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Research Article

Cyto-systematical study of the genus *Senna* Mill. in diverse geographical locations of Uttar Pradesh

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Abstract

Senna Mill. is one of the most typical genus of caesalpinioideae, with wide geographical distribution and diversification. The research work was planned with an objective of taxonomical characterization and assessment of the meiotic behaviour of wild accessions belonging to four species of *Senna* Mill. from different locations of Allahabad and its adjoining areas UP, India. The four species selected for the cytological study were *Senna alata*, *Senna occidentalis*, *Senna siamea* and *Senna tora*. Young floral buds were fixed and all meiotic phases were analysed. Most of the analysed species showed regular meiotic behaviour, some irregularities were also observed to take place with the change in location. An in-depth cytological assessment revealed induction of high frequency of chromosomal anomalies such as micronuclei in *S. siamea*, while normal meiotic phases were observed at various stages in *S. occidentalis* and *S. tora* while stickiness at metaphase and univalent formation, respectively. Cytomictic behaviour and intercellular fusion were visible among micromeiocytes of *S. tora*.

Key words: Caesalpinioideae, Chromosome number, Meiotic behaviour, *Senna* Mill., Taxonomical characterization.

INTRODUCTION

Senna Mill. is one of the most illustrative genus of Caesalpinioideae, with wide geographical distribution. Large variation in the floral pattern and embryogenesis of subfamily Caesalpinioideae (Fabaceae Lindl.), it is taking places in a basal position in phylogenetic trees. The taxonomical hierarchy of the genera *Cassia* L. *Sensu stricto*, *Chamaecrista* Moench and *Senna* Moench and Mill are included in the Subtribe Cassiinae, tribe Cassieae, and scientists commonly used seed proteins, morphological, vegetative and reproductive characters for the taxonomical studies and classification purpose (Lewis *et al.*, 2005).

An ontogenetic characteristics, molecular systematic, and cytological parameters were also used for taxonomical and classification of these genera (Biondo *et al.*, 2005a, 2005b). It is divided into six sections: *Psilorhegma*, *Chamaefistula*, *Senna*, *Peiranisia*, *Paradyction* and

Astroites. Ethano-botanical +point of view some species belong to this genus which was reported particularly for significant medicinal values as well as biochemical extraction of plant material (Tona *et al.*, 2004), For conservation and production purpose, species from this genus were suggested for yielding of quality timber as well as restoration of degraded land (Arato *et al.*, 2003). Marazzi *et al.* (2006) reported that most of the sections are polyphyletic by the critical examination of the DNA strands of different chloroplast gene as *rpS16*, *rpL16*, *matK*. Species of *Senna*, have much more diversity in terms of their chromosomal count, diploid chromosome number $2n = 28$, is base chromosome count in most of the species of *Senna* rather than this base count in chromosome $2n = 22$, 24 and 26 are also reported (Souza & Benko-Iseppon 2004; Biondo *et al.*, 2005a; Matos *et al.*, 2011). Although there are also records of polyploidy as $2n = 42$, 56 and 112 in *Senna rugosa*,

$2n = 56$ in *S. aversiflora*, and $2n = 52$ and 104 in *S. gardneri* (Matos *et al.*, 2011) along with this diploidy event are also reported in which chromosome number $x = 11$, 12 and 13 , whereas the basic number of chromosome of most of the species of *Senna* $x = 14$ (Matos *et al.*, 2011). The consensual basic number for the genus is $x = 14$, which is derived from $x = 7$, the accepted basic number of the subtribe Cassiinae, to which *Senna* belongs (Souza and Benko-Iseppon 2004). Karyomorphological and meiotic behavior of some *Senna* sp. were also reported previously in cytogenetic studies (Biondo, Miotto and Schifino-Wittmann 2005a, 2005b; Biondo, Miotto, Schifino-Wittmann *et al.*, 2005). In India, people have different dietary habits from region to region and foodstuffs used for daily consumption mainly originated based on the farm as well as forests. These are important sources of nutrients and medicinal values for them. Management of food and natural resource in a sustainable manner is essential in recent climate change scenarios. Huge economic value and wild occurrence of less familiar crops like *Senna* play an important role in having a good source of secondary metabolites as sennosides, glycosides and other nutrients. These metabolites and nutrition play a significant role in the reduction of malnutrition and cure of disease. *Senna* has considered one of the important wild crops that have much diversified occurrence, every part of this plant can be useful, in which leaves of this plant are astringent, bitter, sweet, acrid, thermogenic, cathartic, depurative, liver tonic, skin disorders (Balasankar *et al.*, 2013). Although more of the work has been done little is known regarding intra- and interspecific variation in *Senna* at cyto-geographical level. In this work, chromosome

