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Chromosomal variations and genetic diversity in subpopulations of *Senna alexandrina* Mill. from Western Thar, India

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Abstract. Homologous recombination promotes genetic diversity by exchanging genetic material between homologs, ensuring unique combinations of alleles in offspring. Karyomorphology of the chromosomes can prove to be an efficient tool to reveal the true nature of plant species at genetic level. In this context, our study analyzed the karyomorphology and male meiosis in a medicinal herb, Senna (*Senna alexandrina* Mill., Syn. *Cassia angustifolia* Vahl.), family Fabaceae which is known for its significant polymorphic variations. By observing chromosomal variances, we aimed to shed light on the underlying genetic variations responsible for the observed polymorphism. All the accessions of Senna examined in this study exhibited a diploid chromosome number of 2n = 28. We found variations in the chiasma frequencies of almost all the accessions, particularly concerning the observed number of bivalents, quadrivalents, position of centromere and the presence of the B- chromosome at meiosis-I. Amongst the four accessions studied, two displayed reduced pollen stainability, which seems to be correlated with a lower frequency of chiasmata and the influences of the collection sites, that was confirmed by the regression analysis. Further, RAPD analysis also confirmed the variations in DNA homologous sequences recorded by the presence of variable length of the fragments in all accessions. All the results collectively underscored the existence of genetic diversity within the subpopulation of *Senna alexandrina* Mill. & may help to comprehend the broader evolutionary processes within the Fabaceae family.

Keywords: Cytogenetic analysis, Karyotype, Male meiosis, Mitosis, Polymorphism, *Cassia*, Subpopulation variations.

INTRODUCTION

Cytogenetic analysis particularly plays significant role in taxonomy, genetic abnormalities and genetic diversification, as karyotypes can examine genetic differences between subpopulations of the same species having distinct traits (Young et al. 2012; Jha and Halder 2023). Chromosomes, as the tangible carriers of Mendelian factors, have held a significant role in plant systematics because alterations in their number and structure have consistently been associated with the evolution and formation of plant species (Gill and Husaini 1982). The chromosomal variations reflect the sources of genetic

variation within populations at morphological, physiological, and biochemical level arise from gene expression influenced by the environment with root cause mutation (Chesnokov et al. 2020; Nonić and Šijačić-Nikolić 2021). Plant karyotype research holds significant importance in deciphering the origin and evolution of plant species, understanding molecular phylogeny, and elucidating floristic geography (Lucas et al. 2021). In medicinal plants, cytological analysis is crucial for ensuring the proper identification of herbs in medicinal drug preparations (Deakin et al. 2019; Nonić and Šijačić-Nikolić 2021) and for various other applications, including studying genetic disorders, understanding chromosomal abnormalities, and conducting research in fields such as evolutionary biology and genetics (Deakin et al. 2019; Vitales et al. 2020). In most cases, variations such as differences in chromosome length, arm ratio, position, and the presence of secondary constructions, provide enough information to differentiate individual chromosomes. However, at a more precise level, molecular markers allow us to assess and quantify genetic diversity within populations, species, or germplasm collection for understanding the genetic structure, evolutionary history, and potential adaptability of organisms (Marsjan and Oldenbroek 2006; Omondi et al. 2016).

Meiosis, a pivotal reproductive process, involves homologous chromosome pairing, synapsis, recombination, and segregation, effectively halving the chromosome number to maintain the species' diploid count in the zygote. In normal meiotic processes, pollen mother cells exhibit 100% pollen viability, regular bivalent formation, and normal cytokinesis (Pagliarini 2000; Kaur and Singhal 2019).

The paraphyletic genus *Senna* Mill., belongs to the subfamily Caesalpiniodeae of the family Fabaceae and comprises approximately 350 species that are distributed worldwide (Pellerin et al. 2019, Kumar et al. 2021). *Senna* species are extensively utilized in Africa, Asia, Europe, and Latin America for medicinal purposes and have gained recognition for their antimicrobial, anti-diabetic, anti-malaria, anti-inflammatory properties, which have been documented in traditional medicine practices (Resende et al. 2013; Oladeji et al. 2021). The genus *Senna* is predominantly characterized by a diploid chromosome number of 28, although alternative numbers such as 22, 24, 26,52, and 56 have been reported for specific species (Irwin and Truner 1960; Rasende et al. 2013; Cordeiro and Felix 2017; Nguyen et al. 2021).

Senna alexandrina Mill., syn. *Cassia angustifolia* Vahl. (Indian Senna or Egyptian Senna) is a native species to Saudi Arabia, and widely distributed in tropical and subtropical regions (Kumar et al. 2022). It is a under shrub plant height of 1-2 m having pinnately compound leaves with 4-8 pairs leaflets. Senna holds significant value in Ayurveda and is extensively used as a febrifuge, for splenic enlargement, typhoid, cholera, anemia and laxative purposes (Laghari et al. 2011; Shaily et al. 2023). India is the largest producer and exporter of Senna leaves, pods, and sennosides concentrated in the global market (Saudan 2018). Its leaves and pods possess important purgative properties used in medicine (Nayan et al. 2021).

Here, we present the karyomorphological and male meiosis studies on a subpopulation set of *Senna alexandrina* Mill. in four different accessions sites. Our aim is to gain insights into the genetic diversity within the species that is reflected and verified by the morphological variations in the species in its native environment.

