REPRODUCTIVE BIOLOGY OF *SCHIMA WALLICHII* (DC.) KORTH. AND *SCHIMA KHASIANA* (DYER) BLOEMB.

By

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IN FULFILMENT OF THE DEGREE OF

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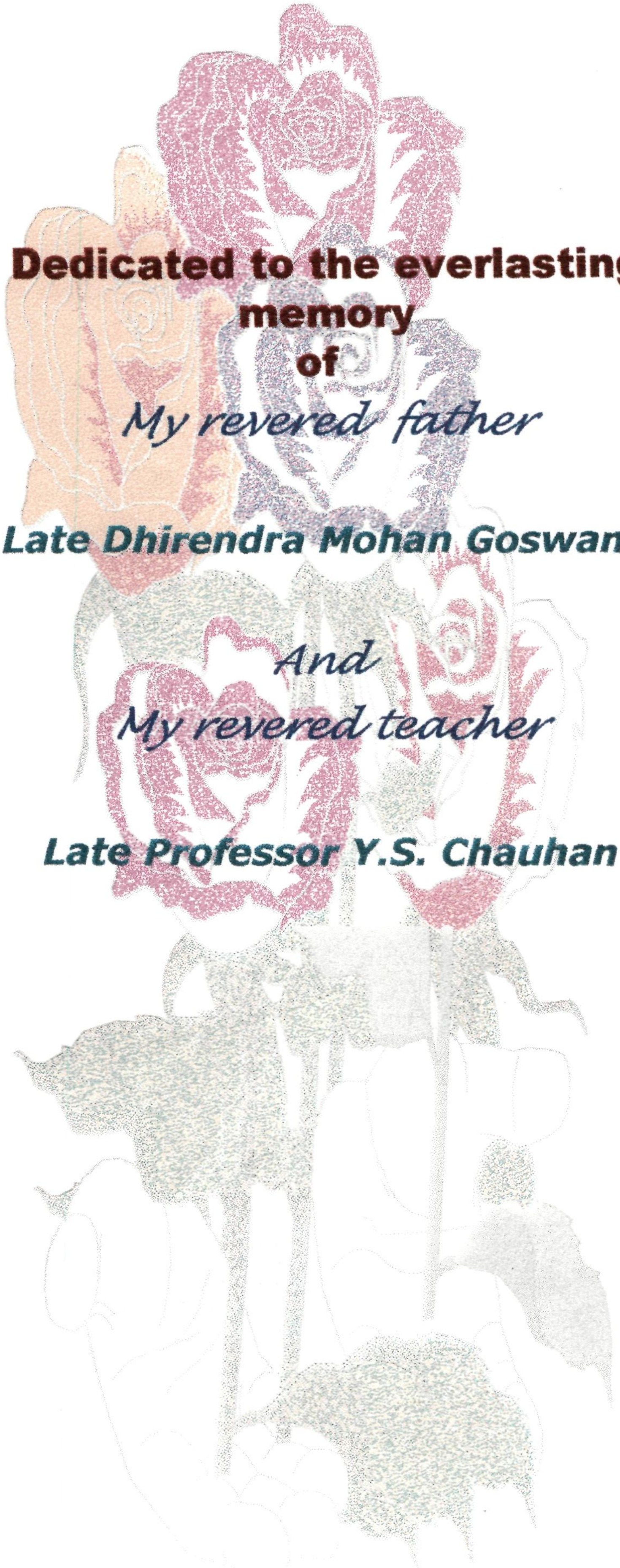

Dedicated to the everlasting memory of

My revered father

Late Dhirendra Mohan Goswami

And
My revered teacher

Late Professor Y.S. Chauhan

NORTH-EASTERN HILL UNIVERSITY
SHILLONG

CERTIFICATE

I, Sanjiban Goswami, hereby, declare that the subject matter of the thesis entitled "Reproductive biology of *Schima wallichii* (DC.) Korth. and *Schima khasiana* (Dyer) Bloemb." is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Botany.