number and meiotic behavior of different populations of four *Senna* species from the different geographical regions of Allahabad was assessed. In addition to this, differential morphological and cytological characteristics among the four species *Senna alata*, *Senna occidentalis*, *Senna seamea* and *Senna tora* was also discussed.

MATERIALS AND METHODS

Species of the genus *Senna* which was reported by K. K. Khanna (BSI) as angiospermic plant of Uttar Pradesh. A checklist of total of fourteen well flourish species (Table 1) and chromosome counts were published in the literature work presented here with respect to their author and reference from the Chromosome Counts Database (CCDB, Rice *et al.*, 2015). Plant collection and identification of *Senna* Mill. was obtained from the different geographical areas of Uttar Pradesh State, India. The plant materials were identified and authenticated at the Botanical survey of India central region, Prayagraj.

Cytogenetic analysis was performed on four species of the genus *Senna* growing in Allahabad and its adjoining area in Uttar Pradesh, India, to determine their floral morphological variance and meiotic behavior.

Table 2 gives an account of the geographical locations and identification of four sp. of *Senna* Mill. Floral buds are fixed from different accessions of the four studying *Senna* Mill species.

The samples were fixed in Carnoy's fixative (3 Ethanol: 1 glacial acetic acid) immediately after harvesting and stored at -4 instead of -20°C . Meiocyte suspensions were

Table 1. Chromosome records of species of the genus *Senna* and their respective references.

Taxon name	Chromosome number (2n)	References
<i>Senna alata</i> (L.) Roxb.	28	Biondo <i>et al.</i> (2005a); Resende <i>et al.</i> (2013); Souza & Benko- Iseppon (2004)
<i>Senna artemisioides</i> (Gaud. ex DC.) Randell	28	Rice <i>et al.</i> (2015)
<i>Senna auriculata</i> (L.) Roxb.	28	Rice <i>et al.</i> (2015)
<i>Senna didymobotrya</i> (Fresen.) Irwin & Barneby	28	Rice <i>et al.</i> (2015)
<i>Senna italica</i> Mill.	28	Rice <i>et al.</i> (2015)
<i>Senna multijuga</i> (L.C.Richard) Irwin & Barneby	24	Biondo <i>et al.</i> (2005a); Resende <i>et al.</i> (2013)
<i>Senna obtusifolia</i> (L.) Irwin & Barneby	24,26,28	Biondo <i>et al.</i> (2005a); Souza & Benko-Iseppon (2004); Rice <i>et al.</i> (2015)
<i>S.oblongifolia</i> (Vogel) H.S.Irwin & Barneby	28	Biondo <i>et al.</i> (2005a); Souza & Benko-Iseppon (2004); Rice <i>et al.</i> (2015)
<i>S. occidentalis</i> (L.) Link	24, 28, 26,	Biondo <i>et al.</i> (2005a); Matos <i>et al.</i> (2011); Rice <i>et al.</i> (2015)
<i>Senna pallida</i> (Vahl) Irwin & Barneby	28	Rice <i>et al.</i> (2015)
<i>Senna sophera</i> (L.) Roxb.	28	Rice <i>et al.</i> (2015)
<i>Senna surattensis</i> (Burm.f.) Irwin & Barneby	32	Rice <i>et al.</i> (2015)
<i>S. siamea</i> (Lam.) H.S.Irwin & Barneby	28	Resende <i>et al.</i> (2013); Souza & Benko-Iseppon (2004); Matos <i>et al.</i> (2011)
<i>S. tora</i> (L.) Roxb.	26	Rice <i>et al.</i> (2015)

Table 2. Species of the genus *Senna* Mill. with sampling localities.

