5. CYTOLOGICAL VARIATIONS OF *S. obtusifolia* PROVENANCES

In a specific taxon, chromosomal complements are constant in their number and morphology. Any difference in them, particularly at the species level, can help in the study of evolutionary trends and phylogenetic position of that species. Thus, their detailed study regarding the count as well as morphological appearance not only helps us in mere identification of the species, but also helps to identify the presence of intraspecific diversity, if any. Every chromosomal complement in a genome along with their morphological attributes taken together can be typified as 'karyotype'. Thus, every individual representing a characteristically distinct set of chromosomes refers to its karyotype. Karyotype study comprises the phenotype of the somatic chromosomes (Levitzky, 1931). The main target of karyotype in a population or a species is to identify the polymorphism at the level of karyotypic study. It helps in the recognition of the intraspecific and interspecific diversity of cytogenetic variation and evolution. Karyotypic analysis comprises of the detailed microscopic study of the chromosomal complements of a specific taxon or a species regarding their number, size, position of individual chromosomes and their morphological features of the chromosomal landmarks such as location of the centromere and the presence of secondary constriction along with the symmetry and asymmetry of them (Heslop-Harrison and Schwarzacher, 2011). According to Weiss-Schneeweiss et al., (2008), diversification and speciation of different taxa play an important role by contemplating various significant information regarding the comparisons of karyotype in different taxa on the mechanism and pattern of karyotypic evolution. In Situ hybridization (ISH) patterns with repeated DNA sequences are sometimes included in karyotypic study (Mirzaghaderi and Marzangi, 2015). Sizes of the chromosomal complements along with their detailed landmarks are assessed and accurately reproduced in the Ideograms. Ideograms are representation of the chromosomal complements of the genome set diagrammatically in a relatively accurate manner depicting their relative size and their landmarks and the homologous groups. Ideograms provide various chromosomal information and help to visualize the genome at a glance.

Intraspecific cytological variations among the different species of the genus Senna is reported since 1980 (Coleman and Demenzes,1980). Polyploidization such as diploids and tetraploids are quite prevalent in the genus (Resende et al., 2013; Cordeiro et al., 2017). Various inter- and intraspecific variations are quite common in the genus (Goldblatt, 1981; Biondo et al., 2005; Rice et al., 2015; Cordeiro et al., 2017). Cytological study of *S. obtusifolia* by reviewing banding pattern (CMA/DAPI analysis) was reported by Cordeiro et al. (2017). Karyomorphological features of *S. obtusifolia* were also demonstrated by Souza and Benko-Iseppon (2004).

In the present study, karyotypic analysis of *S. obtusifolia* of twenty accessions was carried out. Ideograms of *S. obtusifolia* of all the accessions were prepared for finding intra-specific variation among them.

5.1 MATERIALS AND METHODS

5.1.1 Materials

5.1.1.1 Collection of Seed

Sufficient amount of fully ripen pods (around 50 pods of each provenance) of *S. obtusifolia* of all the twenty accessions were collected from the healthy plants for the cytological studies. All the seeds from these pods were stored and handled separately for performing karyotype experiments.

5.1.2 Methods

5.1.2.1 Germination of Seed

Few healthy seeds of each provenance were taken separately for germination and treated for 5-7 minutes with concentrated Sulphuric acid for the process of scarification. The seed coat of *S. obtusifolia* are very hard which necessitates the scarification of the seeds for germination. Scarified seeds were washed thoroughly for around 10-15 minutes to remove any traces of acid, under running tap water. Then the seeds were kept in sterile moist petri plates with soft tissue paper in dark for 12 hours and then placed in a normal photoperiod for proper germination. Root tips were found to emerge from the seeds after around 24 hours. These root tips were used for karyological studies.

5.1.2.2 Treatment of Excised Root or Tissue

The root tips from the seeds were excised on attaining a length of about 0.75 cm which have shown as the ideal time for its harvest. Excised roots, of a length cut of about 3 mm from the apex were pre-treated at 10℃ with PDB or 1,4 Dichlorobenzene. Pretreatment of 8 hours showed the best result and was standardized after several experimental trials. Harvesting of the root tips for pre-treatment between 8 pm to 10 pm IST showed effective number of arrested metaphase cells.

5.1.2.3 Fixation of Tissue

After pre-treatment, root tips were taken and thoroughly washed with distilled water and dipped into the solution of 1:3 aceto-alcohol for fixation of tissues at a metabolic stage. In 1:3 aceto-alcohol, the root tips were kept for 3-4 hours for proper fixation and then were transferred to 70% ethyl alcohol with one or two changes. The root tips can be kept in 70% ethyl alcohol for any duration or long-term storage, till before staining and squashing.

5.1.2.4 Staining with Orcein

- The root tips were taken out from 70% ethyl alcohol and dipped in 45% acetic acid for 10-15 minutes.
- From 45% acetic acid solution, the root tips were dipped into 2% Aceto-Orcein: 1N HCl (9:1).
- Root tips immersed into the stain were warmed at 50°C-60°C for 3-4 times (3-4 seconds in each spell), so that the heat should not rise to any point to prevent the root tips from boiling.
- The root tips were kept for 8 hours in the stain for the chromosomes to get stained and at the same time the clarity of the cytoplasm is also maintained.

5.1.2.5 Squashing

With 45% acetic acid, root tips were squashed after proper staining. About 1-2 mm of the root tip from the apex with darkly stained part was used for squashing to get the best results.

5.1.2.6 Observation and Photography of Chromosome

The plates showing well scattered chromosomes arrested at metaphase stage were identified, observed under microscope and taken snapshot for each of the twenty provenances under study.

5.1.2.7 Karyotype and Ideogram Generation

Images taken from the microscopic observation were imported into a karyotype analysis application DRAWID (Kirov et al., 2017) for measuring the length of chromosomes and generating the ideograms for all the accessions. With the help of this application, position of the centromere and the presence of NOR in the chromosomes, if any, were marked in the scanned metaphase plates to get the length of short (S) and long (L) arms of all the chromosomes of a haploid complements. Then the ideograms were generated for each accession separately.

5.1.2.8 Karyotype Asymmetry Analysis

Karyotype asymmetry analysis for all the accessions were done according to method proposed by Peruzzi and Eroğlu (2013). This method is one of the most approachable methods for determining chromosome asymmetry. Following Levan et al., (1964), chromosome type was classified. To find any chromosome asymmetry and variations, the following parameters were estimated:

- n: Number of Haploid chromosome
- S: Length of short arm
- L: Length of long arm
- CL: Length of chromosome (= L + S)
- CI: Centromeric index (= S/CL)
- HCL: Total length of chromosome of haploid complements (= ∑ CL)
- RL: Relative length percentage (= CL/HCL × 100)
- A: Degree of karyotype asymmetry (= $1 - (\sum (L_i - S_i) / (L_i + S_i))/n$ where i = 1 to n)
- M_{CL}: Average of CL of a haploid complement
- S_{CL}: SS of CL of a haploid complement:
- A_2: Interchromosomal asymmetry index (= S_{CL}/M_{CL})
- CV_{CL}: Coefficient of variation of CL
- M_{CA}: Average centromeric asymmetry (= A × 100)
- CM: Chromosome Morphology

- NOR: Nucleolus Organizer Region

Stebbins (1971) suggested quali-quantitative methods to estimate twelve kinds of karyotype asymmetry, into four categories (1-4) as per the longest and smallest chromosomes ratio complements combined in three classes A, B, and C as per the increased proportion of chromosomal complements with arm ratio < 2:1.

5.1.2.9 Asymmetry Analysis

Karyotype asymmetry analyses were performed according to the process of Peruzzi and Eroğlu (2013). It is the most modern and well-defined method for approaching asymmetry analysis. Asymmetry analyses were performed through scattered diagrams. Scattered diagrams were prepared to find significant interchromosomal asymmetry, if any. In the scattered diagram, CV_{CL} was plotted in the x-axis and M_{CA} was plotted in the y-axis.

