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Microsporogenesis, structure and viability of pollen in *Canscora decurrens* Dalzell a potent medicinal plant

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Abstract

The entire plant of *Canscora decurrens* (syn. *C. diffusa*)^[15] is used in ancient and modern medicine for its broad spectrum pharmacological activities like anti-tumour, anti-microbial, anti-leukemic etc. Its potential role in treatment of central nervous system related disorders has been established undoubtedly. For its use on commercial scale, selection and production of high yielding cultivars by modern biotechnological approach is desired. For this it is imperative to know the pollen development (Microsporogenesis) and pollen viability. Knowledge of structure of pollen can add to pharmacognostic features for authentication of herbal material. Present study on meiosis, fluorescence microscopy and Scanning electron microscopy analysis indicated normal meiosis and pollen mitosis with 19 bivalents in *C. decurrens*. Prominent, gradual deposition of callose around pollen mother cell was observed. Pollen grains are elliptical and tricolpate with good viability.

Keywords: *Canscora decurrens*, Central nervous system activity, microsporogenesis, scanning electron microscopy, callose, pollen viability.

1. Introduction

Genus *Canscora* includes potent medicinal plants showing a plethora of pharmacological activities. Ethnobotanical studies indicated that since ancient times, these plants have been used by Ayurvedic practitioners for activities like anti-tumour, anti-microbial, anti-leukemic and its potential role in treatment of central nervous system -related disorder has been established undoubtedly^[1, 6]. Classical studies on routine phytochemical and ethano pharmacological properties have been carried out on *C. decussata*^[3, 11]. Recently a comprehensive account of pharmacological activity of *C. Diffusa* has been reported^[2].

Entirely new modern approach of genetic analysis and cultivar improvement is taken by the authors to evaluate the potential of *C. decurrens* for its futuristic biotechnological exploitation in pharmaceuticals. The first step in this direction is to study the structure, development (microsporogenesis) and viability of pollen grain in *C. decurrens*. Microsporogenesis is a complex process which includes active cell division activities that marks the start of haplophase. It is a key stage in plant's life cycle as it is associated with chromosome and chromatid segregation generating genetic and morphological diversity in plant species. This mode of cell division in anthers gives rise to pollen mother cell (2n) that develops into mature pollen grains (n). It comprises 3 sequential stages namely premeiosis, meiosis and post meiosis^[4]. Although each stage is controlled independently, a complete meiotic cycle is highly integrated process to ensure production of normal fertile male gametes for successful pollination. The quantity and quality of pollen produced by a plant determines the reproductive success. However many factors including environmental adversity, genetic instability or inbreeding depression may lead to pollen sterility. Further mutations may cause abnormal meiotic products causing pollen sterility. Investigations on normal and abnormal meiotic process give an insight to understand structure and development of pollen and its potential use in molecular plant breeding and mutation studies. Present account on pollen meiosis is a part of induced mutation studies in *C. decurrens* (2n=38)^[13]. Pollen meiosis culminates into production of haploid pollen grains. Identification, characterisation and morphological studies of pollen grain either by light or scanning electron microscopy are important for taxonomy, pharmacognosy and breeding purposes. In *C. decurrens*, as the whole plant is used for medicinal purpose, pollen morphology can prove useful pharmacognostic feature in identification of this medicinal plant material. Development of normal, viable pollen grain and their capacity to germinate decides the success of fruit and seed development. Knowledge on pollen viability for any

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plant species is prerequisite for mutation breeders and commercial growers. This paper gives a comprehensive account of different stages of meiotic cycle, structure and viability status of pollen grain in *C. decurrens*.

2. Material and methods

For meiotic studies, young flower buds were collected from the plants growing under natural condition and fixed in Cornoy's fixative for 24 hours then transferred to 70% alcohol and stored at 4 °C. Anthers were squashed to release pollen mother cells and stained with acetocarmine.

To study the development and deposition of callose, flower buds were treated with 0.05% Aniline blue (1M Phosphate buffer, pH:8) for 15 minutes, mounted in 15% Glycerol^[12] and observed under Fluorescence microscope (Leica DM2500, Mercury lamp 50V, 365 nm-420 nm). Photographs were taken by Leica DFC 450-C.

For pollen morphology, matured pollen grains were processed in acetocarmine for light microscopy (Olympus No.10L 551). While for scanning electron microscopy studies pollen grain were sputter coated with thin layer of Gold-Palladium and the specimens were then studied and photographed by SEM inspects (D8858).

