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Studies on Reproductive Biology of *Terminalia Pallida* Brandis, An Endemic Plant of Eastern Ghats (Andhra Pradesh)

K. Michael David[1], G. V. Ranga Reddy[2], S. Sunitha[3], P. Helen Kumary[4], S. Sailaja[5], B. Bhavani[6]

[1,2,3,4,5,6]Department of Botany, KVR Government College for Women (A), Kurnool, AP

ABSTRACT:
Plants which need conservation, the information about their reproductive features will be useful for designing mathematical models and augmentation programmes and makes conservation efforts successful. The studies can provide information for preserving seedling longevity, pollen viability and prolonging dormancy in seed banks and pollen banks. These studies are also useful for the conservation, management and recovery of threatened species. An attempt has been made to study the reproductive and pollen biology of *T. pallida* an important endemic plant growing in Tirumala hills.

Keywords: *T. pallida*, *in vitro* germination, pollen viability, Pollen to Ovule ratio, Pollen biology, Reforestation programs.

INTRODUCTION:

All conservation approaches have to be based on the in-depth study of plant reproductive biology. Understanding reproductive biology of plants is of immense practical importance for the conservation of biodiversity and the control of invasive species. Reproductive features like gamete development, pollination, endosperm and embryo development etc., can provide important clues regarding the reproductive constraints of plants that need conservation.

The best way to go about conserving trees or any forest diversity resource is to make a scientific study of reproductive biology of plants, animals and microbes of forests. Most importantly, the reproductive biology of forest plants needs to be focused to start with, since the animal bio-resources depend directly or indirectly on plant resources.

Studies on reproductive biology will not only help in developing genetic potential of rare species but also help in the restoration and re-introduction of rare species which are raised by micro propagation techniques. Reproductive characters such as seed dispersal, germination capacity, survival rate of seedlings, age at flowering, reproductive life span, number of flowers and seeds refer to a set of responses that allow a species to adapt to a particular environment.

For plants which need conservation, the information about their reproductive features will be useful for designing mathematical models and augmentation programmes and makes conservation efforts successful. The studies can provide information for preserving seedling longevity, pollen viability and prolonging dormancy in seed banks and pollen banks. These studies are also useful for the conservation,

management and recovery of threatened species. Hence an attempt has been made to study the reproductive and pollen biology of *T. pallida* an important endemic plant growing in Tirumala hills.

Terminalia pallid is an endemic and endangered medicinal tree species native to the Tirumala hills in the Eastern Ghats of Andhra Pradesh. This tree is recognized for its therapeutic applications in traditional Ayurvedic medicine and plays a crucial role in plant regeneration studies. As a threatened species, *Terminalia pallida* highlights the importance of conservation efforts in biodiversity hotspots within its native habitat, emphasizing its significance both ecologically and medicinally

T. pallida populations growing on rocky areas at Akasaganga, Papanasanam, Japalitheertham, Srivari mettu and Talakona in Tirumala Hills of the Eastern Ghats (lat. 13°40′N, long. 79°19′E and altitude 2443 ft) were selected for study. The study included different aspects of reproductive and pollen biology and to obtain basic information that may contribute to reproductive biology of plants

T. pallida is a semi-evergreen tree. The flowers are borne in simple terminal and axillary spikes. Each spike produces flowers acropetally over a period of 5–6 days. The flowers are pedicillate, pale yellow in colour and bisexual. The calyx tube is ovoid and divided into five triangular valvate lobes. Petals are absent. There are ten stamens, arranged in two whorls of five each and inserted inside the distal part of the calyx tube; filaments are 4 mm long and incurved in bud and anthers exerted from calyx tube after anthesis. The anthers are dithecous, fertile and cream-coloured. A nectariferous disc is present on the summit of the ovary and it is enveloped by massive silky hair. The ovary is inferior; unilocular with one are two pendulous anatropous ovules. The style is simple and stigma is cream white, 2 mm long, wet and shiny. The fruit is a drupe which is obovoid, faintly 5 ridged when dry and glabrous.