5.2 RESULTS AND DISCUSSIONS

Twenty different accessions of *S. obtusifolia* were selected for karyotype analyses. All the twenty accessions of the species showed a diploid chromosomal complement of 2n = 26 in all the provenances. The haploid set of chromosomes of all the accessions revealed eleven metacentric and two sub-metacentric chromosomes with a very close length pattern in decreasing order. Karyotype analyses of the haploid chromosomal complements of the twenty accessions were represented in Table (5.1 - 5.20). Figure (5.1 - 5.20) represent ideograms of haploid set of chromosomes (n = 13) and the corresponding karyotype plate of all the accessions of *S. obtusifolia.* Karyotype of all the twenty accessions of *S. obtusifolia* collected from twenty different provenances showed the presence of 2n = 26 chromosomes as diploid complement represented in Table (5.1 - 5.20).

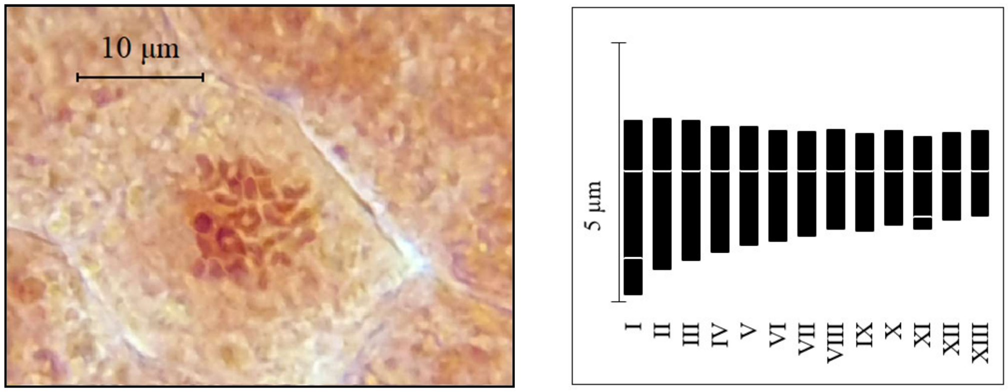


Figure 5.1 Metaphase karyotype plate and ideogram from the germinated root tip of the accession ASN (Asansol)

Table 5.1 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession ASN (Asansol)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.27	0.94	3.21	11.80	0.29	sm	Present
II	1.84	0.98	2.82	10.38	0.35	sm	
III	1.66	0.94	2.60	9.57	0.36	sm	
IV	1.54	0.82	2.36	8.68	0.35	sm	
V	1.42	0.83	2.26	8.29	0.37	sm	
VI	1.32	0.74	2.06	7.59	0.36	sm	
VII	1.20	0.70	1.90	7.00	0.37	sm	
VIII	1.09	0.74	1.83	6.71	0.40	m	
IX	1.09	0.68	1.77	6.52	0.38	sm	
X	0.96	0.71	1.68	6.16	0.43	m	
XI	1.03	0.60	1.62	5.97	0.37	sm	Present
XII	0.89	0.67	1.56	5.72	0.43	m	
XIII	0.81	0.71	1.52	5.60	0.47	m	

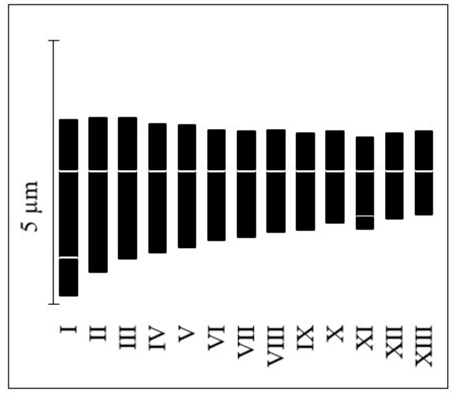


Figure 5.2 Metaphase karyotype plate and ideogram from the germinated root tip of the accession BDG (Bandhavgarh)

Table 5.2 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession BDG (Bandhavgarh)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.31	0.94	3.25	11.74	0.29	sm	Present
II	1.87	0.97	2.83	10.23	0.34	sm	
III	1.69	0.93	2.62	9.46	0.35	sm	
IV	1.55	0.83	2.38	8.61	0.35	sm	
V	1.41	0.83	2.24	8.09	0.37	sm	
VI	1.32	0.75	2.08	7.51	0.36	sm	
VII	1.23	0.73	1.96	7.09	0.37	sm	
VIII	1.10	0.77	1.87	6.74	0.41	m	
IX	1.13	0.69	1.82	6.57	0.38	sm	
X	1.01	0.75	1.76	6.35	0.43	m	
XI	1.04	0.63	1.67	6.04	0.38	sm	Present
XII	0.92	0.71	1.62	5.87	0.44	m	
XIII	0.84	0.74	1.58	5.69	0.47	m	

Figure 5.3 Metaphase karyotype plate and ideogram from the germinated root tip of the accession BNP (Bishnupur)

Table 5.3 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession BNP (Bishnupur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.29	0.94	3.23	11.75	0.29	sm	Present
II	1.89	0.98	2.87	10.44	0.34	sm	
III	1.65	0.97	2.61	9.52	0.37	sm	
IV	1.53	0.86	2.39	8.71	0.36	sm	
V	1.42	0.84	2.26	8.24	0.37	sm	
VI	1.29	0.75	2.04	7.45	0.37	sm	
VII	1.24	0.72	1.96	7.15	0.37	sm	
VIII	1.13	0.75	1.88	6.84	0.40	m	
IX	1.10	0.69	1.79	6.51	0.38	sm	
X	0.96	0.73	1.69	6.15	0.43	m	
XI	1.02	0.61	1.63	5.95	0.38	sm	Present
XII	0.89	0.68	1.57	5.71	0.43	m	
XIII	0.81	0.72	1.53	5.58	0.47	m	

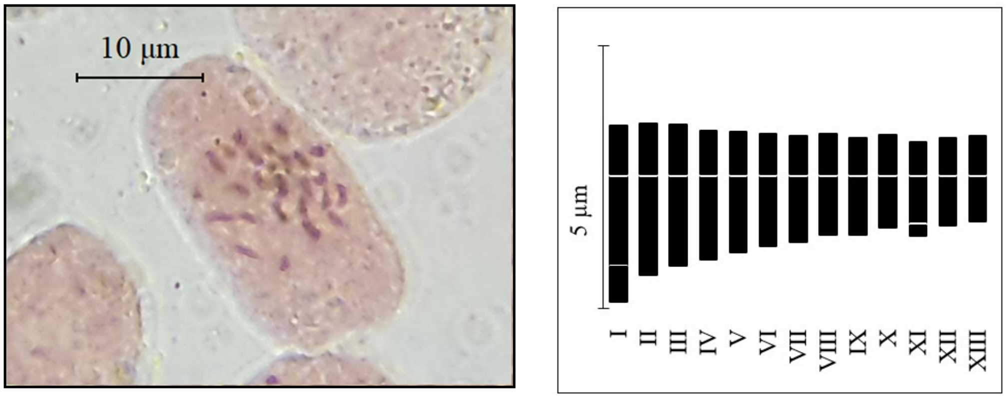


Figure 5.4 Metaphase karyotype plate and ideogram from the germinated root tip of the accession BPR (Bolpur)