Species	Accession number Locality	Latitude	Altitude
<i>Senna alata</i> (L.) Roxb.	1-George town Allahabad	25°27'30.7"N	81°51'06.9"E
	2-Basti	26°48'50.4"N	82°45'46.8"E
	3-Handia	25°17'42.4"N	82°58'16.2"E
<i>S. occidentalis</i> (L.) Link	1- Siddharthnagar	27°16'36.0" N	83°01'36.7"E
	2-Jhansi	25°30'46.3" N	78°31'58.3"E
	3- Saidabad	25°22'13.1" N	82°06'51.6"E
<i>S. siamea</i> (Lam.) H.S.Irwin & Barneby	1-Allahabad University	25°27'42.0"N 25°25'00.2"N	81°51'11.4"E
	2-Hanumanganj	25°23'14.2" N	82°01'29.9"E
	3-Varanasi		82°53'48.7"E
<i>S. tora</i> (L.) Roxb.	1-Phaphamau	25°32'21.6"N	81°53'34.1"E
	2- Babatpur	25°26'38.0"N	82°51'17.6"E
	3- Bhadohi	25°15'42.0"N	82°35'15.4"E

obtained from buds with sizes previously defined through squashing with the 1% acetic-carmin stain technique. The cell suspension was prepared according to Viccini *et al.* (2005). Carmine-stained slides were evaluated under Nikon Research Electron microscope using Olympus PCTV Vision views were recorded from each slide. The meiotic process was analyzed in 5000 meiocytes per species.

RESULTS AND DISCUSSION

The taxonomy and nomenclature of *Senna* Mill. species are quite complex and fascinating. Due to the large similarity range in their morphological characters, they are not easily differentiated from closely related species *i.e.* presence of more similarity causes misidentification and misinterpretation of the plant. Generally, environmental changes affect the morphological characters and the changes are not constant from species to species and differ from place to place. Now it is possible to single out differences on the basis of floral morphology, which is authentic and less affected by environmental factors in comparison to the vegetative part of plants. **Plate 1** represents differences in the floral morphology and plant habits among the four species of *Senna* in a wild condition. Characterization of species at the genetic level fosters efficient conservation, maintenance and utilization of the existing genetic diversity. The four species of *Senna* were studied extensively through morphological, taxonomical and cytological facets.

Habit of the plant is a shrub like the stem is erect, with glabrous surface and it attains the height of approximately 2-2.5 meters. Leaves are pinnated comprising of 8-14 pairs of leaflets. Leaves are sub- sessile having a length of about 38-72 cm. The leaf shape is broadly elliptical oblong, obtuse at apex, obliquely subcordate at base; having upper surface glabrous. Stipules are auriculated and sub- persistent in behavior (**Table 3**). Flowers are arranged in long, erect, apparently terminal pedunculate racemes. Yellow coloured conspicuous bracts are present. Sepals are petaloid and obovate. Stamens are not equal in length where 7 of them are fertile while 3 are staminodes.

Plants are undershrubs, annual 0.5-2 m height. Stem and branches are angular, striated with reddish purple texture. The stem is glabrous all around except for the young part. Leaves are 10-20 cm long having a single gland at the base of the petiole. Pinnatifid leaf has 3-5 pairs of the leaflet, the shape is ovate and acuminate at apex uppermost pair of leaflets has single gland. Stipules are ovated acuminate in shape (**Table 3**). Flowers are composed in axillary corymbose arrangement forming a terminal panicle inflorescence. Sepals are five, distinct with a pink colour having oblong/ obtuse form petals are bright yellow in colour having distinct veins, of unequal size. Seven fertile stamens are present among which 3 lower ones are longer than the lateral ones 3 staminodes are also present (**Plate 2 and Table 4**).

Table 3. Morphological analysis of species of *Senna* Mill.

