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Research Article

REPRODUCTIVE BIOLOGY OF *DOLICHOS LABLAB* L. (FABACEAE)

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ABSTRACT
Dolichos lablab is an ancient legume crop widely grown throughout the world for its vegetable or pulse for human consumption or as animal forage or feed. It is a semi-erect, bushy, perennial herb, cultivated as an annual. It flowers during the months of September to February. Large numbers of small flowers are arranged in long recemes. Flowers open between 11.00am to 04.00pm. Anther dehiscence occurs before flower opening. The stigma becames receptive during 08.00am to 07.00pm on the day of flower opening. The stigma is wet, papillate and the style is solid. Pollen viability percentage in TTC was found to be 94.89%. The percentage of *in vivo* pollen germination was recorded as 38.80% which was found to be increased to 40.48 and 54.25% on the second and third day respectively. Flowers are visited by several insects. However, *Xylocopa*, ants, thrips, butterflies are the main visitors.

***Keywords*:** *Dolichos Lablab, Pollen Viability, Pollen Germination*

INTRODUCTION
Many flower visiting insects and other animals are engaged in a remarkable mutualism with the plants. As bees, butterflies and other pollinators forage for resources found within flowers, such as pollen, nectar or more rarely other substances such as scents or resins, their bodies contact sexual organs of plants (Waser, 2001). Insect pollinators, especially honeybees play an important role in pollination of many cultivated and wild plants. These helps in increasing the quality and quantity of agricultural produce (Sihag, 1984).
D. lablab is a field bean usually grown in dry lands as a rain-fed crop. This bean must have been one of the most ancient among cultivated plants- possibly more than 3,000 years old. Its wild forms are found in India and this country was probably the place of its origin. From India it is likely that it was introduced into china, Western Asia and Egypt. The garden varieties are rare in Northern India, but common in Central, Western and Southern India and in Bengal. Important aspects such as phenology, floral biology, anthesis, stigma receptivity, pollen viability, pollination biology and breeding system in this crop species was undertaken.

MATERIALS AND METHODS
Present study was undertaken on *D. lablab* plants cultivated at Botanic Garden, Department of Botany, Amravati University (MS). Flowering phenology and floral morphology was observed in the field. Anthesis, time of anther dehiscence and stigma receptivity was observed using a hand lens. Pollen viability test was done by using TTC. Fresh pollen were collected in sterilized petridishes just before anthesis, 10% tetrazolium salt solution was prepared in 50% sucrose solution and added at the time of preparation of slides for observations under microscope. *In-vitro* pollen germination was checked by hanging drop culture technique after Brewbaker & Kwack (1963). Pollen germination and pollen tube growth in pistil were studied using the aniline blue fluorescence method suggested by Shivanna and Rangaswamy (1992). Flowers were surveyed for visitors at different times of the day, from early morning to late afternoon. The behaviour of visitors was notes and photographs were taken.

RESULTS AND DISCUSSION
Plant - *D. lablab* is a semi-erect, bushy, perennial herb, cultivated as an annual. Leaves alternate, Tri-foliolate, petioles 2-5 cm long, petioles densely hairly, leaflets broadly ovate. Flowers large and white in colour, pedicels 2-5 mm long, slender. Stamens diadelphous (9+1), free, long. The inflorescence is long recemes, upto 5-20 cm long with 4-15 flowers.

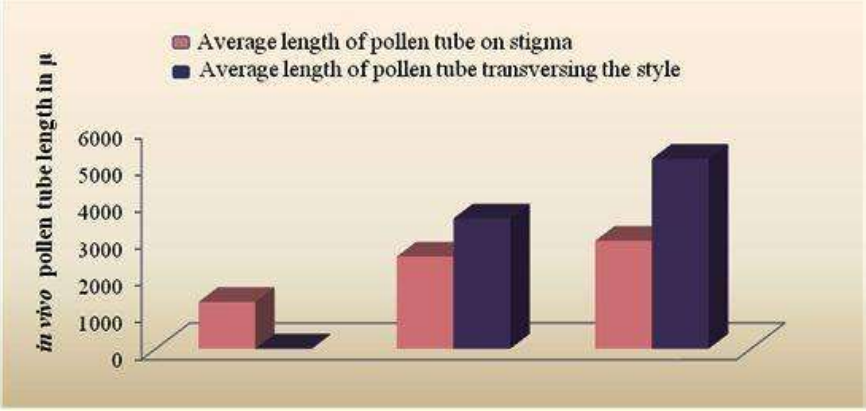
Flowering phenology- Observations on *D. lablab* were carried out during the month of September to February. The plant started blooming from the month of September and the flowering peak came in October to December. The last flower was observed in the month of February.

