RESEARCH ARTICLE

Realization of Paleopolyploidy through Cytogenetic and Phylogenetic Characterization of Ten Species of *Senna* Mill., Fabaceae-Caesalpinioideae

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Abstract

Taxonomically and phylogenetically *Senna* species complex is quite intriguing having several status and origin related issues. Keeping this view in mind and scope of cytological investigation 10 species of same genus viz. *S. alata, S. alexandrina, S. italica, S. obtusifolia, S. occidentalis, S. polyphylla, S. siamea, S. sulfurea, S. surattensis* and *S. tora* were analysed in cytological frame and comprehensive data/ information, is generated. The basic chromosomal analysis revealed regular meiotic behavior despite the occurrence of some irregularities. The regular occurrence of multivalents especially quadrivalents at diplotene as well as diakinesis stage and unavailability of individual having x=7 chromosome number possibly indicates paleopolyploid origin of worked out taxa. Individual anther basis pollen analysis provides some clues regarding on going evolutionary processes in the same genus. Considered cytological parameters based cluster analysis and their comparison with molecular marker based phylogenetic analysis of earlier workers revealed the efficacy of used parameters in phylogenetic characterization of *Senna* species complex.

Keywords: Cytology, Genome Size, Meiosis, Paleopolyploidy, *Senna.*

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Introduction

Cytological characters are often regarded as of predominance importance in taxonomy since chromosomes are more closely connected with mechanism of heredity than other features employed in taxonomy (Stebbins and Zohary, 1959; Guerra, 2012). Phylogenetically chromosome number can be a plesiomorphic characteristic of a large clade or a recurrent trait which arose independently in two or more clades. It may show direction of evolutionary change, indicates which groups are derived from other or at least which derivations are not possible (Jones and Jopling, 1972). The cytological findings are equally useful in defining the base number, aneuploidy, paleopolyploidy, and neopolyploidy nature of particular taxa, interpreting flora, interrelationship between floras, endemism, conservations, geographical and geological composition (Darlington and Wylie, 1955; Favarger, 1960; Reese, 1961).

The genus *Senna* Mill.*,* is a member of subtribe *Cassiinae* (Caesalpinioideae, Leguminosae, Fabaceae) considered as monophylectic, dervided from subdivision of the complex genus *Cassia* L. comprising of 350 species of trees, shrubs and sub-shrubs (Cordeiro and Felix, 2018). Nearly 80% of *Senna* species are distributed in the American continent and rest are in tropical Africa, Madagascar, Australia, Southern Asia, and the Pacifc Islands (Marazzi *et al*., 2006). Commercial importance of the genus lies in its diverse medicinal applications effective in skin ailments, gastrointestinal disorders, inflammation and visual problems. Further its species are also used in an alternative source of commercial gum, pet food and coffee. Some members of genus also valuable for remediation of degraded areas in conservation efforts (Singh *et al.,* 2013; Pawar and Lalitha, 2014). Taxonomically and phylogenetically *Senna* species complex is quite intriguing having several issues. The taxonomic issues involving in this genera mainly due to high morphological similarity (Machado, 1983; Boonkerd *et al*., 2005) or due to inadequate exploration of different dimentions which have taxonomic and phylogenetic values. Among these dimentions cytological approach is quite important but the cytologoical work on this genus is available in sporodic form and mainly limited to record chromosome number. In the view of above facts, scope and need of comprehensive information the present investigation is designed to study the ten species of *Senna* in cytogenetic and phylogenetic frame.

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Materials and Methods

The present study incorporates 10 species of genus *Senna* Mill. viz. *S. alata* (L.) Roxb., *S. alexandrina* Mill., *S. italica* Mill., *S. obtusifolia* (L.) H.S. Irwin & Barneby, *S. occidentalis* (L.) Link, *S. polyphylla* (Jacq.) H.S. Irwin & Barneby, *S. siamea* (Lam.) H.S. Irwin & Barneby, *S. sulfurea* (Collad.) H.S. Irwin & Barneby, *S. surattensis* (Burm.f.) H.S. Irwin & Barneby and *S. tora* (L.) Roxb. collected from various locations of central India. The species were analysed for various meiotic, mitotic and pollen charecterstics as per the following protocols:

Meiotic Parameters

Unopened flower buds were collected in the forenoon and fixed in Carnoy's fixative (6 Ethanol: 3 Chloroform: 1 Acetic acid v/v), kept at room temperature for 24 hrs and stored in 70 % ethyl alcohol. The anthers were sqashed in 2% acetocarmine. Fresh slides were carefully examined from each collection to determine meiotic pairing behaviour at diplotene and diakinesis and chromosome number at anaphase stages. Pairing configuration, chiasma frequency/PMC (CFPPs), chiasma frequency/chromosome (CFPCh), percentage of rings/pollen mother cell (% rings), percentage of rods/pollen mother cell (% rods) were recorded and tabulated in a table using standard methodology and formulae (Baysal, 1973). Meiotic index (MI) was calculated from 500 tetrad from per accession using methodology of Love (1951). The pollen mother cell diameter (PMC-D) and volume calculated as per methodology adapted for InD & InV, respectively.

