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REPRODUCTIVE AND POLLEN BIOLOGICAL STUDIES OF *TERMINALIA BELLERICA*, Roxb. (COMBRETACEAE)

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ABSTRACT

Terminalia bellerica is a deciduous tree widely distributed in tropical semi-evergreen and moist deciduous forests. Flowers of *T. bellerica* have ten stamens, which remain inside the bud and anthesis is carried out at different times of the day. Pollen grains are yellow in colour, medium and spherical, aperture is trizonocolporate and exine is reticulate. The pollen: ovule ratio is about 19240: 1. Optimum germination was seen in BBM + 17.5% sucrose. After 16 hr of anthesis, the pollen grains lost their viability and there was no fruit set. The current findings will be useful in studying pollen – pistil interactions, gene flow and heterozygosity of the *T. bellerica* populations.

KEYWORDS *T. bellerica*, in vitro germination, pollen viability, Reforestation programs.

INTRODUCTION

India is recognized as one of the megabiodiversity countries of the world and nurtures enormous plant diversity. It is estimated that as many as 5285 species of angiosperms belonging to 140 genera are endemic to the country (BSI 2001). However, this plant wealth is eroding at a fast pace due to habitat loss, fragmentation, over exploitation, invasion of exotics, pollution and climate change (Rathcke et al). The growing awareness of the importance of plant diversity and the rapid decline that has come to notice, have given an unprecedented impetus for monitoring and conservation. In such conditions understanding the pollen and reproductive biology of particular species is important for developing a forest improvement strategy (Bosch, 1992).

Pollen biology is one of the most important and exciting area of plant reproductive biology and plays an important role in crop improvement programs. The ability of pollen to perform its function of delivering male gametes to embryo sac is referred to as pollen viability and the period for which the pollen remains viable varies greatly from species to species. Besides pollen biology, stigma receptivity and pollen pistil interactions also play a vital role in successful pollination and fertilization. These studies provide clues for finding barriers to fertilization. Study of basic mechanisms of reproductive biology of the species is very important to understand how to maximize the yield and quality of the seed produce, to improve desired traits and to develop new varieties. These studies are also useful for the conservation, management and recovery of threatened species.

There are 250 Species in the genus Terminalia. About 7 species grow in Tirumala hills among which T. bellerica, Roxb. (Myrobalanus bellerica) (Thandra, Thani) is a large decidious tree usually with straight tall bole which grows heigh upto 18-24 m with clear bole of 6-9 m and a girth of about 2.4m. The bark is blueish or ash grey in colour, with numerous fine longitudinal cracks and yellowish inside. Leaves are arranged spirally or crowded at the ends of the branchlets. They are broadly elliptic, rounded or obtuse, more rarely acuminate at apex and margin is entire. Flowers are in cream colour, small, scented, produced in axillary solitary or paired spikes and are bisexual. The calyx contains 5 triangular, valvately arranged sepals. There are 10 stamens arranged in two series, the outer 5 are alternate to sepals and the inner 5 are opposite to them. The ovary is inferior, unilocular with 1-2 pendulous ovules and stigma is small. Fruit is a drupe which is globular in shape and develops 5 angles when dry and wings are absent. Flowering & Fruiting: May to August.

Terminalia bellirica seeds have an oil content of 40%, whose fatty-acid methyl ester meets all of the major bio-diesel requirements in the USA, Germany and European Union. In traditional Indian Ayurvedic medicine its fruits are used in the popular Indian herbal rasayana treatment called triphala. The stem bark extract is diuretic and cardio tonic. The tonic made from fruit is digestive and laxative. It is also used in rheumatism, swellings, opthalmia, bronchial asthma, trachyphonia, diseases of throat, Oedema, worm infections, leucorrhoea, venereal diseases and hypertension.

MATERIALS AND METHODS

The study was conducted on the pollen and reproductive biology of T. bellirica between January to December 2015 and intensive exploration trips were made to Tirumala forest during this period.

Flowering Phenology

The flowering was started in May 2015 in T. bellirica and continued upto August 2015. Peak flowering was observed in the month of July 2015. A flower opened for three days but remain receptive for less than one day. 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. Size of pollen grains was measured under light microscope using ocular and stage micrometer.

Pollen Studies

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. 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 by hanging drop method. Pollen external morphology was studied by following the acetolysis (Erdtman, 1963) method and through Scanning Electron Microscopy (SEM) photographs.

For detection of starch in pollen grains (Jensen, W.A. 1962), 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 solutionswere used.

STIGMA RECEPTIVITY

Alpha-naphthyl acetate test was used to determine stigma receptivity.

ANATOMYICAL STUDIES

Anatomical studies were carried out to observe the development of anther, embryo sac, embryo, endosperm and fruit wall etc. Flowers were collected at different stages of development and fixed in FAA for at least 24 hours before processing. Dehydration was done in graded series of tertiary butyl alcohol. Embedding was done near 58 ^{0} C in thermostat using thin flakes of paraffin wax. Soon after 6 hrs, the vials were kept inside an oven at 62° C and then sections were cut at a thickness of 5-10 µm. Mayor's egg albumen was used as an adhesive. The sections were stained with Toluidine blue, Acetocarmine, Safranin and Fast green. Photomicrographs of different parts were taken.