MATERIALS AND METHODS

Plant materials

The germplasm of four accessions of *Senna angustifolia* Vahl. were obtained from different sources-the IHCM accession from NBPGR-CAZRI, Jodhpur (26.263611,72.995352), the IHGA accession from the private institute of Herbal Heritage, Sonamukhi Nagar, Jodhpur (26.193664-73.001885), and the RAU-1 and RAU-2 accessions were obtained from Swami Keshwanand Rajasthan Agriculture University, Jodhpur (28.075225,73.344524). Vouchers of these accessions were submitted to the Department of Botany, JNVU, Jodhpur, and BSI Jodhpur. The seeds were collected during the kharif season for two consecutive years and stored in a cool place. Following the experimental design, the seeds were treated and germinated in the nursery soil at the Botanical Garden, Department of Botany, JNVU, Jodhpur. Phenological records were made for all four accession numbers, including observations on the plant's habit, habitat, height, leaf size, shape, blooming period, cluster of pods, number of seeds per pod, and fruiting time.

Seed germination of F_0 generation

The seeds of *S. alexandrina* were obtained from wild populations (considered here as the F_0 generation). The seeds were first subjected to a fungicide treatment using 0.1% sodium Bavistin for 7-8 minutes to minimize fungal growth. Afterward, they were rinsed thoroughly four times with autoclaved distilled water to remove any residual fungicide. The sterilized seeds were then placed in disposable petri plates containing pre-moistened soil.

These plates were incubated in a controlled environment with a constant temperature of 25 ± 2°C and maintained in darkness to encourage germination.

Mitotic chromosome preparation and staining

After the seed germination, the root tips of appropriate length (0.5-1.0 cm) were excised in the morning between 7:30 am to 8:00 am and immediately pretreated with 0.025% colchicine (HiMedia © India) for three hours at room temperature to arrest cytological stages. After the pretreatment, the root tips were washed multiple times with distilled water, carefully dried by absorbing the moisture, and subsequently fixed in Carnoy's fluid (1 part of glacial acetic acid mixed with 3 parts of 95% ethanol (v/v)) for at least 24 hours at 4°C. The tips were stored in 10% ethanol at 10° C in a refrigerator for long-term use.

The stored root tips were hydrolyzed with 0.1 N HCl for 2-4 minutes at 60 ± 2°C and then washed with distilled water. The softened root tips were stained with 0.5% leuco-basic fuchsin (HiMedia) and subsequently squashed in 1% aceto-carmine (HiMedia) to obtain cytological observations.

At least five clear preparations of metaphase stage of each accession were analyzed to prepare karyotypes. The slides were observed under a light microscope (Olympus BX 60). The average length of the short (p) and long arm (q) of each chromosome was measured using the software Sigma (Pro v. 3 software and ImageJ software).

Male meiosis

Young flower buds were collected in the morning between 7:30 am to 8:00 am and immediately fixed in Carnoy's solution and kept for approximately 24 hours at room temperature. The fixed anthers were then separated from all non-anther parts by using a clean fine needle on the surface of alcohol-washed glass slide. The anther lobes were then squashed onto the glass slide using 1% acetocarmine. A total of 25-30 pollen mother cells (PMCs) at the diplotene/diakinesis and metaphase I stages were observed to record chromosome associations and chiasmata frequencies. It was noted that minor differences existed between the diakinesis and metaphase I stages in terms of associations and chiasma frequency. Therefore, observations from both stages were compiled together, considering an equal number of cells from each stage. Additionally, 15-20 PMCs were analyzed at anaphase I and II to study the distributional pattern of chromosomes/chromatids.

Microphotography

Microphotography was conducted using a Trinocular Research Microscope (Olympus, model BX60F) to capture photomicrographs of the cytological preparations. The photographs of the chromosomes were further analyzed using DRAWID software for ideogram development.

Genomic DNA isolation and PCR amplification for RAPD markers amplification

Total genomic DNA was extracted from 2 g fresh young leaves (2-3 weeks old) of F1 generation of all accession of *Senna alexandrina* by using a modified CTAB method (Lodhi et al. 1994). The extracted DNA was treated with RNase to eliminate RNA impurities and then DNA integrity of the isolated DNA was visualized on Agarose gel (0.8%). After quantification with a spectrophotometer (Thermo Scientific ND-2000), the purified DNA was served for PCR-based amplification. Six out of 20 applied arbitrary RAPD primers (Operon Biotechnologies, Alabama USA) were found suitable for the DNA fingerprint of two accessions IHCM and RAU 1 (Table 1).

PCR amplification was conducted using a programmable thermal cycler (Mycle Bio RAPD 96 Well Gradient Machine) for 35 cycles. The reaction mixture (25 µL) included 2 µl of template DNA (25 ng/µl), 2.5 µL of random primer (IDT Technologies, USA), 4.0 µL of 10 mM dNTPs (Biogene), 1 unit of Taq polymerase (Geni, Bangalore), 2.5 µL of 10X reaction buffer (GeNei), 0.3 µL of 1.5 mM $MgCl_2$, and 15.7 µL of nuclease-free sterile water. The amplification conditions were as follows: initial denaturation at 92°C for 1 minute, annealing at 37°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The reaction ended with an indefinite hold at 4°C.

The 25 µL of amplified PCR product were mixed with 5 µL of gel loading dye (0.25% bromophenol blue, 25% Xylene eynol and 30% glycerol) and then loaded on 1.5% agarose gel electrophoresis along with ladder of 1 kb in 1X TBE buffer at pH 8.2 containing 0.5 µL of Ethidium bromide. The results of the gel run were photographed at Gel-doc UV visualizer with kodak digital Camera. The electrophoretogram of the results of electrophoresis gel analysis was scored based on the presence (1) or absence (0) of bands for all RAPD primers with respect to two accessions.