Mitotic Parameters

Healthy root tips harvested from germinated seeds were fixed in 1: 3 (acetic acid: ethanol) in between 1:15 -1:35 PM, kept at room temprrtaure for 24 hours and preserved in 70% ethanol. Well spread mitotic slides were analysed for following parameters:

i. **Interphase nuclear diameter and volume (InD & InV):** 10 randomly selected nuclei were scored from well spread squash of root tip under 100 X objectives under by using Leica Microscope DM 3000. Mean diameter of two adjacent nuclei was obtained by measuring at right angle of each other. Interphase nuclear volume was calculated using the formula $4/3\pi r^3$, where r is radius of the nuclei.
ii. **Nucleolous diameter and volume (NuD & NuV):** NuD and NuV were recorded as per the previous protocol for the InD and InV. The measurements were taken at prophase stage.
iii. **4C DNA content:** 4C DNA content was measured from Nikon Eclipse 200 microscope equipped with micro spectrophotometer using monochromatic light of 550 nm following the method of Sharma and Sharma (1980). In-situ DNA was calculated on the basis of optic density, which was converted in to pg using Van Hof's (1965) 4C nuclear DNA values (67.1pg) for *Allium cepa* var. Deshi as standard (Mohanty and Das 2006a).
iv. **Genome size (GS):** Genome size of different species was calculated on the basis of methodology adapted by Mohanty and Das (2006 b).

Pollen fertility and pollen germination % (PoF & PoG)

Pollen fertility was estimated through acetocarmine (2%) stainability tests, well filled pollen grains with stained nuclei were noted as fertile while shrivelled and with unstained or poorly stained cytoplasm were counted as sterile (Pollen fertility = Number of fertile pollen/ Number of total pollens observed × 100). The pollen germination percentage was assessed using the sucrose solution (30%) method.

Statistical analysis

The data collected minimum in 3 replicates and were analysed statistically as per the following methods:

i. **Analysis of variance (ANOVA):** Two Way ANOVA worked out for assessment of variability at intra and interspecific level.
ii. **Pearson correlation analysis:** Pearson correlation analysis was used to assess relative association among the variables. The $p < 0.05$ treated as significant and $P < 0.01$ treated as highly significant.
iii. **Similarity matrix and cluster analysis:** For the study of similarity between species/ linkage between species, the observed data of of each species were entered into binary matrix as discrete variable (1) for the value above the median value and (0) for below the median value of respective parameter and the matrix were subjected to further analysis. Scores of individual phenotypic characters were used to create data matrix. A dendrogram was constructed based on Nei and Li's coefficient with unweighted pair group method and arithmetic average analysis (UPGMA) using Fig Tree Version 1.3.1 software. All statistical were performed on STATISTICA (version 6.0).

Results

Chromosome Number and Meiotic Behaviour

The chromosome number of 10 species of genus *Senna* were worked out and 2n=28 was recorded as most common chromosome number. In general, in analysed species meiotic course was found normal but at notable extent multivalent, especially qudrivalents were also observed. The meiotic indices was found maximum for *S. sulfurea* (93.88 ± 0.88) minimum for *S. polyphylla* (73.68 ± 1.42). Percentage of ring and rod bivalents between species ranged between 7.94 ± 1.89 to 73.01 ± 1.89 and 26.89 ± 1.90 to 91.29 ± 1.56 respectively. The chiasma frequency/ PMC and chiasma frequency / chromosome recorded highest for *S. occidentalis* (22.29 ± 0.44, 0.79 ± 0.01) and lowest for *S. siamea* (8.75 ± 0.66, 0.31 ± 0.02) (Table 1, Fig. 1).

InD & InV, NuD & NuV, PMC-D & PMC-V

Values for aforesaid parameters differed significantly in the studies species. InD & InV noted highest for *S. occidentalis* (10.22 ± 0.01µm, 557.31 ± 2.64µm^3) and minimum for *S. italica* (6.18 ± 0.03 µm, 123.71 ± 1.89 µm^3). NuD & NuV observed maximum for *S. occidentalis* (4.50 ± 0.05 µm, 47.75 ± 1.70 µm^3) and lowest for *S. siamea* (1.73 ± 0.04 µm, 2.75 ± 0.22 µm^3). PMC-D & PMC-V

Table 1: Observations recorded on various cytological parameters studied in *Senna* species.