Species	Plant height (m)	Leaf length (cm)	Leafletes (pair)	Glands Present(+), absent(-)	Stipule Present(+), absent(-)
<i>S. alata</i>	2-2.6	38-72	8-14	(-)	(+)
<i>S. occidentalis</i>	0.5-2.2	10-20	3-5	1, (+)	(+)
<i>S. siamea</i>	4-8.5	15-30	6-16	(-)	(-)
<i>S. tora</i>	0.3-1.5	5-12	3	2, (+)	(+)

This species of senna is a medium sized tree attaining a height of 4.5 - 8 meters height. The bark is a prominently dark brown or blackish brown texture with evergreen leaves. Leaves are 15-30 cm long and pinnatifid leaf bearing 6-16 pairs of leaflets. The shape of the leaf is oblong to ovate oblong, emarginated with a small bristle form sinus, gland absent (**Table 3**). Flowers are arranged in corymbose where 2-3 flowered racemes form a panicle. Pedicels are variable as 2-4 cm long. The

shape of sepals is elliptical ovate while petals are bright yellow in colour, obvate, broadly elliptic. Anterior petal is clawed. Androecium comprises of stamens which are 10 in the number of unequal sizes. Filaments of longer stamens nearly 3mm long. Style is glabrous (**Plate 2 and Table 4**).

The species is a herb or under shrub. It is annual erect, 0.2-1-2 meters tall. The stem is flexous. Leaves are 5-10



Plate 1. Photographic representation of Senna Mill. plant. (A) *S. alata*; (B) *S. occidentalis*; (C) *S. siamea*; (D) *S. tora*. Bar = 3

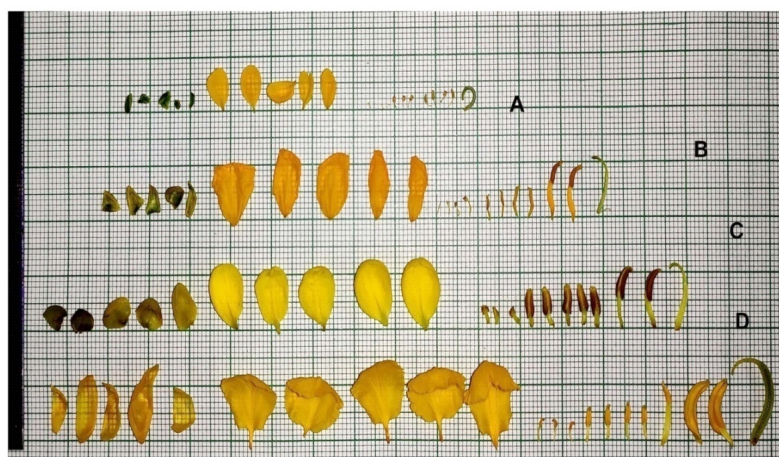


Plate 2: Comparative floral morphology of Senna sp. A: *Senna tora*; B: *Senna occidentalis*; C: *Senna siamea*; D: *Senna alata*.

Table 4. Floral estimation of species of *Senna* Mill. with their respective sampling localities.

Species	Sepals, 5 (mm)	Petals, 5 (mm)	Stamens	Carpel, 1 (mm)
<i>S. alata</i>	8-14 , petaloid	12-20, spatulate	7 fertile, 3 staminode	18-20, curved
<i>S. occidentalis</i>	3-8 , pink	10-13	7 fertile, 3 staminode	9-12, straight
<i>S. siamea</i>	5-10, hairy at margin	10-12, upper clawed	10 unequal	12-14 slightly curved at tip
<i>S. tora</i>	2-5	7-9	7 fertile unequal	5-6 highly curved

cm long having 3 pairs of leaflets. Leaf shape is obvate, obtuse, rounded, glabrous surface due to presence of hair on the upper surface, appressed hairy beneath. 2 glands are present in between the two lowest pairs of leaflets. Stipules are present that are linear, acute hairy (Table 3). Flowers are present on 0.5-1 cm long peduncle in an axillary arrangement. Sepals are ovate in shape, 6-8 mm long while petals are 8-10 mm long. Stamens are unequal in length and 7 in number (Plate 2 and Table 4).

In the present study, cytological work was executed from the collected buds of different sp. of *Senna*. Meiotic disturbance in a wild plant at different geography was considerably encountered at metaphase and anaphase as mention in Fig.1 and 2.

