

Reproductive Biology of *Abelmoschus esculentus*. III. Emasculation and Temporal Course of Pollen Tube Growth in Pistil

SUDHIR CHANDRA¹ and S P BHATNAGAR²

¹Department of Botany, Kumaun University, Nainital

²Department of Botany, University of Delhi, Delhi 110007

(Received 20 August 1979)

Temporal growth of pollen tubes and development of ovules have been studied using flowers emasculated 16 to 18 hr before opening, prior to anther dehiscence. The data were recorded from intact as well as excised pistils.

Pollen grains germinate within 5 to 10 min after landing on the stigma. They are polysiphonous and the pollen tubes show branching at any level in the pistil. The time taken by pollen tubes to reach the ovary varies from 2 to 10 hr depending upon the environmental conditions. The rate of growth of the tube follows a sigmoid curve in the pistil, being fastest 20 to 30 min after pollination. Generally nucellar cells in the micropylar region disintegrate to form a passage before the entry of the pollen tube. The tube enters the embryo sac between the synergid and the egg and persists until 10 or 11 days after pollination.

Key Words : *Abelmoschus*, Emasculation, Pollen tube, Pistil, Temporal growth, Polysiphonous

Introduction

Investigations on pollen tube growth in pistil tissues have generally been studied in near and distant crosses of hybrid plants. However, such studies are lacking in self-pollinated plants. During a study of reproductive biology of *Abelmoschus esculentus* (Bhatnagar & Sudhir Chandra 1976 a,b, 1978, 1979, Sudhir Chandra 1969, 1970, 1976 a, b, 1977, Sudhir Chandra & Bhatnagar 1974, 1975 a, b, c, 1976 a, b, c, 1979) the course of

pollen tube development in pistil tissues was followed in relation to time.

Materials and Methods

Raising the plants: Seeds of *Abelmoschus esculentus* 'Pusa Sawani' were obtained from the Plant Introduction Division, Indian Agricultural Research Institute,* New Delhi. Plants were raised in the Botanical Garden

*Presently National Bureau of Plant Genetic Resources

of the University of Delhi in the last week of May. About 800 plants were used for study and all the existing fruits were removed.

Emasculation and Pollination: Buds were emasculated, prior to anther dehiscence, 16 to 18 hr before flower opening. A deep longitudinal slit was made on either side of the bud (figure 1A). The two calyx halves were pulled apart but not separated from the bud (figure 1B). A transverse slit was made at the base of the corolla connecting the two longitudinal slits made earlier (figure 1B). With the help of a pair of tweezers, the side of corolla where a transverse slit had been made, was removed by pulling it downwards (figure 1C). If the corolla was not pulled downwards, there was the possibility of one or two anthers at the top of the staminal column getting crushed against the stigma. Half of the staminal column became separated along with the corolla (figure 1C). The other half of the corolla bearing the remaining part of the staminal column was removed holding the apex with tweezers and pulling it downwards (figure 1D). Such buds in which the anthers had already dehisced or had become punctured during handling were discarded. The emasculation procedure took about 90 to 100 sec. and usually 75% of the buds thus emasculated were found suitable for cross-pollination.

Hand pollination was done between 6 and 10 A.M. The buds were bagged to ensure natural self-pollination. For hand self-pollination, the anthers from emasculated flowers were used. The staminal columns were numbered with respect to source flower and were placed (along with attached corolla halves) in paper bags and stored in a desiccator over CaCl_2 overnight at laboratory temperature (ca. 28°C). They were used for pollination in the morning. For cross-pollination, the stigmas of emasculated flowers were covered by pollen from dehis-

cing anthers of flowers from different plants.

Preparation of Material for Study of Pollen Tube Growth: The growth of pollen tube was studied in intact as well as excised pistils (diffuse day light 100–200 Lux; at $22 \pm 2^\circ\text{C}$, and 50–60% relative humidity). The latter pistils were kept in the holes of a wax-float with their stalks dipping in water.

To trace pollen germination and pollen tube growth in the stigma, the latter was dissected and examined. Subsequent pollen tube growth was traced in preparations of the stigmas and the styles. The styles were prepared by removing the cortical tissues and exposing the stigmatoid tissues (*see* Sudhir Chandra 1970).

Pollen tube growth was also studied in serial sections. For this the material was fixed in FAA and processed through the embryological procedures (*see* Jensen 1963).

Results

As observed under field conditions, only the lower and lateral portions of stigma show the presence of pollen grains a little before anthesis. When the entire surface of the stigma becomes covered by pollen it gives a glistening appearance. As the pollen grains settle between the stigmatic hairs, the surface lustre of the stigma disappears. The spinules of the pollen grains (figure 2A) help them to adhere to the stigma.