Table 5.4 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession BPR (Bolpur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.30	0.92	3.21	11.78	0.29	sm	Present
II	1.87	0.95	2.83	10.37	0.34	sm	
III	1.67	0.96	2.63	9.65	0.37	sm	
IV	1.55	0.85	2.41	8.83	0.36	sm	
V	1.38	0.83	2.21	8.11	0.38	sm	
VI	1.31	0.75	2.06	7.56	0.36	sm	
VII	1.21	0.73	1.94	7.12	0.38	sm	
VIII	1.09	0.75	1.84	6.75	0.41	m	
IX	1.09	0.67	1.75	6.43	0.38	sm	
X	0.96	0.73	1.69	6.20	0.43	m	
XI	1.00	0.60	1.60	5.88	0.38	sm	Present
XII	0.86	0.69	1.55	5.70	0.44	m	
XIII	0.80	0.73	1.53	5.63	0.48	m	

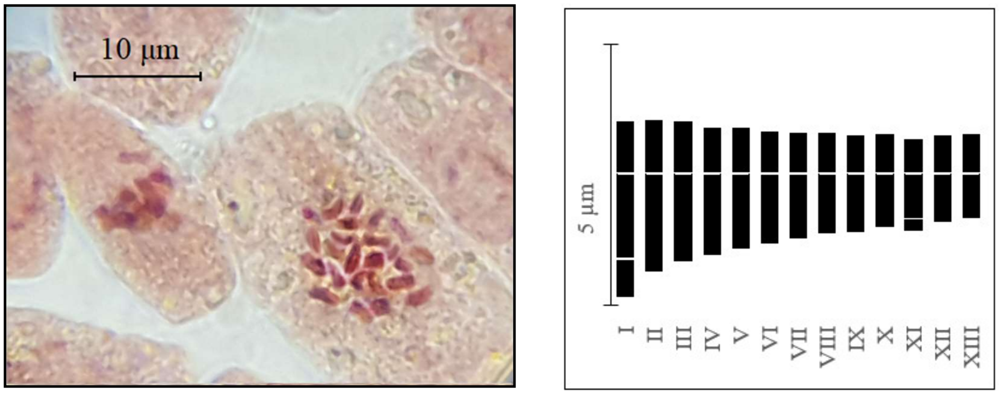


Figure 5.5 Metaphase karyotype plate and ideogram from the germinated root tip of the accession DHD (Dahod)

Table 5.5 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession DHD (Dahod)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.32	0.93	3.25	11.69	0.29	sm	Present
II	1.87	0.98	2.86	10.29	0.34	sm	
III	1.70	0.96	2.67	9.60	0.36	sm	
IV	1.58	0.84	2.42	8.72	0.35	sm	
V	1.43	0.82	2.25	8.11	0.37	sm	
VI	1.31	0.77	2.09	7.51	0.37	sm	
VII	1.23	0.73	1.97	7.08	0.37	sm	
VIII	1.10	0.77	1.87	6.73	0.41	m	
IX	1.10	0.70	1.80	6.48	0.39	sm	
X	0.97	0.75	1.72	6.20	0.43	m	
XI	1.07	0.62	1.69	6.08	0.37	sm	Present
XII	0.92	0.70	1.62	5.84	0.43	m	
XIII	0.84	0.73	1.57	5.66	0.46	m	

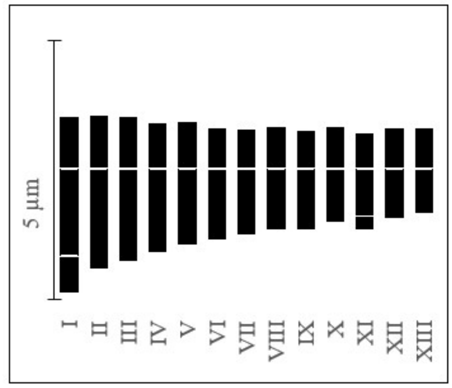


Figure 5.6 Metaphase karyotype plate and ideogram from the germinated root tip of the accession DHN (Dehradun)

Table 5.6 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession DHN (Dehradun)

Chromosome Pair	**L (µm)**	**S (µm)**	**CL (µm)**	**RL (%)**	**CI**	**CM**	**NOR**
I	2.28	0.95	3.23	11.8	0.29	sm	Present
II	1.85	0.99	2.84	10.38	0.35	sm	
III	1.64	0.95	2.59	9.47	0.37	sm	
IV	1.54	0.83	2.37	8.67	0.35	sm	
V	1.40	0.84	2.24	8.20	0.38	sm	
VI	1.32	0.75	2.07	7.58	0.36	sm	
VII	1.22	0.73	1.95	7.13	0.37	sm	
VIII	1.11	0.73	1.83	6.70	0.40	m	
IX	1.09	0.69	1.78	6.50	0.39	sm	
X	0.99	0.71	1.70	6.21	0.42	m	
XI	1.02	0.62	1.64	6.01	0.38	sm	Present
XII	0.89	0.69	1.58	5.77	0.44	m	
XIII	0.82	0.71	1.53	5.58	0.46	m	

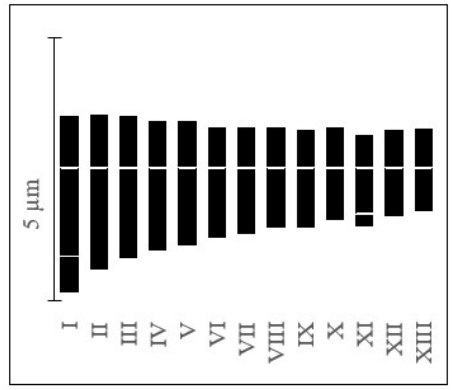


Figure 5.7 Metaphase karyotype plate and ideogram from the germinated root tip of the accession DNG (Devendranagar)

Table 5.7 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession DNG (Devendranagar)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.31	0.95	3.25	11.62	0.29	sm	Present
II	1.90	0.99	2.89	10.33	0.34	sm	
III	1.74	0.96	2.70	9.63	0.35	sm	
IV	1.58	0.84	2.42	8.66	0.35	sm	
V	1.44	0.86	2.30	8.21	0.37	sm	
VI	1.34	0.74	2.08	7.45	0.36	sm	
VII	1.24	0.72	1.97	7.02	0.37	sm	
VIII	1.14	0.76	1.90	6.81	0.40	m	
IX	1.13	0.69	1.82	6.49	0.38	sm	
X	0.99	0.75	1.74	6.21	0.43	m	
XI	1.08	0.63	1.70	6.08	0.37	sm	Present
XII	0.92	0.73	1.65	5.89	0.44	m	
XIII	0.83	0.74	1.57	5.60	0.47	m	

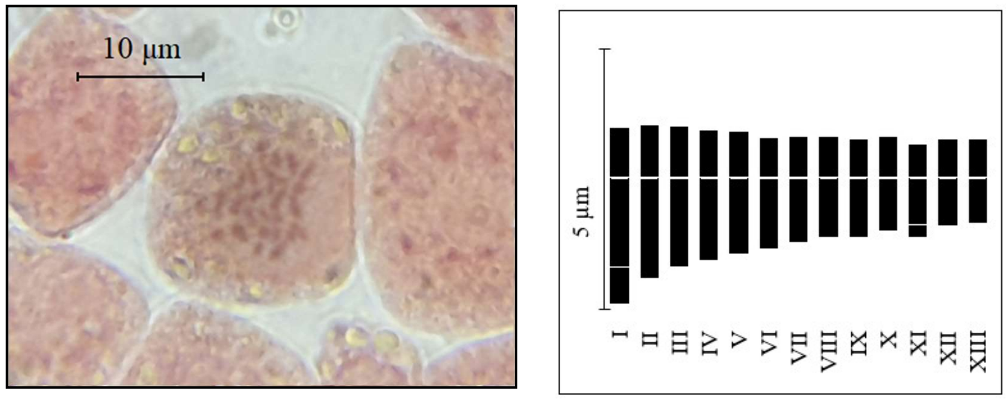


Figure 5.8 Metaphase karyotype plate and ideogram from the germinated root tip of the accession GBP (Gobindapur)