Karyotype analysis

The karyotype comparison was followed according to Battaglia's (1955) classification as metacentric/

Table 1. Polymorphism detected by six RAPD arbitrary markers in *Senna alexandrina*.

Primer code	Sequence of oligo 5'–3'	Range of fragment Size (bp)		Total no. of bands per primer		Polymorphism (%)	Jaccard similarity coefficient
		IHCM	RAU1	IHCM	RAU2		
OPA10	GTGATCGCAG	300-1000	175-700	4	6	80	0.25
OPX-12	TCGCCAGCCA	325-900	175-750	7	6	92.85	0.077
OPX-18	GACTAGGTGG	200-700	350-1000	4	4	83.33	0.091
OPX-07	GAGCGAGGCT	300-650	225-500	3	3	85.71	0.17
OPX-04	CCGCTACCGA	200-525	100-900	6	7	86.67	0.143
OPB-18	CCACAGCAGT	325-1300	150-900	5	7	88.89	0.125

median [V], submetacentric/submedian [L], subtelocentric/ [J], and telocentric [I] based on an arm ratio of 1:1>1:1<1:3>1:3<1:0 and 0:1 respectively. For karyotype depiction, we used IdeoKar software (version 1.2) to generate ideograms. The degree of symmetry was estimated using Stebbins's (Stebbins 1971) scheme by recognizing three degrees of difference between the largest to the smallest chromosome of the complements, and four degrees with respect to the proportion of sub-telocentric chromosomes.

Statistical analysis

We employed various techniques to assess significant phenotypic and cytogenetic variations within the *Senna alexandrina* population. We selected 9-15 individuals from each accession sites. These individuals were then subjected to detailed phenological characterization, recording height, number of branches, leaves, leaflets, pods, seeds, and leaflet size. Additionally, we computed the mean frequency and range of chiasmata, the terminalization coefficient, and pollen stainability for all accessions. To identify statistically significant differences between sites, we compared the mean values of these measurements using one and two-factor ANOVA, multiple regression, and Tukey's post hoc statistics with the R programming language and related packages. Furthermore, we conducted PCA analysis for different RAPD primers, examining their clustering patterns within sites based on band size.

RESULTS

Morphological characteristics

Senna alexandrina is observed as a small shrub, reaching a height of approximately 51 cm. Accession IHGA showed a smaller height compared to others. The stem of the plant is erect, smooth, and pale green. It displays long spread branches bearing 5-8 jugate leaflets, which are usually oval or lanceolate in shape and glabrous (Fig 1). The pod size was ~ 3.5 cm (Fig. 1-table). The RAU 1 accession exhibited a larger pod size, while the IHGA accession had a higher number of pods. Seed counts per pod, however, did not show significant variation. Morphological differences appear to be influenced by environmental factors, which may also contribute to chromosomal and DNA sequence variations.

Karyotypic variations

The karyotypic analysis of different accessions of *Senna alexandrina* revealed notable variations in the number and type of chromosomes. All the studied accessions exhibited a chromosome count of 2n = 28, comprising fourteen pairs of homologous chromosomes arranged in descending order of length within the complements (Fig. 2).

Among the 28 chromosomes in the IHCM accession, eight pairs were metacentric, six pairs were submetacentric. No heteromorphic pairs or nucleolar chromosomes were observed. The resulting karyotypic formula was 16V+12L (Fig. 2). Whereas in the IHGA accession, out of the 28 chromosomes, 9 pairs metacentric, 4 pairs submetacentric, and 1 telocentric were found. No heteromorphic or nucleolar chromosomes were observed in any of the complements. The karyotypic formula obtained was 18V+8L+2I (Fig. 2). In the case of the RAU-1 accession exhibited 9 pairs of metacentric chromosomes, 4 pairs of submetacentric chromosomes, and one pair of telocentric chromosomes among its 28 chromosomes. No heteromorphic or nucleolar chromosomes were observed. The karyotypic formula obtained for this accession was 14V+12L+2I (Fig. 2). In the case of RAU-2, the number of chromosomes analyzed was 28, confirming the same chromosomal complement as the other accession. Among these chromosomes, seven

Accessions	Plant height (cm) Mean ±SD	Leaf size /plant Mean ±SD (cm)	Leaflet number/ leaf Mean ±SD	Pod number/plant	Pod Size Mean ±SD	Seeds/ Pod Mean ±SD (cm)
IHCM	50.71 ± 5.31	25.70 ± 3.07	6.14±1.02	23.71±5.31	3.43±0.27	6.14 ±1.29
IHGA	48.57 ± 6.07	24.79 ± 3.32	6 ±1.04	25.85 ± 1.91	3.47±0.35	6.35±1.15
RAU-1	51.42± 3.95	23.5 ± 4.09	6.14±0.94	22.14±2.56	3.58±0.38	6.07±1.59
RAU-2	51.71 ± 4.80	21.41± 3.65	6.07±0.83	24.28±1.43	3.44± 0.25	6.42±1.40
Average	**51.71 ± 4.80**	**23.70± 3.40**	**6.08±0.96**	**24 ± 2.01**	**3.48±0.31**	**6.25±0.18**

Figure 1. Phenotypical observations in *Senna alexandrina*. The attached table highlights morphological variations across accessions, including measurements of plant height, branch number, and leaflet size.

pairs were metacentric, five pairs were submetacentric, and two pairs were telocentric in nature. No heteromorphic or nucleolar chromosomes were observed in any of the complements. The karyotypic formula obtained was 14V+10L+4I (Fig. 2).