The pollen grains germinate within 5 min of their landing on the surface of the stigma (figure 2B). At the time of germination the pollen measure from 162 to 222 μm (figures 2A, B) in diameter. On an average four pollen tubes are produced from each grain simultaneously (figure 2A). The diameter of the emerging pollen tubes is about 25 μm (average of 10 pollen tubes).

Studies during mid-July indicated that pollen tubes can reach the base of the style in 9 to 10 hr after pollination. In this period

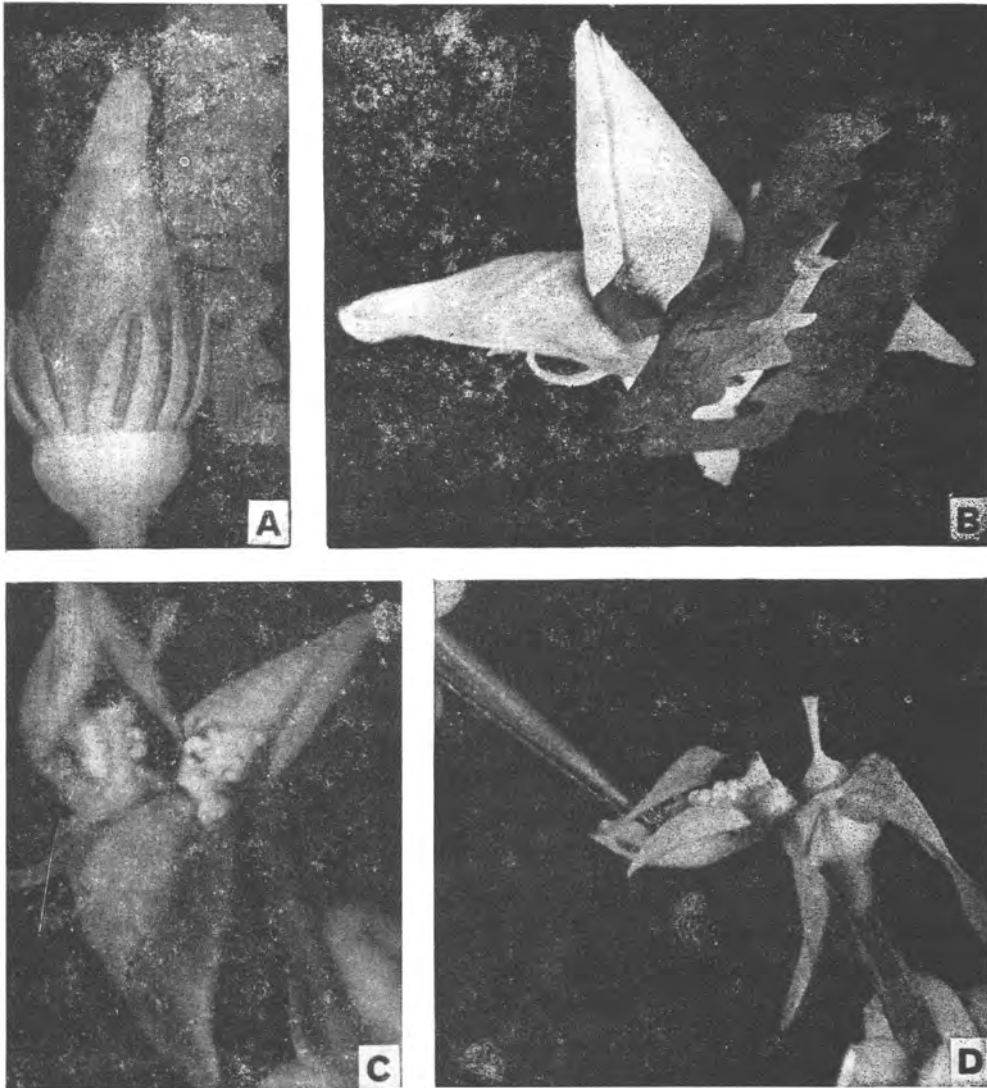


Figure 1 A-D Emasculation technique. *A*, Twelve hours before anthesis a deep longi-incision is made in the bud by a blade ($\times 2$); *B*, The two halves of calyx are pulled apart abaxially followed by a deep trans-incision at the base of the corolla ($\times 2$); *C*, One half of the corolla with staminal column is pulled downwards at the point of incision with the help of a pair of forceps ($\times 2$); *D*, The other half of the corolla with the staminal column is then removed. Note the intact calyx half ($\times 1$)