Table 5.8 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession GBP (Gobindapur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.28	0.94	3.22	11.79	0.29	sm	Present
II	1.90	0.97	2.86	10.48	0.34	sm	
III	1.68	0.95	2.63	9.64	0.36	sm	
IV	1.54	0.86	2.40	8.8	0.36	sm	
V	1.43	0.84	2.27	8.32	0.37	sm	
VI	1.29	0.74	2.03	7.44	0.37	sm	
VII	1.22	0.72	1.95	7.13	0.37	sm	
VIII	1.10	0.72	1.82	6.68	0.40	m	
IX	1.11	0.68	1.79	6.55	0.38	sm	
X	0.95	0.72	1.67	6.12	0.43	m	
XI	1.02	0.59	1.61	5.91	0.37	sm	Present
XII	0.88	0.67	1.55	5.67	0.43	m	
XIII	0.79	0.71	1.50	5.48	0.47	m	

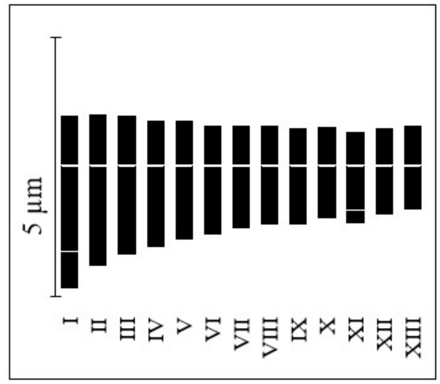


Figure 5.9 Metaphase karyotype plate and ideogram from the germinated root tip of the accession HDR (Haridwar)

Table 5.9 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession HDR (Haridwar)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.28	0.95	3.23	11.68	0.29	sm	Present
II	1.88	0.99	2.87	10.38	0.34	sm	
III	1.73	0.95	2.67	9.67	0.35	sm	
IV	1.56	0.89	2.45	8.85	0.36	sm	
V	1.41	0.84	2.25	8.13	0.37	sm	
VI	1.34	0.75	2.10	7.58	0.36	sm	
VII	1.22	0.71	1.94	7.00	0.37	sm	
VIII	1.09	0.76	1.85	6.70	0.41	m	
IX	1.11	0.71	1.82	6.58	0.39	sm	
X	0.96	0.74	1.70	6.16	0.43	m	
XI	1.06	0.61	1.67	6.02	0.36	sm	Present
XII	0.89	0.69	1.58	5.72	0.44	m	
XIII	0.81	0.73	1.53	5.54	0.47	m	

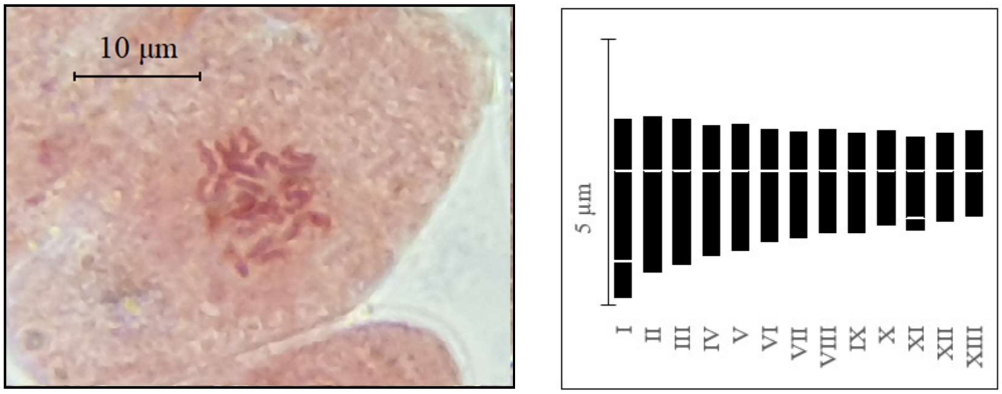
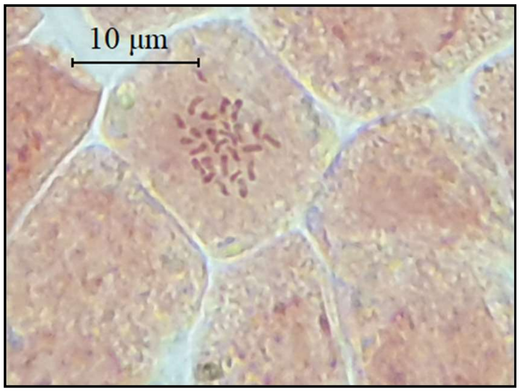


Figure 5.10 Metaphase karyotype plate and ideogram from the germinated root tip of the accession JBP (Jabalpur)

Table 5.10 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession JBP (Jabalpur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.31	0.96	3.27	11.61	0.29	sm	Present
II	1.89	1.01	2.90	10.32	0.35	sm	
III	1.71	1.00	2.71	9.61	0.37	sm	
IV	1.60	0.85	2.46	8.72	0.35	sm	
V	1.45	0.85	2.30	8.18	0.37	sm	
VI	1.33	0.78	2.11	7.49	0.37	sm	
VII	1.24	0.76	2.01	7.13	0.38	sm	
VIII	1.14	0.76	1.91	6.77	0.40	m	
IX	1.13	0.72	1.85	6.58	0.39	sm	
X	1.00	0.75	1.75	6.22	0.43	m	
XI	1.07	0.63	1.70	6.03	0.37	sm	Present
XII	0.91	0.70	1.61	5.73	0.44	m	
XIII	0.85	0.73	1.58	5.61	0.46	m	



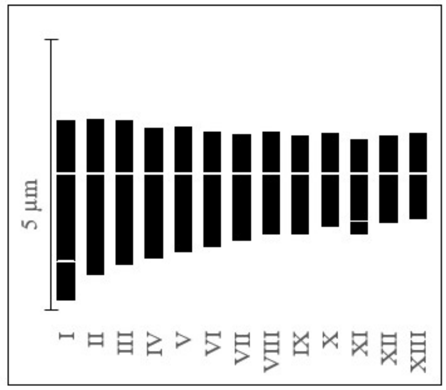


Figure 5.11 Metaphase karyotype plate and ideogram from the germinated root tip of the accession JPR (Jaipur)

Table 5.11 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession JPR (Jaipur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.32	0.92	3.24	11.78	0.29	sm	Present
II	1.88	0.97	2.85	10.37	0.34	sm	
III	1.66	0.94	2.60	9.47	0.36	sm	
IV	1.54	0.87	2.41	8.76	0.36	sm	
V	1.43	0.85	2.28	8.29	0.37	sm	
VI	1.32	0.73	2.05	7.44	0.36	sm	
VII	1.21	0.74	1.95	7.08	0.38	sm	
VIII	1.10	0.75	1.85	6.72	0.40	m	
IX	1.09	0.69	1.78	6.46	0.39	sm	
X	0.99	0.74	1.73	6.30	0.43	m	
XI	1.06	0.60	1.66	6.04	0.36	sm	Present
XII	0.88	0.70	1.58	5.74	0.44	m	
XIII	0.83	0.70	1.53	5.56	0.46	m	