The rate of chromosomal aberrations was documented in the form of Total Abnormality Percentage (TAB) in Table 5. Maximum abnormality per cent was obtained in the case of *Senna siamea* (4.62 ± 0.11) and minimum in the case of *Senna occidentalis* (2.40 ± 0.35), whereas *Senna alata* and *Senna tora* showed 3.26 ± 0.11 and 4.18 ± 0.32 TAB per cent, respectively (Fig. 3). In the present study, different types of metaphasic abnormalities such as stickiness, scattering, unorientation, univalent whereas, anaphasic abnormalities like stickiness, anaphasic unorientation, laggard, bridge were recorded. Some other abnormalities which include cytomixis, micronuclei were also recorded.

Pollen mother cells (PMCs) of *Senna alata* and *Senna occidentalis* showed normal pairing of their bivalents in diakinesis stage. In addition to the normal pairing of their bivalents, still other meiotic abnormalities were observed in *Senna alata* plants in both first and second meiotic divisions. The profound abnormalities included were cytomixis and chromosome stickiness at different accessions in which cytological investigations of *Senna alata* showed that cytotoxic channels were a usual abnormality in this plants population. In the majority of cells (Fig.1, J & K), significant frequencies of the PMCs depicted cytotoxic flow between 3 cells and 4 cells. Cytomixis is defined as the migration of chromatin between adjacent cells through cytoplasmic connection channels and was first recorded by Kornicke (1901) PMCs of *Crocus sativus*. Early prophase was seen with apparently double the chromatin and with two nucleoli flow, which indicates that the maximum PMCs formation must have resulted from cell fusion or cytomixis in premeiotic conditions. These syncytes formations involve the fusion of two or more PMCs, usually in the early prophase of the first meiotic division. Disorder in cytokinesis during the premeiotic division, abnormal spindle formation and direct cell fusion (Pagliarini *et al.*, 2000) is the forceful mechanism for the formation of syncytes. It was also observed that the presence of micronuclei in *Senna siamea* (Fig. 2. O & Q), micronuclei originate either from acentric chromosomal fragment or sometimes the whole chromosome left behind at anaphasic stage of cell division that is unable to incorporate into the nucleus.

Table 5. Total Abnormality Percentage (TAB%) and various Chromosomal Abnormality in *Senna* species

Species	Metaphasic abnormalities % (Mean \pm SE)				Anaphasic abnormalities % (Mean \pm SE)				Cytomixis	MN	Other	TAB% (Mean \pm SE)
	St	Sc	Un	Uni	Ast	Aun	Lg	Br				
<i>Senna alata</i>	0.39 \pm 0.23	0.25 \pm 0.12	0.26 \pm 0.13	0.26 \pm 0.13	-	0.26 \pm 0.13	0.25 \pm 0.12	0.26 \pm 0.13	2.04 \pm 0.12	-	0.26 \pm 0.13	4.18 \pm 0.32
<i>Senna occidentalis</i>	1.00 \pm 0.17	0.23 \pm 0.11	0.33 \pm 0.18	-	0.23 \pm 0.11	0.33 \pm 0.18	0.22 \pm 0.11	-	-	-	0.23 \pm 0.12	2.40 \pm 0.35
<i>Senna siamea</i>	0.77 \pm 0.22	0.26 \pm 0.13	0.26 \pm 0.13	0.26 \pm 0.13	0.24 \pm 0.12	0.26 \pm 0.13	0.50 \pm 0.10	0.35 \pm 0.20	-	1.50 \pm 0.13	0.26 \pm 0.13	4.62 \pm 0.11
<i>Senna tora</i>	0.23 \pm 0.12	0.27 \pm 0.13	0.39 \pm 0.02	0.82 \pm 0.30	0.24 \pm 0.12	0.37 \pm 0.22	0.26 \pm 0.13	0.26 \pm 0.13	-	-	0.24 \pm 0.12	3.26 \pm 0.11

Note: St- Stickiness; Sc- scattering; Un- Unorientation; Uni- Univalent; Ast- Anaphasic stickiness; Aun- Anaphasic unorientation; Lg- Laggard; Br- Bridge; MN- Micronuclei; TAB- Total abnormalities percentage