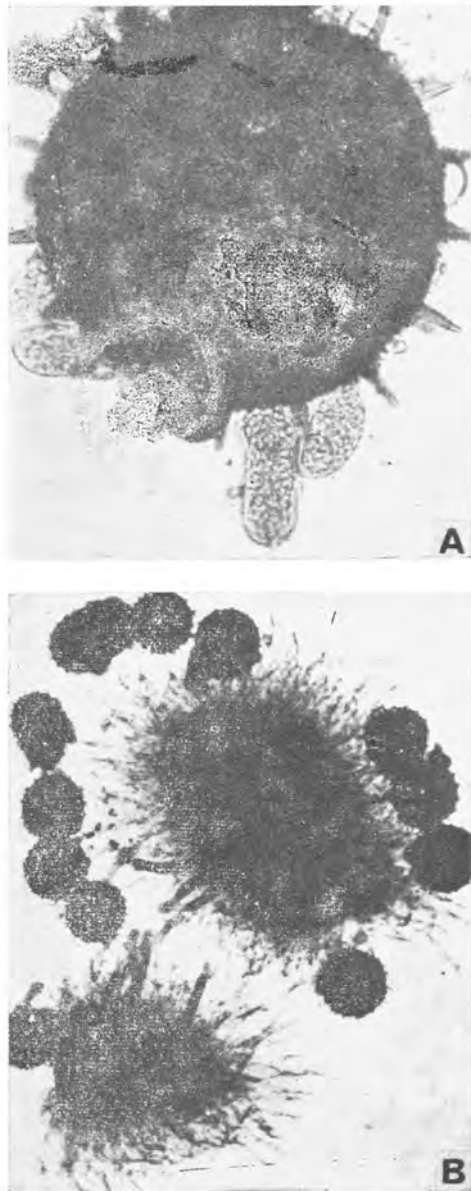


Figure 2 A-B Emergence and growth of pollen tube. *A*, Pollen grain showing polysiphonous condition ($\times 325$); *B*, A small portion of stigma showing numerous germinating pollen grains ($\times 45$)

no detailed analysis or record of pollen tube growth was made. However, at the end of July, pollen tubes were seen at the base of the style even within 2 or 3 hr after pollination (we thought that it may have been due to chance pollination at the time of emasculation). But in August the pollen tubes were seen at the base of the style even one hour after pollination. A detailed analysis of pollen tube growth was made in August and the distance travelled by the pollen tube in stigma, style and ovary under natural and cultural conditions has been described below (figures 6).

Pollen Tube Growth in Stigma, Style and Ovary (Under Intact Conditions)

Ten minutes after pollination the length of the pollen tubes is approximately one and half times the diameter of the pollen grain (ca. $300 \mu\text{m}$; figures 3A,5,6). In the next 10 min they elongate rapidly attaining a length of up to $2,750 \mu\text{m}$. By this time they enter the common stigmatoid tissue of the style (figure 3B). A linear rate of growth is exhibited until they reach the base of the style 60 min after pollination (figures 3C-E, 5,6). But the rate of growth in the ovary is comparatively slow. It takes about 3 hr to traverse the $10,000 \mu\text{m}$ long ovary (figure 6). The growth curve of the pollen tube in the pistil follows the sigmoid pattern.

Pollen Tube Growth in Stigma and Style (in Excised Pistils)

The rate of pollen tube growth is very slow until 20 min after pollination and the tube length is only about twice as long as diameter of the pollen grain (figures 5, 6). Within the next 10 min the pollen tubes enter the common stigmatoid tissue and begin to grow at a fast rate so as to reach the base of the style 2 hr after pollination (figures 5,6).