Flowering and Fruiting: April to August.

MATERIAL AND METHODS

The study was conducted on the pollen and reproductive biology of *T. pallid b*etween January to December 2024 and intensive exploration trips were made to Tirumala forest during this period.

Flowering Phenology

Flowers in several inflorescences were tagged and anthers were periodically collected to examine morphological changes under microscope in order to determine the pattern of anthesis and pollen shedding.

Pollen Studies

Size of pollen grains was measured under light microscope using ocular and stage micrometer.

A fully mature anther, just before dehiscence was squashed in a glass test tube containing 0.9 ml of ethanol (70%) + 3 drops of methylene blue (0.5%) + 4 drops of detergent and transferred into a calibrated tube and filled up to 1ml with the same ethanol detergent solution. The suspension was stirred for 60-90 seconds and the preparation was separated into 6 samples of 10 µl each and number of pollen grains was counted with the help of a haemocytometer.

The Pollen –Ovule ratio was determined by the number of ovules per flower divided by total number of pollen grains per flower. Buds and flowers were fixed in 70% ethanol. Ovule quantity was calculated using Anderson and Symons's method. (Anderson and Symons, 1989).

Pollen viability was tested by standard methods using the stains like 2, 3, 5- Triphenyl Tetrazolium Chloride (T T C), Benzidine test (King, 1960), methylene blue and Fuchsine test (Schwendiman) and acetocarmine test.

In vitro pollen germination was conducted using sucrose in Brewbakers medium. Various sucrose concentrations (2.5% to 25%) were used to detect the optimam level required for pollen germination. Pollen germination was determined by hanging drop method. The cultures were sealed with Vaseline to prevent evaporation of the culture medium and the percentage of pollen germinaton and length of pollen tubes were assessed.

Pollen external morphology was studied by following the acetolysis method and through Scanning Electron Microscopy (SEM) photographs. Acetolysis(Erdtman, 1963) was practiced to empty the grains of protoplasmic content and involves the treatment of pollen grains with acetolysis mixture.

For detection of starch in pollen grains (Jensen, W.A. 1962), Iodine - Potassium Iodide (IKI) solution was prepared by dissolving, 0.2 gm Potassium Iodide in as little water as possible. 1 gm of iodine was added and the volume was made up to 100 ml with distilled water. Fresh and mature pollen grain samples were immersed in the IKI solution and examined under a microscope.

For testing Lipids in pollen grains (Vaisssiere, B.E.1991) sudan III, IV solution was prepared by adding excess of Sudan IV to saturated solution of Sudan III in 70% ethanol. The dye was stored in a dark bottle (while using for few weeks) Pollen sample from mature anthers was immersed in a drop of freshly made stock of Sudan IV and examined within 2-3 min. after the application of the dye.

Nectar Studies

To assess nectar constituents paper chromatography studies were carried out (Baker and Baker 1983).

Stigma Receptivity:

10 mg of α-naphthyl acetate powder was dissolved in acetone in a vial that will hold more than 20 ml. of fluid. To this, 5 ml of phosphate buffer (0.1 M, pH 7.0) was added and the tightly stoppered vial was shaken for about 10 min, until the initial “milky” colour of the fluid began to break up. Then fast blue B salt was added and shook so that everything was well mixed. After filtering, the stain was applied directly on a selection of stigmas taken at different stages of the flower life-span, until they are completely immersed for 2-5 min. The stigmas were washed in distilled water and were observed under a dissecting microscope.