The karyotypic details of the examined accessions of *Senna alexandrina* revealed a combination of metacentric (V), submetacentric (L), and telocentric chromosomes. Across all four accessions examined, in IHGA and RAU-I, the longest pair of chromosomes in the karyotype (designated as the I pair) exhibited submetacentric, and in IHCM and RAU–2 it was observed to be metacentric (Fig. 2).

Male meiosis, associations and chiasma frequency

The accessions of *Senna* from the different locality sites (Table 2 and Fig. 3) showed variations in chromosome patterns at different meiosis stages. RAU-1 site accession showed highest numbers of bivalent (96.67±2.18) and pollen viability in form of stainability % (97.71±0.45) of pollen grain. We observed chromosome associations in the range of 89% to 96%. The accessions RAU1 and RAU2 displayed higher associations compared to IHCM and IHGA. There was the occurrence of quadrivalent formation, which happened in about 3.33% of cases in RAU1 but was absent in RAU2. Additionally, RAU2 showed higher pollen stainability, indicating potentially lower genetic diversity compared to RAU1 and the other two. A one-way ANOVA showed that the differences in pollen viability across the levels of % occurrence of Chiasmata are highly significant ($F(1, 10) = 34.6$, $p < 0.001$). This result provides strong evidence that pollen stainability is influenced by the level of Chiasmata (Fig. 4a). These findings indicate that pollen stainability varies significantly across different levels of Chiasmata per PMC per accession collection sites (Fig. 4a-c). Comparatively, a high mean value of chiasmata frequency has been observed in RAU-2 which 27.03 chiasmata per PMC was recorded. The lowest mean value for chiasmata was recorded as 24.8 in IHGA. The remaining accessions had values ranging between these two. No. of chiasmata generally observed per bivalent was one or two. The maximum association of chromosome was found as 12 ± 2 II + 2 ± 2 I in maximum cells however quadrivalent occurrence was frequent in IHCM and IHGA (Table 2).

Meiosis configuration

S. alexandrina- IHCM

The meiotic configuration of *S. alexandrina* IHCM was characterized by a predominant presence of bivalents in the Pollen Mother Cells (PMCs) while few shows both bivalents and univalents. However, a few cells exhibited quadrivalent associations, suggesting chromosomal pairing anomalies or structural rearrangements. The gametic number reported in all the cells of this accession was n=14. The mean percentage of total bivalents was 91.42±1.42 with the range 12.73±0.11 (12-13