Floral biology– The flowering is initiated in the month of September and continues up to February. Anthesis and anther dehiscence is most important event in the process of flower development. Flowers open generally two days after anther dehiscence. The anthesis was observed during 11.00 am to 04.00 pm. Anther dehiscence occurs before flower opening. The stigma receptivity of flower varies from few hours to few days (Dafni, 1992). The stigma becames receptive during 08.00 am to 07.00 pm on the day of flower opening. The loss of stigma receptivity coincided with the petal colour change and withering of flower. Non-specific esterases were present on the receptive surface of stigma. Stigma morphology of each plant species has attained much significance in taxonomy (Heslop – Harrison, 1975). The structural and physiological features of the stigmatic surface vary considerably between families and these even within families and sometimes these characteristics are related to the operation of the breeding system. The comparative morphology and physiology of the stigma can throw light on the important aspects of reproductive biology (Heslop-Harrison and Shivanna, 1977). During the present study, the stigma is wet, papillate and knob shaped. The style was found to be solid with central core of transmitting tissue. According to Johri (1984) and Shivanna (2002), the solid type of style mostly present in dicotyledons with a core of transmitting tissue traverses the whole length of style.

The pollen viability percentage was recorded 94.89% in TTC. The viability decreased gradually during the day of anthesis. The best pollen germination occurs in 25% sucrose solution was 47.07% with 280.44 µ average length of pollen tube. *In vivo* pollen germination and growth of pollen tube were observed on the opening day of flower. The percentage of pollen germination was recorded as 38.80% which was found to be increased to 40.48 and 54.25 % on the second and third day respectively. In Pistil, pollen tubes were observed growing beyond upper region into the ovulate part of the ovary. Four to eight pollen tubes were grow into the stylar tissue with 5166 µ average length of pollen tube. Similar observations on pollen germination of *Clitoria ternatea* plants have been recorded by Tidke and Patil (2000). According to them, on the day of anthesis, the percentage of *in vivo* pollen germination was 18.78 and 10.83%, which increased to 32.04 and 26.34%, respectively, in the white and blue colour morphs on the next day.

Table 1: *In vivo* pollen germination in *D.lablab*.

Period after flower opening	First day	Second day	Third day
Total no. of stigmas observed	5	5	5
Total pollens on stigma	75	189	195
Pollen grains germinated on stigma	26	72	106
Percentage of germinated pollen	34.66	39.68	54.35
Pollen tubes traversing the style	-	2	4
Average length of pollen tube in style (µm)	-	3542.4	5166