Pollen viability test were carried out using pollen grains from fresh flowers. They were (appx 1000) either mounted in aceto-glycerin mixture or cultured in BK (Brewbaker & Kwack) medium by sitting drop method^[9]. Pollen fertility was estimated by counting the fully stained pollen grains with

normal shape as fertile and shrivelled, unstained PG as sterile. In *in vitro* culture condition the pollen grains showing initiation of pollen tube growth were considered as fertile PG. Photomicrographs of pollen mother cells and germinating pollen grains were taken from freshly prepared slides using Nikon D1300.

3. Results and Discussion

3.1 Meiotic study

Meiotic behaviour was analysed in more than 1000 PMCs. A wide range of meiotic stages were found in the anthers within same flower. (Fig1. A) Prophase I is marked by initiation of condensation and pairing of homologous chromosomes which can be seen from Leptotene (Fig1. B) to Pachytene (Fig1. C). These stages are also characterised by presence of prominent nucleolus (n). Most of the cells showed regular meiotic behaviour with discrete 19 bivalents at diplotene (Fig. D) and Diakinesis (Fig1. E). Metaphase-I (Fig1. F) show normal behaviour of chromosomes. Here the bivalents get aligned on meiotic spindle on equatorial plate. Rod and V-shaped bivalents are common but ring bivalents are also seen. (Fig1.G) There is a regular disjunction at anaphase-I (Fig1. H). Telophase -I marks the end of Meiosis I (Fig1. I) Different stages of Meiosis II viz. Metaphase-II, (Fig1. J), Anaphase II (Fig1. K) and telophase-II (Fig1. L) Show normal meiotic behaviour. At the end, cytokinesis gives rise to pollen tetrad (Fig1. M) and finally haploid microspores. (Fig1. N)

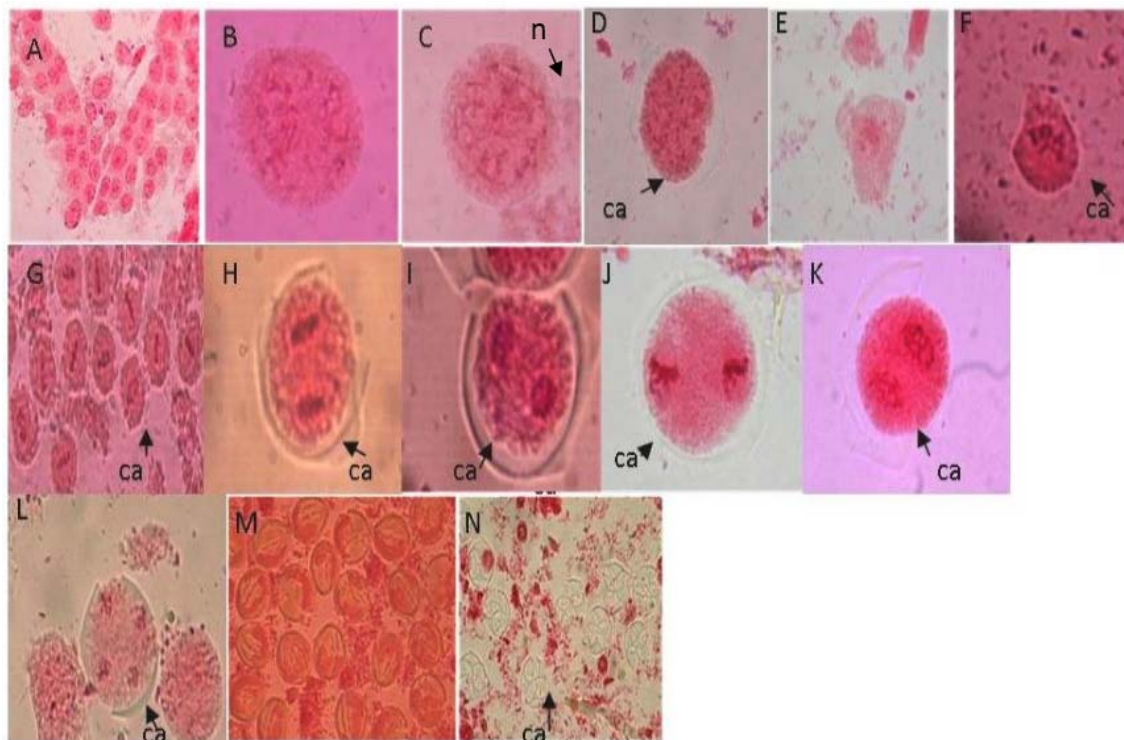


Fig 1: Different stages of pollen meiosis in *Canscora decurrens*. (A) Premeiosis (B) Leptotene (C) Pachytene (D) Diplotene (E) Diakinesis (F) V and Rod shaped bivalent (G) Metaphase I (H) Anaphase I (I) Telophase I (J) Metaphase II (K) Anaphase II (L) Telophase II (M) Pollen grain (N) Callose carcasses ; ca-callose, n-nucleolus

