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POLLINATION BIOLOGY OF OKRA (*ABELMOSCHUS ESCULENTUS* L. MOENCH)

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ABSTRACT
Okra [Abelmoschus esculentus (L.) Moench] breeders have long recognized the importance of alien germplasm as sources of genes for biotic and abiotic stress resistance in crop improvement programs. However potential uses of these alien germplasm in improvement of desirable traits are often limited by pre or post-fertilization barriers. In our study, pollination biology was studied with respect to the behavior of pollen and pollen tubes of okra. Fluorescein diacetate and Aniline Blue method were used to access the pollen viability and growth of pollen tube in pistil. Highest pollen viability was observed within one hour after anthesis which drastically declined with time in okra. Normal growth of pollen tubes with higher rate of success of fertilization was observed upon selfing in A. esculentus.

Keywords: Abelmoschus, Aniline blue, Okra, Pollination biology

INTRODUCTION
The cultivated okra [*Abelmoschus esculentus* L. (Moench), Malvaceae] is an important multipurpose vegetable grown throughout the all environmental conditions in India. Okra has an ideal ratio of essential nutrients, proteins, fats and carbohydrates in fruits and therefore, popular as a valuable supplementary food in the tropical diet (Patil et al. 2013). Over past ten years the average productivity of okra increased by only 3.1 mt/ha (in 1991-92 productivity was 8.5 mt/ha) while, in 2010-11 productivity has raised to only 11.6 mt/ha (NHB database-2011), which is less than the productivity (15mt/ha) realized through the trails in India. The low productivity in okra is attributed to poor seed replacement due to the limited availability of quality seed and high incidence of pests (jassids, white fly and borers) and yellow vein mosaic virus (YVMV) which severely affects the crop resulting in low production (Jambhale and Nerkar 1981).

Genetic resistance involving interspecific crosses in okra have been exploited commercially time to time for YVMV and other abiotic stress. Hence hybrids are very much admired in this crop and the hybrid seed production is based on traditional breeding techniques such as hand emasculation and hand pollination. Any hybrid seed production program of vegetable crop requires basic knowledge of its pollination biology (Tyagi 2002). An understanding of the biological nature of the incompatibility systems that prevent hybridization and/or seed development is most essential for the successful hybridization and introgression between okra and its wild relatives. Hence, in the present study, pollen viability, pollen tube germination and its growth, fertilization pathway was observed.

MATERIAL AND METHODS
The study was conducted on Pusa Sawani, popular growing cultivar in India. Emasculation of unopened flower buds was done by removing the petals and undehisced anthers with a small knife in the afternoon between 4:00 and 6:00 pm and was covered with paper bags. The pistil from self-pollinated flowers were collected at 1, 2, 4, 8, 12 and 24 hours after pollination (HAP) and fixed in FAA solution (5% formalin: 5% acetic acid: 90% ethanol) for 12h and then transferred in 70% ethanol for further process. Pistils were stained with 0.001% Aniline blue dissolved in 0.1% K3PO4 solution (Kho and Baer 1968) for at least 15 minutes. Pollen viability was observed by FDA (Fluoroscein di-acetate) test. The observations were carried out under Leica DM 5000B fluorescent microscope at 390 to 420nm with a 450nm emission filter. Quantification of fertilization barriers was done as pollen grains observed on stigma (Phase I), percentage of pollen germination and further growth of pollen tube subsequent to stigma (Phase II), style and up to the ovary (Phase III) and inside the ovary (Phase IV).

RESULTS AND DISCUSSION
The flowers were hermaphrodite, with terminal style and papillate stigma having 5 lobe. The time of anthesis varied with cultivar, temperature and humidity. In the present study, flowers fully opened at 9:00 to10:00am and closed by 3:00 to 5:00pm on the same day in the month of November to January at the temperature ranging from 28-30^{0}C. The dehiscence of anther was transverse and occurred 15-30min after anthesis and pollens were found viable only for 1hr after anther dehisces and pollen viability declined further with time. This indicates that morning time is quite effective to increase rate of success of crossing efforts in okra.

The pathway of the events which contributed to the successful fertilization in *A. esculentus* was traced with the help of Aniline Blue Florescent (ABF) method. Pollen grains germinated quickly on the papillate stigma and

produced pollen tubes in a polysiphonous manner. Pollen adhesion, hydration, germination and penetration of pollen tube into the stigma were found to be completed within an hour after deposition of pollen on stigma. During next 6 hrs pollen tubes travel through the transmitting tissue and after 8 hrs reaches the ovary. This reproductive pathway have been divided in four phases (I, II, III, IV) depicting pollen tube growth on stigma (phase I), in the style (phase II), entry into the ovary (phase III) and growth inside the ovary (phase IV) as shown in Figure 1. This finding can help to trace the pre- and post-fertilization barriers reported by Abdullah et al., (2000) and Tyagi (2002). Also, present study suggest that morning time, especially 8:00 am to 9:00 am is most suitable time for conducting crossing experiments in okra. Knowledge of pollination biology play a crucial role in planning of breeding programs. Therefore, present research work, definitely, boost the efforts of okra breeders to develop new okra varieties.

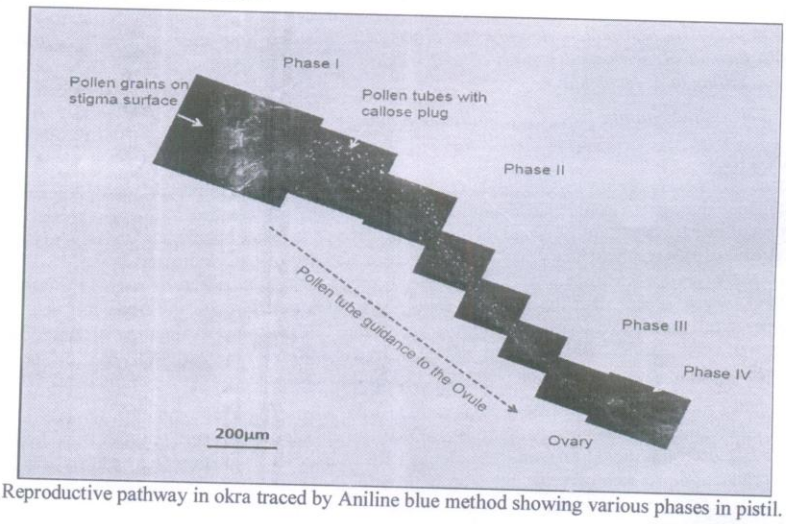

Figure 1 Reproductive pathway in okra traced by Aniline blue method showing various phases in pistil.

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