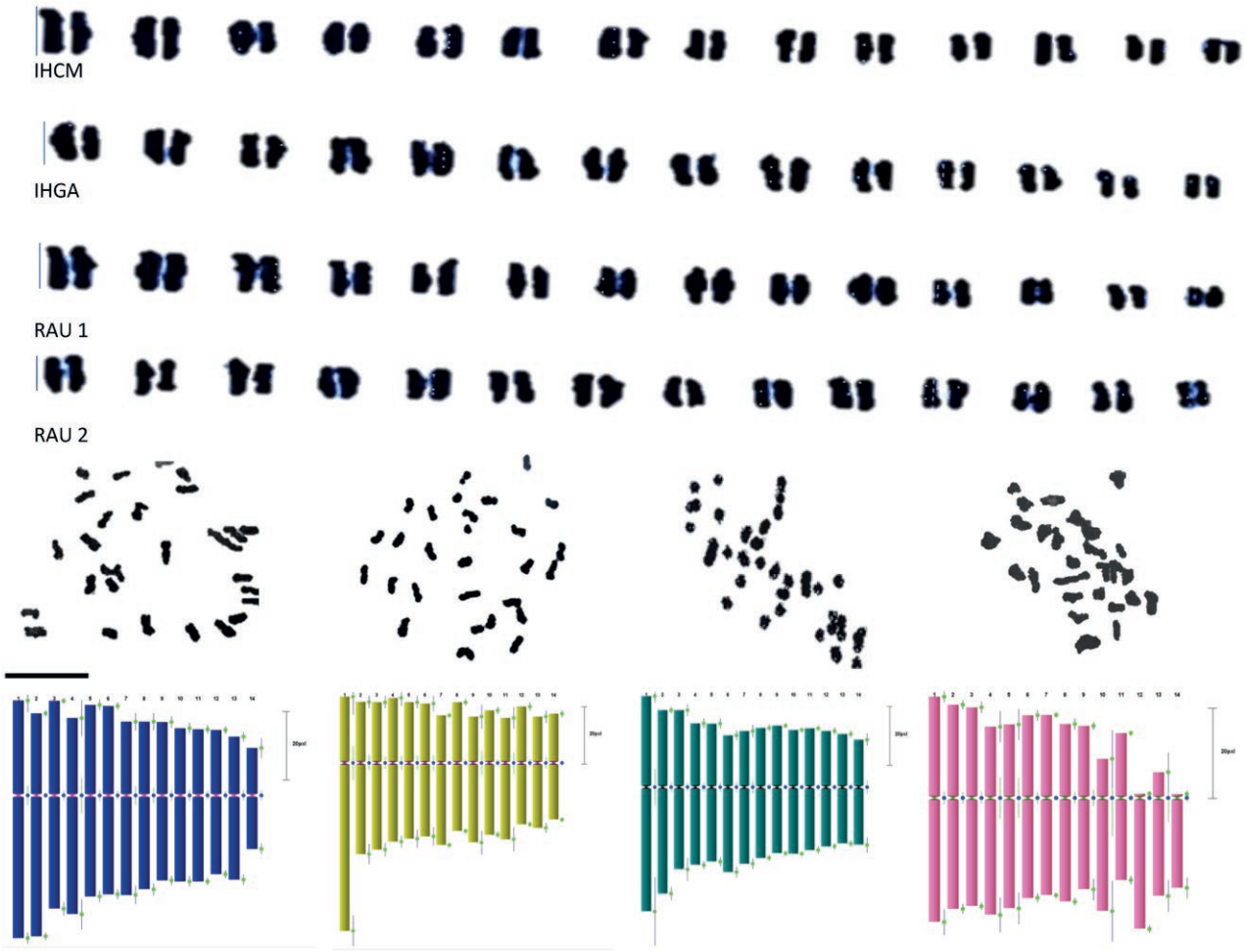

Figure 2. Mitotic karyotype of *Senna alexandrina* from four accession site. Mitotic spread and ideogram show that all four accession have the majority of the metacentric and submetacentric chromosome. RAU-2 had telomeric chromosomes as ideogram showed. Scale bar= 10 µm.

bivalents per cell), out of which 11.93±0.23 were of ring and 0.8 ±0.2 ~ 1 were rod-type bivalents. On average, there were 9.5±2.06 univalents per cell. The approximate chromosomal association per cell recorded was 1 IV+12 II+2 I (Table 2).

S. alexandrina- IHGA

In the IHGA accession, the mean percentage value of total bivalents was ~ 77%, out of which ~69% ring and ~ 25% were rod-type of bivalents, their number ranged between 8-14 in the observed PMCs. Few PMC cells also showed the presence of 1 or 2 quadrivalents (21.43±7.14 % abundance in per cell where quadrivalent present) with 5% occurrence in total observed PMCs. On average there were 13.09±4.12 % univalent per cell. Each cell on average may show chromosome associations 1 IV+11 II+ 2 I out of 14 gametic number. (Table 2).

S. alexandrina- RAU–1

The majority of the PMCs analyzed had shown a high frequency of bivalents along with a few cells showing a mixture of the quadrivalents or multivalent and univalents. The pollen viability was about 92% in this accession. The occurrence of total bivalents was 11-13 out of 14 maximum possible bivalents, out of which 10-13 were ring (12.8±1.58) and 0-3 (1.76±0.35) were rod type. On average 1.26±0.94 univalents (5.66±0.99%) were seen in the cells with 0-4 range. Each cell on average showed chromosome associations with 1 IV+12 II+1 I (Table 2, Fig. 3).

S. alexandrina- RAU–2

In this particular accession site, all of the examined PMCs exhibited almost fourteen bivalents (Fig. 3), with no observed quadrivalent or multivalent associations. The mean total number of bivalents was 13.53±0.31,

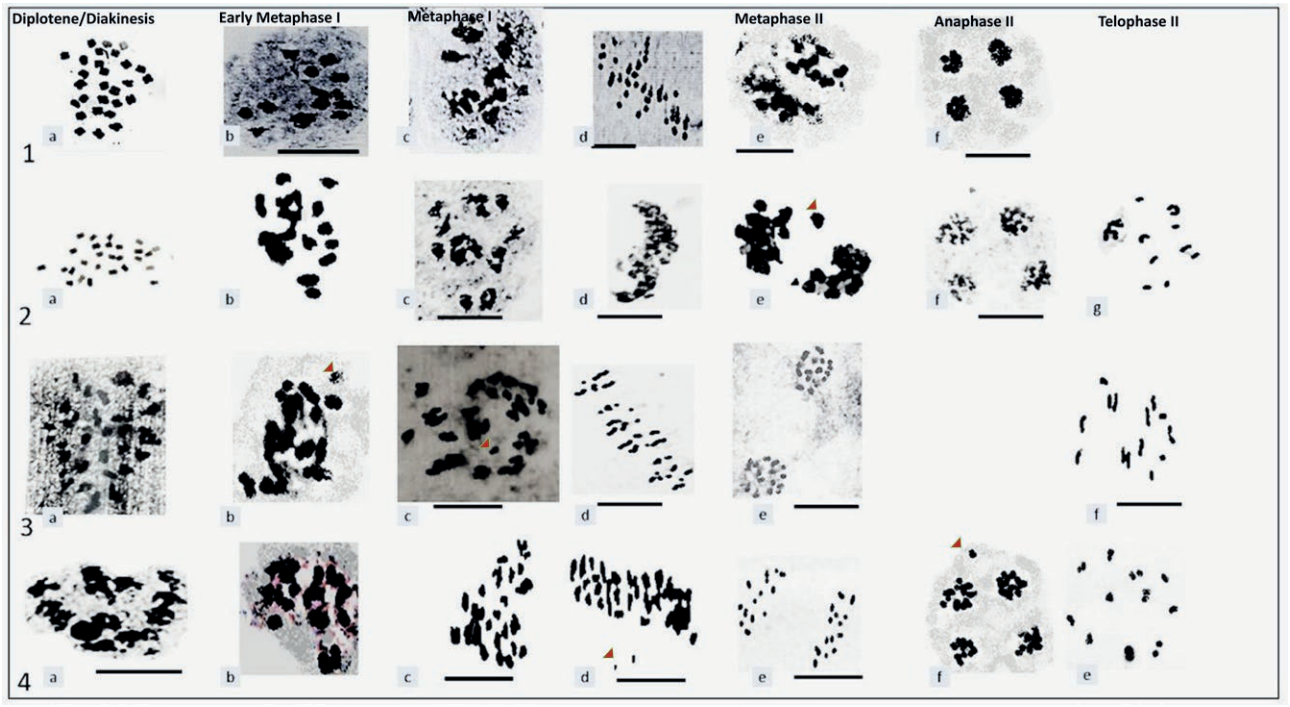

Figure 3. Meiotic chromosome behavior: Different stages of Meiosis in Pollen Mother Cells of *Senna angustifolia*. 1. IHCM, 2. IHGA, 3. RAU-1 and 4. RAU-2. B chromosome can be observed as a separated non-homologous part of DNA (arrow). Some chromosome was attached to nucleolus as a nucleolus organizing chromosomes (diakinesis stage 4a). One PLC at telophase (chromosome no. 14) showed nonseparated chromosome. Scale bar= 10 µm.

