INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-2, April-June-2012 Coden : IJABPT Copyrights@2012 ISSN : 0976-4550

Received: 20th Feb-2012 *Revised: 27th Feb-2012* *Accepted: 10th Mar-2012*

Research Article

STUDIES ON THE REPRODUCTIVE AND POLLEN BIOLOGY OF *TERMINALIA ARJUNA*, W&A (COMBRETACEAE)

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ABSTRACT: *Terminalia arjuna* is a deciduous tree widely distributed in tropical semi-evergreen and moist deciduous forests. Flowers of *T. arjuna* 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 tri zonocolporate and exine is smooth. The pollen: ovule ratio is about 15,400 : 1. Optimum germination was seen in BBM + 12.5% 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. arjuna* populations.

Key words: *T. arjuna, in vitro* germination, pollen viability, Pollen to Ovule ratio, Pollen biology, Reforestation programs.

INTRODUCTION

Establishment of forest plantations is currently of great interest in India because of rapid depletion of the natural forest. In order to enhance the forest cover and to meet increasing human needs for timber, firewood, fodder, herbs and other forest products, many restoration strategies were developed. These afforestation programs have been vigorously implemented in various parts of India by Government, Non-Government organizations and private entrepreneurs. Most reforestation and afforestation programs use seedlings as planting materials, with improved quality planting materials obtained from rigorous genotype selection. Hybridization is another method that holds potential for improving planting materials in foresting. In either case, understanding the pollen and reproductive biology of particular species is important for developing a tree improvement strategy (Bosch, 1992).

Reproductive success of hermaphroditic plants is determined by both the quantity and quality of the gametes and offspring produced. Pollen grains are reduced male gametophytes which, upon pollination, produce pollen tubes that grow through the pistil to effect fertilization and seed set. Most estimates of the reproductive success of hermaphroditic plants are based solely on the haploid contribution through the female function, ovule and seed production (Wheller and Guris, 1992), because of the difficulties inherent in estimating haploid contribution through male function. The production and the dispersal of pollen have both biological and genetic implications for the quantity and genetic value of the seed produced. Hence pollen biology is of immense significance in tree improvement programmes as it determines gene flow and heterozygosity of the population, and these in turn determine genetic variability.

There are 250 Species in the genus *Terminalia.* About 7 species grow in Tirumala hills among which *T. arjuna* is important medicinally which is commonly distributed along streams, dry water course, also planted as an avenue tree. It is an evergreen tree, often buttressed with outer bark flaking off in pieces and inner smooth, white. The bark powder of *Terminalia arjuna*, an indigenous plant has been found to have antianginal, decongestive and hypolipidemic effect. Because of its astringent properties, its powder or decoction is good for application on wounds. It helps in their healing.

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The bark extracts is cardio-tonic, thus providing nutrition to heart muscles and strengthen them. It normalizes the disturbed rhythms of heart, reduces the heart rate and stress and nervousness of the heart. It helps in reversing the hardening of the blood vessels. Bark of *T. arjuna* is used in chronic fever, Poly-urea, obesity, skin diseases, dysentery symptomatic hyper-tension, edema, blood clotting, diuretic and for cirrhosis of liver. Fruit is used in treating leucorrhoea, leprosy, pain ulcers and worm infection. Recently Bark extract of *T. arjuna is* being used for treating cardiac patients in AIMS, New Delhi (Moulik).

MATERIALS AND METHODS

The study was conducted on the pollen and reproductive biology of *T. arjuna* between January to December 2009 and Intensive exploration trips were made to Tirumala forest during this period.

Flowering Phenology

The flowering was started in April 2009 in *T. arjuna* and continued upto September 2009. No flowering was observed in the months of January, February, March and December. Peak flowering was observed in the month of July 2009. 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. 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 carriedout (Baker and Baker 1983).

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STIGMA RECEPTIVITY

Alpha-naphthyl acetate test:

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.

