± 0.18	0.79 ± 0.16	13.60	1.70	0.88 ± 0.07	1.86
C. globosa	1.44 ± 0.28	1.25 ± 0.31	20.59	2.69	0.79 ± 0.12	1.44
C. vestita	0.77 ± 0.09	0.74 ± 0.09	12.14	1.51	0.96 ± 0.03	1.40

SD – standard deviation, TCL– total chromosome length of the complement, MCL– mean chromosome length of the complement, r – mean arm ratio, R– ratio between the largest and the smallest chromosomes of the complement.

TABLE 2**:** Evaluation of karyotype asymmetry in *Crotalaria* species using different methods.

Taxon	2n	Karyotype formula	CV_{CL}	CV_{CI}	AI	TF%	Karyotype category
C. clarkei	16	8m	18.13	3.15	0.57	45.83	1A
C. filipes var. *trichophora*	16	8m	20.13	4.22	0.84	46.76	1A
C. globosa	16	1sm + 7m	13.11	10.40	1.36	43.87	1A
C. vestita	16	8m	12.54	1.34	0.16	48.85	1A

CV_{CL} – The coefficient of variation in chromosome length, CV_{CI} – The coefficient of variation of the centromeric index, AI – Asymmetry index, TF% – The total form per cent.

lowest was found in *C. vestita*, whereas TF% value was highest (48.85%) in *C. vestita* while it is lowest (43.87%) in *C. globosa* (Table 2).

DISCUSSION

Crotalaria has two basic chromosome numbers i.e., x = 7 and 8; x = 7 is found in very few species such as *C. incana* with 2n =14 (Gupta & Gupta 1978). Despite several studies on karyomorphology of Indian *Crotalaria* in the past, chromosome numbers are known only for 28% of the taxa and are mostly based on x = 8 (Almada et. al 2006). In India, *Crotalaria* is represented by six sections with 102 species 3 subspecies, 19 varieties, and 4 formae. *Calycinae* to which the present species belong is the largest section comprising 47 species, 16 varieties and 4 formae of these 21 species 10 varieties and 2 formae are endemic. Under *Calycinae*, karyotypes of 14 species 7 varieties and 3 formae have not been cytologically evaluated. According to Almada et al. (2006) and Windler (1974) the section *Calycinae* is characterised by the presence of a large number of polyploid species. In India, the section shows two polyploid species namely, *C. ferruginea* (2n = 48) (Mangotra & Koul 1979, 1991) and *C. juncea* (2n = 32, 64) (Ansari 2008, Bhaumik 1975, Koul et al. 2000) while remaining species have 2n = 16. The species analysed in the present investigation have 2n = 16, as reported in

other species by Kumari & Bir (1990) and Mangotra & Kaul (1991).

The genus shows uniformity in chromosomes as well as sufficient interspecific karyotypic differences that allow for a species characterization (Fernando et al. 2005). The total chromosome length of haploid (TCLH) is real determinant rather than the chromosome numbers as most of the species of *Crotalaria* show consistent diploid chromosome number of 16 (Kumari & Bir 1990). In the most specialized sections of the genus *Crotalaria*, karyotype evolution points to a chromosome reduction and lower karyotype symmetry index (Boulter et al. 1970, Oliveira & Aguiar-Perecin 1999). Gupta & Gupta (1978) analysed karyotypes of 27 species of the genus *Crotalaria* and they found that karyotype asymmetry has a low order. Chromosomes having median centromeres are primitive than chromosomes with arms of unequal length and so the symmetrical karyotypes are more primitive than the asymmetrical ones (Vimala et al. 2021). Karyomorphological study of eight species of *Crotalaria* was carried out by Chennaveeraiah & Patil (1973). They reported that symmetrical karyotypes are found in species having simple leaves, suggesting that these species are primitive. The four species investigated here are unifoliate and showed predominance of chromosomes with the centromere in a median position showing gradual decrease in length from largest to the smallest pair.