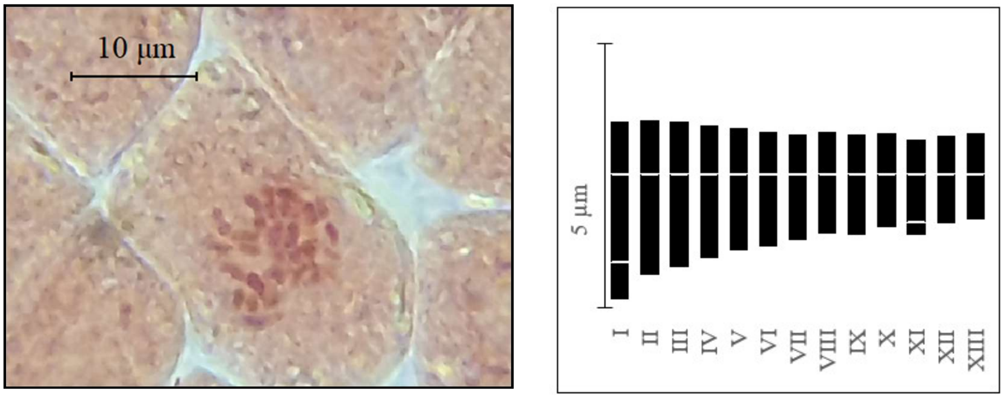


Figure 5.12 Metaphase karyotype plate and ideogram from the germinated root tip of the accession JRM (Jhargram)

Table 5.12 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession JRM (Jhargram)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.29	0.95	3.24	11.82	0.29	sm	Present
II	1.88	0.94	2.82	10.30	0.33	sm	
III	1.66	0.97	2.63	9.60	0.37	sm	
IV	1.54	0.85	2.39	8.71	0.36	sm	
V	1.38	0.83	2.22	8.09	0.38	sm	
VI	1.31	0.74	2.05	7.49	0.36	sm	
VII	1.23	0.73	1.95	7.13	0.37	sm	
VIII	1.11	0.75	1.85	6.77	0.40	m	
IX	1.08	0.69	1.77	6.45	0.39	sm	
X	0.98	0.71	1.69	6.17	0.42	m	
XI	1.05	0.61	1.65	6.04	0.37	sm	Present
XII	0.92	0.69	1.60	5.86	0.43	m	
XIII	0.80	0.72	1.52	5.56	0.47	m	

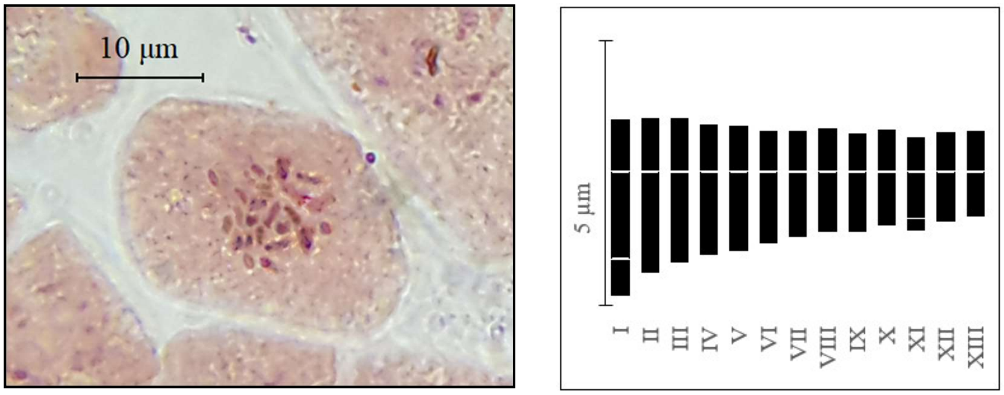


Figure 5.13 Metaphase karyotype plate and ideogram from the germinated root tip of the accession KLN (Kalyani)

Table 5.13 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession KLN (Kalyani)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.26	0.95	3.22	11.65	0.30	sm	Present
II	1.88	0.97	2.86	10.35	0.34	sm	
III	1.69	0.97	2.66	9.64	0.37	sm	
IV	1.55	0.86	2.41	8.73	0.36	sm	
V	1.46	0.82	2.28	8.26	0.36	sm	
VI	1.32	0.73	2.04	7.40	0.36	sm	
VII	1.19	0.73	1.92	6.96	0.38	sm	
VIII	1.10	0.77	1.88	6.81	0.41	m	
IX	1.11	0.69	1.81	6.55	0.38	sm	
X	0.98	0.75	1.73	6.25	0.43	m	
XI	1.04	0.61	1.65	5.99	0.37	sm	Present
XII	0.90	0.71	1.60	5.80	0.44	m	
XIII	0.81	0.74	1.55	5.62	0.48	m	

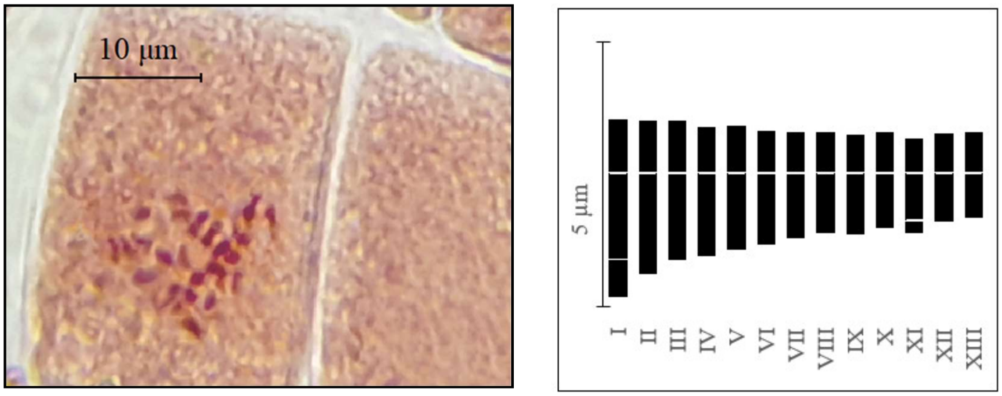


Figure 5.14 Metaphase karyotype plate and ideogram from the germinated root tip of the accession KPR (Kharagpur)

Table 5.14 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession KPR (Kharagpur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.31	0.95	3.26	11.65	0.29	sm	Present
II	1.89	0.95	2.84	10.15	0.33	sm	
III	1.70	0.95	2.65	9.49	0.36	sm	
IV	1.57	0.85	2.41	8.64	0.35	sm	
V	1.45	0.84	2.29	8.20	0.37	sm	
VI	1.34	0.74	2.09	7.47	0.36	sm	
VII	1.23	0.74	1.97	7.06	0.38	sm	
VIII	1.13	0.78	1.91	6.85	0.41	m	
IX	1.13	0.72	1.85	6.61	0.39	sm	
X	1.01	0.75	1.77	6.33	0.43	m	
XI	1.07	0.61	1.68	6.02	0.36	sm	Present
XII	0.91	0.71	1.62	5.79	0.44	m	
XIII	0.84	0.77	1.60	5.74	0.48	m	

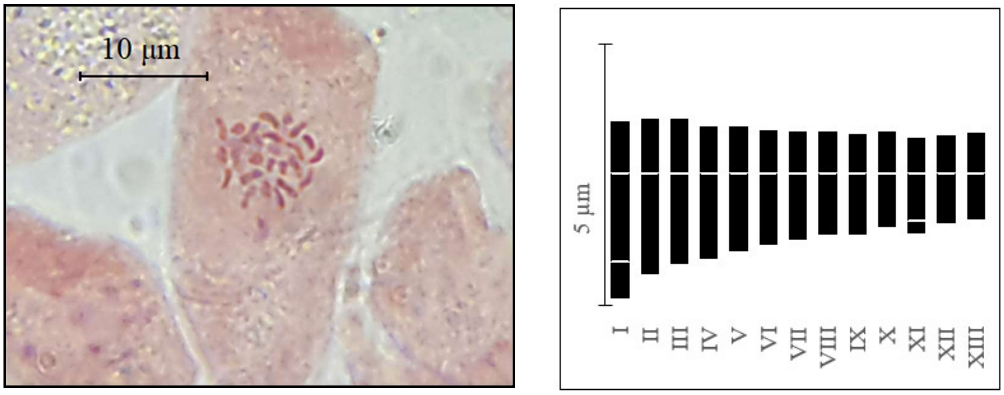
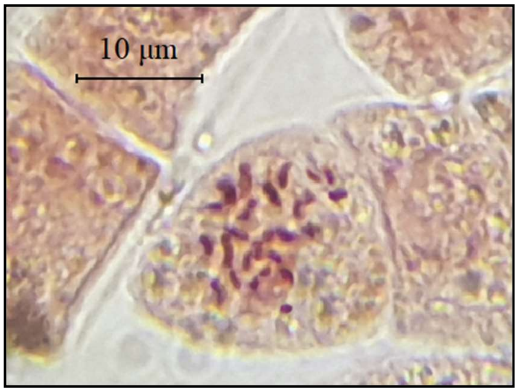