3.2 Fluorescence microscopic analysis

Keeping pace with these activities there is a progressive development of callose wall around each microsporocyte (microsporal callose) which can be seen as shiny transparent envelop in the cells without Aniline staining (ca in Fig1. D to N). After staining with Aniline blue callose shows prominent fluorescence. Callose wall deposition started in early stages of

meiosis in discrete patches (Fig 2. A) And develops progressively to form a continuous thick callose wall (Fig2. B to D). The cytokinesis is simultaneous and microspores are arranged in tetrahedral (Fig2. F) or rhomboidal (Fig2. G) Configuration. Intersporal callose thickening appears as thin strands (in Fig2. F) And develops centripetally finally leading to matured tetrad with thick walled callose. (Fig2. G and H)

The pollen grains released from tetrads show very thin continuous callose wall (Fig2. I) which protects the pollen tube during subsequent growth. Callose deposition during microsporogenesis is universal feature and is reported to have protective function to ensure perfect, viable meiosis [14] and is

linked to aperture pattern definition [10]. Because of very small size of chromosomes, abnormal meiotic details could not be observed. Hence overall meiosis in *C. decurrens* is described as perfectly normal.

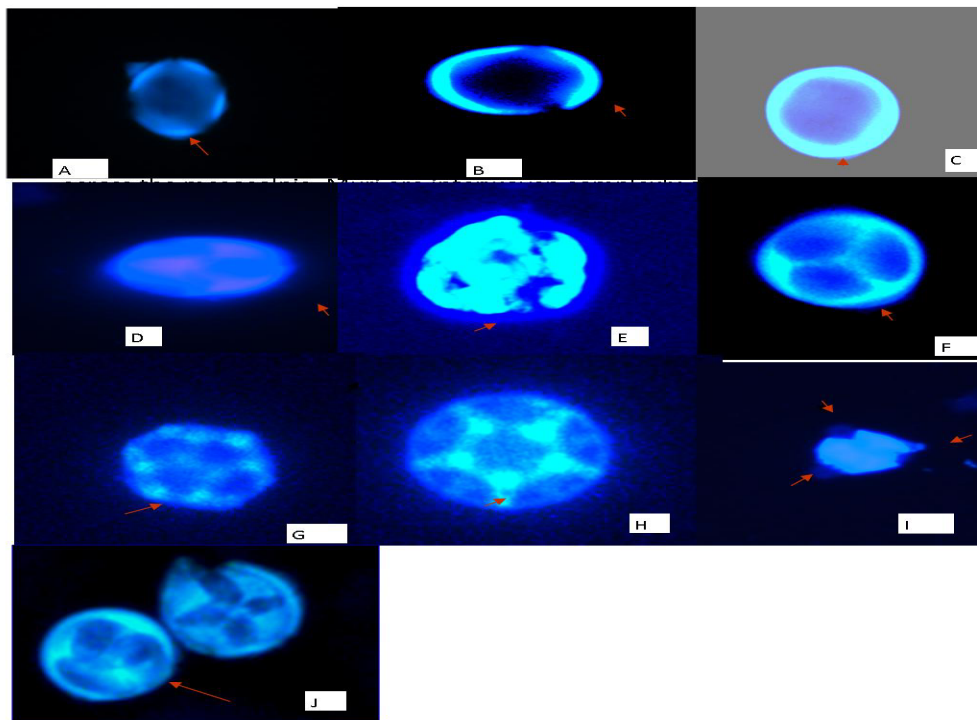


Fig 2: Callose development during microsporogenesis (A) Initiation of callose (B) Later stages of callose wall formation (C, D and E) Complete microsporocyte callose wall (F) Intersporal callose wall formation during tetrad stage. (H) Rhomboidal arrangement of microspore in tetrad (I) Lateral focus of tetrad (J) Pollengrain showing thin callose wall (K) Remaining callose carcasses indicate Callose.

3.3 Scanning electron microscopic analysis

Scanning electron microscopy images revealed that the pollen grains are medium sized. The equatorial length and breadth 182.81 μ and 20.31 μ respectively (mag. 500x, Fig 3. A). At the pole the PG is tapering with 21.876 \times 17.37 μ dimension

(mag. 11109x, Fig 3. B). It gives the PG elliptic (porate) shape. It is described as convex in equatorial view and obtuse in polar view. The pollen grain is tricolpate (Fig 3. C to D) having three medianly arranged colpi. Length of colpus is 19.68 μ . (Fig 3.A)