For the evaluation of karyotype asymmetry and heterogeneity many authors have used the parameters viz., CV_{CL}, CV_{CI}, AI and TF%. These parameters showed greatest sensitivity in characterising chromosome morphology (Eshetu et al. 2013, Lavand et al. 2019, Paszko 2006, Sharma et al. 2012). The relative variation in chromosome length in a complement is assessed by CV_{CL} parameter. Highest value of CV_{CL} was found in *C. filipes* var. *trichophora* and lowest CV_{CL} was observed in *C. vestita* (Table 2). The relative variation in centromere position in a chromosome set can be evaluated by using coefficient of variation for the centromeric index CV_{CI}. *C. globosa* has highest value of CV_{CI}, while *C. vestita* is characterised by lowest value of CV_{CI} (Table 2). The asymmetry index (AI) gives a measure of the heterogeneity of chromosome length and centromeric position in a karyotype. Increase in value of AI is associated with karyotype asymmetry and if the AI value decreased it indicates greater karyotype symmetry (Paszko 2006). *C. globosa* has highest AI value and represents a comparatively asymmetrical karyotype, while *C. vestita* has lowest AI value and represents a symmetric karyotype (Table 2). TF% is used to describe karyotype asymmetry and to determine karyotypic relationship between species. *C. vestita* has highest TF% value and *C. globosa* shows lowest TF% value. Based upon the TF% values it is suggested that except *C. globosa*, karyotypes of remaining species are symmetric. Our findings are in concurrence with those of Boro & Das (2020). According to Stebbins' (1971) classification, karyotypes of these four species fall under 1A category. On the basis of available information, most of the species of *Crotalaria* including the present ones have symmetrical karyotypes having metacentric chromosomes.

ACKNOWLEDGEMENT

The authors are grateful to the Head, Department of Botany, Shivaji University, Kolhapur for providing necessary facilities.

REFERENCES

ALMADA R D, DAVINA J R & SEIJO J G 2006 Karyotype analysis and chromosome evolution in southernmost South American species of *Crotalaria* (Leguminosae) *Bot J Linn Soc* **150** 329–341

ANSARI A A 2008 *Crotalaria L in India* Bishen Singh Mahendra Pal Singh Deharadun

ANSARI A A & CHAUHAN V 2020 *Crotalaria L in India: A Supplement* Bishen Singh Mahendra Pal Singh Deharadun

BHAUMIK G H 1975 Chromosome studies of some Indian species of *Crotalaria Sci & Cult* **41** 521–523

BORO N & DAS B N 2020 Karyomorphology in two species of the genus *Phlogacanthus* Nees of Assam: some new karyological insights *J Genet* **99** 1–5

BOULTER D, DERBYSHIRE E, FRAHM-LELIVELD J A & POLHILL R M 1970 Observations on the cytology and seed proteins of various African species of *Crotalaria* L (Leguminosae) *New Phytol* **69** 117–131

CHENNAVEERAIAH M S & PATIL B C 1972 Chromosome number in the genus *Crotalaria* Linn *Proc Indian Sci Congr Assoc 59 (III)* pp 351–352

CHENNAVEERAIAH M S & PATIL B C 1973 Chromosome number and karyotype study in eight species of *Crotalaria Cytologia* **38** 73–79

DATTA R M & BISWAS P K 1963 Karyotypic study in the genus *Crotalaria Caryologia* **16** 701–705

ESHETU F, KIFLE D, NINA R, SEBSEBE D & OLWEN G 2013 Karyotypes in Ethiopian *Aloe* species (Xanthorrhoeaceae): Asphodeloideae) *Kew Bull* **68** 599–607

FERNANDO T P, ELOIR G P, CLAUDIA T P & PEDRO M R 2005 New cytogenetic information of two populations of *Crotalaria incana* L (Leguminosae-Papilinoidae) *Cytologia* **70** 207–212

GUPTA R & GUPTA P K 1978 Karyotypic studies in the genus *Crotalaria* L *Cytologia* **43** 357–369

KOUL K K, NAGPAL R & SHARMA A 2000 Temperature influenced variation in the chromosomal behaviour of male and female sex cells in Sunn hemp (*Crotalaria juncea* Linn Fabaceae) *Caryologia* **53** 113–120

KUMAR V & SUBRAMANYAM B 1987 *Chromosome atlas of flowering plants of the Indian subcontinent: dicotyledons* Botanical Survey of India Calcutta

KUMARI S & BIR S S 1990 Karyomorphological evolution in Papilionaceae *J Cytol Genet* **25** 173–219

LAVAND P R, GAIKWAD S V, GURAV R V & SHIMPALE V B 2019 Karyomorphological studies in three species of *Argyreia* Lour (Convolvulaceae) from India *Nucleus* **62** 71–75

LEVAN A, FREDGA K & SANDBERG A 1964 Nomenclature for centromeric positions on chromosomes *Hereditas* **52** 201–220

MANGOTRA R & KOUL A K 1979 Chromosome number in *Crotalaria ferruginea* Grah ex Benth *Sci Cult* **45** 252

MANGOTRA R & KOUL A K 1991 Polyploidy in genus *Crotalaria Cytologia* **56** 293–296

MANGOTRA R & KOUL A K 1991 Base number in genus *Crotalaria*–evidences from meiosis *Nucleus* **34** 158–161