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Shillong

Sanjiban Goswami

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(Sanjiban Goswami)

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INTRODUCTION

India is rated among the few mega-diversity centres in the world. However, in spite of the occurrence of different variety of plant forms, the actual forest cover is only 64.20 million ha which works out to 19.52 percent of the 323 million ha total geographic area of the country; The National Forest Policy (1988) fully reflects the concern for the protection of forests as well as improvement in their productivity and aims at one third of the total land area to be under forest or tree cover. Tropical forests provide a wealth of timber and non-timber products, many of which have been traditionally used by local people for generations. The current high rate of deforestation threatens the genetic resources of these species, reducing the ability of these forests to meet local requirements. The challenge is to increase the quality and quantity of productivity per unit area. Now-a-days, indigenous species are increasingly being planted for social forestry and environmental stability. Sufficient information is available on the choice of species for different regions and plantation zones. A number of species currently in use in plantations programmes in the country. Prominent among these species are *Dalbergia sissoo*, *Tactona grandis*, *Alianthus excelsa*, *Anthocephalus cadamba*, *Gmelina arborea*, *Casuarina equesitifolia* etc. and different species of *Acacia*, *Albizzia*, *Prosopis*, *Terminalia*, *Bauhinia*, *Cassia*, etc. Analysing the yield in a number of plantations, it was observed that poor yield in many plantations is due to poor quality of seeds.

Perennial habit of trees, phenomenon of juvenility, seasonal flowering, long generation time and limited flowering periods, difficulty in experimental manipulation due to large plant size are some of the reasons why research in reproductive biology of trees has lagged behind (Sedgley and Griffin, 1989).

Flowering of tree crops is a highly complex process, which involves many developmental stages. These stages must proceed successfully for the realization of yield potential. There have been few studies of floral induction in trees (Hartly, 1970; Menzel, 1984; Sedgley, 1985; Westwood, 1978).

A number of physiological features associated with the reproductive process can influence the breeding system of a plant species. These features generally result in a breakdown or abnormality in the sequence of flower and fruit development. Floral development in addition to floral initiation is influenced strongly by temperature. Flowering in most woody perennials does not appear to be under photoperiodic control except *Rhododendron, Picea, Hibiscus and Malus* (Moss, 1969). In these trees, the differentiation of the generative tissues appears to be particularly sensitive to temperature.

Low fruit set in nature may be largely due to a high incidence of self-pollination and a high degree of self-incompatibility, but several other causes such as resource limitation and position of fruit within inflorescence, may also be involved (Bawa and Webb, 1984). The low fruit to flower ratio is generally found in hermaphrodite plants, which exhibit self-incompatibility (Sutherland, 1986).

There is an ever-increasing demand for timber as a raw material for construction, fuel wood, furniture and industrial fiber. It is estimated that forest in developing countries have declined by nearly half during last century and that 11 million hectares of tropical forest are currently being cleared for alternative uses such as agriculture (Smith, 1985).

The National Commission on Agriculture in its report estimated the requirements of industrial timber to be of the order of 47-64 million cubic meters. Apart from this, the additional requirements for wood will be to the tune of 48-66 million cubic meters. This gains added significance as the tree species are of immense value for their timber and fuel wood purposes and as such, large scale plantation of fast-growing trees are being raised for the above purpose.

The production of high percentage of viable seeds with a capacity to germinate quickly is an ideal pre-requisite for the proliferation of such tree species.

Of late, there has been renewed thrust to impart greater impetus to the various aspects of reproductive biology with a view to adopt either mono- or mixed cultures of trees or both. Such studies encompass a broad spectrum of features viz., phenology, floral biology, pollen-pistil interactions, pollen viability, pollen germination and seed development resulting in the healthy raising of tree seedlings.

The North-Eastern region of India is a treasure house of plant resources and occupies a unique geographical position in terms of agricultural and industrial production potential. The high rainfall and considerable variation in ecological conditions prevailing in the region has resulted in an environment in which a wide range of plants can be grown for the production of medicinal, aromatic (perfumery) materials, timber wood, fuel wood, essential oils etc. Despite varied flora in North-Eastern region, not many efforts have been made to utilize the forest resources especially from industrial point of view.

The pentropical family Theaceae is one of the largest families of Theales (s.l.) and includes 25 to 30 genera (see Tsou, 1995). Many of its members are common trees or shrubs in lowland forests. The genus *Schima* occurs only in tropical and sub-tropical Asia (Tsou, 1997). The number of species of *Schima* varies from one (Mabberley, 1987) to 30 (Wu, 1984). Keng (1978) reduced all Malayan *Schima* species into *Schima wallichii*. Mabberley (1987) took this single species to represent the whole genus. Morphological, anatomical and embryological features support a multispecies *Schima* (Tsou, 1997). However, members of Theaceae, to which *Schima* belongs, besides having no means of vegetative reproduction, have multilocus gametophytic incompatibility and hence rely on pollen-eating insects for reproduction (Richards, 1986).

