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CYTOLOGICAL STUDIES OF *ADHATODA* L. SPECIES AND *BARLERIA* L. SPECIES

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ABSTRACT
Four selected plants types namely *Barleria cristata* L., *Barleria prionitis* L., *Adhatoda beddomei* L. and *Adhatoda vasica* L. have been studied for chromosome number and chromosomal associations, chiasma frequency, tetrads of microspores and pollen grains. In all the four plant types, the chromosome numbers remain more or less same (2n = 32 or 34). However, there is minute difference in the frequency of univalents in both species of *Barleria* (*B. cristata* = 2.6, *B. prionitis* = 4.4). Both the species of *Adhatoda* differ from the two species of *Barleria* in having the linear type of tetrads. Overall tetrads of *Adhatoda* species are quite smaller than that of *Barleria* species. Both viable and nonviable pollen grains are larger in *Barleria* species in comparison to those of *Adhatoda* species. The pollen viability among the selected four species does not show much variation but the pollen morphology is quite distinct from each other.

INTRODUCTION

Acanthaceae is a large panatropical family of about 250 genera and 2,500 species (Airyshaw, 1973). The genus *Barleria* L. and *Adhatoda* L. belong to this family. *Barleria* L. is a panatropical genus of herbs and shrubs comprising about 300 species occurring mainly in tropical parts of Africa and Asia (Balkwill and Balkwill, 1997), *Barleria* is an erect, prickly perennial shrub, growing during September to December and *Adhatoda* is an evergreen herb and its following in January to March throughout the year. *A. vasica* (Adusa) is a ayurvedic medicine which mostly used in brothchitis, leprocy, blood disorders, heart troubles, thirst, asthma, fever, vomiting, loss of memory, leucoderma, jaundice, tumers etc. *B. prionitis* (Vajardanti) have shown potent activity against respiratory syncytial virus *in vitro* and mouth washed used to relieve toothache and treat bleeding gums. Both plants are used in medicinal applications. *Barleria* and *Adhatoda* have received attention from several authors (Nees, 1847, Anderson, 1864, Bentham, 1876, Clarke, 1885, Gamble, 1956).

Very little attention has been paid towards their cytological analysis of these economically important plants. The contribution by Narayanan (1951), De (1964; 1966), Joseph (1964), Kaur (1966), Kaur and Nizam (1970), Datta and Methi (1968; 1970), Sareen and Sanjota (1976), Daniel and Balkwill (2000) and Daniel (2000). In the present study, we have selected the four species to study the various cytological aspects as mentioned in abstract.

MATERIALS AND METHODS

Flowering occurs from October-January in *Barleria cristata* L. and from September-December in *Barleria prionitis* L. However in both species of *Adhatoda* flowering periods remains the same (January-March).

Flower buds of different sizes were collected from the selected plants species during their flowering seasons, at the relative temperature and humidity appropriate size of bud was taken. They were fixed in Carnoy's fluid I° for 24 hrs, then transferred to 70% alcohol for storage.Their anthers were put on the slide and stained in 1% acetocarmine solution. A cover slip was placed over the solution. After pressing and heating different pollen mother cell (PMC's) were seen with different meiotic stages. Microphotography was done from temporary prepared slides at different magnifications (x100-450 μm).

RESULTS AND DISCUSSION

Observation about cytological characters have been present in Table 1 and 2; Fig. 1, 2 and 3(a to h). Normal meiotic division was observed in all the selected species.

The four plant species namely *Barleria cristata* L., *Barleria prionitis* L., *Adhatoda beddomei* L. and *Adhatoda vascia* L. were studied for their chromosome number, chromosomal association, chiasma frequency, tetrads of microscopores and pollen grains.

The chromosome number in both of *Barleria* species were 2n = 32 (Fig. 3c). The chromosome associations were concerned, there were minute differences in the frequency of ring or rod bivalents, whereas the ring association was maximum in *B. prionitis* is 6.8 ± 0.251. However rod association was maximum in *B. cristata* (9.2 ± 1.327; Fig. 3d). The frequency of univalents was maximum in *B. prionitis* (4.4 ± 2.439). However there was no change in chiasma frequency per chromosome at both species level (Table 1; Fig. 1). We had