MIRZAGHADERI G & MARZANGI K 2015 Ideokar: an ideogram constructing and karyotype analyzing software *Caryologia* **68** 31–35

OLIVEIRA A L P & AGUIAR–PERECIN M L R 1999 Karyotype evolution in the genus *Crotalaria* (Leguminosae) *Cytologia* **64** 165–174

PASZKO B 2006 A critical review and a new proposal of karyotype asymmetry indices *Plant Syst Evol* **258** 39–48

RAINA S N & VERMA R C 1979 Cytogenetics of *Crotalaria* 1 Mitotic complements in twenty species of *Crotalaria* L *Cytologia* **44** 365–375

SHARMA S K, KUMARIA S, TANDON P & SATYAWADA R R 2012 Comparative karyomorphological study of some Indian *Cymbidium* Swartz 1799 (Cymbidieae Orchidaceae) *Comp Cytogenet* **6** 453–465

STEBBINS G L 1971 *Chromosomal evolution in higher plants* Edward Arnold London

VERMA R C, KESAVACHARYULU K & RAINA S N 1984 Cytogenetics of *Crotalaria* IX Mitotic complements in 19 species *Cytologia* **49** 157–169

VIMALA Y, LAVANIA S & LAVANIA U C 2021 Chromosome change and karyotype differentiation –implications in speciation and plant systematics *Nucleus* **64** 33–54

WINDLER D R 1974 Chromosome numbers for native North American unifoliolate species of *Crotalaria* (Leguminosae) *Brittonia* **26** 172–176

YARADUA S S 2018 A review of the genus *Crotalaria* L (Crotalarieae Fabaceae) *IJSRP* **8** 316–321

Journal of Cytology and Genetics 2021 VOL. **22** (NS): 13−20

RESEARCH ARTICLE

IMPACT OF CONSANGUINITY ON FAMILY CLUSTERING OF MALE INFERTILITY IN SOUTH KARNATAKA

G. SREENIVASA [1], P. T. CHAITHRA [2] AND MALINI S. SUTTUR[3]*

[1]Department of Studies in Zoology, Davangere University, Davangere 577 007

[2]Department of Genetics and Genomics, University of Mysore, Manasagangotri, Mysuru 570 006

[3]Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysuru 570 006

*For correspondence. Email:ssmalinisri@yahoo.co.in

(Received 2 July 2021, revised accepted 30 July 2021)

SUMMARY Consangunious marriages are strongly favoured in the state of Karnataka amounting to about 30% and the phenomenon is associated with sterility, stillbirth, prenatal losses and genetic disorders. Male infertility results from multifactorial etiology which is one of the frequently associated factors of consanguinity. This study is an attempt to understand the impact of consanguinity on infertility in the maternal and paternal families of the proband. The present study covers 34 (14.7%) of infertile male subjects with consangunious marriages out of 231, and 51 (22.07%) infertile males with history of parental consanguinity. The result of the study displays the statistical association of azoospermic, oligospermic and teratozoosperic conditions with consanguinity and family history of infertility. It is evident that both parental and proband consanguinity are significant ($p < 0.05$) and is suggested that family based investigation plays a key role for ruling out the molecular mechanism in causing the abnormal sperm characters.

Keywords: Consanguinity, male infertility, sperm characters.

INTRODUCTION

Consanguineous marriages are a common practice from ages in many parts of the world with variable rates specific to ethnicity, geographical region, religion and culture (Jaber et al. 1998). Consanguinity has been regularly practised in the state of Karnataka, where the frequency is around 30%. Due to the recent advances in the demographic and socio-economic conditions, consanguinity has decreased in some parts of South India except Karnataka (Krishnamurthy & Adinarayana 2001). Association of consanguinity with increased sterility, higher rate of abortions, stillbirths, prenatal losses, neonatal death and congenital anomalies has been reported by earlier studies (Bromiker 2004, Hann 1985, Jaber et al. 1998, Kulkarni & Kurian 1990). With the ongoing global epidemiological transition from communicable to non-communicable diseases, consanguineous marriage bagged considerable attention, and considered as a major etiological factor in the prevalence of genetic disorders (Bittle 2005).

Infertility, a multi-factorial disorder is the inability of a sexually active, non-contraceptive couple to achieve pregnancy in one year, and

approximately 15% of the couples are confronted with inability to conceive after 2 y of unprotected intercourse (World Health Organization 1999). It is a worldwide problem affecting people of all communities, though the cause and magnitude vary with geographical location and socio-economic status. However, because of the advances in the field of genetics, it is now realized that a significant percentage of male infertility cases, particularly those with severe pathological conditions, are due to genetic abnormalities. In the majority of cases, infertility is due to the inability of the male partner to produce spermatozoa of sufficient number (oligozoospermia), with adequate motility (asthenozoospermia), or normal morphology (teratozoospermia) or because of combinations of all these defects.