Figure 5.15 Metaphase karyotype plate and ideogram from the germinated root tip of the accession LKP (Lakshmikantapur)

Table 5.15 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession LKP (Lakshmikantapur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.29	0.93	3.22	11.82	0.29	sm	Present
II	1.89	0.95	2.84	10.4	0.33	sm	
III	1.69	0.93	2.61	9.58	0.35	sm	
IV	1.54	0.82	2.36	8.68	0.35	sm	
V	1.39	0.83	2.21	8.12	0.37	sm	
VI	1.30	0.72	2.02	7.42	0.36	sm	
VII	1.18	0.74	1.92	7.05	0.38	sm	
VIII	1.10	0.74	1.84	6.75	0.40	m	
IX	1.10	0.69	1.79	6.56	0.38	sm	
X	0.98	0.71	1.69	6.19	0.42	m	
XI	1.03	0.60	1.63	5.99	0.37	sm	Present
XII	0.91	0.68	1.58	5.80	0.43	m	
XIII	0.81	0.72	1.53	5.62	0.47	m	



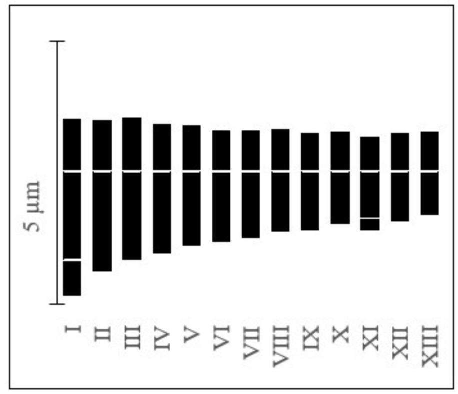


Figure 5.16 Metaphase karyotype plate and ideogram from the germinated root tip of the accession MGL (Mangalore)

Table 5.16 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession MGL (Mangalore)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.30	0.96	3.26	11.75	0.29	sm	Present
II	1.88	0.99	2.87	10.34	0.34	sm	
III	1.69	0.96	2.65	9.55	0.36	sm	
IV	1.58	0.84	2.42	8.75	0.35	sm	
V	1.43	0.86	2.30	8.28	0.38	sm	
VI	1.31	0.75	2.06	7.44	0.36	sm	
VII	1.24	0.74	1.98	7.15	0.37	sm	
VIII	1.11	0.77	1.88	6.79	0.41	m	
IX	1.10	0.70	1.79	6.47	0.39	sm	
X	0.98	0.74	1.71	6.18	0.43	m	
XI	1.04	0.62	1.66	5.99	0.38	sm	Present
XII	0.91	0.69	1.59	5.75	0.43	m	
XIII	0.83	0.72	1.54	5.57	0.46	m	

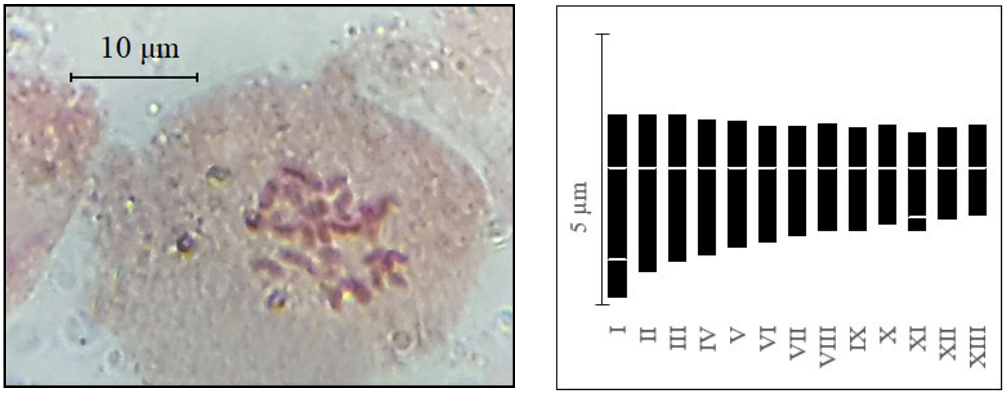
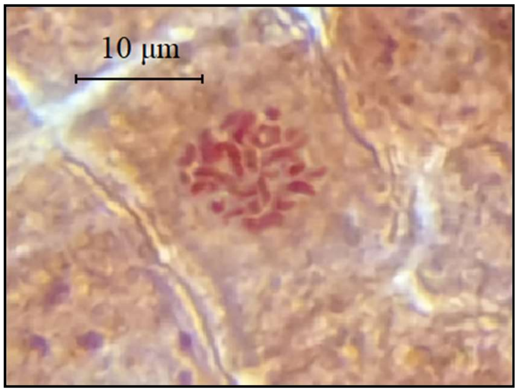


Figure 5.17 Metaphase karyotype plate and ideogram from the germinated root tip of the accession NGP (Nagpur)

Table 5.17 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession NGP (Nagpur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.26	0.96	3.22	11.75	0.30	sm	Present
II	1.87	0.94	2.81	10.24	0.33	sm	
III	1.62	0.95	2.57	9.38	0.37	sm	
IV	1.54	0.82	2.36	8.62	0.35	sm	
V	1.41	0.84	2.24	8.19	0.37	sm	
VI	1.31	0.75	2.06	7.52	0.36	sm	
VII	1.20	0.73	1.93	7.04	0.38	sm	
VIII	1.11	0.74	1.85	6.76	0.40	m	
IX	1.12	0.69	1.81	6.59	0.38	sm	
X	1.01	0.72	1.73	6.31	0.42	m	
XI	1.06	0.60	1.66	6.07	0.36	sm	Present
XII	0.89	0.70	1.60	5.83	0.44	m	
XIII	0.82	0.74	1.57	5.72	0.47	m	



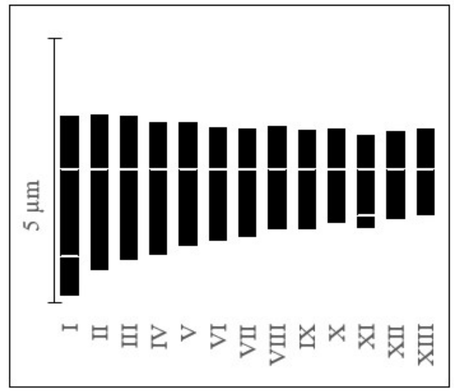


Figure 5.18 Metaphase karyotype plate and ideogram from the germinated root tip of the accession PRL (Purulia)

Table 5.18 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession PRL (Purulia)