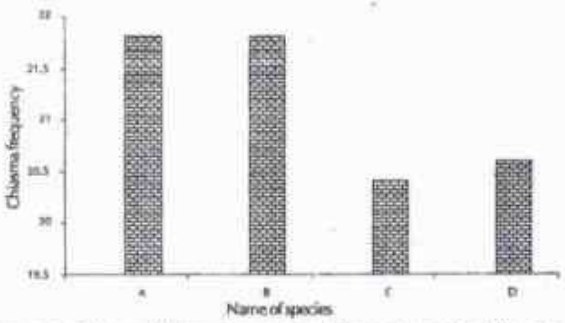

Figure 1: Data on chiasma frequency of charomosome in *Adhatoda* species and *Barleria* species

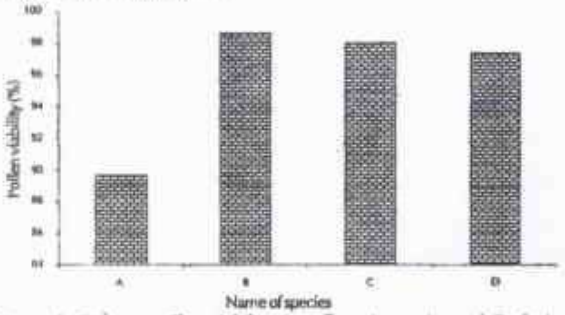

Figure 2: Data on pollen viability in *Adhatoda* species and *Barleria* species A – *Adhatoda beddomei* L; B – *Adhatoda vasica* L; C – *Barleria cristata* L; D – *Barleria prionitis* L.

also studied morphology of tetrads of microspores, as well as pollen grains along with their pollen, viability in *Adhatoda* species and *Barleria* species (Table 2). The average sizes of different types of tetrads were maximum in *B. cristata* in comparison to *B. prionitis* (Table 2). However, the average size of viable, non-viable and average pollen viability (%) was quite similar in both species of *Barleria* (Table 2; Fig. 2).

In the present investigation it was observed that the similar chromosome number *i.e.* (2n = 34) in *Adhatoda vasica*, *Adhatoda beddomei* (Fig. 3a; 3b) Cytology of *Adhatoda beddomei* and *Adhatoda vasica* reveals similarity in the selected parameters characteristics (Table 1), in *A. vasica* maximum size of different types of tetrads was observed in comparison to *A. beddomei* (Table 2). In *A. vasica* average size of viable pollen grains (67.921 × 42.001μm) and non-viable pollen grains (54.611 × 37.125 μm) were measure larger. Pollen viability 98.60% was maximum in *A. vasica* in comparison to *A. beddomei* (Table 2).

Adhatoda vasica was somewhat distinct from that of *A. beddomei* in these characteristics. Both the species of *Adhatoda* differ from two species of *Barleria* in having the linear type of tetrads. Overall tetrads of *Adhatoda* species were quite smaller than that of *Barleria* species. Pollen morphology of all the four selected plant species was quite distinct from each other. They were round in *B. cristata* (Fig. 3g) in comparison to triangular type in *B. prionitis* (Fig. 3h).