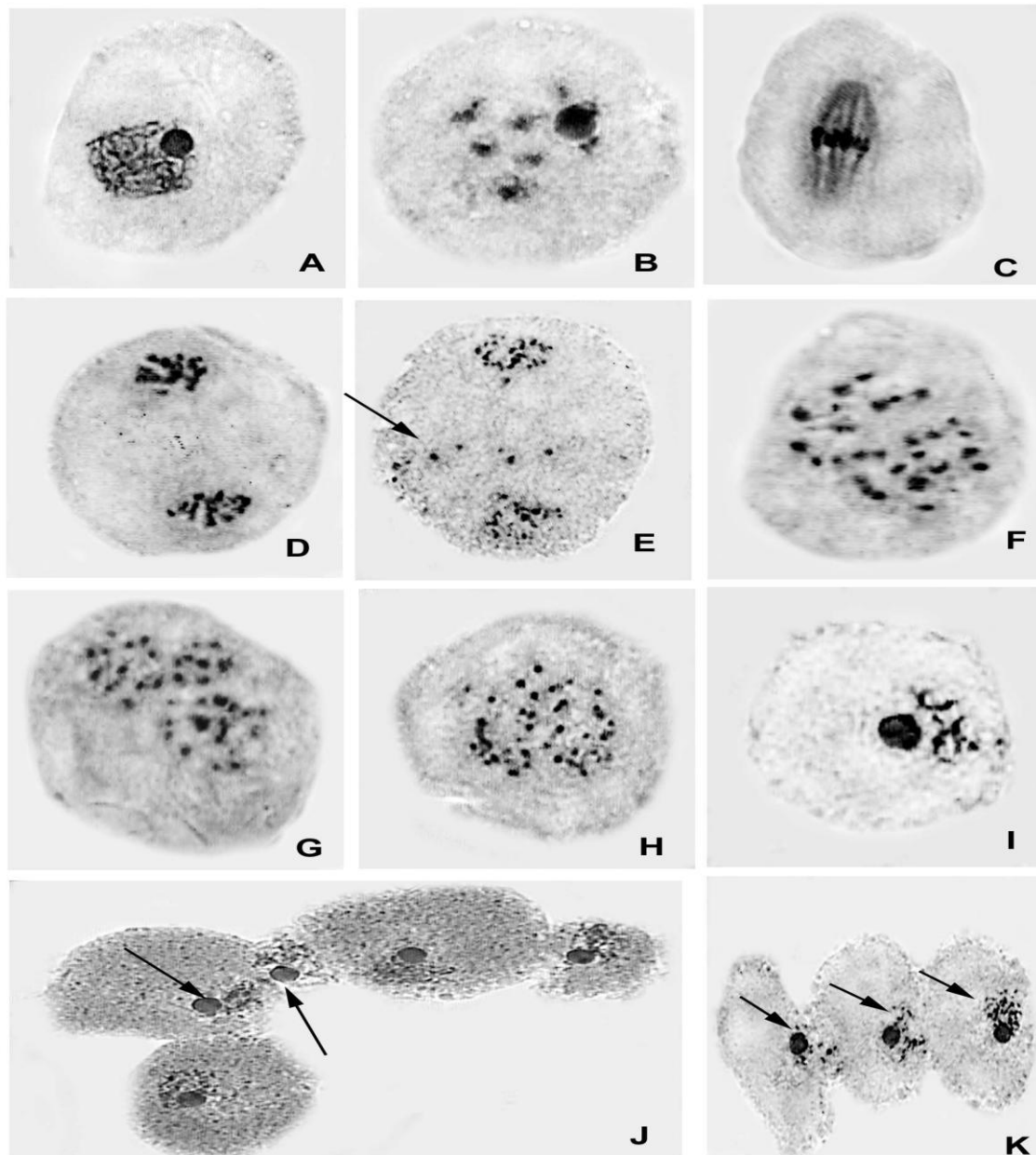


Fig. 1. Cytological studies in *Senna* sp. A to E- Meiotic configuration of *Senna occidentalis*. A: Pachytene stage; **B:** Diakinesis stage; **C:** Sticky metaphase I with prominent spindle fibers; **D:** Unorientation at Anaphase I; **E:** Laggard formation at anaphase I. **F to H- Meiotic configuration of *Senna tora*. F:** Early anaphase II; **G:** Unorientation at Anaphase II; **H:** Univalent formation **I to K- Meiotic configuration of *Senna alata*. I:** Prophase I; **J:** Cytometric flow between 4 PMC; **K:** Channel formation among 3 PMCs.