Chromosome Pair	L (μm)	S (μm)	CL (μm)	RL (%)	CI	CM	NOR
I	2.31	0.93	3.24	11.69	0.29	sm	Present
II	1.89	0.96	2.85	10.29	0.34	sm	
III	1.64	0.96	2.61	9.40	0.37	sm	
IV	1.58	0.86	2.44	8.79	0.35	sm	
V	1.45	0.84	2.29	8.25	0.37	sm	
VI	1.30	0.75	2.05	7.41	0.37	sm	
VII	1.22	0.76	1.97	7.12	0.38	sm	
VIII	1.14	0.76	1.89	6.83	0.40	m	
IX	1.12	0.71	1.82	6.57	0.39	sm	
X	1.00	0.73	1.72	6.22	0.42	m	
XI	1.04	0.63	1.67	6.03	0.38	sm	Present
XII	0.92	0.70	1.62	5.85	0.43	m	
XIII	0.82	0.71	1.54	5.55	0.46	m	

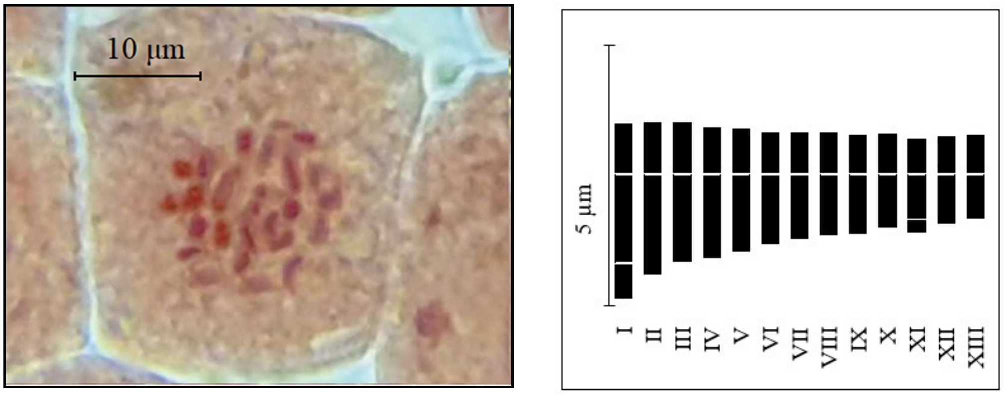


Figure 5.19 Metaphase karyotype plate and ideogram from the germinated root tip of the accession RPR (Raipur)

Table 5.19 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession RPR (Raipur)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.31	0.93	3.24	11.69	0.29	sm	Present
II	1.89	0.96	2.85	10.29	0.34	sm	
III	1.64	0.96	2.61	9.40	0.37	sm	
IV	1.58	0.86	2.44	8.79	0.35	sm	
V	1.45	0.84	2.29	8.25	0.37	sm	
VI	1.30	0.75	2.05	7.41	0.37	sm	
VII	1.22	0.76	1.97	7.12	0.38	sm	
VIII	1.14	0.76	1.89	6.83	0.40	m	
IX	1.12	0.71	1.82	6.57	0.39	sm	
X	1.00	0.73	1.72	6.22	0.42	m	
XI	1.04	0.63	1.67	6.03	0.38	sm	Present
XII	0.92	0.70	1.62	5.85	0.43	m	
XIII	0.82	0.71	1.54	5.55	0.46	m	

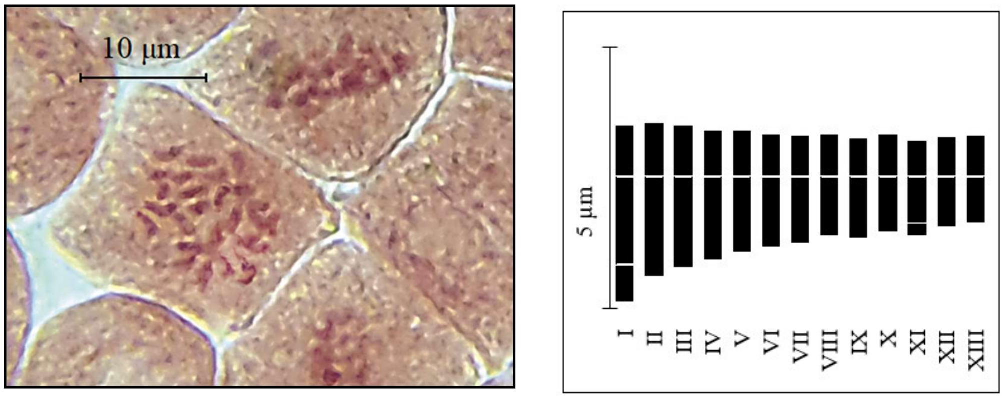


Figure 5.20 Metaphase karyotype plate and ideogram from the germinated root tip of the accession TNG (Tatanagar)

Table 5.20 Chromosome morphometry of *S. obtusifolia* (2n = 26) of the accession TNG (Tatanagar)

Chromosome Pair	L (µm)	S (µm)	CL (µm)	RL (%)	CI	CM	NOR
I	2.29	0.94	3.23	11.66	0.29	sm	Present
II	1.88	0.99	2.87	10.36	0.34	sm	
III	1.68	0.94	2.62	9.46	0.36	sm	
IV	1.60	0.85	2.45	8.84	0.35	sm	
V	1.43	0.84	2.27	8.20	0.37	sm	
VI	1.32	0.78	2.10	7.58	0.37	sm	
VII	1.22	0.76	1.98	7.13	0.38	sm	
VIII	1.11	0.75	1.86	6.70	0.40	m	
IX	1.13	0.69	1.82	6.58	0.38	sm	
X	1.00	0.74	1.74	6.28	0.43	m	
XI	1.05	0.60	1.65	5.96	0.36	sm	Present
XII	0.91	0.68	1.59	5.72	0.43	m	
XIII	0.82	0.72	1.54	5.54	0.47	m	

Table 5.21 Chromosome morphometry variations of *S. obtusifolia* of the twenty accessions.

Accession	**HCL(μm)**	**A**	**A2**	**CV_{CL}**	**M_{CA}**
ASN	27.19	0.382	0.242	38.245	24.242
BDG	27.68	0.379	0.240	37.897	24.031
BNP	27.45	0.374	0.236	37.424	23.638
BPR	27.25	0.370	0.235	37.027	23.458
DHD	27.78	0.377	0.239	37.732	23.912
DHN	27.35	0.377	0.238	37.743	23.812
DNG	27.99	0.381	0.242	38.084	24.215
GBP	27.30	0.380	0.241	37.988	24.095
HDR	27.66	0.372	0.236	37.231	23.552
JBP	28.16	0.375	0.237	37.521	23.659
JPR	27.51	0.379	0.240	37.924	24.030
JRM	27.38	0.378	0.240	37.839	23.955
KLN	27.61	0.372	0.235	37.160	23.531
KPR	27.94	0.378	0.240	37.813	24.043
LKP	27.24	0.384	0.244	38.363	24.375
MGL	27.71	0.375	0.237	37.528	23.691
NGP	27.41	0.379	0.240	37.896	24.017
PRL	27.71	0.378	0.239	37.827	23.917
RPR	28.03	0.378	0.239	37.780	23.921
TNG	27.72	0.381	0.241	38.060	24.100
SD	0.277	0.0035	0.0024	0.3410	0.2471

The diploid set of all the provenances showed 4 chromosomes with NOR (nucleolar organizer). The chromosome number I is contributing as the longest chromosome in all the sets as revealed from the ideograms with secondary constriction in the long arm. Another set of chromosomes of this genome also have a secondary constriction corresponding to the chromosome number XI of the ideograms. Nine chromosomes of the haploid set (n = 13) were found to be sub-median type (sm) and the rest four were median type (m). The chromosomal complements having (9 sm and 4 m) are in accordance with the findings of Souza and Issepon (2004). The average length of the chromosomes of the haploid set for all the accessions varied from 3.276 µm to 1.496 µm. Comparative study of the karyotype of all the twenty accessions revealed minor variations among them only regarding the length of total genomic length. The size of the longest chromosome corresponds the chromosome number I of the ideogram was found in the accession of Jabalpur (JBP) of length 3.276 µm and the size of the shortest one of the chromosome complement I showed from the provenance Asansol (ASN) is found to be 3.210 µm while the longest length of the chromosome complement XIII i.e., the shortest one of the Ideogram was found to be 1.603 µm in the Kharagpur (KPR) and the shortest one of length 1.496 µm of the provenance Gobindapur (GBP) among all the accessions. This suggests the presence of quite a subtle amount of variations, though very minor, on the level of 10^{-1} µm to 10^{-2} µm among the provenances. The greatest total length of genome of the haploid set of the chromosomal complements was found to be 28.160 µm, the highest among all the provenance Jabalpur (JBP), on the contrary, the total lowest value was recorded as 27.190 µm in the Asansol (ASN) provenance. Thus, a negligible variation was observed amongst all the provenances while processing the results of karyotypes digitally by DRAWID.