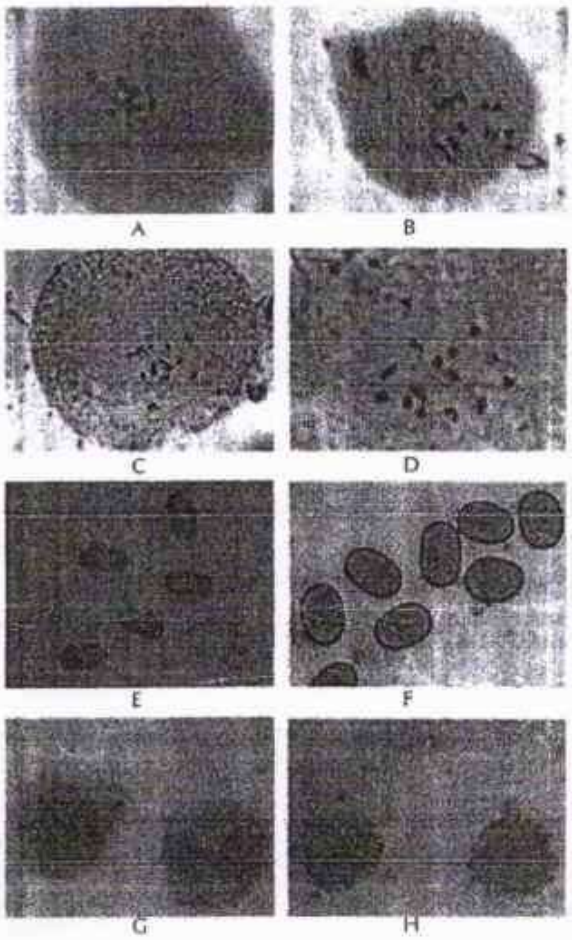

Figure 3a to h: PMC showing (A-D) A-17 II at M I (X450) in *A. beddomei* L., B - Rod and Ring bivalents in *A. vasica* L. (X450), C – 16 II at MI (X 450) in *B. cristata* L., D – Rod and Ring bivalents in *B. prionitis* L. (X450), Viable pollen grains (E-H)E- *A. beddomei* L. (X 100), F – *A. vasica* L. (X400), G – *B. cristata* L. (X 400), H – *B. prionitis* L. (X 400)

Table 1: Data on chromosomal characteristics of *Adhatoda* spp and. *Barleria spp*

Name of plant	Chromosome number	Chromosomal association bivalent (range / average ± S.E.) ring	rod	Univalent (range / average ± S.E.)	Chiasma frequency / chromosome (range/ average ± S.E.)
1. *Adhatoda beddomei* L.	2n = 34	6-7/6.6±0.107	7-10/8.6±0.464	2-6/3.6±1.00	21-23/21.8±0.251
2. *Adhatoda vasica* L.	2n = 34	6-8/6.4±0.466	9-10/8.0±0.986	2-4/3.2±0.430	20.23/21.8±0.609
3. *Barleria cristata* L.	2n = 32	4-7/5.6±0.466	7-12/9.2±1.327	2-4/2.8±0.430	20-21/20.4±0.107
4. *Barleria prionitis* L.	2n = 32	6-8/6.8±0.251	6-9/7.0±0.717	2-8/4.4±2.439	18-22/20.6±1.00

Table 2: Data on tetrads of microspores and pollen grains in *Adhatoda* species and *Barleria* species

S. No.	Plant species character	*Adhatod beddomei* L.	*Adhatoda vasica* L.	*Barleria cristata* L.	*Barleria prionitis* L.
1.	*Average size of tetrahedral tetrads	33.982 × 20.051 ± 2.946 × 2.125	51.131 × 40.320 ± 2.171 × 1.005	92.191 × 89.392 ± 3.514 ± 2.310	89.953 × 82.580 ±3.491 × 2.122
2.	*Average size of isobilateral tetrads	35.160 × 21.191 ± 1.914 × 1.629	49.031 × 40.022 ± 1.316 × 0.715	95.10 × 85.090 ±3.614 × 1.319	91.524 × 85.163 ±2.795 × 1.341
3.	*Average size of rhomboidal tetrads	33.80 × 21.050 ± 1.012 × 1.716	45.92 × 31.721 ± 1.901 × 0.918	91.012 × 89.101 ± 2.392 × 1.362	87.09 × 79.580 ± 2.469 × 1.829
4.	*Average size of linear tetrads	39.093 × 20.054 ± 1.992 × 0.901	56.613 × 32.060 ± 0.091 × 1.055	Absent	Absent
5.	*Average size of viable pollen grains	44.83 × 31.531 ± 2.946 × 2.325	67.921 × 42.001 ± 3.256 ± 0.925	115.850 ± 8.084	110.250 × 120.252 ± 23.03 × 23.03
6.	*Average size of non viable pollen grains	36.751 × 26.250 ± 4.845 × 1.744	54.611 × 37.125 ± 5.582 × 3.256	93.03 × 83.360 ± 14.86 × 12.75	91.72 × 87.910 ± 8.373 × 7.431
7.	*Average pollen viability (%)	89.63	98.60	98.01	97.25
8.	Shape of pollen grains	Rod	Oval shape	Round	Triangular

*Size measures in micrometer per unit area (per)

Similar *Adhatoda beddomei* had rod shape pollen grains (Fig. 3e) as compared to oval shape as observed in *A. vasica* (Fig. 3f). Both viable and nonviable pollen grains were larger in *Barleria* species in comparison to those of *Adhatoda* species.The pollen viability among the selected four species did not show much variations (Fig. 2).

2n = 32 as chromosome number in *B. cristata* and *B. prionitis* had been observed. There were many reports about similar chromosome number reported in species of genus *Barleria* (Kaur, 1966, Govindrajan, 1984, Sagoo and Bir, 1982, 1986, Roy, 1990). Kaur (1966) found that the genus *Barleria* was polybasic in nature (x = 15, 16, 19, 20).