RESULTS

Flowering was started from May 2015. Peak flowering was observed in the month of July 2015 with a mean number of 433. The production of flowers was remarkably low in the month August (414.8) which also coincided with the ending of flowering season. Pollen grain size was measured in fresh pollen grains collected immediately after anther dehiscence. Pollen size was found to be maximum in July (30.07 ±0.54 X 29.31 ±0.27 µm) which coincided with the peak flowering. Pollen count was maximum (19,240) in July and pollen- Ovule ratio was found to be 19240: 1.

Pollen viability was noticed to be maximum between 4 AM to 12 Noon. On the whole, the percentage of viability varied with the same sample in different staining techniques. In T. bellerica 72.72% viability was observed in TTC (2, 3, 5 Triphenyle Tetrazolium Chloride), 78.87% in Benzidine, 73.95% in Methylene blue and Fuchsine and 73.95% in Acetocarmine.

The percentage of germination varied in Brewbaker's medium with various concentrations of sucrose and also in sucrose solutions used alone (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.

Maximum germination was noticed after 8 – 10 hrs of inoculation in BBM containing 17.5% sucrose concentration. Pollen grains commenced germination 3 hours after dusting, but the maximum pollen germination was obtained after 12 hr of incubation.

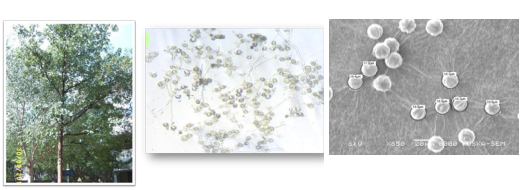

Figure 2:A: T. bellerica Natural habitat. B: Pollen germination at17.5 %sucrose in BBM. C: Pollen Scanning Electron Micrograph

Percentage of germination and rate of pollen tube growth showed an identical behavior. Pollen tube growth was also maximum in BBM containing 17.5% sucrose and measured 6050.8 ±88.59µm. Pollen grains were medium, radially symmetrical, Spherical, trizono, colporate type of apertures, Exine is reticulate. Pollen grains of of T. bellerica turned to red colour, when tested, indicating the presence of lipids.

The ovules are pendulous, anatropous and bitegmic (Fig. 5 K). Micropyle is narrow and zig - zag, formed by both integuments. Chalazal megaspore develops into a polygonum type of embryosac.The development of embryo is Asterad type.

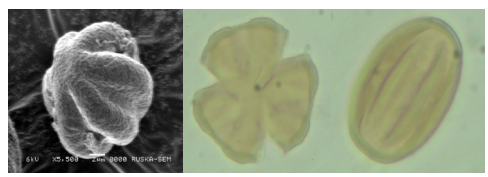

Figure 3: D: Pollen Scanning Electron Micrograph. E: Acetolysed pollen Polar view. F: Acetolysed pollen -Equatorial view

DISCUSSION

Studies of pollen viability and fertility are important for breeding programs. Pollen germination studies are essential for the estimation of the quantity of the pollen grains required

for controlled pollination. Artificial germination of the pollen grains is sure test of pollen fertility, which is important for undertaking any breeding program. The medium components required for pollination of different plant species varies (Vasil, 1960). The present investigation shows that 17.5% of sucrose in BBM was the optimum medium for the germination of pollen grains of T. bellerica. Pollen germination and tube elongation are two distinct processes differing

in their sensitivity to different concentrations of the medium. In many instances due to hyper or hypo nutrition the percentage of pollen germination and length of tube were conciderably reduced. Bursting of pollen increased and occasionally the pollen tubes were observed to eject their contents. In addition to this was various pollen deformities viz. bloating or 'bulla' formation resulting in the swelling of the tip of the pollen tube. Pollen tube grown in a coiled manner was also observed frequently which was due to unstraight tube wall.

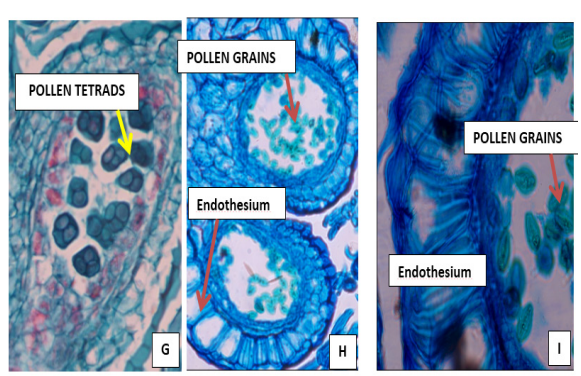

Figure 4: G: Anther T. S. showing Microspore Tetrads. H: Anther TS Showing Endothesium. I: Mature anther Locule T. S.

The factors affecting pollen viability, like the duration for which anthers continue shedding pollen and the range of environmental factors to which they are exposed are critical for

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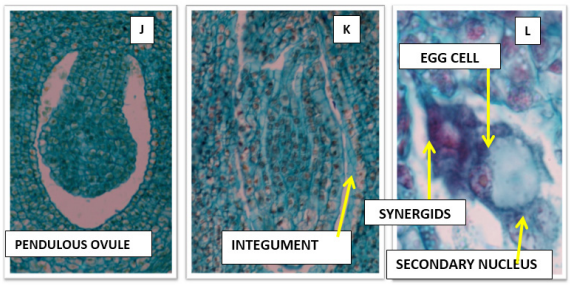

Figure5: J: L.S of young ovule; K: L.S of mature ovule. L: Egg apparatus

cross-pollinated species of T. bellerica where it has been observed that the stigma is receptive for 10 hrs after anthesis and anthers continue shedding pollen from anther dehiscence.

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