Earlier studies have reported that several genes are involved in regulating spermatogenesis which are located on the Y chromosome. The most frequent molecular genetic cause of infertility in man involves microdeletion of the long arm of the Y chromosome, which is associated with spermatogenic failure (Chan 2007, Maduro 2002). Such deletions are manifested in a variety of defects with respect to sperm morphology, including defects of the sperm head and sperm tail (Baccetti et al. 2001). A few of the conditions, such as azoospermia, oligospermia, asthenospermia or morphological defects may be heritable, and they may cluster in families and communities depending upon the level of consanguineous marriages in the general population (Fuster 2003, Helgason 2008). Recent studies suggest that consanguinity is highly correlated with rare genetic sperm defects. (Bacetti et al. 2001, Latini et al. 2004). These include a wide range of syndromes that have high impact on sperm morphology and motility which may be transmissible to the male offspring. It is considering this that the present study was carried out aiming at analysing the pattern of inheritance, influence of consanguinity on male infertility and family clustering among the families of infertile males from Mysuru.

MATERIALS AND METHODS

Ethical clearance was obtained from the University of Mysore and the concerned health authorities as well as consent from the control group for the present double blinded study carried out in the Department of Studies in Zoology, University of Mysore, Mysuru, Karnataka, India. The study was conducted during 2008-2012, Semen samples of 231 confirmed infertile subjects were collected from different IVF centers, hospitals and clinics in Mysuru. The routine analysis of semen profile included measurement of volume, pH, sperm count, sperm motility and sperm morphology. The spermeogram was conducted in the study group as per the WHO guidelines. Genetic registry was collected from the subjects, which includes family and reproductive history and life style factors. Pedigrees were constructed by using progeny software (version 6).

OBSERVATIONS

Based on the sperm character/profile, the infertile subjects were categorized into 7 subgroups wherein 27.2% were azoospermic, which represents the highest number of cases recorded in the present study, followed by teratozoospermia (17.7%), and idiopathic condition (2.1%) which is the least recorded (Table 1). Further, 14.7% of

TABLE 1: Distribution of different infertile subgroups with respect to sperm characters and different degrees of consanguineous marriages.

Infertile conditions	No. of subjects (%)	No. of proband consanguinity	No. of parental consanguinity	No. of pedigrees showing both parental and proband consanguinity
Oligospermia	76 (23.9)	9	20	3
Azooapermia	63 (27.2)	12	11	2
Teratozoospermia	41 (17.7)	7	8	4
Asthenospermia	20 (8.6)	0	4	0
Aspermia	12 (51)	3	4	2
Oligoasthenoterato-zoospermia	13 (5.6)	2	4	1
Idiopathic	6 (2.5)	1	0	0
Total (N=231)	231	34(14.7%)	51(22.07%)	12 (5.19%)

TABLE 2: Family history and consanguineous marriages in 231 patients with male infertility.

Family information	Subjects (231)		Control (100)		Odds ratio (lower; upper)	P value
	No.	(%)	No.	(%)		
Proband consanguinity	34	14.7	4	4	0.241 (0.83; 0.070)	0.001*
Parental consanguinity	51	22.07	10	1 0	0.392 (0.190; 0.809)	0.011*
Both proband and their parental consanguinity	12	5.19	2	2	0.372 (0.082; 1.690)	0.202
Paternal history of infertility	10	0.3	2	2	0.451 (0.97; 2.097)	0.310
Maternal history of infertility	4	1.7	1	1	0.573 (0.063; 5.194)	0.421
Sibling family history of infertility	2	0.8	0	0	0.001 (0.01; 0.01)	0.997

*P is significant at 0.05 levels.

TABLE 3: Families showing infertile history.

Infertile condition	No. of paternal families having history of infertility	No. of maternal families having history of infertility	No. of families having abortion/stillbirth	No. of siblings with infertility
Oligospermia	4	2	5	1
Azooapermia	1	0	2	0
Teratozoospermia	4	1	8	1
Asthenospermia	0	0	1	0
Aspermia	0	0	0	0
Oligoasthenoterato-zoospermia	0	1	0	0
Idiopathic	1	0	0	0
Total (N = 231)	10 (4.3%)	4 (1.7%)	16 (6.9%)	(0.86%)