Schima wallichii (DC.) Korth. and *Schima khasiana* (Dyer) Bloemb. are timber trees of commerce restricted to eastern Himalayas, N. E. region of India, Bangladesh, Myanmar, Nepal, Bhutan and China. Meghalaya, the tiny hilly state of North-Eastern region is blessed with a rich diversity of flora and fauna. Among the various tree species known to grow in this region, important tree species namely *Schima wallichii* is endemic to North-Eastern region and *Schima khasiana* is endemic to Meghalaya (see Chauhan *et al.*, 1996). *Schima wallichii* trees occur in plains and on the hills between 1200-1700 m altitude. *Schima khasiana* trees do not occur in plains and confined between 1200-2000 m altitude. In Meghalaya they are present mostly in sacred forests at Jowai, Sohrarim, Pongtung etc. These two pioneer species of sub-tropical broad-leaved forests of North-Eastern India are useful for afforestation and ecorestoration of degraded lands of this region. However, natural regeneration of *Schima wallichii* and *Schima khasiana* suffers due to high seed sterility (50%), poor seed germination and high (80 – 90%) seedling mortality (Boojh, 1981). In *Schima khasiana*, the distance from the parent tree decreases seed predation and increases germination. Germination of seeds in case of *Schima khasiana* has been found to be better in disturbed strands. An alteration in forest microclimate and microsite characteristics, consequent upon the exposure of the forest floor to insolation, favoured both seed production and germination in the shade-intolerant *Schima khasiana* (Barik *et al.*, 1996). High survivorship and high growth rates of *Schima khasiana* seedlings in large gaps is indicative of the regeneration niche preferred by this species (Rao *et al.*, 1997).

Schima wallichii is an out breeding species and is also inefficient reproductively (Chauhan *et al.*, 1996). The species suffers from more than 50% seed sterility and approximately 80 – 90% seedling mortality (Boojh, 1981). The investigations on pollen viability indicate that only one-third of pollen is viable (Chauhan *et al.*, 1996).

Keeping above facts in view, the present work has been done on phenology, floral biology, pollination mechanism, pollen-pistil interaction and seed development in the important timber-yielding trees of North-Eastern region of India.

REVIEW OF LITERATURE

Studies in floral biology are largely concerned with how flowers function to promote pollination and mating. The role of pollination in governing mating patterns in plant populations inextricably links the evolution of pollination and mating systems (see Lloyd and Barrett, 1996). Floral biology provides necessary background information, which may be utilized, for the production of fertile seeds, raising of healthy seedlings and the trees. Many tree species display the phenomenon of irregular bearing. Some fruit crops such as mango (*Mangifera indica*) and most timber species show less regular periodicities in fruit and seed production (Monselise and Goldschmidt, 1982; Owens and Blake, 1985). The irregular bearing (inhibition of floral initiation, abscission of flower buds and premature fruit shed) in various tree has been correlated with the depletion of carbohydrate level in the trunk, branches and root (Smith *et al.*, 1986; Takeda *et al.*, 1980) and growth regulators in plants (Luckwill, 1980; Harshemesh *et al.*, 1986).

A number of physical features associated with the reproductive process can influence the breeding system of a plant species. These features generally result in a breakdown or abnormality in the sequence of flower and fruit development. There have been relatively few studies of floral induction in tree crops (Hartley, 1970; Menzel, 1984; Sedgley, 1985; Westwood, 1978).

Phenological studies include observations on different phenophases such as shoot growth, leafing, bud-initiation, bud-break, flowering, fruit development, seed dispersal, seed germination and seedling establishment in nature. The concept and significance of phenological studies have been discussed by Leith (1970) and Leith and Radford (1971). These studies are important for a better understanding of ecological adaptations of individual species and also from the point of germplasm collection. Boes *et al.* (1994) studied floral phenology and morphology of black cottonwood (*Populus trichocarpa).* Semalty and Sharma (1996) studied phenology and floral biology of *Acer caesium*. Diekman (1996) has studied relationship between flowering phenology of perennial herbs and meteorological data in deciduous forests of Sweden.

Temperature influences floral bud initiation and development of flower in plants (Sedgley and Griffin, 1989). Barner and Christiansen (1960) reported that fluctuating temperature adversely affected the fertility of developing buds in *Larix* by disrupting meiosis. Flowering in most woody perennial does not appear to be under photoperiodic control except *Rhododendron, Picea, Hibiscus* and *Malus* (Moss, 1969). In these texa, the differentiation of the generative tissue appears to be particularly sensitive to temperature.

