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# Meiotic chromosome behavior of *Haworthia limifolia*

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**Abstract:** *Haworthia limifolia* is a xerophytic plant belonging to the family Liliaceae and is indigenous to Africa. It is use extensively for its medicinal properties like antibacterial, antifungal properties and used for the treatment of sores, superficial burns, as a blood purifier and to promote pregnancy in women and cattle's. In present investigation chromosomal behavior of *H. limifolia* in meiosis was studied. In diplotene stage chiasmata was observed showing the possibilities of genetic recombination. Chromosome clumps were observed in diakinesis indicating sticky nature of chromosomes. Meiotic abnormalities like stickiness, precocious movement, formation of bridges and laggards were also reported in both meiosis I and II. A fairly high percentage of pollen sterility that is 73.41% was recorded resulting in failure of fruit formation. This plant could be designated as facultative apomict (Swanson, 1957) as the only means of reproduction found was asexual or vegetative.

**Key words:** Meiosis; *Haworthia limifolia*; Liliaceae.

## Introduction

*Haworthia limifolia* is a xerophytic plant belonging to the family Liliaceae and is indigenous to Africa. Various species of genus *Haworthia* including *Haworthia limifolia* have been introduced to India and are being grown for their horticultural and medicinal values. *Haworthia limifolia* is used extensively for its medicinal properties as it contains Lectin and Lectin like derivatives with antibacterial and antifungal properties[1,2]. Traditionally it is used for the treatment of sores, superficial burns, as a blood purifier and to promote pregnancy in women and cattle[1]. In recent years, *Haworthia* have attracted the attention of geneticists and cell biologist with respect to meiotic process. The medicinal value and male sterility of *Haworthia limifolia* make it important for cytogenetic investigations. Despite recent biochemical and taxonomical studies in *Haworthia*, cytogenetical studies in *Haworthia limifolia* has progressed slowly due to the occasional flowering and abnormal meiosis. Since cytogenetical studies are very important not only for understanding plant reproduction and fertility but also for a better understanding of genome constitution. Thus, present study aims to provide a detailed understanding of structure and behavior of meiotic chromosome of *Haworthia limifolia*.

### Objective of research

Detailed analysis of meiotic chromosome behavior especially of prophase I chromosome of *Haworthia limifolia* is inadequate because of the extremely sticky nature of the chromosomes. So it was found necessary to perform the meiotic analysis of *Haworthia limifolia*.

## Material and Methods

Fresh flower buds of appropriate size were collected from plants of *Haworthia limifolia* and fixed in Carnoy's fluid (3 ethanol: 1 acetic acid) for 24 hours and then they were transferred to 70% alcohol and stored. Flower buds of varying sizes were arranged in watch glass containing fixative. Individual bud was transferred on to a clean glass slide and a single anther was dug up from the bud. A small drop of aceto-carmine stain was placed over the anther and it was then gently tapped to release pollen mother cells into the stain. Anther wall was then removed from the slide and cover slip was placed carefully on it. The slide was then gently warmed to improve staining of the chromosomes. The preparation was then squashed gently using thumb pressure. Well stained and squashed cells were examined for the structure and behavior of chromosomes at different stages of meiotic division and the appropriate stages were microphotographed. Pollen stain ability test was performed to test pollen fertility and sterility. More than three thousand pollen grains were examined for pollen fertility and pollen sterility percentage. Darkly stained pollen grains were considered as fertile while lightly stained pollen grains were considered as sterile[3]. Percentage pollen fertility and percentage pollen sterility were determined by using following formula:

$$\text{Percentage pollen fertility} = \frac{\text{No. of fertile pollen grains}}{\text{Total no. of pollen grain studied}} \times 100$$

$$\text{Percentage pollen sterility} = \frac{\text{No. of sterile pollen grains}}{\text{Total no. of pollen grain studied}} \times 100$$

## Results

In *Haworthia limifolia* since the chromosomes are of extremely sticky in nature, meiotic prophase was not clearly observed. However, in some slides leptotene stage was observed with quite tenous chromosome thread (Fig.1A). Zygotene stage showed well dispersed and conspicuous chromatin threads (Fig. 1B). In Pachytene stage more condensed chromosomes with beaded appearance

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were observed (Fig.1C). Diplotene stage was identified by more condensed and thicker chromosomes showing 14 bivalents with variable chiasmata (Fig. 1D). In Diplotene and Diakinesis stage, well separated bivalents and chiasmatic region were difficult to observed clearly due to high frequency of chromosome clumping at these stages. At Metaphase I, 14 heavily condensed chromosomes were found (Fig. 1E and 1F). At Anaphase I, homologous chromosomes migrated towards poles were clearly observed (Fig. 1G). At Metaphase II, chromosomes appeared more condensed. At Anaphase II, chromatids started separating and migrating towards the poles perpendicular to the 1st nuclear division forming a tetrad each containing haploid no of chromosomes (Fig. 1I). Telophase II was identified by the presence of clumped chromosome mass at poles (Fig. 1J). This formed a tetrad which later developed into four mature pollen grains (Fig. 1K). Percentage pollen fertility in *Haworthia limifolia* was found 26.58% whereas percentage pollen sterility in *Haworthia limifolia* was found 73.41%.