Sagoo and Bir (1986) had reported meiotic studies in certain members of acanthaceae including *Barleria* species. They found some relationship and made comparison between plant habit and incidence of polyploidy in perennial and shrubby species to annual and harbacious ones. However the observations on the chromosome number of *Barleria* species revealed their diploid nature inspite of being shrubby in nature.

Ranganath (1981) concluded from his morphological and cytological studies in some members of acanthaceae n = 17 as chromosome number in *Justicia adhatoda* of section *vasica* of family acanthaceae.

Maheshwari (1952) had carried out cytological investigation in *Adhatoda vasica* Nees. He reported 17 bivalent at diakinesis. Metaphase was normal two spindles may be arranged parallel or at right angles to each other.

Daniel (2000) and Daniel and Balkwill (2000) also reported similar chromosome number in some genera of family acanthaceae including two species of genus *Justicia*. In the present investigation it was also observed the similar chromosome number i.e. (2n = 34) in *Adhatoda vasica* L., *Adhatoda beddomei* L.

CONCLUSIONS

We have concluded the similar result of present study carried out shows that there was no change by different authors in above said cytological characters while they were growing in different habitats.

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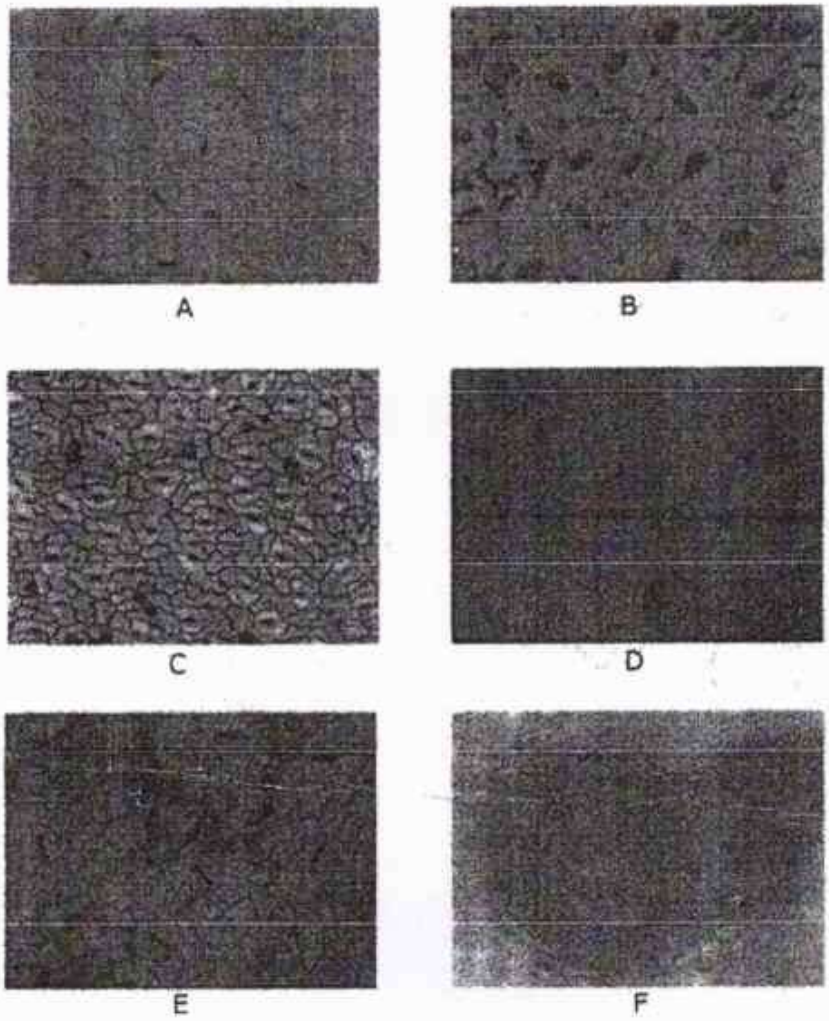

Fig. 2(A-F) : Lower surface of leaf for epidermal studies showing A,B & C - Paracytic type of stomata in ***T.peruviana L.*** (var. orange, yellow,white). D&E - Anomocytic type of stomata in ***C.roseus L., T.divericata L.*** . F- Stomatal crypt in ***N. indicum L.***