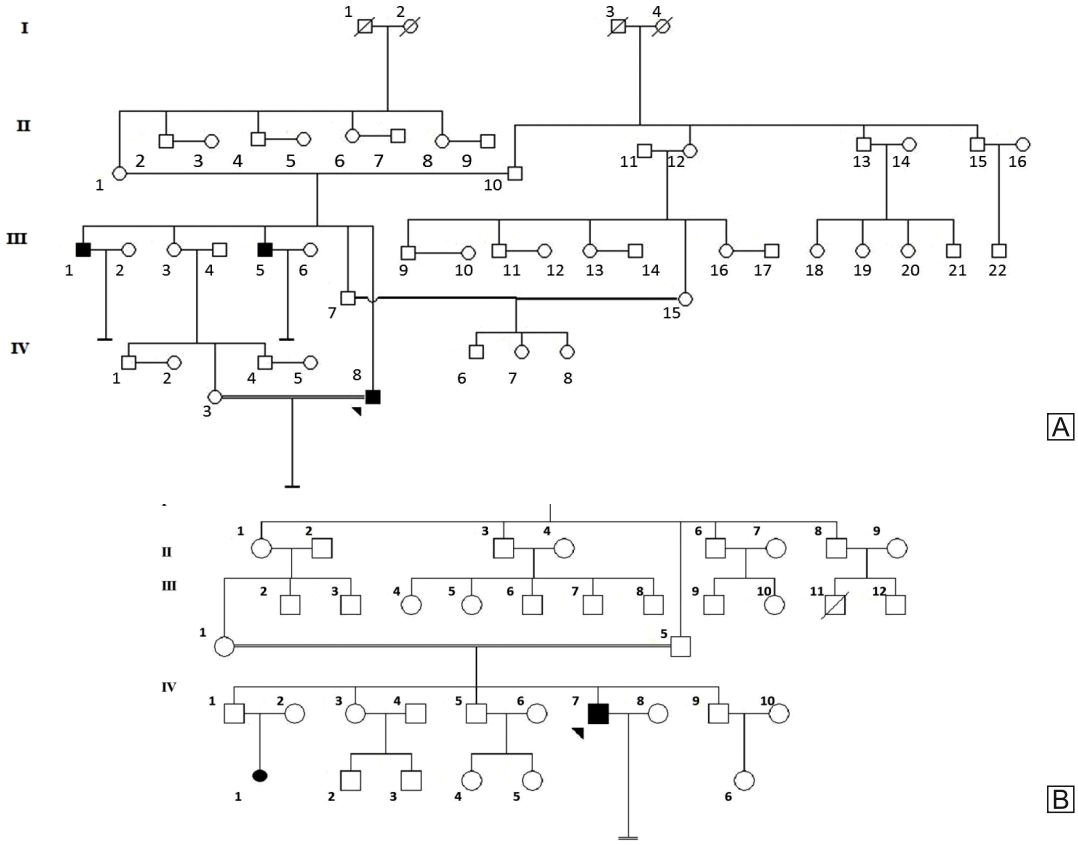


Fig. 1: Pedigrees of families of infertile men (A) Proband with consanguineous marriage and having family history of infertility (B) Parental consanguinity. Roman numbers on left side of the figure indicate the number of generations; Arabic numbers below the symbols denote the number of individuals in the generation. The filled arrow symbol represents the proband.