**Table 1:** Percentage pollen fertility and sterility in *Haworthia limifolia*

| Total no. of pollen grains studied | No. of fertile pollens | No. of sterile pollen | % pollen fertility | % pollen sterility |
|---|---|---|---|---|
| 3314 | 881 | 2433 | 26.58 | 73.41 |

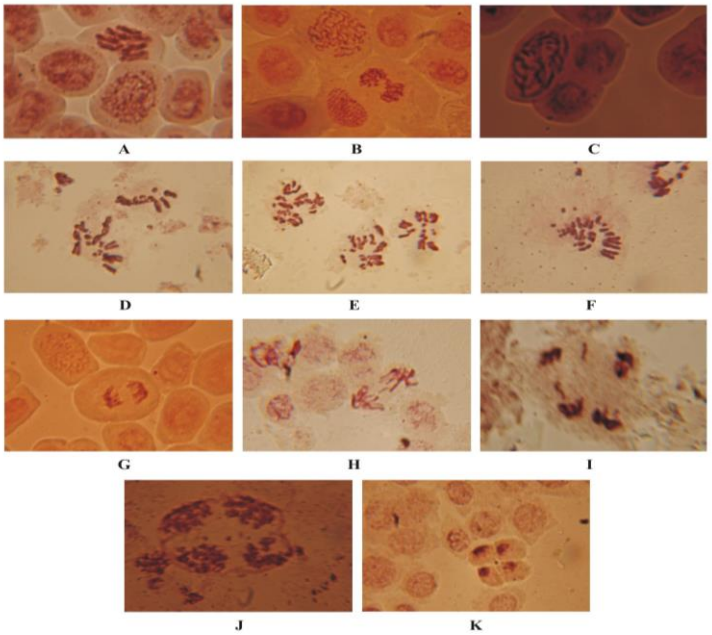


**Fig. 1 :** Meiotic stages from acetocarmine - stained squash preparations of pollen mother cells of *Haworthia limifolia*. **A.** Leptolene, showing unsynapsed chromatin threads; arrow indicates heterochromatin; **B.** Zygotene, showing well dispersed and conspicuous chromatin threads; **C.** Pachytene, showing thick and beaded chromosomes; **D.** Diplotene, showing fourteen bivalents with chiasma; **E-F.** Metaphase I, showing fourteen bivalents; **G.** Anaphase I, showing chromosomes migrating towards the pales; **H.** Late Anaphase I, chromosomes have moved to the poles; **I.** Anaphase II showing four groups of chromatids two in each diad; **J.** Early Telophase II with lagging bivalent; **K.** Formation of tetrads.

**PLATE - I**

## Discussion

Present study was carried out with an aim to prepare the chromosomal atlas of meiosis of *Haworthia limifolia* and to provide a basic outline for chromosomal structure and behaviour of meiotic chromosomes in *Haworthia limifolia*. Due to the higher stickiness of chromosomes prophase stage was difficult to observe. However, chromosome could be identified as fine long threads in leptotone stage. Process of synapses occurred at zygotene stage which made chromosomes appeared as beaded structure. Although this stage was difficult to decifer. In pachytene stage chromosomes become more condensed and exhibited beaded appearance. In diplotene stage, shorter, thicker and heavily stained chromosomes with chiasmata were observed. Chiasmata are the regions where physical exchange occur between non sister chromatids of homologous[4]. Chiasmata frequency is regarded as a good estimate of the level of genetic recombination in species[5]. Chismata analysis presents the primary source of information on meiotic recombination that leads to the new combination of alleles. Chromosome clumps were observed in diplotene and diakinesis and showed the sticky nature of chromosomes of *H. limifolia*. At metaphase I, bivalents became condensed enough to be more prominent. Chromosome pairing and disjunction at anaphase I and distribution of chromatids at anaphase II were clearly observed. Chromosomes separation and migration to the poles perpendicular to the plane of first nuclear division forming a tetrad which later developed into four mature pollen grains were reported in *H. limifolia* as reported earlier in *Aloe vera* (L) Burn f. by A.K. Chaudhari and B.R. Chaudhary, 2012[6].

Meiotic abnormalities like stickiness, precocious movement, formation of bridges and laggards were also reported in both meiosis I and II resulting in a fairly high percentage of pollen sterility that is 73.41% so this plant could be designated as facultative apomict as the only means of reproduction found was asexual or vegetative[7]. The vegetative propagation may be a mechanism to exploit a ecological conditions which is done by suppressing sexual reproduction through failure of meiosis and fertilization together, avoiding segregation and recombination[8]. In *H. limifolia* failure of fruit formation is because of the failure of meiosis resulting in a high pollen sterility and of self-incompatibility as suggested by Berger (1908) for *Aloe* species[9].

## Conclusion

**Research Highlights:** Chromosomal atlas of meiosis of *H. limifolia* was obtained. Main highlights of research are In diplotene stage chiasmata was observed showing the possibilities of genetic recombination.

Chromosome clumps were observed in diakinesis showing the stickiness of chromosomes of *H. limifolia*.

Meiotic abnormalities like stickiness, precocious movement, formation of bridges and laggards were also reported in both meiosis I and II. A fairly high percentage of pollen sterility that is 73.41% was recorded resulting in failure of fruit formation. This plant could be designated as facultative apomict (Swanson, 1957) as the only means of reproduction found was asexual or vegetative.

**Limitations:** The plant of *H. limifolia* was collected locally from nurseries at Ranchi (Jharkhand, India) and their cytological studies were performed in the laboratory of University Department of Botany, Ranchi University, Ranchi.

**Recommendations**
The cytological analysis gives an authentic data for the preparation of chromosomal atlas

**Funding and policy aspects**
Preparation of chromosomes atlas of medicinally important plants *H. limifolia*.

**Justification of research**
Chromosomes atlas

**Authors contributions and competing interest**
The authors declare that this is the original research work and they have no financial interest in making the chromosome atlas.

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