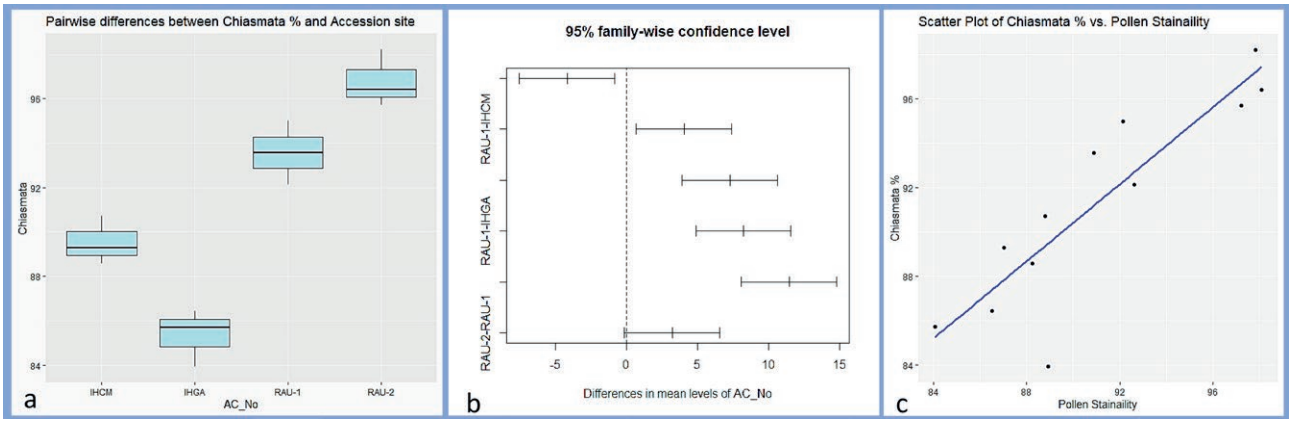

Figure 4. Post hoc analysis of Chiasmata frequency, terminalization and pollen stainability in *Senna alexandrina* with accession sites.: a. Pairwise comparison between chiasmata frequency and accession sites. Variation in chiasmata frequency significantly grouped as per site. b. Confidence level of Tukey HSD among groups. c. Correlation between pollen stainability and chiasmata frequency. Analysis: Software R using ANOVA (aov), regression (lm), plot (ggplot2). P value =0.05.

with a range of 13-14. Out of these bivalents (mean 96.67±2.18%), 13.63±0.32 (97.38 ± 2.29%) were ring type and 1.31±0.23 (11.90±2.97%) were identified as rod type. On average, 3.38±0.75% univalents were present per cell. Each cell demonstrated a chromosome association of 13-14 II+1-2 I (Table 2).

Meiotic association in anaphase I/II and pollen stainability

The no. of chiasmata ranged between 23-28 per PMC with an average of 25.9 out of which about 20 were terminalized with coefficient of 0.78. The percentage of pol-

Table 2. Meiosis chromosome observation in *Senna alexandrina* : Chiasmata %, terminalization co-efficient, chromosomes configurations, and pollen viability.

Ac. No.	Chiasmata Occurrence Mean ± SD %	Chromosome no. with Chiasmata/ cell Mean ± SD	Terminalization Coefficient (Tc) Mean ± SD	Quadrivalent/ PMC Mean ± SD % (Range)	Bivalent / PMC Mean ± SD % (Range)	Univalent /PMC Mean ± SD % (Range)	Pollen viability / flower Mean ± SD %
IHCM	89.52±1.09	25.20±0.26	0.72±0.07	1.90±0.82 (1.33±0.57)	90.95±0.82 (12.73±0.11)	9.52±1.19 (2.66±0.33)	88.01±0.91
IHGA	85.36±1.29	24.47±0.59	0.78±0.06	4.76±1.64 (3.33±1.15)	76.67±7.86 (10.73±1.10)	13.09±4.12 (4.05±0.41)	86.51±2.43
RAU-1	93.57±1.43	25.50±0.85	0.79±0.02	3.38±0.82 (2.67±0.57)	88.22±3.81 (12.35±0.53)	5.66±0.99 (1.59±0.27)	91.89±0.91
RAU-2	96.79±1.29	27.20±0.30	0.82±0.00	0	96.67±2.18 (13.53±0.31)	3.38±0.75 (1.23±0.30)	97.71±0.45

Single and two factor ANOVA and multiple-regression among the groups of IHCM, IHGA, RAU-1 and RAU-2.