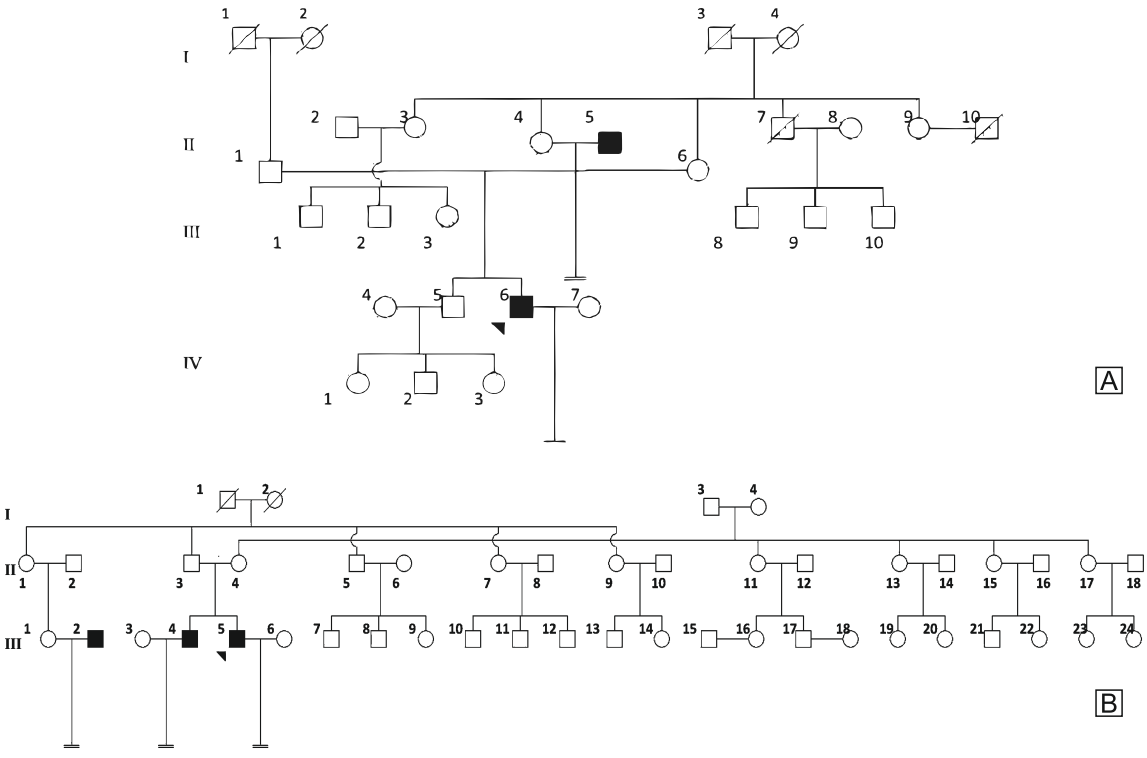


Fig. 2: Pedigrees of families of infertile male. (A) Proband without consanguineous marriage and having family history of infertility (B) Infertile siblings. Roman numbers on left side of the figure indicate the number of generations; Arabic numbers below the symbols denote the number of individuals in the generation. The filled arrow symbol represents the proband.

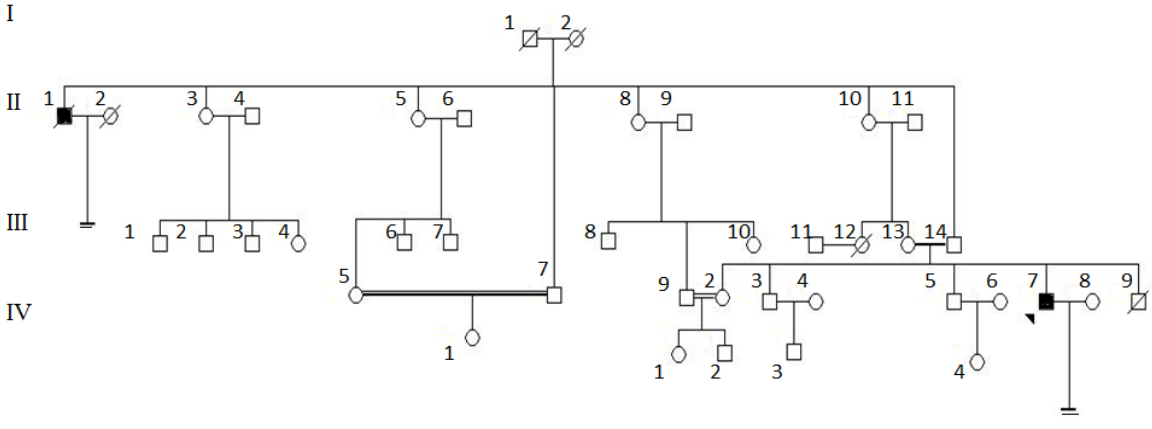


Fig. 3: Pedigrees of families of infertile men showing family history of infertility in parental consanguinity. Roman numbers on the left side of the figure indicate the number of generations; Arabic numbers below the symbols denote the number of individuals in the generation. The filled arrow symbol represents the proband.

the probands depicted consanguinous marriages among which the frequency of subjects with azoospermic condition was the highest (12%) followed by oligospermia (9%), teratozoospermia (7%). In the study group, 22.07% of the families represented history of parental consanguinity. The frequency of parental consanguinity with respect to motility, was observed in azoospermic (11), oligospermic (20) and teratozoospermic (8) cases. Parental as well as proband consanguinity were observed in 5.91% of the families, while in the idiopathic subjects, parental consanguinity was not documented. Family history of infertility and its association with consanguinity with respect to control subjects are depicted in Table 2. The infertile proband and parental consanguinity demonstrated significant odd ratio for the association of consanguinity and the clustering of infertility or infertile conditions in the family. The maternal and paternal family history of infertility with respect to proband infertile conditions are given in Table 3. The family history of male infertility and the pattern of inheritance with respect to consanguineous and nonconsanguineous marriages are depicted in Figs 1–3.