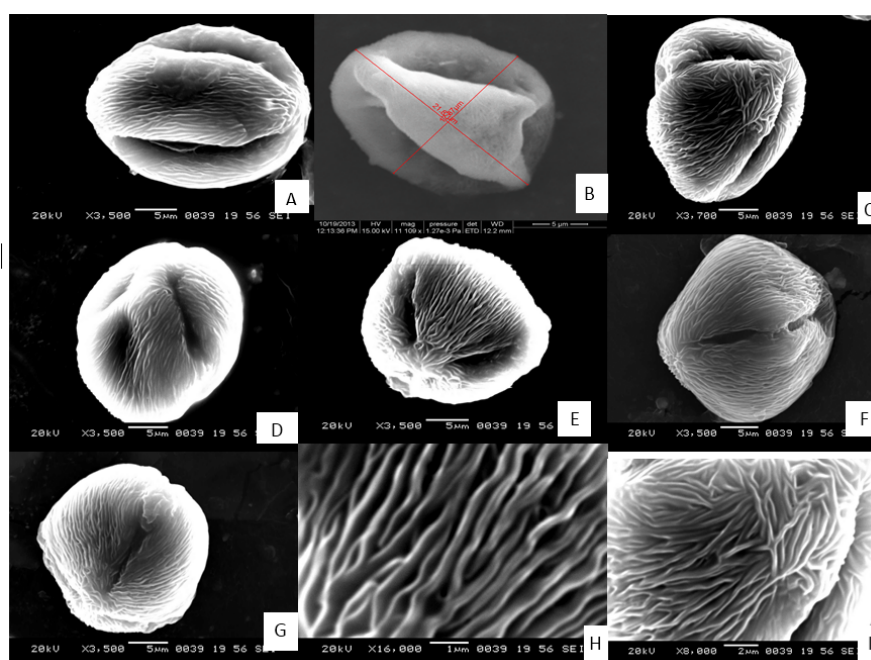


Fig 3: SEM of mature pollen grain. (A) Equatorial view showing colpi (B) Tricolpate pollen grain polar view, (C) lateral view, (D) equatorial view (E) Subpolar view showing mesocolpi (F), (G) Striate-reticulate pollen wall. (H) Interwoven short muri.

Pollen grain sculpturing is striate-reticulate with numerous short muri running across the mesocolpia. Muri are interwoven complexly especially at the poles. (Fig 3. F, G and H). Species closely related to *Canscora* invariably show three colpi pollen grains [8]. However three colpi pollen grains in *C. decurrens* are reported for the first time. Striate-reticulate exine sculpturing in *C. decurrens* is in accordance with earlier reports of family *Gentianaceae* on *Centaurium* and *Eustoma*.

3.4 Pollen viability test

High percentage (81%) of stained pollen grains with normal morphology were recorded indicating high pollen viability in

this population. This reflects the regular meiotic behaviour observed during microsporogenesis. *In vitro* germination is most widely method of testing pollen viability [7]. Pollen tube growth was observed in 65% pollen grains (Fig 4.C) however certain percent of pollen grains were inviable which failed to stain and showed abnormal morphology (Fig4. A and B). This pollen inviability is not likely due to cytological abnormalities but a number of factors like pollen age, temperature and humidity can contribute to pollen infertility [5].

Survey of literature indicated that there are no report on cytological studies and pollen viability. Hence of the present study is first of its kind

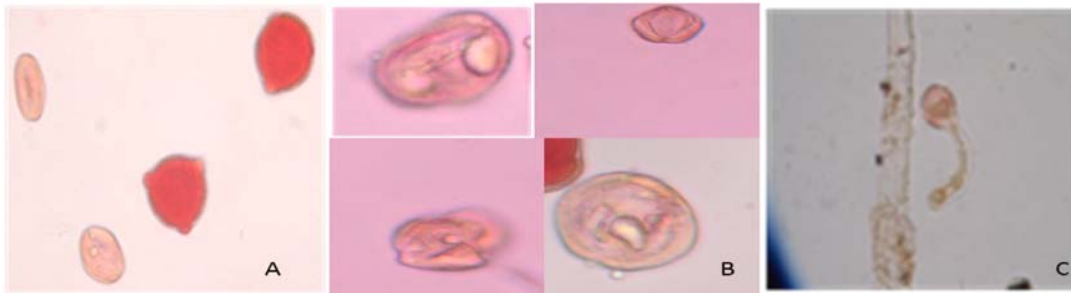


Fig 4: Pollen viability (A) Sterile and fertile pollen grain (B) Abnormal, sterile pollen grain (C) Pollen tube germination.

4. Conclusion

The present study lay the basis to understand the chromosome number and their behaviour during cell division which is foremost requirement for the program of cultivar improvement by mutation. The tricolpate, striated- reticulate pollen grain provide an important phamo cognistic feature for authentication of medicinally important *C. decurrens* plant.

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