Species / parameters	*InD µm*	*InV µm³*	*NuD µm*	*NuV µm³*	*PMC-D µm*	*PMC-V µm³*	*PoS µm*	*PoF %*	*MI %*	*% rings*	*% rods*	*CFPPs*	*CFPCh*	*4C-DNA (pg)*	*Genome Size (Mb)*
S. alata	9.56 ± 0.31	456.14 ± 4.47	4.33 ± 0.10	42.65 ± 3.20	22.38 ± 0.02	5852.00 ± 17.39	63.06 ± 0.28	90.74 ± 1.47	91.21 ± 0.98	73.01 ± 1.89	26.89 ± 1.90	17.77 ± 0.26	0.63 ± 0.01	33.21 ± 0.02	16025.43
S. alexandrina	7.51 ± 0.11	223.00 ± 10.25	2.22 ± 0.39	5.75 ± 0.32	27.10 ± 0.05	10430.02 ± 61.03	37.14 ± 0.37	82.89 ± 1.42	84.00 ± 1.09	20.29 ± 0.79	79.71 ± 0.79	13.83 ± 1.18	0.49 ± 0.04	22.10 ± 0.02	10701.85
S.italica	6.18 ± 0.03	123.71 ± 1.89	1.92 ± 0.02	3.70 ± 0.13	22.08 ± 0.02	5622.00 ± 21.63	51.84 ± 0.02	84.39 ± 2.87	84.42 ± 1.17	22.09 ± 1.90	77.90 ± 1.90	16.99 ± 0.22	0.60 ± 0.01	6.55 ± 0.05	3160.38
S. obtusifolia	9.31 ± 0.18	422.30 ± 15.62	3.77 ± 0.04	27.95 ± 0.94	21.15 ± 0.45	4970.08 ± 322.81	52.07 ± 0.33	86.33 ± 2.31	93.14 ± 0.73	25.76 ± 2.31	74.23 ± 2.31	15.96 ± 0.31	0.57 ± 0.01	26.73 ± 0.02	12897.23
S. occidentalis	10.22 ± 0.01	557.31 ± 2.64	4.50 ± 0.05	47.75 ± 1.70	29.01 ± 1.09	12919.06 ± 122.81	85.82 ± 0.32	87.75 ± 1.17	92.21 ± 0.82	58.76 ± 3.15	41.24 ± 3.15	22.29 ± 0.44	0.79 ± 0.01	12.25 ± 0.03	5912.23
S. polyphylla	8.98 ± 0.03	348.43 ± 23.41	3.14 ± 0.01	16.43 ± 0.24	20.25 ± 0.02	4346.58 ± 13.35	45.31 ± 0.17	78.12 ± 0.03	73.68 ± 1.42	21.24 ± 0.37	78.76 ± 0.37	10.83 ± 0.43	0.37 ± 0.02	25.29 ± 0.05	12205.64
S. siamea	7.28 ± 0.19	203.17 ± 17.17	1.73 ± 0.04	2.75 ± 0.22	20.18 ± 0.13	4291.31 ± 80.81	52.77 ± 2.36	76.40 ± 4.09	74.86 ± 4.09	21.59 ± 0.62	78.42 ± 0.62	8.75 ± 0.66	0.31 ± 0.02	35.34 ± 0.14	17053.16
S. sulfurea	8.38 ± 0.02	307.51 ± 02.87	4.12 ± 0.04	36.61 ± 1.24	24.44 ± 0.63	7665.96 ± 610.88	36.06 ± 0.50	92.16 ± 1.24	93.88 ± 0.88	26.75 ± 2.94	72.24 ± 3.10	18.24 ± 0.59	0.74 ± 0.02	20.24 ± 0.06	9764.12
S. surattensis	8.37 ± 0.15	307.91 ± 17.33	2.04 ± 0.03	4.50 ± 0.22	23.47 ± 0.21	6774.55 ± 191.12	39.49 ± 0.73	88.26 ± 1.59	81.28 ± 1.75	27.72 ± 1.41	72.28 ± 1.41	17.37 ± 1.24	0.62 ± 0.04	22.32 ± 0.09	10767.79
S. tora	9.12 ± 0.04	396.70 ± 5.41	3.51 ± 0.10	22.82 ± 2.09	20.85 ± 0.51	4377.36 ± 106.32	53.38 ± 1.22	86.18 ± 2.73	93.14 ± 0.73	7.94 ± 1.89	91.29 ± 1.56	14.02 ± 0.26	0.54 ± 0.01	28.22 ± 0.22	13936.21

Abbreviations: InD: Interphase nucleus diameter, InV: Interphase nucleus volume, NuD: Nucleolous diameter, NuV: Nucleolous volume, PMC-D: Pollen mother cell diameter, PMC-V: Pollen mother cell volume, PoS: Pollen size, PoF: Pollen fertility, MI: Meiotic Index, CFPPs: Chiasma frequency/pollen mother cell, CFPCh: Chiasma frequency/chromosome.

