IV. MATERIAL & METHODS
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COLLECTION OF MATERIAL:

Ten species of Plantago were selected for the present study. The seeds were collected from various sources as these species do not grow in Vidarbha region either wild or in the cultivated fields. They were grown in the experimental plots of Botanical garden of Vidarbha Mahavidyalaya, Amravati, for field study. Voucher specimens are deposited in the Herbarium of Vidarbha Mahavidyalaya, Amravati.

TABLE: List of Plantago species and their sources

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Species</th>
<th>Wild/ cultivated</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>P. coronopus L.</td>
<td>wild</td>
<td>G.A.U. Anand, Gujrat</td>
</tr>
<tr>
<td>5.</td>
<td>P. major L.</td>
<td>wild</td>
<td>University of Jammu</td>
</tr>
<tr>
<td>10.</td>
<td>P. exigua Murrey</td>
<td>wild</td>
<td>seeds of Cuminum cyminum.</td>
</tr>
</tbody>
</table>
A. KARYOMORPHOLOGICAL STUDIES

The seeds were germinated in sterile petridishes on wet filter papers. Healthy and young root-tips of about 2 mm length were cut between 10.30 & 11.30 a.m. Staining procedure was adopted from Sharma and Sharma (1965) with slight modification at pretreatment stage. Various pretreatment agents such as Colchicine, p-dichlorobenzene, 8-Hydroquinolene, aesculin, α-bromonaphthalene were tried. However the best results were obtained by using 1:1 mixture of α-bromonaphthalene and aesculin with a little bit of Saponin as pretreatment agent. A drop of α-bromonaphthalene was added to 2 ml water and was thoroughly shaken and a few crystals of Saponin were added for complete saponification. Aqueous solution of aesculin was prepared separately by adding a little quantity of aesculin powder to 2 ml water just to produce a blue tinge. These two mixtures were added together and were shaken well. Healthy roots tips of about 2 mm length were kept in the above freshly prepared pretreatment agent for 5 minutes at 4°C, and then for 2 hours at 14-16°C (Singh, 1978).

After pretreatment the roots tips were washed with tap water, fixed in Carnoy's Fluid I for 24 hours and preserved in 70% alcohol. Hydrolysis was carried in 1N HCl at 60°C for 2-3 minutes. The hydrolysed root tips were kept in mordant (4% Iron alum) for ten minutes, then washed thoroughly in
water and stained in 1\% haematoxylin for five minutes. Squashes were prepared in 45\% acetic acid. Slight warming before mounting resulted in better spreading of the tissue. Polar metaphases were selected for the karyotype study. Homologues were decided on the basis of absolute length, arm ratio and index number. Karyotype analysis was done following Levan et al. (1964), Adhikary (1974) and Huziwara (1955).

Morphometric values were calculated with the help of the following formulae:

1. Index number ($r_1$)\[= s/l\]
2. Arm ratio ($r_2$)\[= l/s\]
3. Centromeric index (i)\[= \frac{100 \times s}{c}\]

Here 'l' represents the length of the long arm; 's', the length of the short arm, and 'c', the absolute length of the chromosome.

4. Relative length\[= \frac{\text{Length of a chromosome}}{\text{Length of the longest chromosome}} \times 100\]

5. Total chromatin length (TCL\%)\[= \frac{\text{Total length of a chromosome}}{\text{Total length of chromosomes}} \times 100\]

6. Total form value (TF\%)\[= \frac{\text{Total length of short arms}}{\text{Total length of chromosomes}} \times 100\]

The chromosome pairs were arranged in a decreasing order of absolute length and accordingly the pair numbers
were written in Roman letters. Chromosomes were arbitrarily grouped into the following five categories on the basis of their absolute lengths.

A  - 5 to 5.99 μm
B  - 4 to 4.99 μm
C  - 3 to 3.99 μm
D  - 2 to 2.99 μm
E  - 1 to 1.99 μm

Karyodiagrams were sketched in such a way that long arms of all the chromosomes were pointed towards the lower side and the short arms towards upper side, irrespective of the position of satellites or secondary constrictions.

Centromere positions of the chromosomes were determined as per Adhikary's terminology (1974). Most of the earlier investigators used Levan's terminology (1964), for this purpose. For discussing the present results with those of earlier workers, Adhikary's terminology was applied for convenience.

Following points were taken into consideration while writing the karyotype formula.

1. Chromosome pairs were placed in groups like, A,B,C,D and E according to their absolute lengths.
2. Satellited chromosomes were marked by putting letters 'SAT' against them.
3. Brackets read the centromeric position of the respective pairs.
Graphs were plotted for arm ratio, relative length, TCL\% and centromere index against chromosome pairs for each species. Histogram depicting total form value (TF\%) for ten species was also given. Degree of asymmetry among ten species of Plantago was decided on following 2-way system of classification of karyotype asymmetry (Stebbins, 1971).

B. MEIOTIC STUDIES -

The young spikes were fixed in Carnoy’s Fluid II (one part glacial acetic acid + 3 parts Chloroform + 6 parts absolute alcohol at 7 a.m. for 24 hours and preserved in 70\% alcohol. Smears of anthers were prepared in 1\% acetocarmine (Smith, 1947). PMCs were scored and chiasma frequency at diplotene, diakinesis and metaphase I was calculated. Sequence of various stages of meiosis was diagrammatically presented. Meiotic irregularities were also recorded. Permanent preparations were made by applying CO₂ gas freezing technique and procedure of dehydration by using acetic acid and Butyl alcohol grades (Bowen, 1955).

Observations on meiosis of P. afra and P. exigua could not be presented due to the limited number of plants growing on the field, which were not enough for meiotic studies. P. major could not reach the flowering stage as it could not sustain high temperature of the atmosphere.
C. **POLLEN STUDIES** -

Smears of mature anthers were made in 1:1 mixture of glycerine and 2% acetocarmine. The pollen grains which exhibited unstained empty portion inside the exine were scored as sterile and those having red coloured cytoplasm with distinct nucleus inside the exine, as fertile. Pollen fertility percentage was calculated by examining the pollens from at least five different spikes. Size difference between fertile and sterile pollen was also measured and camera lucida sketches were made.

D. **MORPHOLOGICAL STUDIES** -

For morphological studies fresh as well as FAA preserved plant materials were used. The macro and micromorphological characters of the respective species were studied. Camera lucida sketches were made wherever necessary. Systematic description of each species was written by observing the plant specimens except *P. major* as it survives only for a short period in the field.

The taxa investigated here are serially numbered as mentioned in the list on page number 26. These numbers are maintained throughout the work for illustrations.