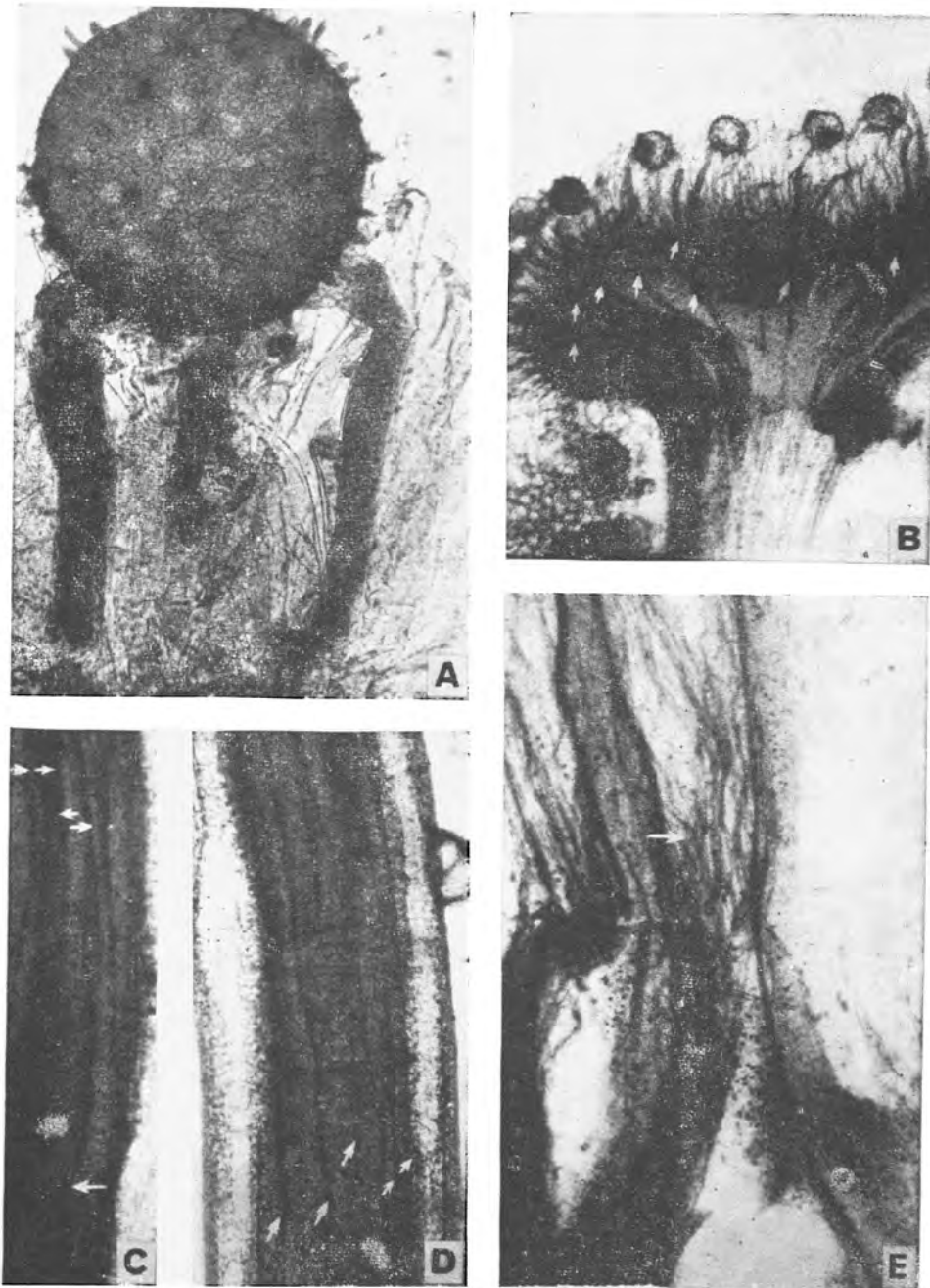


Figure 3 A-E Pollen tube growth. *A*, A pollen grain with three pollen tubes (10 min after pollination) ($\times 250$); *B-E* pistil mounts. *B*, A stigmatic lobe showing passage of pollen tubes in between stigmatic papillae and their entry into stigmatoid tissue ($\times 20$); *C*, Mid-portion of style showing pollen tubes (30 min after pollination) ($\times 20$); *D*, Mid and lateral-portion of style showing pollen tubes (50 min after pollination) ($\times 20$); *E*, Portion of pistil showing the pollen tube entering the ovary ($\times 20$)