Table 2: Pollen characteristics at individual stamen basis

Species/Stamen no.		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
S. alata	PoS	62.18±0.78	63.34±0.54	65.13±1.22	62.96±0.42	64.33±0.75	63.37±0.58	63.33±0.59			
	PoF	96.93±0.53	96.00±0.55	93.05±1.33	93.80±0.93	92.33±1.17	91.18±1.67	88.94±1.16		Sterile	
	PoG	25-35%		10-18%							
S. alexandrina	PoS	35.19±0.53	37.67±0.51	37.00±0.34	37.05±0.51	36.99±0.41	38.27±0.48	37.79±0.52			
	PoF	88.12±0.09	86.22±1.42	86.00±0.91	80.90±1.29	78.37±0.91	80.80±1.85	79.88±2.02		PNF	
	PoG	35-45%		30-35%							
S. italica	PoS	55.43±1.31	52.20±0.81	51.75±0.68	50.49±0.88	50.64±0.82	53.63±0.76	50.92±0.89			
	PoF	95.33±0.90	94.42±0.43	87.37±0.59	82.30±0.83	82.24±1.84	80.78±1.85	82.35±2.78		PNF	
	PoG	30-40%		15-20%							
S. obtusifolia	PoS	50.10±1.19	53.25±0.90	52.02±1.49	53.90±1.43	52.83±0.98	53.77±0.89	53.11±1.06			
	PoF	94.00±0.67	92.79±0.57	92.27±0.71	82.34±0.91	84.66±0.93	82.77±1.02	82.33±1.98		Sterile	
	PoG	30-40%		35-40%							
S. occidentalis	PoS	84.74±2.36	86.77±0.66	83.81±1.14	87.38±1.17	86.32±0.81	85.60±1.21	84.57±1.13			
	PoF	92.33±0.28	92.71±0.53	91.76±0.59	86.69±0.93	86.65±1.07	86.35±0.82	84.86±1.27		PNF	
	PoG	30-40%			20-25%						
S. polyphylla	PoS	45.21±0.10	45.23±1.42	44.11±0.21	44.23±0.21	43.23±0.89	44.60±0.82	42.45±1.23			
	PoF	78.17±0.67	78.23±1.21	76.45±0.34	79.23±0.32	78.12±0.17	76.12±0.43	77.23±0.91		Sterile	
	PoG	20-30%			15-20%						
S. siamea	PoS	49.89±1.67	49.41±0.61	55.76±3.04	55.5±0.19	60.99±1.42	61.58±1.49	62.55±1.03	46.02±1.03	44.52±0.62	42.56±1.10
	PoF	87.21±1.15	87.98±0.36	86.82±0.42	84.22±1.32	82.05±1.34	80.26±0.51	81.22±1.98	59.44±1.68	56.33±0.82	58.45±1.00
	PoG	20-25%		20-25%					15-17%		
S. sulfurea	PoS	34.93±.43	36.63±0.76	36.14±0.45	37.14±0.47	37.39±0.27	35.31±0.65	36.48±0.38	35.91±0.58	35.94±0.67	36.19±0.51
	PoF	96.27±0.04	94.81±0.37	93.95±0.21	95.66±0.23	95.24±0.54	93.33±0.81	94.81±0.53	88.61±0.51	86.40±0.77	69.59±0.61
	PoG	10-12%			10-12%				2.0-5.0%		
S. surattensis	PoS	41.20±0.58	40.53±0.93	41.88±1.39	39.46±1.11	41.33±1.34	35.84±0.64	36.73±0.61	36.26±1.45	41.12±1.26	40.54±0.67
	PoF	90.10±0.80	91.89±0.72	91.86±1.12	91.28±1.62	90.27±2.68	86.30±2.77	88.24±0.92	89.51±0.49	87.40±2.18	75.00±4.73
	PoG	20-25%							20-25%		
S. tora	PoS	51.60±1.42	51.91±1.01	51.64±1.15	54.01±1.37	52.88±0.96	54.40±0.87	52.89±0.89			
	PoF	95.21±0.60	93.42±0.51	92.44±0.83	82.30±0.82	83.88±0.94	80.80±1.85	82.02±2.19		Sterile	
	PoG	25-30%			10-12%						

Colour yellow for large sized anthers; greem for medium size anthers, crimson for small sized anthers or staminodes.
Abrevations: PoS; Pollen size; PoF: Pollen fertility; PoG: Pollen germination %; PNF: Pollen not formed ; Sterile: Pollen formed but all are sterile.

recorded maximum for *S. occidentalis* (29.01 ± 1.09 µm, 12919.06 ± 122.81 µm^3) and minimum for *S. siamea* (20.18 ± 0.13 µm, 4291.31 ± 80.81 µm^3) (Table 1).

C-nuclear DNA content and genome size

Significant variation of nuclear DNA content was observed among the species. Minimum values for nuclear DNA content and genome size calculated for *S. italica* (6.55 ± 0.05 pg, 3160.38 Mb) while highest for *S. siamea* (35.34 ± 0.14 pg, 17053.16 Mb) (Table 1).

Pollen size and pollen fertility

In worked out species pollen size ranged between 36.06 ± 0.50 µm in *S. sulfurea* to 63.06 ± 0.28 µm in *S. alata* whereas pollen fertility (%) ranged between 76.40 ± 4.09 to 92.16 ± 1.24 recorded for *S. siamea* and *S. sulfurea* respectively (Table 1).

Pollen size, pollen fertility and pollen germination % at individual stamen basis

Among worked out species pollen size not significantly varied with type of stamen whereas pollen fertility significantly varied with type of stamen except for *S. polyphylla*. Pollen germination percentage showed significant variation with type of stamen except for *S. obtusifolia*, *S. occidentalis* and *S. surattensis* (Table 2).