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^o C , 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

The flowering in *T. arjuna* was observed almost throughout the year. It started from April 2009 and continued up to September. Peak flowering was observed in the month of July 2009. There were 631.2 ± 13.91flowers in one inflorescence. The production of flowers was remarkably low in the month of September (533.8 ± 12.8) which also coincided with the ending of flowering season (Fig.1). Pollen grain size was measured in fresh pollen grains collected immediately after anther dehiscence. Pollen size was found to be maximum in July (28.55 ± 0.5 X 14.565 ± 0.26). The maximum size of pollen grains coincided with the peak flowering. Pollen count was maximum in the month of June. (15,546 ± 947.5) and pollen- Ovule ratio was found to be 15,400: 1.

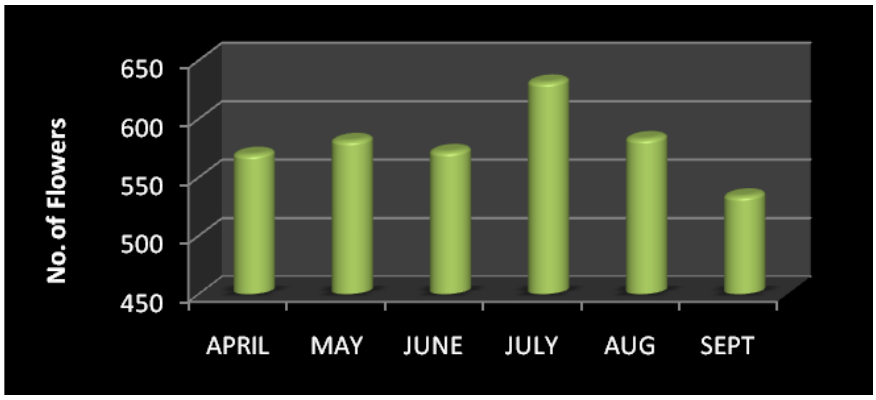

Fig.1: Flowering Phenology of *T.arjuna*

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. *arjuna* 73.95% viability was observed in TTC (2, 3, 5 Triphenyle Tetrazolium Chloride), 88.67% in Benzidine, 73.95% in Methylene blue and Fuchsine and 73.91% in Acetocarmine.

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 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.

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Table 1. Pollen germination in different concentrations of Sucrose in BBM

	5%	7.5%	10%	12.5%	15%	17.5%	20%	22.5%	25%
T. arjuna	37.47 ±0.635	46.795 ±1.902	56.38 ±2.351	68.02 ±3.584	52.29 ±2.64	46.45 ±1.29	42.21 ±2.86	32.74 ±1.016	16.61 ±1.59

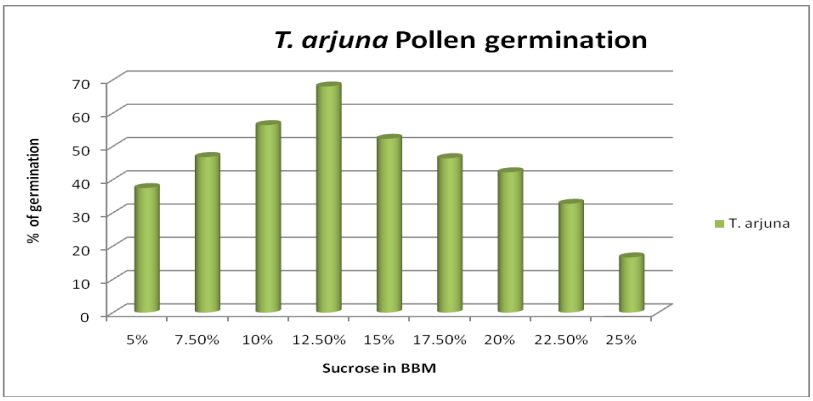

Fig 2. Pollen germination in different concentrations of Sucrose in BBM

Maximum germination was noticed after 8 – 10 hrs of inoculation in BBM containing 12.5% sucrose concentration (Table 1 and Fig. 2). Pollen grains commenced germination 3 hours after dusting, but the maximum pollen germination was obtained after 12 hr of incubation. Percentage of germination and rate of pollen tube growth showed an identical behavior. Pollen tube growth was also maximum in BBM containing 12.5% sucrose and measured 6594.9 5± 106.39 µm. Pollen grains were medium, Prolate, Tri zonocalporate with smooth exine (psilate) (Fig.3). Pollen grains of of *T. arjuna* turned to red colour, when tested, indicating the presence of lipids. Nectar showed to contain glucose and fructose.