DISCUSSION

The population of India is unique in its size and in the level of subdivision, with 15 major languages and six main religions (Bhasin et al. 1992). The Indo-European speaking Hindu people in the northern states avoid marital unions between biological kins, because of prohibition on consanguineous marriages believed to exist from the very distant past of 200 BC (Kapadia 1976, Sanghvi 1966). But, in South India, there is a long tradition of uncle-niece marriage and union between a man and his maternal uncle's daughter (Sastri 1976). Male infertility in general is a multi-factorial etiology, and different sperm morphological conditions tend to cluster in families. The multi-factorial etiology involves the effect of environmental toxins, including pesticides, diesel and petroleum exhaust and heavy metals. Furthermore, systemic disorders, like hypothalamic-pituitary disease, testicular cancers, and germ cell aplasia and genetic factors including aneuploidies and single-gene mutations are the major etiological factors of infertility. Previous studies provide data with respect to positive association between consanguinity and male factor infertility and diverse inheritance patterns (Gianotten et al. 2004, van Golde et al. 2004). However, the present results strongly correlate positive association between consanguineous marriages and male infertility (Table 3).The present study also reveals that the affected proband with oligospermic, azoospermic and teratozoospermic conditions to be due to high level of parental consanguinity, followed by the condition which is similar to the findings quoted by Anika et al. (2007). Globozoospermia is a rare form of teratozoospermia with the incidence of < 0.1% in male infertile patients, which is mainly characterized by round-headed spermatozoa that lack an acrosome, which originates from disturbed spermatogenesis which is suspected to be induced by a genetic factor. It results from the homozygous mutation in the spermatogenesis-specific gene *SPATA16* non-syndromic male infertility condition in humans, caused by an autosomal gene defect, and this could also mean that the identification of other partners like *SPATA16* could elucidate acrosome formation.

A few of the pedigrees depicting male infertility conditions among the siblings which might have occurred due to variation in the

genetic material especially located on the Y chromosome. The human Y chromosome harbours genes meant for normal spermatogenic activities such as *SRY, AZF, RBM, DAZ, USP9Y, TSPY, DFFRY, CREM, MIS, UTY (* Xiao-Wei et al. 2002). Microdeletion in the Y chromosome causes impairment in testicular development and spermatogenesis (Forista Carlo et al. 2001). Autozygosity by descent was demonstrated in families in the ~11 cM region on chromosome 11q13.1, of the gene which codes for channel protein flanked by markers D11S1765 and D11S-4139 (Matthew et al. 2009), and these microdeletions contribute the recessive genetic factors to the etiology of male infertility (Inhorn 2009). However, a few studies also showed that a few of the genes located on chromosome 11 such as *WT1* and *SOX9* in the 17 chromosome, *DAZLA* on 3 and *FSHR* on chromosome 2 which play an important role during spermatogenesis. In the present study, a few of the pedigrees showed male infertile proband with family history of spontaneous abortion (SBA). A few of the earlier studies also reported that this kind of SBA is also caused due to Y chromosome microdeletion and chromosomal anomalies as contributing factor from the male partner. The prevalence of the Y chromosome microdeletions in the proximal AZFc region was reported to be higher in male partners of females with recurrent pregnancy loss (Dewan et al. 2006). Matthew et al. (2009) revealed insertion mutation in *CATSPER1* which suggested that mutations in the CATSPER family of calcium channel subunits should be considered as candidate genes in cases of male infertility. This was demonstrated in 2 four-generation consanguineous Iranian families segregating non-syndromic autosomal-recessive male infertility. Sperm defects and reduced fertility were reported through the clinical analysis of semen from individuals of the study group which correlates with the findings of the present study. The rarity of similar reports might reflect difficulties in diagnosing and characterizing male infertility.

CONCLUSIONS

Clinical semen analysis is an effective tool for the determination of abnormalities in spermeogram. Clinical evaluation of fertility status in males with history of consanguinity should not be commonly limited to routine semen analysis as it bears serious medical, social and economic implications for the communities. Consanguineous marriages facilitate more chances of receiving the factors which may cause male infertility, but the molecular variation in those affected families is hidden. Pedigree analysis and the degree of consanguinity can be considered as a screening tool for male infertility. Educational programmes have to be organized to increase awareness of the potential danger of consanguineous marriages and its risk on genetic disorders including infertility.

ACKNOWLEDGEMENT

Authors thank the UGC-RFSMS for financial support.