Pollen analysis at individual anther basis indicates that out of ten species, seven species are staminode bearing. Off which in four species namely *S. alata*, *S. obtusifolia*, *S. polyphylla* and *S. tora* staminodes observed as complete vegetative structure, as they are devoid of any event of microsporogenesis process whereas in remaining three species viz. *S. alexandrina*, *S. italica* and *S. occidentalis* staminodes not found as complete vegetative structure as in them microsporogenesis takes place and pollen grains are formed but they are sterile.

Analysis of variance

F-value estimates indicate that variation at intraspecific level is non significant (except for pollen size) whereas at interspecific level is highly significant (Table 3).

Pearson corelation analysis

Inter corelation analysis of observed parameters indicates that in different species of *Senna* InD was significantly correlated with INV(0.989**), NuD (0.842*) and NuV (0.810*) ;InV with NuD (0.859*), NuV (0.850*) and Pos (0.654*); NuD with NuV (0.980**) and MI (0.731*); NuV with PoF (0.639*), MI (0.724*), % Rings (0.690*), % Rods (0.699*), CFPPs (0.644*), CFPCh (0.669*); PMC-D with PMC-V (0.994**), CFPPs (0.670*), CPPCh (0.639*); PMC-V with CFPPs (0.642*); PoS with % Rings (0.651*), % Rod (- 0.649*); PoF with MI (0.829*), CFPPs (0.837*) and CFPCh (0.896**); MI with CFPPs (0.714*) and CFPCh (0.781*), 4C DNA with GS(1.00**); % Rings with % Rod (-1.00**); CFPPs with CFPCh (0.978**) (Table 4).

Similarity matrix and cluster analysis

Similarity matrix between *S. alata* and *S.* occidentalis, *S. sulfurea* and *S. surattensis* noted highest (0.857) while lowest for *S. surattensis* and *S. tora* (0.00). Moderate values were found

Table 3: F values for observed parameters (Two way ANOVA)

Parameters	*Replication (n = 5)*	*Between species (n = 10)*
InD	0.53	311.28*
InV	1.28	86.27*
NuD	0.264	276.49*
NuV	0.62	102.7*
PMC-D	1.559	66.89*
PMC-V	2.01	43.86*
PoS	1.85	334.78*
PoF	9.74*	109.04*
MeI	2.11	31.97*
% Rings	1.71	85.25*
% Rods	1.88	89.69*
CFPPs	2.47	38.08*
CFPCh	0.25	5.36*

F value< table value at 5% point of F e^2 non significant; F> than significant; F> table value at 1 % point of e^2 highly significant.

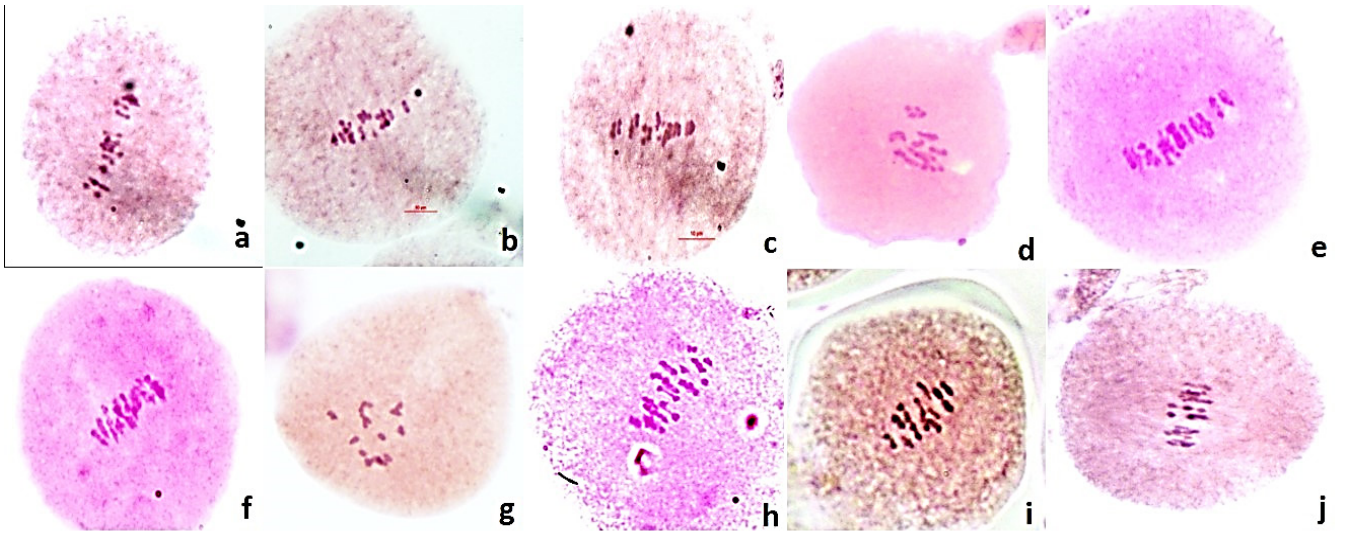

Fig. 1: Meiotic chromosomes in *Senna* (n=14, 2n=28) showing presence of ring and chain quadrivealents: *a. S. alata*; *b. S. obtusifolia*; c. *S. surattensis*; d. *S. alexandrina*; e. *S. italica*; f. *S. sulfurea*; g. *S. occidentalis*; h. *S. polyphylla*; i. *S. tora*; j. *S. siamea*

Table 4: Pearson correlation analysis among worked out parameters.