In general, plant growth inhibitors such as Cycocel, Alar, TIBA reduce vegetative growth and promote flowering in angiosperm tree species (Cathey, 1964; Jackson and Sweet, 1972; Luckwill and Silva, 1979; Ramirez and Hood, 1984; Embree *et al.*, 1987). Plant growth regulators control sex expression in plants. In *Morus nigra, Morus alba* and *Morus latifolia* application of 'Ethrel' can induce the production of female flowers on male plants (Jaiswal and Kumar, 1980).

Scanning electron microscopy is increasingly used in the study of the reproductive biology of plants (Heslop-Harrison and Shivanna, 1977; Matsubara, 1980; Sedgley, 1981; Sedgley and Blesing, 1982; Uwate *et al.*, 1982; Cresti *et al.*, 1982; Owens and McGrath, 1984; Owens *et al.*, 1984; Kreitner and Sorensen, 1985; Sedgley *et al.*, 1985). In several species structure of stigma (surface structure, papillae morphology, receptor sites), pollen germination and pollen tube growth on the stigma have been studied using scanning electron microscope (Konar and Linskens, 1966; Jensen and Fisher, 1969; Heslop-Harrison *et al.*, 1975; Heslop-Herrison and Shivanna, 1977; Matsubara, 1980; Tilton and Horner, 1980; Owens and Kimmins, 1981; Cresti *et al.*, 1982; Owens and McGrath, 1984; Owens *et al.*, 1984).

Biochemical investigations of developing plant parts help to understand the physiological processes associated with the development. Histochemical investigations are better suited for the purpose (Hegde and Andrade, 1982). However, investigations dealing with histochemical changes occurring at different stages during the anther and ovule development in the angiosperms are inadequate (Prasad, 1977; Shah, 1982, 1983). The physiology of female gametophyte development in *Capsicum annum* is influenced by histochemical constituents: polysaccharides, DNA, RNA, proteins ascorbic acid etc. (Panchaksharappa and Prabhakar, 1978). Histochemical investigations of embryological events in *Nigella sativa* revealed that the frequent occurrence of infertile embryosac could be due to the lack of nucleic acids which are essential for megasporogenesis (Jalan, 1978).

Living organisms possess several hundred macromolecular proteinaceous substances, which function as biological catalysts. These specialized proteins are synthesized within a cell and are specific in reaction. The basic level of metabolic control exists at the enzyme level which are produced through translation inside the cell and function under specific, well defined range of pH, substrate, temperature, co-factors, concentrations etc. (Malik and Singh, 1980). A number of enzymes are found with in the cell in multiple molecular forms termed isozymes or isoenzymes (Markert and Moller, 1959). Several workers have attempted to investigate the role of different enzymes in the intricate process of development and differentiation (Nitsan, 1962; Scandalios, 1974; Arora *et al.*, 1974; Arnison and Boll, 1974; Nash and Davies, 1975; Wolter and Gordon, 1975; Grasso and Alicchio, 1981; Asins *et al.*, 1983; Kumar and Goswami, 1985). Peroxidases and esterases have been used frequently for development and differentiation studies, separation of cultivars, genetical diversity etc. (Scandalios, 1974; Arora *et al.*, 1974; Wolter and Gordon, 1975; Wehner *et al.*, 1976; Kuhns and Fretz, 1978; Payne and Koszykowski, 1978; Subhash *et al.*, 1980; Vamos Vigyazo, 1981; Brown and Munday, 1982; Tripathi *et al.*, 1982; Asins *et al.*, 1983; Abbott *et al.*, 1984).

Discussing the hormonal regulation of growth and development in higher plants Galston and Davies (1969) concluded that four different hormones (gibberllin, auxin, cytokinin, ethylene) can control the peroxidase. Singh and Singh (1978) found higher level of peroxidase in dwarf varieties in comparison to tall varieties of wheat. Discussing the role of cytokinin and peroxidase interaction Galston and Davies (1969) stated that, in the pith cells of geranium, kinetin promotes the formation of single peroxidase band, which is inhibited by auxin. They further established that kinetin and auxin interact in the control of peroxidase activity as they do in the control of growth. Rychter and Lewak (1971) while studying the peroxidase of apple embryo examined the effect of various growth hormones. They found benzyladenine to be stimulatory for the appearance of two fast moving peroxidase bands. Jain *et al.*, (1969) while working on the effect of growth regulators on abscission and IAA-oxidising enzyme system of dwarf bean seedlings observed inhibitory effect of kinetin on IAA level. Cytokinin promoted peroxidase activity in lentil roots and barley coleoptiles (Darimont *et al.*, 1971), in sugar cane (Gaylor and Glasziou, 1969) and in etiolated maize seedlings (Sharma *et al.*, 1976). In case of *Lens* roots, increase in peroxidase activity was observed after the application of kinetin. Mapson and Wardale (1972) while working on the aspect of involvement of peroxidase in ethylene biosynthesis found that the IAA leads to the production of ethylene, initiating oxidation. Different aspects of ethylene biosynthesis related to auxins and peroxidase have been discussed in a detailed review by Galston and Davies (1969).