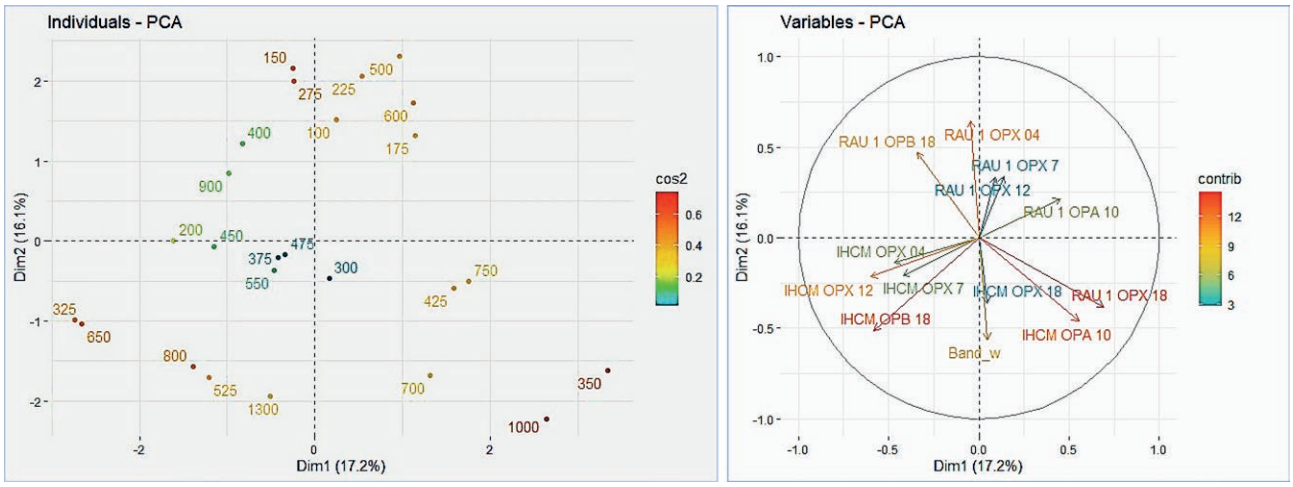

Figure 5. RAPD arbitrary markers-based polymorphism in two accessions of *Senna alexandrina*. -RAU 1 and IHCM. a), shows the clustering of all bands resulted RAPD markers. b), PCA cluster analysis significantly grouped accession and bands as per their variability. Cos2 = quality of representation of the variables of the principal components, contrib= contribution-based clustering.

len stainability was highest (97%) in RAU-2, and it was lowest (85%) in IHGA (Table 2).

Polymorphic bands amplified by RAPD markers

Out of four accession two were selected for RAPD analysis. The analysis of genetic variability in *Senna alexandrina* accessions IHCM and RAU-2 using six arbitrary primers (Table 1) resulted in the amplification of a total of 34 and 33 polymorphic bands, respectively. Polymorphic bands indicate genetic variability between the two accessions. The number of bands varied across different primers in IHCM and RAU-2 (Fig. 5).

Among the primers used, the highest number of polymorphic bands were amplified with primer OPX 12 and OPX 04 in the IHCM accession, and OPX 04 and OPB 18 in the RAU-2 accession. On the other hand, the lowest number of bands were amplified with primer OPA 10 and OPX 07 in IHCM, and OPX 18 and OPX 07 in RAU-2 (Table 2). The highest polymorphism was found for primer OPX-12 for these two accessions. The PCA analysis showed that the arbitrary RAPD primers OPX 04, OPX07, OPX12, and OPB 18 can be used to identify the intra species variations. However, advanced versions of DNA sequencing may give high potential results, but at preliminary level RAPD can provide evidence about genetic changes in a population. Dendrogram (Fig. 6) based on these primers were clustered RAUI for OPB18 and OPX04 against remaining band showing high similarity and less magnitude of the dif-

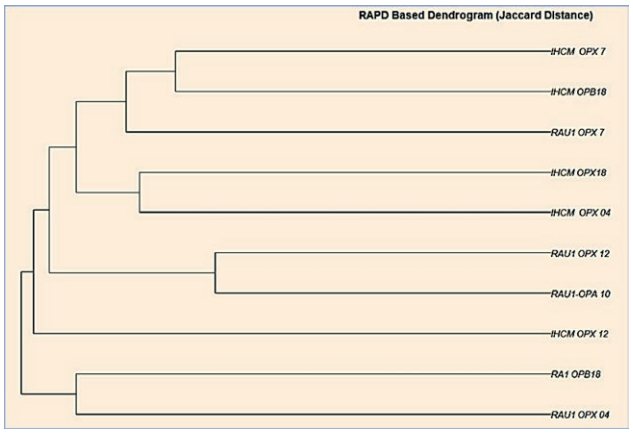

Figure 6. UPGMA Hierarchical clustering of *Senna alexandrina* accessions (RAU 1 and IHCM) based on RAPD band presence/absence patterns (binary data). Jaccard Distance –Jaccard distance metric used to measure similarity (proportion of shared bands). Shorter branch lengths indicate higher similarity in the proportion of shared bands.

ference as per the Jaccard coefficient. The amplified bands might share a high degree of sequence similarity.

DISCUSSION

Cytological evidence strengthens the understanding of the evolutionary origin of a species, it provides chromosome number, length, type, ploidy, and distribution pattern of specific sequences in the whole genome within a population of a species. Such information is useful in identifying the phylogenetic relationship among the related species (He et al. 2022). The present investigation involves a representative collection of four accessions of a sub-population Senna (*Senna alexandrina* Mill.) a natural laxative medicinal plant from different areas. All confirmed the somatic chromosome number as 28, without any indication regarding the existence of polyploidy/aneuploidy or any numerical variation in the natural populations. In our observations except for a few exceptional cases (±1), almost all the cells analyzed had shown the 2n number of chromosomes as 28 which confirms previous observations published on *Cassia angustifolia* Vahl. (Irwin and Turner 1960; Elaine et al. 2005; Cordeiro and Felix 2017; Nguyen et al. 2021). However, a chromosome number=26 for *Senna* plants has also been reported (Kumar et al. 2024a).