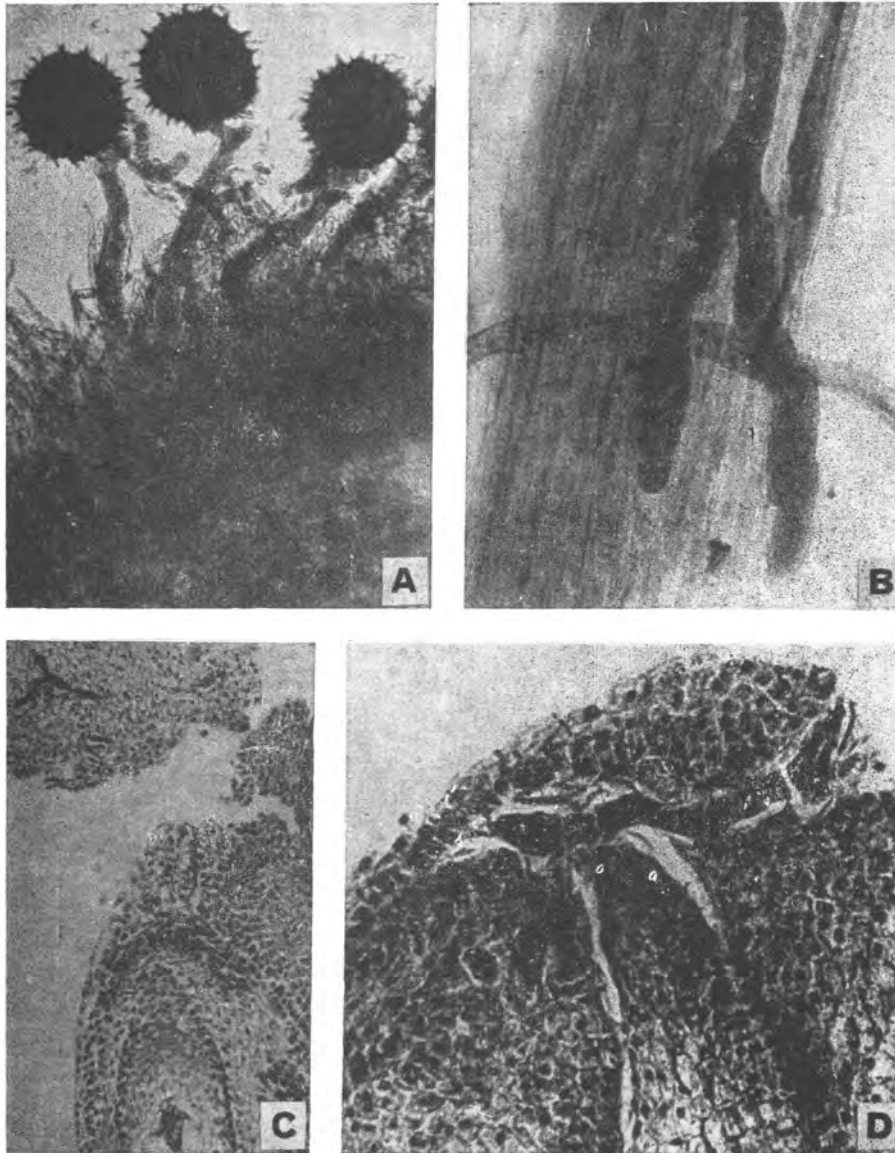


Figure 6 A-D Pollen tube branching at different regions of pistil. *A*, On Stigma ($\times 25$); *B*, in style ($\times 210$); *C*, In placenta near the ovule (*see* arrow) ($\times 120$); *D*, In the micropyle and nucellar cap ($\times 300$)

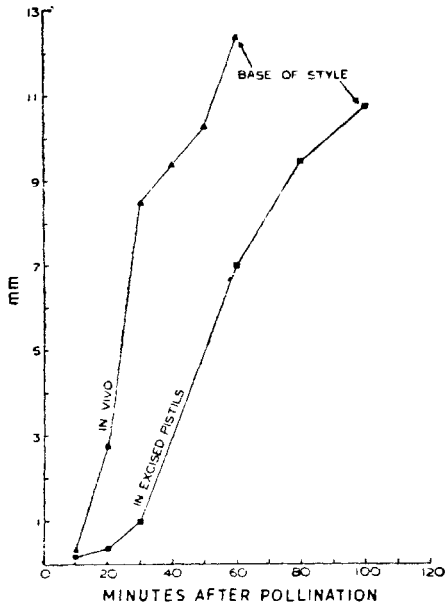


Figure 5 Growth of pollen tubes *in vivo* and in excised pistils. Each value is an average of 8 measurements

Mode of Pollen Tube Entry into the Pistil

The pollen tubes traverse over the surface and between the stigmatic hairs (figure 3B), and then enter the stigmatoid tissue corresponding to the lobe. They grow almost straight downwards in the stigmatoid tissue (figures 3 C,D) and rarely change their course in this region; the entire stigmatoid tissue is occupied by pollen tubes (figure 3E). The pollen tubes may branch in the pistil close to the pollen grain, in the stigmatic tissue (figure 4A), style (figure 4B), placenta (figure 4C), or even in the micropyle or the nucellus (figure 4D). Out of over 400 ovules examined, branching of the pollen tube inside the ovule was observed only in 5. Extirpation experiments suggest that