Graph No 1: *In vivo* pollen tube length in *D. lablab*

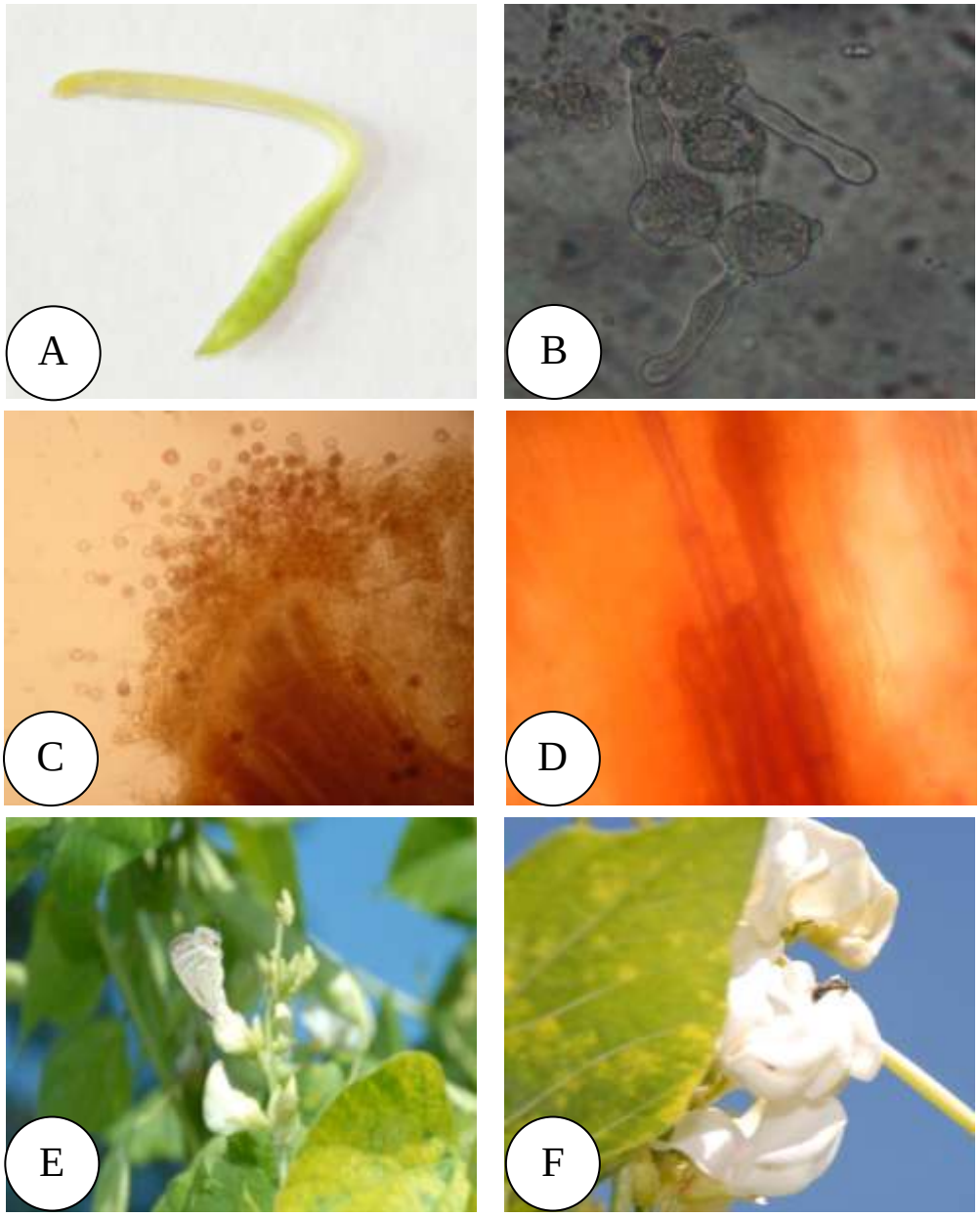
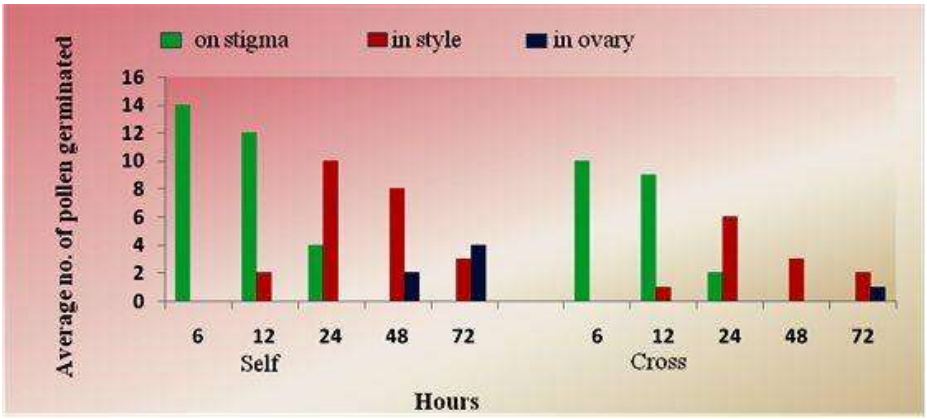


Figure 1A-F: A. Receptive stigma of *Dolichos lablab*, B. *In vitro* pollen germination, C. Pollen germination on stigma, D. Pollen tube growth in style, E. Visiting the flower–Moth, F. *Ceratina* spp.

***Pollination biology*-** The observations were made on flower visitors during the month of September to February. The flowers were observed at different time and days. Insects visited the flowers from the time of anthesis till flowers remained open, for a period of more than 24h. *Xylocopa*, ants, thrips, butterflies were the main visitors. However, bee species such as *Apis dorsata, A. cerana, A. florae, Amegilla* sp. were the main pollinators as they were loaded with the pollen on their mouth parts and transferred the pollen on virgin stigma of fresh open flowers. They were most active in the mornings on clear sunny days. Tidke and Patil (2000) state that the pollinators choose flowers on the basis of colour varies with the diversity, the reward levels of the available flowers, the availability of other perceptible signals and the foraging habits of pollinators. Dafni and Neal (1997) also reported that the importance of the size and shape of flowers in the attraction of pollinators has along been recognized. The blossom colour in many species associated with reward level (Gottsberger, 1971).

Breeding system- Small fruit production as compared to flowers may be due to delayed receptivity or unavailability of pollinators or pollen competition and pollen ovule ratio (Battacharya and Mandal, 2004). According to Sunnichan *et al.*, (2004) the fruit set under open pollination is poor and is highly variable from tree to tree. Several factors may be responsible for the low fruit set under open-pollination (Tandon *et al.*, 2003). During the present investigations, development of fruit and number of seeds were observed after self- and cross-pollination. The size and shape of the fruits and seeds varied considerably. Mature fruits were collected with 15 total numbers of seeds after self-pollination whereas small fruits with 10 total numbers of seeds were collected after cross-pollination. Self-pollinated flowers showed 80 percent fruit set whereas cross-pollinated flowers showed 60 percent fruit set. The percent fruit set and total number of seeds from self-pollination were higher than those from cross-pollination indicating that dominant mode of reproduction was found to be self-pollination.



Graph No. 2: Pollen tube growth in pistils of *D. lablab* after self- and cross-pollination.

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