Parameters	*InD*	*InV*	*NuD*	*NuV*	*PMC-D*	*PMC-V*	*PoS*	*PoF*	*MI*	*4C-DNA*	*GS*	*% Rings*	*% Rods*	*CFPPs*
InD	1													
InV	0.984**	1												
NuD	0.842*	0.859*	1											
NuV	0.810*	0.850*	0.980**	1										
PMC-D	0.210	0.283	0.269	0.354	1									
PMC-V	0.236	0.312	0.275	0.364	0.994**	1								
PoS	0.532	0.654*	0.499	0.597	0.305	0.366	1							
PoF	0.399	0.443	0.618	0.639*	0.402	0.334	0.112	1						
MI	0.469	0.539	0.731*	0.724*	0.354	0.312	0.310	0.829*	1					
4C-DNA	0.250	0.152	0.039	-0.001	-0.521	-0.512	-0.155	-0.231	-0.175	1				
GS	0.254	0.155	0.042	0.000	-0.524	-0.517	-0.154	-0.229	-0.167	1.000**	1			
% Rings	0.524	0.598	0.576	0.690*	0.398	0.411	0.651	0.467	0.316	0.014	0.005	1		
% Rods	-0.528	-0.602	-0.586	-0.699*	-0.399	-0.411	-0.649	-0.479	-0.328	-0.016	-0.006	-1.00**	1	
CFPPs	0.403	0.506	0.574	0.644*	0.670*	0.642*	0.459	0.837*	0.714*	-0.589	-0.590	0.595	-0.601	1
CFPCh	0.386	0.475	0.611	0.669*	0.639*	0.600	0.358	0.896*	0.781*	-0.551	-0.550	0.508	-0.517	0.978**

Abbreviations: InD: Interphase nucleus diameter, InV: Interphase nucleus volume, NuD: Nucleolous diameter, NuV: Nucleolous volume, PMC-D: Pollen mother cell diameter, PMC-V: Pollen mother cell Volume, PoS: Pollen size, PoF: Pollen fertility, MI: Meiotic Index, GS: Genome size, CFPPs: Chiasma frequency/Pollen mother cell, CFPCh: Chiasma frequency/chromosome. Ns-*p> 0.05, * p<0.05, **- p<0.01.*

for *S. alata* and *S. obtusifolia* (0.815), *S. alata* and *S. polyphylla* (0.609), *S. alata* and *S. sulfurea* (0.696), *S. alata* and *S. tora* (0.667), *S. obtusifolia* and *S. occidentalis* (0.720), *S. obtusifolia* and *S. polyphylla* (0.700), *S. obtusifolia* and *S. tora* (0.762), *S. occidentalis* and *S. sulfurea* (0.667), *S. polyphylla* and *S. siamea* (0.615) *S. polyphylla* and *S. tora* (0.824). The cluster analysis showed that species were assembled into two major clusters and each cluster divided into two groups. The first cluster- I consisted of 06 species; *S. alata, S. occidentalis*, *S. obtusifolia, S. polyphylla, S. tora, S. siamea,* where *S. seamea* present as out group. Cluster-II comprises of 04 species of which *S. alexandrina*, *S. italica* in one clade while *S. sulfurea* and *S. surattensis* present in other (Table 5; Fig. 2).

Discussion

In present investigation the most common chromosome number is 2n=28 which is in consonance with the findings of earlier workers (Biondo *et al*., 2005; Codeiro and Felix, 2018; Resende *et al.,* 2013; Rice *et al.,* 2015). However, under present investigation the meiotic pairing analysis at diplotene and diakinesis showed presence of multivalents predominantly quadrivalents in all the species (Fig. 1). Presence of quadrivalents and absence of any report on individual having 2n=14 on one hand favours the establishment of x=7 as basic chromosome number and otherhand supporting the idea of plaeopolyploidy in the genus during the due course of evolution.

Earlier reports on the genus indicate that that genus is polybasic in nature and x = 6, 7, and 8 are possible basic chromosome number of same (Irwin and Turner, 1960; Pantulu, 1960; Bir and Kumari, 1977). Meiotic anomalies like presence of multivalent and secondary chromosomal association is most common in all the PMCs analysed. Analysis of meiotic anomalies like structural changes of chromosome sometimes provide clue of various evolutionary processes operative in the taxa. They also play a crucial role in creating effective reproductive barrier between the species. Presence of multivalent, qudrivalents might indicate that at least partial homology of chromosome extended to some non homologous pairs that are probably