Polyploidy was observed in some the accessions of *S. obtusifolia* was quite interesting. Presence of polyploidy in this species was also reported by Resende et al., (2013) and Cordeiro et al., (2017). The Karyotype plate showing polyploidy were represented in Figure 5.22.

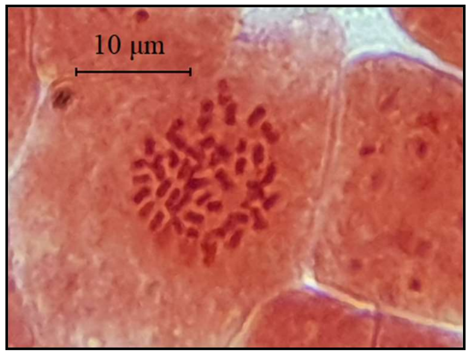


Figure 5.21 Polyploidy of S. obtusifolia

Karyotype characterization is a different approach for studying the symmetry /asymmetry in *S. obtusifolia.* Levitzky (1931) developed the karyotype asymmetry concept for the first time which was carried later in "Chromosomal evolution in higher plants", a masterpiece by Stebbins (1971). Estimating karyotype asymmetry was proposed by Peruzzi and Eroğlu (2013) using a quali-quantitative method.

The heterogeneity occurred in the complements of chromosomes regarding their sizes, considering "Interchromosomal Asymmetry" was studied by various researchers proposing quantitative estimation methods, as for A2 index (Romero-Zarco, 1986) and CV_{CL} (Watanabe et al., 1999; Paszko 2006). Variations in the size of chromosomal complements even in minor ranges were determined by CV_{CL} in a statistically accurate manner (Peruzzi and Eroğlu, 2013).

In the present study of *S. obtusifolia* assessing, the inter-chromosomal heterogeneity or asymmetry is followed by the method of Stebbins (1971). Karyotype of all the twenty accessions of *S. obtusifolia* belongs to the type 2B of Stebbins, suggesting the absence of inter-chromosomal asymmetric variations among the karyotypes of the species from different provenances. Type 2B of Stebbins suggested moderate nature of asymmetry among the karyotype.

Intrachromosomal Asymmetry, the most debated and the complex one for quantitative estimation of the chromosomal complements is based on the centromeric position. For representing karyotype asymmetry among the species, bi-dimensional scatter plots were the most accurate and best way for determining variation. In asymmetry analysis, the intersection point of X-axis and Y-axis estimators represent the asymmetry among the species. According to Peruzzi and Eroğlu (2013) the two asymmetry estimators CV_{CL} and M_{CA} were the best way for representing scatter plot.

The values of karyotypic asymmetry (Figure 5.21) though having finer differentiations among twenty different accessions of *S. obtusifolia* hold considerable significance collected from the twenty provenances. Here, in Figure 5.21, the overall karyotype asymmetry comparisons followed according to the proposal of Peruzzi and Eroğlu, (2013) for determining the karyotypic asymmetry among twenty different accessions of *S. obtusifolia.*

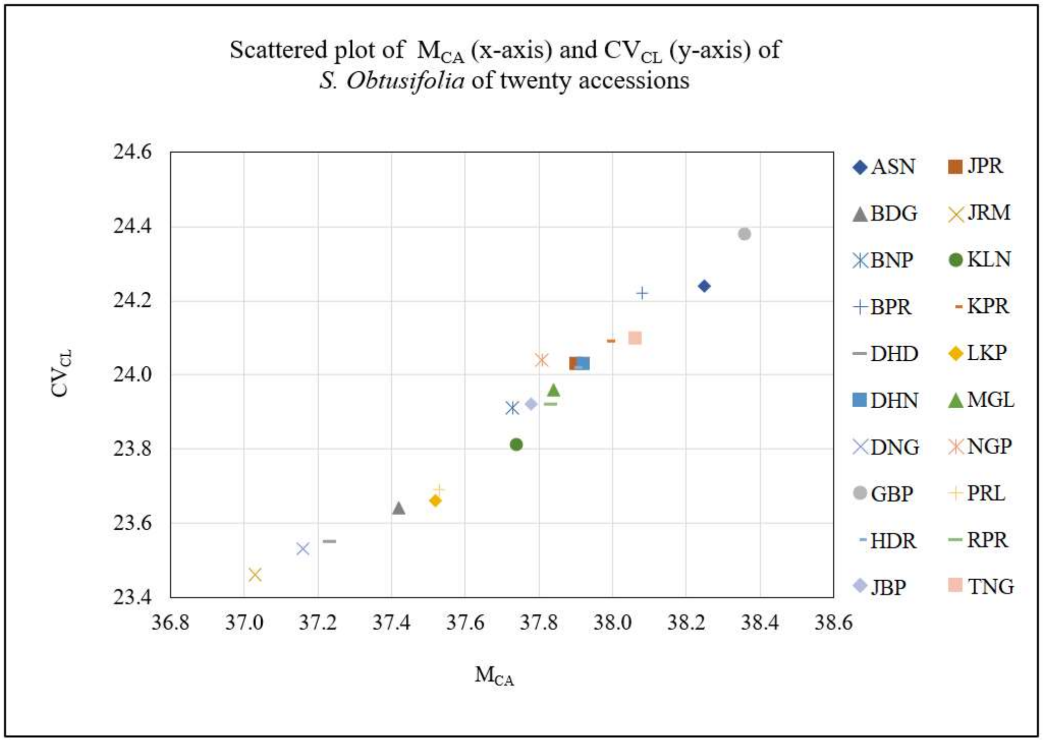


Figure 5.22 Karyotype asymmetry of S. obtusifolia of twenty accessions

The finer differences in the values were quite significant in representing variations among the accessions of *S. obtusifolia,* collected from the twenty different provenances. This information holds quite importance in understanding the cytological variations. Though very minor, it may have some impact on the intraspecific diversity of the species. In this study, it is quite apparent that the distant provenances differ more, although require more sufficient evidences for claiming emphatically.

Twenty accessions of *S. obtusifolia* collected from twenty different provenances were assessed for karyotypic analyses. In every analysis, 26 chromosomes were observed comprising of a diploid set which comply with the findings of other researchers like Cordeiro et al., (2017), Souza and Iseppon, (2004), Rice et al., (2015). The presence finer granulations with delimiting chromocentres and diffused chromatin staining in all the accessions corroborated with the study of Souza and Benko- Iseppon (2004). The presence of diploid set 2n = 26 chromosomes suggested that the species have a basic number X = 13 (Biondo et al., 2005; Rice et al., 2015; Cordeiro et al., 2017). The chromosomal structure and morphology in all the accessions are also similar with the presence of and NOR present in four chromosomes which is accordance with the study of Souza and Benko- Iseppon (2004). The variations in the total length of the haploid chromosomes in all the accessions of *S obtusifolia* showed very minor variations among the species collected from different provenances. The study of karyotype asymmetry analyses from the twenty accessions also revealed minor variations among the provenances that might hold some importance in the intraspecific levels.