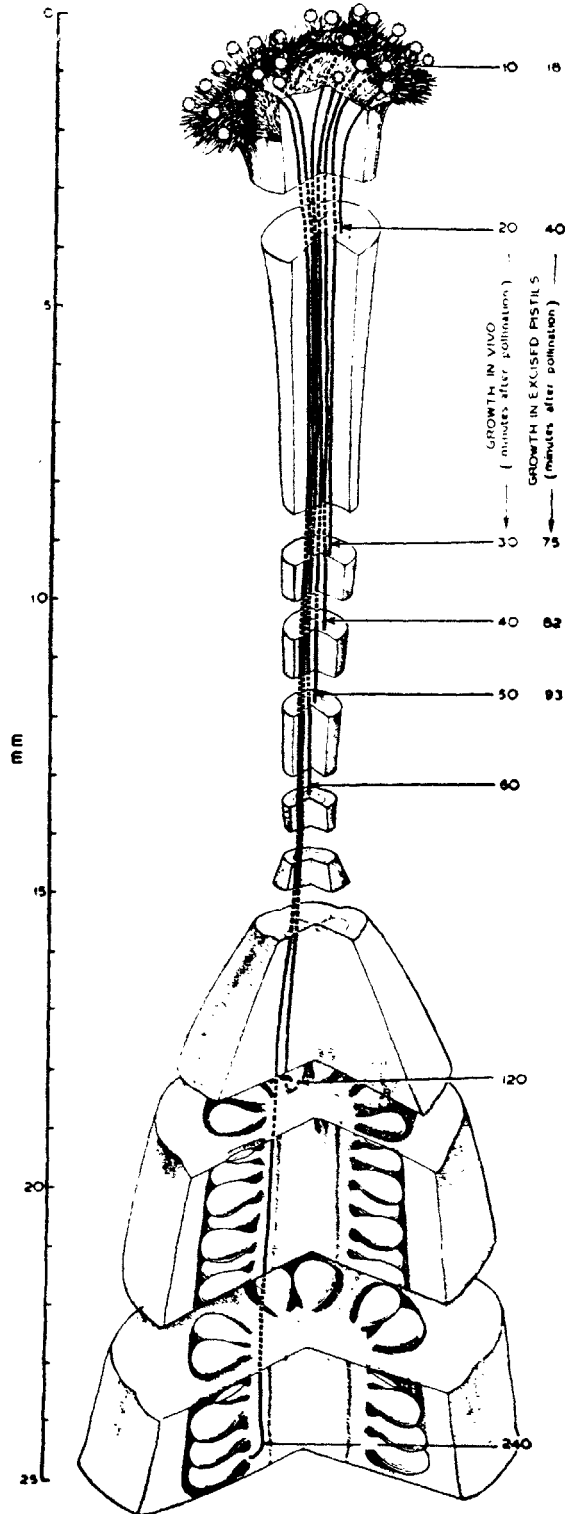


Figure 6 Three-dimensional diagram of the pistil showing the extent of pollen tube growth. In excised pistils pollen tube growth was followed until 100 min, while *in vivo* upto 240 min of pollination. Each value is an average of 8 observations

Fig. 6

pollen tube transfer takes place in the transmitting tissue (stylar region). Histological studies failed to reveal any intercarpellary transfer in the region of the ovary.

Although pollen tubes have been shown to take 3 hr to reach the base of the ovary, it is difficult to specify which ovule would be entered first. Occasionally ovules showed considerable synchrony in pollen tube entry. Frequently, ovules in one or two locules failed to receive any pollen tube. Rarely, all the ovules in a locule received them. No instance in which all the ovules in an ovary have received pollen tubes was observed.

Although the time taken for the pollen tubes to enter the ovules was between 2 to 10 hr after pollination, instances in which this was extended to 43 hr were noted. Prior to the entry of the pollen tube into the ovule the nucellar cells generally dissolve to form a passage. The dissolution of nucellar cells may start even when the pollen tube is in the style. However, in a few instances no dissolution of nucellar cells occurs even when the pollen tube is seen in the vicinity of the ovule.

The penetration of the pollen tube in the embryo sac between one of the synergids and the egg causes the displacement of the egg apparatus.

Pollen tubes persist in the ovule until after the dicotyledonous stage of the embryo (11 days after pollination).

Discussion

The pollen tube growth in the stigmatic and stylar regions shows a sigmoid curve. It slows down in the lower part of the pistil. The reasons for the retardation of growth of pollen tubes are little understood. If the pollen tubes are attracted towards the ovules as a result of chemical stimulus, it is strange that their growth instead of being speeded up is slowed down. Probably lack

of nutrients in the lower portion of the pistil coupled with mechanical resistance offered by tissues traversed by the pollen tubes may be responsible for retardation of the rate of pollen tube growth.

The time interval required for the pollen tube to enter the ovules varies from 2 to 10 hr in *Abelmoschus*. This may be due to seasonal variability with regard to relative humidity (10 hr in July and 2 hr in August). In *Hibiscus* (Cochis 1964) and *Gossypium hirsutum* and *G. arboreum* (Pundir 1967), which are also malvaceous taxa, the time interval is 9 and 48 hr, respectively. In *Gossypium hirsutum* the time required was reported to be 15 hr according to Joshi (1962), and 24 to 48 hr according to Pundir (1967). In the present study delayed pollen tube entry up to 43 hr has been observed.

Polysiphonous condition which is of common occurrence in Malvaceae (see Maheshwari 1950) and the branched pollen tubes reported in *Gossypium* (Joshi 1962), also occur in *Abelmoschus*. In the latter plant the maximum number of pollen tubes emigrating from one grain was seven, although as many as 10 and 14 have been noted in *Althaea rosea* and *Malva neglecta* respectively (Stenar 1925). The peculiar feature in *Abelmoschus esculentus* is the ability of the pollen tube to branch at any place during its growth in the pistil.