Such kinds of micronuclei appear due to the genotoxic effect of clastogen or aneugen (Ventura-Camargo *et al.*, 2011). Thus, geographical climate can also induce this type of abnormality. The micronuclei might have been the result of acentric fragmentation and chromosome laggard during anaphase (Leme and Marin-Morales,

2009). In *Senna occidentalis*, meiotic behaviour was considered regular (**Fig.1**) based on the analysis of at least 100 meiocytes per phase of the cell cycle. In results normal pairing of 14 bivalent in diakinesis (**Fig. 1 B**) and normal chromosome segregation in anaphase I and II were observed. Chromosome stickiness and adherence

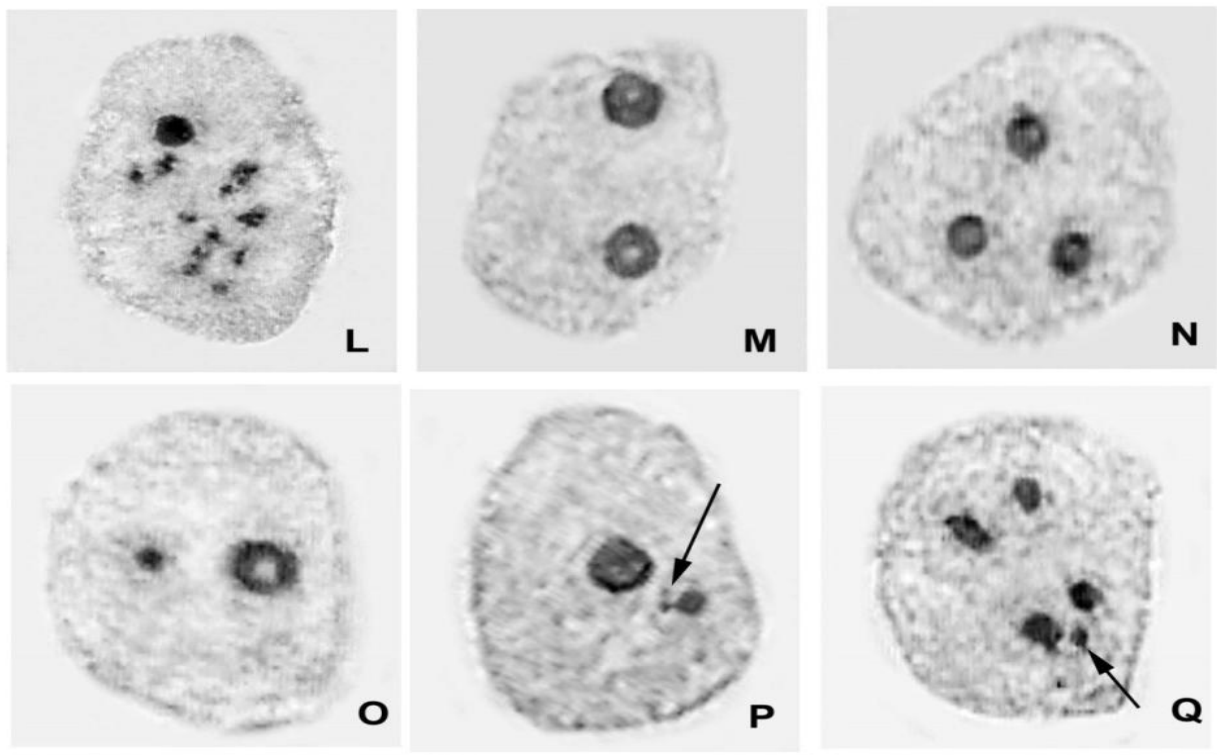


Fig. 2. Cytological studies in *Senna* sp. L-Q Meiotic configuration of *Senna siamea*. L: Diakinesis; M: Telophase I; N: Tri-nucleated cell; O: Micronuclei; P: Nuclear budding; Q: Micronuclei at Telophase II