It was noticed that the stigmas of *T. arjuna* were receptive to pollen excised from fully mature buds and opened flowers only. The stigmas are receptive from 2 hrs upto 10 hrs of anthesis. *i.e.* The mature buds remain receptive for 8 hrs after anthesis.

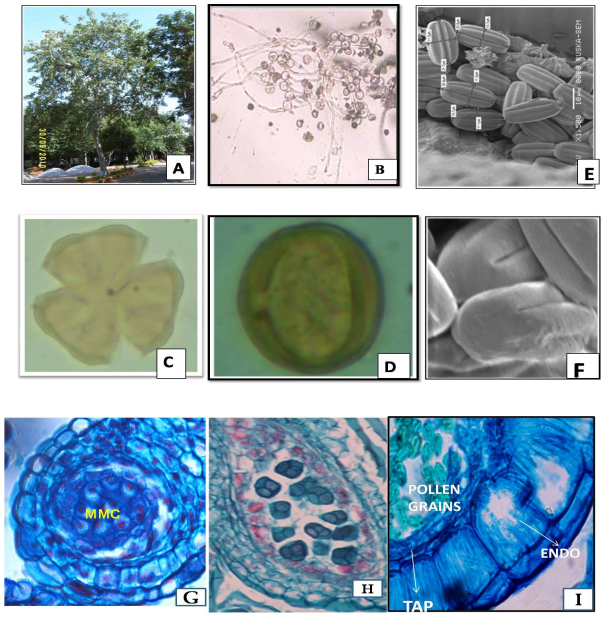

Fig3. *T. arjuna* in natural habitat, its pollen biology section of anthers Legends to the plates

A. *T. arjuna* Natural habitat. B. Pollen germination at12.5 %sucrose in BBM. C. Acetolysed pollen Polar view. D. Acetolysed pollen -Equatorial view, E. Pollen S.E.Micrographs F. Pollen Exine. G. Anther T. S. showing Microspore Mother Cells. H. Pollen Tetrads. I. Mature anther Locule T. S.

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Flower anatomy

Anther wall is differentiated into outer epidermis; with very thin walled stomium.The endothecium developed fibrous thickenings as anther matured except at stomium (Fig 3) . There are one to three middle layers in the anther wall at microspore mother cell stage. Tapetum is single layered and secretory type. In an anther about to dehisce, both the tapetum and middle layers get crushed and distorted. The tetrahedral arrangement of microspores is common.

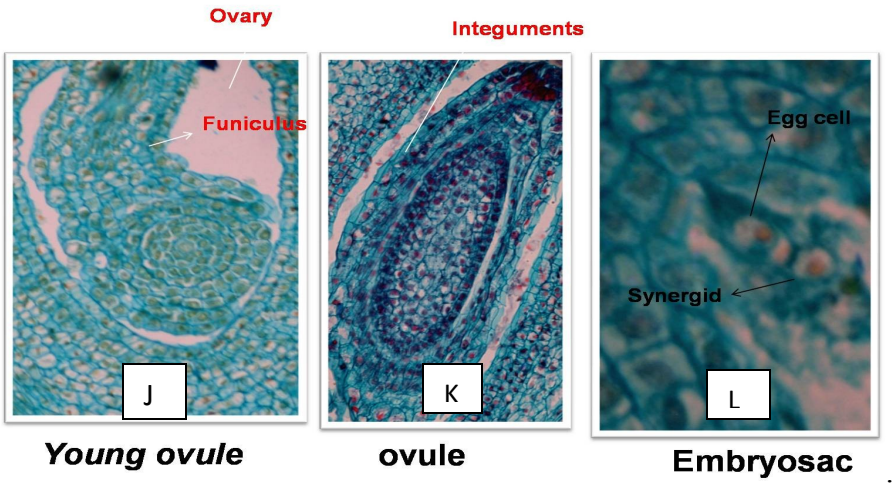

Fig. 4 .J. L.S of young ovule; K. L.S of mature ovule. L. Egg apparatus. Tap:Tapetum, Endo: Endothecium.

The ovules are pendulous, anatropous and bitegmic (Fig. 4). 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

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 12.5% of sucrose in BBM was the optimum medium for the germination of pollen grains of *T. arjuna.* 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.

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 cross-pollinated species of *T. arjuna* 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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