The gynoecium in *Abelmoschus esculentus* is eu-syncarpous following the terminology of Carr and Carr (1961). Usually the pollen tubes show a direct descending growth in *Abelmoschus* so that they enter the locules corresponding to the stigmatic lobes. Only pollen tubes issuing from pollen grains located on the central top of the stigma (inner margins of the stigmatic lobes) show random distribution of entry into the locules. Intercarpellary transfer of pollen tubes in the eu-syncarpous gynoecium of *Abelmoschus* is in contrast to the situation

in the paracarpous gynoecium in *Reseda* in which the dual construction of the stigmas and placentae enables the pollen tubes to pass to ovules belonging to several carpels. Intercarpellary transfer of pollen tubes has also been observed in cotton (Doak 1937).

The factors responsible for the movement of pollen tubes to the ovules are enigmatic. In recent years the theory of chemotropic response has received considerable attention. Although chemotropic response to the ovules and placenta has been shown in *Narcissus* and *Antirrhinum* (Brink 1924), *Oenothera* (Schneider 1956), and *Lilium* (Rosen 1961, Welk et al. 1965) existence of a gradient in the chemotropic factor is yet to be demonstrated. The present investigation on *Abelmoschus* and that on *Gossypium hirsutum* (Jensen 1969) suggests that ovules are probably involved in attracting the pollen tubes. In both these plants it was noted that the nucellar cells in many ovules breakdown and form a passage even when the pollen tubes are still in the stylar region.

Despite the time lag of 3 hr required by pollen tubes to travel from apex to the base of the ovary, ovules at any position in the ovary may receive them first and the order of their entry into the ovules does not depend upon the position of the ovules in the locule. This is in conformity with the observations on cotton (Ayyar & Ayyangar 1932, Iyengar 1938). However, Ayyar and Ayyangar noted that in a variety of cotton the first pollen tubes seemed mainly to enter the ovules of the third position from the top of the ovary and the frequencies were less in all the ovules in even positions. In this context an important observation on

Abelmoschus is that the percentage of pollen tubes entering ovules in positions 1 and 2 is very low as compared to that in respect of ovules in position 3. Thus if a large number of ovaries are not analysed, it would appear that in *Abelmoschus* also ovules at position 3 would show preferential pollen tube entry. Such is not the case.

In *Abelmoschus* all the ovules in an ovary appear mature yet all do not receive pollen tubes. This is in contrast to the situation in multiovulate (tricarpellary and trilocular) *Lilium* (Cave & Brown 1957), in which all the ovules receive pollen tubes. In *Medicago* the depth of pollen tube penetration and the number of pollen tubes entering the ovary are two important factors which influence seed set (Sayers & Murphy 1966) but these factors do not appear to be operating in *Abelmoschus*.

In *Abelmoschus* the pollen tube enters into the embryo sac between the synergids and the egg. In *Gossypium* it is through a degenerate synergid (Jensen & Fisher 1968). In the latter plant the other synergid remains healthy until 2 or 3 days after pollination. In *Abelmoschus* both the synergids disintegrate simultaneously after fertilization.

Acknowledgements

The authors are deeply indebted to Professor B M Johri, FNA and Professor H Y Mohan Ram, FNA for encouragement and facilities. Thanks are extended to Dr N S Rangaswamy and Dr P S Ganapathy for their helpful suggestions. Financial assistance from the Centre of Advanced Study in Botany, University of Delhi to one of us (SC) is gratefully acknowledged.