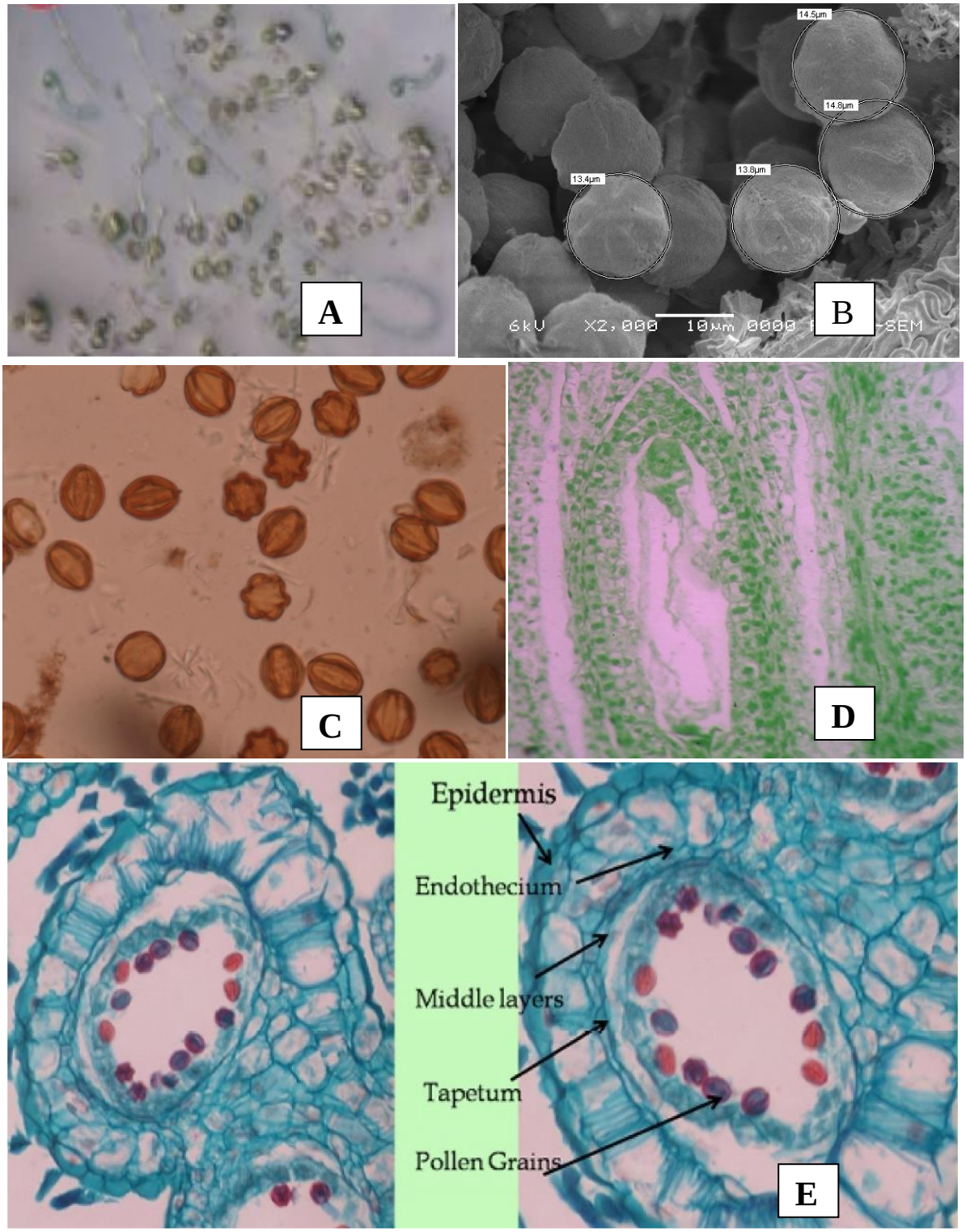
RESULTS AND CONCLUSIONS

Maximum pollen count was noticed in the month of June and July. There were significant differences in the average number of pollen grains per flower. The highest number of pollen grains per flower were observed in the month of July (12640). The variation between maximum and minimum pollen count ranges around 20 % and pollen count was low in the month of August (14 %) that coincided the ending of flowering season.

The maximum size of pollen grains coincided with almost peak flowering. Pollen grain size was measured in fresh pollen grains collected immediately after anther dehiscence. The average size of the pollen grain is (30.04 X 29.978 µm) in July.

The external morphology of the pollen grains was observed from acetolysed pollen grains and by scanning electron microscopic studies**.** They are spherical and the apertures are trizono colpate type. Exine is smooth reticulate and homobrachate. For every one ovule the number of pollen produced are 11, 460.