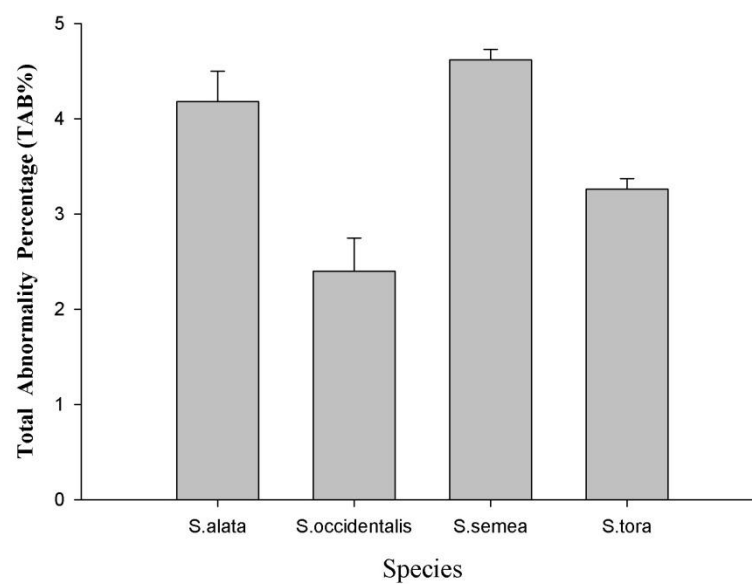


Fig. 3. Total Abnormality Percentage (TAB%) in *Senna* species

in *Senna occidentalis* was the prominent anomaly in the observed result (Fig. 1 C). Stickiness is a cytogenetical abnormality which is also has been widely described in plants. Stickiness was first described by Kornicke (1901) but the term stickiness was first introduced by Beadle (1932) in the aspect of corn chromosomes observed in cells carrying a recessive mutation (Kumar and Singh, 2020). Bridges and laggard formation at anaphase were recorded at high frequency in *Senna occidentalis* among four *Senna* sp. This was the second anomaly in this plant after the stickiness. Chromosomal bridges were another anomaly in both the plants encountered prominently at anaphase. It might have occurred at a high rate of environmental UV-B irradiation, making chromosomal breaks, then these broken chromosomes, healed, producing double centromere chromosome *i.e.* chromosomal bridges. The formation of bridges could be credited to chromosomal stickiness (Kumar and Rai, 2007 and Sikder *et al.*, 2015) and chromosome breakage and gathering (Kumar and Singh, 2002.) that proceed due to loss of genetic material (Sharma and Kumar, 2004). Such chromosomal abnormalities may affect adversely the vigour, fertility and yield of the exposed plant. The species *Senna tora* with $n=13$, suggest the occurrence of either aneuploidy and dispoloidy in the evolutionary history of *Senna* which is previously reported by Souza and Benko-Iseppon (2004) for the genus *Cassia* which includes *Senna* along with *Chamecrista* and *Cassia*.

It can be concluded that present work supports $x=14$ as the basic number for *Senna* in all the genera except *Senna tora* which had observed the basic chromosome number of $x=13$. *Senna siamea* shows abnormality with higher temperature, PMCs shows micronuclei as a prominent anomaly. Despite the considerable rate of chromosomal pairing patterns, cytomixis and cytoplasmic channel flow anomaly was maximum in the accessions of *Senna alata*. The stickiness of chromosomes at metaphase and anaphase stage was considerably high in *Senna occidentalis* compared to the other three species. In conclusion, results indicate towards environmental effect on cytomixis in *Senna alata*, and micronuclei in *Senna siamea* during a definite phase of meiosis (prophase I), although the phenomenon of cytomixis is direct under the control of gene as previously proposed by Mantu and Sharma (1983), the physiological, environmental and climatic condition is certainly influence its manifestation. Higher the total abnormality parentage (TAB%), shows more stress conditions for cell division explained that the geographical condition is more susceptible. *Senna occidentalis* and *Senna tora* having lower TAB% more suitable as well as wild for this geographical location as a comparison to *Senna siamea*. Maximum TAB% recorded in *Senna seamea* explains that the geography conditions where the study was conducted are less favorable for the plant in comparison to other species.

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