References

- Ayyar V R and Ayyangar G S 1932 Studies on the movements of cotton pollen tubes; *Proc. Ass. econ. Biol., Coimbatore* **8** 39-40
- Bhatnagar S P and Sudhir Chandra 1976a Reproductive biology of *Abelmoschus esculentus* VIII. Seed disfunction; *Bot. Jahrb.* **97** 425-430
- and — 1976b Pollination and pollen tube growth in *Abelmoschus esculentus*(L.) Moench.; *Abst. Ist Int. Symp. on Physiology of Sexual Reproduction in Flowering Plants* pp 14-15, (Ludhiana: Punjab Agricultural University)
- and — 1978 Reproductive biology of *Abelmoschus esculentus*. V. Post-pollination development of seed coat, perisperm, micropyle, raphe and funiculus; *Acta bot. Indica.* **6** 36-42
- and — 1979 Pollination mechanism in *Abelmoschus esculentus*; *Abst. Nat. Symp. on Pollination Ecology and Applied Palynology*, pp 22-23 (Hissar: Haryana Agricultural University)
- Brink R A 1924 The physiology of pollen. IV. Chemotropism. Effect on growth of grouping grains, formation and function of callose plugs, summary and conclusions; *Am. J. Bot.* **11** 417-436
- Carr Stella G M and Carr D J 1961 The functional significance of syncarpy; *Phytomorphology* **11** 249-256
- Cave M S and Brown S W 1957 The detection and nature of dominant lethals in *Lilium*. III. Rates of early embryogeny in normal and lethal ovules; *Am. J. Bot.* **44** 1-8
- Cochis T 1964 Studies on problems concerning seed-set in *Hibiscus* spp; Ph.D. Thesis, Louisiana State Univ. *Diss. Abstr.* **25** 4335
- Doak C C 1937 The pistil anatomy of cotton as related to experimental control of fertilization under varied conditions of pollination; *Am. J. Bot.* **24** 187-194
- Iyengar N K 1938 Pollen tube studies in *Gossypium*; *J. Genet.* **37** 69-106
- Jensen W A 1962 *Botanical Histochemistry, Principles and Practice* (San Francisco: Freeman)
- 1969 Cotton embryogenesis: Pollen tube development in the nucellus; *Can. J. Bot.* **47** 383-385
- and Fisher D B 1968 Cotton embryogenesis: the entrance and discharge of the pollen tube in the embryo sac; *Planta* **78** 158-183
- Joshi P C 1962 Morphological, embryological and experimental studies on *Gossypium* L., Ph.D. Thesis, University of Delhi
- Maheshwari P 1950 *An Introduction to the Embryology of Angiosperms* (New York & London; McGraw-Hill Book Co. Inc.)
- Pundir N S 1967 Experimental embryology of *Gossypium arboreum* L. and *G. hirsutum* L., and their reciprocal crosses; Ph.D. Thesis, University of Delhi
- Rosen W G 1961 Studies on pollen-tube chemotropism; *Am. J. Bot.* **48** 889-895
- Sayers E R and Murphy R P 1966 Seed set in alfalfa as related to pollen tube growth, fertilization frequency, and post-fertilization ovule abortion; *Crop. Sci.* **6** 365-368
- Schneider G 1956 Wachstum und chemotropismen von Pollenschlauchen; *Z. Bot.* **44** 175-205
- Stenar H 1925 Embryologische Studien. I. Zur Embryologie der Columniferen. II. Die Embryologie du Amaryllideen; Diss. Uppsala
- Sudhir Chandra 1969 Seed development in *Abelmoschus esculentus* (L.) (Moench).; *Abst. Seminar on Morphology, Anatomy and Embryology of Land Plants* pp 82-83, eds. B M Johri et al. Centre of Advanced Study in Botany, Plant Morphology & Embryology, University of Delhi, Delhi
- 1970 Post-pollination studies in *Abelmoschus esculentus* (L.) Moench and *Triticum aestivum* Linn. Ph.D. Thesis University of Delhi, Delhi
- 1976a Independent initiation of embryo from endosperm in *Abelmoschus esculentus*; *Abst. Ist Int. Symp. on Physiology of Sexual Reproduction in Flowering Plants* pp 62-63 (Ludhiana: Punjab Agricultural University)
- 1976b Additional evidence of influence of ovule position on pollen tube entry and seed set in *Abelmoschus esculentus* var. Pusa Sawani; *Abst. Ist Int. Symp. on Physiology of Sexual Reproduction in Flowering Plant* pp 84-85 (Ludhiana: Punjab Agricultural University)
- 1977 On independent initiation of embryo from endosperm in *Abelmoschus esculentus*; *Geobios* **4** 78-79
- and Bhatnagar S P 1974 Influence of ovule position on pollen tube entry and seed set; *Naturwissenschaften* **61** 688
- and — 1975a Seed development of *Abelmoschus esculentus* (L.) Moench (Malvaceae); *Pl. Systematics & Evolution* **123** 253-262
- and — 1975b Reproductive biology of *Abelmoschus esculentus*. I. Reproductive behaviour, floral morphology, anthesis and pollination mechanism; *Acta botanica Indica* **3** 104-113

- Sudhir Chandra and Bhatnagar S P 1975c Reproductive biology of *Abelmoschus esculentus*. II. Pollination and nucellus; *Biol. Pl.* 17 154-155
- and — 1976a Reproductive biology of *Abelmoschus esculentus*. IV. Ovule, fertilization endosperm and embryo development; *J. Indian bot. Soc.* 55 1-13
- and — 1976b Reproductive biology of *Abelmoschus esculentus*. VII. Growth correlations of fruit, seed and embryo development; *Proc. Indian natn. Sci. Acad.* B42 318-324
- and — 1976c Embryo development in relation to seed and fruit growth in *Abelmoschus esculentus* var. Pusa Sawani; *Abst. 1st Int. Symp. on Physiology of Sexual Reproduction in Flowering Plants* pp 85-86 (Ludhiana: Punjab Agricultural University)
- and — 1979 Pollen stigma interaction in relation to seed set in *Abelmoschus esculentus*; *Abst. Natn. Symp. on Pollination Ecology and Applied Palynology* pp 23 (Hissar: Haryana Agricultural University)
- Welk M, Millington W F and Rosen W G. 1965 Chemotropic activity and the pathway of the pollen tube in lily; *Am. J. Bot.* 52 774-781