REFERENCES

ANIKA H D M DAM, ISABELLE KOSCINSKI, JAN A M KREMER, CELINE MOUTOU, ANNE-SOPHIE JAEGER, ASTRID R OUDAKKER, HERMAN TOURNAYE, NICOLAS CHARLET, CLOTILDE LAGIER-TOURENNE, HANS VAN BOKHOVEN & STEPHANE VIVILLE 2007 Homozygous mutation in SPATA16 is associated with male infertility in human globozoospermia *Am J Hum Genet* **81** 813–820

BACCETTI B, CAPITANI S, COLLODELG, CAIRANOG, GAMBERA L, MORETTI E & PIOMBONI P 2001

Genetic sperm defects and consanguinity *Hum Reprod* **16** 1365–1371

BHASIN M K & NAG S 2002 Consanguinity and its effects on fertility and child survival among muslims of Ladakh in Jammu and Kashmir *Anthropologist Special Issue* **1** 131–140

BITTLES A H 2005 Endogamy consanguinity and community disease profiles *Community Genet* **8** 17–20

BROMIKER R, GLAM-BARUCH M, GOFIN R, HAMMERMAN C & AMITAI Y 2004 Association of parental consanguinity with congenital malformations among Arab newborns in Jerusalem *Clin Genet* **66** 63–66

CHAN P 2007 Practical genetic issues in male infertility management Paper presented at the American Society for Reproductive Medicine Washington DC

DEWAN S, PUSCHECK E E, COULAM C B, WILCOX A J & JEYENDRAN R S 2006 Y-chromosome microdeletions and recurrent pregnancy loss *Fertil Steril* **85** 441–445

FORESTA CARLO, ENRICO MORO & ALBERTO FERLIN 2001 Y Chromosome microdeletions and alterations of spermatogenesis *Endocrine Reviews* **22** 226–239

FUSTER V 2003 Inbreeding pattern and reproductive success in a rural community from Galicia (Spain) *J Biosoc Sci* **35** 83–93

GIANOTTEN J, WESTERVELD G H, LESCHOT N J, TANCK M W, LILFORD R J, LOMBARDI M P & VAN DER VEEN F 2004 Familial clustering of impaired spermatogenesis: no evidence for a common genetic inheritance pattern *Hum Reprod* **19** 71–76

HANN K L 1985 Inbreeding and fertility in a south Indian population *Ann Hum Biol* **12** 267–274

HELGASON A, PALSSON S, GUDBJARTSSON D F, KRISTJANSSON T & STEFANSSON K 2008 An association between the kinship and fertility of human couples *Sci* **319** 813–816

INHORN M C, KOBEISSI L, NASSAR Z, LAKKIS D & FAKIH M H 2009 Consanguinity and family clustering of male factor infertility in Lebanon *Fertil Steril* **91** 1104–1109

JABER L, GABRIELLE J, HALPERN & MORDECHAI S 1998 The impact of consanguinity worldwide *Community Genet* **1** 12–17

KAPADIA K M 1976 *Marriage and family in India* 2nd ed Oxford University Press Calcutta

KRISHNAMURTHY S & ADINARAYANA N 2001 Trends in consanguinity in South India *J Biosoc Sci* **33** 185–197

KULKARNI M L & KURIAN M 1990 Consanguinity and its effect on foetal growth and development: A South Indian study *J Med Genet* **27** 348–352

LATINI M, GANDINI L, LENZI A & ROMANELLI F 2004 Sperm tail agenesis in a case of consanguinity *Fertil Steril* **81** 1688–1691

MADURO M R & LAMBD J 2002 Understanding the new genetics of male infertility *J Urol* **168** 2197–2205

MATTHEW R, AVENARIUS, MICHAEL S, HILDEBRAND, YUZHOU ZHANG, NICOLE C, MEYER, LUKE L H SMITH, KIMIA KAHRIZI, HOSSEIN NAJMABADI, & RICHARD J H SMITH 2009 Human male infertility caused by mutations in the CATSPER1 channel protein *Am J Hum Genet* **1084** 505–510

SANGHVI L D 1966 Inbreeding in India *Eugen Q* **13** 291–301

SASTRI K A N 1976 *A history of South India: from prehistoric times to the fall of Vijayanagar* 4th ed Oxford University Press Madras

VAN GOLDE R J, VAN DER AVOORT I, TUERLINGS J H, KIEMENEY L A, MEULEMAN E J, BRAAT D D & KREMER J A 2004 Phenotypic characteristics of male subfertility and its familial occurrence *J Androl* **25** 819–823

WORLD HEALTH ORGANIZATION 1999 *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction* 4th ed Cambridge University Press Cambridge

XIAO WEI Y U, ZHEN TONG WEI, YU TING JIANG & SONG LING ZHANG Y 2015 Chromosome azoospermia factor region microdeletions and transmission characteristics in azoospermic and severe oligozoospermic patients *Int J Clin Exp Med* **8** 14634–14646