Distinctive variations have been observed in the karyotypes of multiple accessions of *Senna alexandrina*. These differences primarily involve the presence of metacentric (V) or submetacentric (L) chromosomes, although three accessions also displayed the presence of telocentric (I) chromosomes. There is a correlation between the level of ploidy and total haploid chromatin length (Doyle & Coate 2019). Across all four accessions examined, in IHGA and RAU-I, the longest pair of chromosomes in the karyotype (designated as the I pair) exhibited submetacentric, whereas in IHCM and RAU–2 it was metacentric. Variations in total haploid chromatin length or karyotype morphology within different diploid or tetraploid taxa may possibly be attributed to chromosomal rearrangements involving the loss or gain of segments, paracentric inversions, and translocations. Such alterations in the karyotype represent significant evolutionary mechanism that plays a role in the diversification and speciation of angiosperms for ecological adaptation (Weiss-Schneeweiss and Schneeweiss 2012; Lavania and Lavania 2021).

In the meiotic study, the occurrence of meiotic abnormalities in a species that is normally fertile and productive indicates the existence of some homoeostatic mechanism related to survival. The increase in the frequency of chiasmata also points out the capacity for the release of variability by the organism (Osman et al. 2021). The Meiotic behavior, reported in *Cassia flexuosa, Cassia vestita, and Cassia desvauxii* at diakinesis and metaphase I showed chromosome disjunction and segregation was over 99% and pollen fertility was over 92% (Biondo et al. 2006). The distal chiasma was predominant over interstitial chiasma, and they were terminalized at early metaphase I. The total chiasma frequency in PMCs of plants of one species is a stable index of recombination potential which is not dependent on the growing conditions (Strelnikova et al. 2019).

Meiotic behavior is generally regular with a predominance of bivalent pairing in diakinesis and metaphase I, and normal chromosome segregation at anaphase I and II. Some irregularities, such as quadrivalents, multivalents, and univalents at diakinesis and metaphase I, and bridges and unequal segregation at anaphases were observed in some accessions of *Senna splendida, S, multijuga, S. corymbosa* and *S. occidentalies* (Elaine et al. 2005). Interestingly, all the accessions of *Senna angustifolia* had a maximum of bivalents, with rare quadrivalent formation. Some earlier findings also confirmed the fact that chromosomal numerical changes in the genus Cassia (Irwin and Turner 1960; Biondo et al. 2006). A high frequency of multiple chromosomes pairing (multivalent) suggested strong similarities between chromosomes, indicating autopolyploidy whereas 'allo' or 'auto' polyploidy depends on degree of difference between parental genome (the level of bivalent paring) (De Storme et al. 2014). Collection site significantly affecting the chiasmata frequency in chromosomes. Which indi-

rectly correlated with environmental effect on genetic composition.

Another interesting observation is that except for one accession (RAU–2), all the remaining three accessions showed a mixture of bivalent, univalent, and quadrivalent associations. The highest percentage of PMCs with fourteen bivalents (98%) was observed in RAU–2. The least number of bivalents per PMC (75%) was observed in IHGA. Similarly, the highest percentage (17%) of univalents was recorded in IHGA followed by 16% in IHCM, 9% in RAU–1, and 4.5% in RAU–2. Such behavior of chromosomes with regard to their associations at metaphase I is reported earlier in a number of plants from arid regions i.e. *Salvadora, Capparis decidua, and Prosopis cineraria* (Rawat et al. 2007). Environmental factors rather than genetic and epigenetic factors have supposedly played a role in partial disruption of synapses among bivalents. The presence of univalents in various PMCs anyhow did not influence the distributional pattern of bivalents at anaphase I in 3 out of the 4 accessions analyzed. However, in one accession, the distribution was affected leading to the occurrence of lagging univalent.

The presence of extra chromosomal bodies may be linked with meiotic abnormalities. The unpaired and the B chromosomes are reported to be the main cause of the abnormal distribution of chromosomes at anaphase I and that of chromatids in anaphase II (Stebbins 1971). Similarly, the presence of B chromosomes was also observed *Salvadora, and Prosopis cineraria* (Rawat et al. 2007).

Random Amplified Polymorphic DNA (RAPD) is a versatile genetic analysis technique that does not require any prior knowledge of the DNA sequence of the target organism and can be used to study population differentiation and phylogenetic relationships (Ahmed et al. 2012). Nowadays RAPD markers are not popular due to the difficulties in reproducibility rate, however, RAPD markers are still used to access clone fidelity in *in vitro* and *ex vitro* grown plants because of low cost and rapid analysis (Kader et al. 2022). The smallest and the largest size presence of bands in a DNA sample compared to others, may be linked to there is alteration in that particular sequence which further may reflect in the time of DNA homologous pairing. However, to draw a more definitive conclusion and determine the nature of the addition or insert or delete, further investigation and sequencing of this specific DNA region would be necessary. As reported recently, the application of SCOT markers on DNA of *Senna alexandrina Mill.* confirmed the morphological variations at a targeted part of genome (Kumar et al. 2024b).

CONCLUSION

The present study reports chromosome karyomorphology in a subpopulation of *Senna alexandrina*. We observed that all the accessions of the plant had a maximum of bivalents, with rare quadrivalent formation. Our analysis revealed significant differences among accession types in chiasma formation. The regression model of the study showed that pollen stainability is influenced by the total formation of bivalents and chiasma frequency in pollen mother cells. These findings shed light on the factors influencing chiasma formation and contribute to our understanding of genetic recombination in plants.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, we used [https://chatgpt.com/sciSpace] in order to make the sentences clearer and for grammatical correctness. After using this tool/service, we reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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