Pollen viability was noticed to be maximum between 4 AM to 12 Noon and decreased gradually. On the whole, the percentage of viability varied with the same sample in different

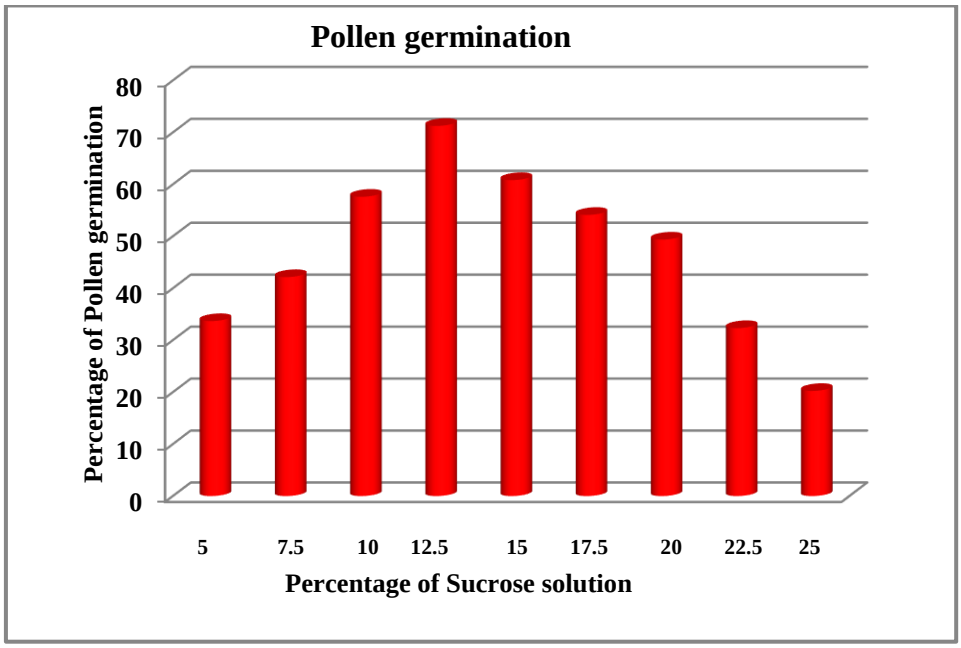


Terminalia pallida **(A) Pollen Germination (B) SEM Photograph of Pollen (C) Acetolysed Pollen (D) Embryosac of *T. palliida* (E) T.S of Mature anther**

staining techniques. In TTC, maximum viability (88.67%) was noticed at 4.00 AM. In benzidine it ranged from 78.87 to 88.6 %; in methylene blue and fuchsin from 72.9 to 83.5 % while in acetocarmine it was 71.4 -85.7 %. FDA test showed the viability of pollen grains to be in between 78.66- 87.2%.

Regarding the time of maximum viability it was between 4.00 AM to 10.00 AM in benzidine, methylene blue + fuchsin, acetocarmine and FDA.

Germination of freshly collected pollen grains was observed at different timings of the day. The percentage of germination varied in Brewbaker's medium with various concentrations of sucrose (5 – 27.5). No germination was observed after 16 hrs of anthesis in all the tested treatments. No germination was noticed both in 2 % and 35% in sucrose either used alone or in combination with BBM.



The *Terminalia* pollen did not germinate at all on a medium without sucrose. Increasing sucrose concentrations up to 12.5 % improved pollen germination percentages. However, germinations decreased with higher sucrose concentrations (25 to30%).

A close examination of cultures in BBM + sucrose solutions in respective plants indicated that the germination of pollen grains got initiated 3 hours after dusting, but the maximum pollen germination was obtained about 8 to 10 hrs after incubation.