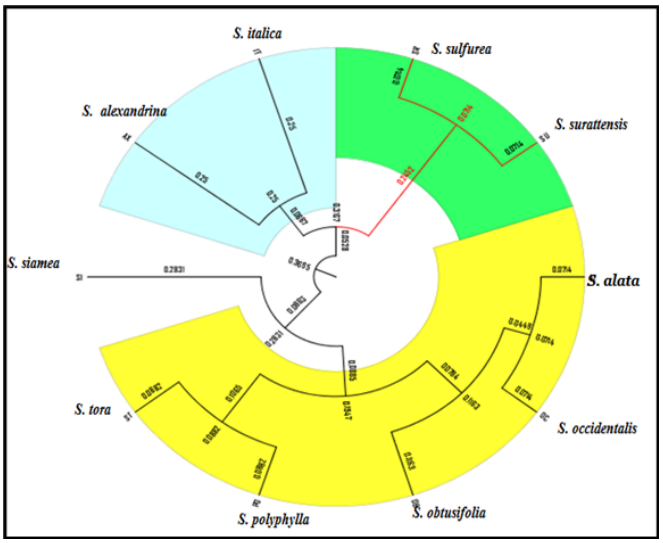

Fig. 2: Cytological parameter based clustering pattern of *Senna* species.

Table 5: Similarity matrix for *Senna* species based on worked out cytological parameters mentioned in Table 4 (Nei and Li/Dice)

Species	*S. alata*	*S. alexandrina*	*S. italica*	*S. obtusifolia*	*S. occidentalis*	*S. polyphylla*	*S. siamea*	*S. sulfurea*	*S. surattensis*
S. alata	1								
S. alexandrina	0.211	1							
S. italica	0.211	0.500	1						
S. obtusifolia	0.815	0.125	0.125	1					
S. occidentalis	0.857	0.353	0.353	0.720	1				
S. polyphylla	0.609	0.167	0.167	0.700	0.381	1			
S. siamea	0.400	0.222	0.222	0.471	0.111	0.615	1		
S. sulfurea	0.696	0.333	0.333	0.400	0.667	0.125	0.154	1	
S. suratteensis	0.571	0.400	0.400	0.222	0.526	0.143	0.182	0.857	1
S. tora	0.667	0.154	0.154	0.762	0.545	0.824	0.571	0.118	0

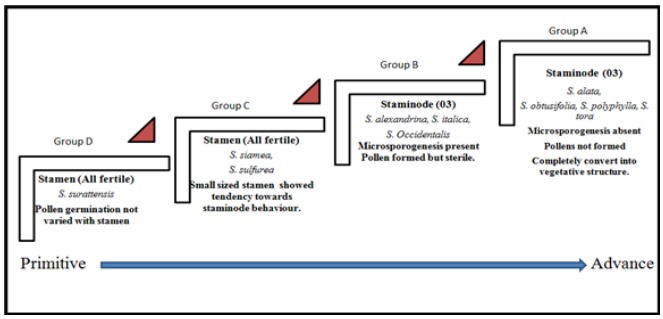

Fig. 3: Showing evolutionary status of the *Senna* species on the basis of presence of stamonide.

either due to hybrid nature of taxon or heterozygosity for reciprocal translocation or paleopolyploidy (Aparicio and Albaladeja, 2003). Our findings also supports the earlier reports on the *Senna rugosa* that polyploidization could be an important phenomenon in the evolution process of *Senna* species (Resende *et al*., 2014). In the process of paleopolyploidization the genetic material is doubled leading to massive cellular changes with increased cell size and altered gene expression. Gradually the process of diploidization results in duplication of genes from large gene families coupled with gene loss altering the expression of genes. Therefore most paleopolyploids, during evolution have lost their polyploid status through a process called diploidization, and are currently considered diploids e.g. baker's yeast, *Arabidopsis thaliana*, and *Glycine max*. Recently, the word paleopolyploid has been applied for species with low chromosome number which until recently were supposed to be diploids, as *Zea mays* and *Arabidopsis thaliana* (Gaut *et al*., 2000; Poggio *et al*., 2005; Yogeeswaran *et al*., 2005; Guerra, 2008). In recent past paleopolyploid origin of several taxa reported in family Magnoliaceae, Platanaceae, Salicaceae and Bromeliaceae (Grant, 1981). Extreme cases of paleopolyploidy are species of monotypic families Strasburgeriaceae (2n=500) with very high chromosome numbers (Oginuma *et al*., 2006), or monotypic genera, as *Voanioala gerardii*, with 2n = about 596 (Johnson *et al*., 1989). Side by side pairing or secondary association of bivalents indicate presence of homology between the bivalents and polyploidy origin of the species (Malgwi *et al*., 1997). The persistant occurance of secondary associations of chromosome is used by many workers in determining base number and polyploidy nature of the species *viz. Oryza sativa* (Nandi, 1936), *Zea mays* (Molina and Naranjo, 1987) and *Ocimum* spp. (Mukharjee and Datta, 2005) etc. In present report there is presence of chain and ring quadrivalents at diplotene and diakinesis stage in all the ten species thereby strongly supporting the idea of polyploid ancestors. Maximum species represents higher magnitude for meiotic index, chiasma frequency/PMC and Chiasma frequency / chromosome. Presence of higher frequency of chiasmata cause excessive restructuring of chromosomes through reshuffling of genetic material which create an array of gametes, all different from each other and from the parental gamete (Zarchi *et al*., 1972). This variation determines the rate of increase in fitness of an organism when exposed to the effect of natural selection. It allows for flexibility of population or family, which can successfully invade new habitats. On the contrary, the presence of low chiasma frequency provides genetic fixicity to an organism which becomes stable or less flexible ecologically. Besides this, chiasma frequency is an indicative of the homology between homologous pair of chromosomes. Higher chiasma frequency means higher degree of homology.

