METHODS

Karyotype analyses have been useful in elucidating the phylogenetic and evolutionary relationship between species and groups of plants. In order to accurately ascertain chromosome homologies and differences, several indices are used by measuring chromosomes as a whole and their arms (Duncan & Smith, 1978). In Dioscoreaceae, the karyotype analysis is all the more difficult due to the presence of very small dot like chromosomes, apparently without centromeres. The centromeres are inconspicuous in some rod shaped chromosomes also, which were described as beta chromosomes by various authors.

Chromosome counting.
Slides were prepared by the technique outlined by Sharma & Sharma, 1980. Squash experiments were carried out on root tips at mitotic metaphase stage. The root tips were obtained soon after sunrise (before 7 a. m.) from plants grown in sawdust in a greenhouse. Roots were pre-treated in pre chilled, saturated solution of para dichlorobenzene for about three hours. The root tips in the cytostatic solution were kept at 20° C for better results.

The pretreated root tips were then washed thoroughly twice in tap water followed by a rinse in distilled water. The material was then fixed in 1: 3 acetic acid – ethyl alcohol mixture(Sharma & Sharma, 1980) for 45 minutes to 1 hour. This was followed by 5 minutes treatment of the specimen in 45% acetic acid. Hydrolysis of the roots with HCl was avoided as it greatly affected the staining properties of the chromosome. The apical portions of the roots were then separated and immersed in 2% aceto – orcein containing traces of 1N HCl for 30 minutes. Squash preparations of these root tips were prepared on slides in 1% aceto – orcein. These slides were then mounted on Leitz light microscope with photographic attachment. The chromosomes were counted in 3-5 cells per slide and in 5-10 root tips per species at magnification of 1000x. Photographs of the selected cells were taken by loading 125ASA, 35mm Kodak film on the camera attachment of the microscope.