In vivo pollen germination studies were carried out to estimate pollen load on the stigma and to study stigma receptivity. Very few pollen grains were observed on stigmas after three to four hours after anthesis (1.24 ± 0.218), but there was significant increase in the number of pollen grains (6.4 ± 0.536 to 13.48 ± 1.29). On the third day after flower anthesis, the pollination success was found to be almost nil due to the deposition of negligible amount of pollen grains. Because of the massive style and trichomes on ovary, visibility of pollen tubes passing through styles is very poor.

Pollen histochemical studies were carried out for the starch contents by immersing the fresh and mature pollen and also already shed pollen samples (Baker and Baker, 1979) into the IKI solution (Jensen, 1962). It was noticed that pollen were poor in starch.

By immersing the fresh and mature pollen sample into the Sudan IV solution, pollen histochemical studies were carried out for lipids. Pollen grains turned to red colour, when tested, indicating the presence of lipids (Vaissiere, 1991).

Stigma receptivity was tested by immersing the excised pistils (of various stages of development) into the reaction solution of alpha – naphthyl acetate. Receptive stigmas reacted positively to the alpha – na-

phthyl acetate solution and got their surfaces stained deep blue – black.

It was noticed that the stigmas are receptive to pollen excised from fully mature buds and opened flowers only. The stigmas are receptive to pollen from 2 hrs up to 18 hrs after anthesis. *i.e.* the mature buds remain receptive for 16 hrs after anthesis.

The transverse section of young anther shows epidermis with thin walled stomium, followed by endothecium. As the anther matures, the endothecium gradually develops fibrous thickenings except in the region of stomium. The number of middle layers varies from one to three. The tapetum is of secretary type and single layered. In an anther about to dehisce both tapetum and the middle layers get crushed and distorted. The tapetal cells are consumed either at uni or binucleate stage of the pollen grains. Sometimes the endothecial cells become richly filled with darkly stained contents because of the presence of tannins and phenolic substances.

In the early stages, the anthers showed a homogenous mass of meristematic cells. Very soon, a filament differentiated below and an anther above from the staminal primordial. The archesporial cells were distinguished from the rest of the anther cells by their larger size, prominent nuclei and deeply stained cytoplasm. The parietal layer formed 3 wall layers below the epidermis. The outermost of these layers formed endothecium in the mature anther and its cells developed fibrous thickenings on their walls when the pollen grains are uninucleate and are quite pronounced at maturity. The cells of the inner most layer enlarged and formed the anther tapetum. The cells of middle layers became crushed between the endothecium and the tapetum during microsporogenesis although their remnants can be seen even in the dehisced anther.The sporogenous tissue which is 10-15 cells long and 6-8 cells wide were found in a fully formed anther. These are enveloped on the outside by the anther tapetum.

The tapetum is uni-nucleate and rich in cytoplasm. The tapetal cells which are at first filled with cytoplasm become vacuolated by the time the microsporocytes are in the Ist meiotic divisions. It was observed that tapetal cells are in uninucleate condition, by the time the tetrads of pollen grains are formed. As the pollen matures, the tapetum shows signs of degeneration and is almost absorbed during maturation of microspores.

The natural fruit set is 6% .The number of fruits per inflorescence does not exceed 12 and most of the fruited inflorescences bear 1–3 fruits. The low natural fruit set and the small number of fruits per inflorescence in *T. pallida* could be attributed to the inherent capacity of the plant, limitation of compatible pollen, flower and fruit predation by a beetle, and nutrient-poor rocky habitat with scanty litter availability. Dry fruits fall to the ground due to abscission at the point of fruit stalk. Wind also causes fruit fall. Fallen fruits serve as food for the rodents and are also susceptible to a fungal species. Rain water is effective in fruit dispersal. As they are carried away by rain water up to a distance of 1 km. The fruits thus dispersed and settled eventually decompose exposing the seed for subsequent germination. However, seed germination and seedling establishment rate appear to be closely related to the nutrient status of the soil. The area in Tirumala being rocky and litter-deficient or free does not seem to favour new recruitment each year for the build-up of *T. pallida* population. Therefore, appropriate measures are required to encourage seed germination and seedling establishment in the natural areas for the effective conservation and management of *T. pallida*. (Solomon Raju *et al.*, 2012)

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