ANOVA showed that numerical values for InD, InV, NuD, NuV, PMC-D and PMC-V varied with species, in general variation at intraspecific level found non-significant and interspecific lavel found significant. Maximum values for these parameters recorded for *S. occidentalis* and minimum for *S. italica*. Moreover, morphologically allied species like *S. tora,* S. *obtusifolia*, *S. sulfurea* and S. *surrettensis*, showed almost similar estimates. This probably signifies the potential of aforsaid cytological parameters in resolving the various taxonomical and phylogenetic issues of angiosperms. There are reports of variability in the DNA content within different starins of a single species and also related species of microalgae with a strong correlation between the absolute Nuclear DNA content and the chromosome number, body size and number of terminal lobes in the microalgae (Poulíčková *et al.,* 2014). Noteworthy variation in 4C nuclear DNA content and genome size were observed among the 10 species of *Senna*. The present observations also show resemblance with earlier reports on genus by Mohanty and Das (2006b). The constancy in the DNA amount at the species level in repeated experiments revealed the stable 4C DNA content for particular species and interspecific diversity of DNA amount has

often been attributed to deletion or addition of highly repetitive DNA sequences rather than AT or GC rich sequences in genome (Matel *et al.,* 1997). However non a significant corelation of InV, NuV, PMC-V with 4C- DNA and GS is found contarray with the findings of Mohanty and Das (2006a) and nucleoskeleton theory which states that DNA content may influence the volume of the nucleous which in turn influences the size of the cell (Gregory, 2005; Jovtchev *et al*., 2006). However, present data supports the earlier theory that genome size is not the determining factor of nuclear size rather it is likely that there is a nuclear-scaling mechanism whereby nuclear volume is proportional to, and determined by, the levels of one or more cellular factors (Harris, 1967; Altman and Katz, 1976). The decrease in DNA value per genome during plant evolution and the high level of species formation in taxon with large DNA values have been reviewed by Grif (2000) and showed that plant taxa with a small DNA value per genome have a high percentage and higher degree of polyploidy.

Individual anther basis pollen analysis signifies the importance of this parameter in assessment of evolutionary status of particular species. Reduction in floral appendeges considered as advanced feature hence species grouped under 'A' section of Fig. 3 found to be most advanced followed by species of section 'B' than section 'C'. *S. surattensis* placed under section 'D' might be most primitive. In maximum species small sized anther are characterized by reduced fertility. In maximum species gradual decrease in stamen size charecterized by reduced fertility than that of large sized stamen signifies shifting of the stamens toward staminode behaviour, which is of evolutionary significance. Presence of staminode signifies reduction of androecium. However, the developmental behaviour of staminode is different in actinomorphic and zygomorphic flowers. In actinomorphic flowers the staminodes replace the staminal whorl and this is an irreversible process whereas in zygomorphic flowers the staminodes are present in different whorls as subset and they once lost can reappear in a lineage as in case of *Senna*. Sometimes staminodes acquire new functions and are lost quickly whereas the nonfunctional staminodes are considered as advanced character appeared only in recently derived taxa (Walker-Larsen and Harder, 2000; Marazzi and Endress, 2008).

Cluster analysis based on aforesaid parameters and significant corelation between InV and PoS, NuV and PoF, PMC-V and Chiasma frequency/ PMC, MI with CFPPs and CFPCh reveled importance of these characters cytotaxonomy and phylogenetic characterization any species. To check the actual efficacy of used parameters in context of phylogeny, cluster diagram based on the observed parameters were compared with the cluster diagrams of earlier workers based on RAPD, ISSR and AFLP data (Acharya *et al*., 2011), RAPD, ISSR and SSR data (Mohanty *et al*., 2010), RAPD marker (Tripathi and Goswami, 2011). Comparative study of our cluster analysis with cluster of earlier workers showed similarity in relative positioning of the species thereby signifying the relevance of the parameters considered under present investigation to be effective in phylogenetic analysis of a species complex and could be a preliminary tool to access the phylogeny of a species complex before proceeding with the expensive molecular tools. Thus, the present phylogenetic assessment of the different species of genus *Senna* is in consonance with the molecular phylogenetic evidences of evolution in the genus *Senna*.

Conclusion

The cumulative informations generated through this work enrich the cytological information available on genus *Senna*. The variations in chiasma frequency distribution, 4C DNA content and genome size can provide clue to overall level of recombination in the species. The studies also give an insight on paleopolyploid nature of species, various evolutionary processes operative in genus *Senna* and provide basic information for researchers working on various aspects of molecular variations and phylogeny of subtribe *Cassiinae*.

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