The first stage in flowering process is floral induction or evocation, when the vegetative meristem becomes programmed to change into a reproductive meristem. Floral initiation is the first morphological change, which can be detected in the bud. In many cases floral bud initiation may occur weeks or months prior to macroscopic appearance of buds. Information about floral bud development are available only for a limited number of tree crops such as *Populus, Tsuga, Magnolia, Eucalyptus, Artocarpus, Picea, Prunus, Acacia, Cassia, Malus, Avocado, Litchi, Bauhinia, macadamia, Myristica, Pyrus,* *Mangifera, Betula, Peach,* (see Fechner, 1972; Owens and Molder, 1974,1975; Thien, 1974; Ashton, 1975; Sinha, 1975; Owens and Molder, 1976, 1979a; Cresti *et al.*, 1978 ; Buttrose *et al.*, 1981; Sedgley, 1985; Dulberger, 1981; Buban *et al.*, 1982; Sedgley *et al.*, 1983; Menzel, 1983, 1984; Ramirez *et al.*, 1984; Moncur *et al.*, 1985; Armstrong *et al.*, 1986; Banno *et al.*, 1986; Scholefield *et al.*, 1986; Shu *et al.*,1987; Macdonald *et al.*,1987; Raseria *et al.*,1987).

Stigma plays an important role in pollen capture, recognition, selection and germination of pollen. There is a great variation in the morphology of stigma (see Heslop-Harrison and Shivanna, 1977; Cresti *et al.*, 1992). The angiosperm stigma may be papillate or non-papillate and at maturity may appear wet or dry depending on the amount of extracellular secretion present. The stigmatic secretion generally contains carbohydrates, proteins, lipids, water, phenolics, aminoacids and alkaloids (Heslop-Harrison and Shivanna, 1977; Heslop-Harrison and Heslop-Harrison, 1985; Knox *et al.*, 1986). A range of enzymes has also been localized; the non-specific esterases are the predominant ones. Cytochemical demonstration of non-specific esterases has become a standard method of localization of the receptive surface of the stigma (Shivanna and Rangaswamy, 1992). Enzymatic activity, including that of esterase is detectable at the surface of the secretion in some species (Vithanage, 1984; Sedgley *et al.*, 1985).

In the dry stigma, extra cellular components are present in the form of a thin extracellular membrane called the pellicle. The pellicle components originate from the epidermal cells of stigma and/or stigmatic papillae and are extruded on to the surface through discontinuities in the cuticle. In some systems, the pellicle shows ATPase and carbonic anhydrase activity (in addition to esterases and phosphatases); the pellicle also binds to lectins and contains arabinogalactans, a group of carbohydrates with adhesive properties (Heslop-Harrison and Shivanna, 1977).

In the wet stigma, extracellular components are present in the exudate. During the early stages of flower development, the wet stigma is comparable to the dry type with a cuticle-pellicle layer. At later stages, secretions produced from the cells of stigma accumulate below the cuticle-pellicle layer; eventually, this layer is disrupted and the exudate spreads on the surface (Shivanna and Sastri, 1981). The amount of exudate that accumulates on the stigma is highly variable; it may be confined to the interstices of papillae or may flood the entire surface. The exudate may be lipoidal, as in *Petunia* and *Oenothera* or aqueous, as in *Lilium*. The lipoidal component is considered to prevent excessive evaporation and wetting by acting as a liquid cuticle. Proteinase inhibitors have been reported on the stigma of *Nicotiana* (Atkinson *et al.*, 1993). The phenolics and proteinase inhibitors have been suggested to give protection against insects and pathogens. The stigmatic exudate has also been reported to serve as a nutrient source for pollinating insects.

In most species, which have been studied in detail, maximum secretion precedes or coincides with flower opening. The stigma of species showing gametophytic control tend to be wet at anthesis in contrast to those of sporophytic species that have dry stigmas (Heslop-Harrison and Shivanna, 1977). During stigma development a cuticle is present over the surface of the stigma. In some species, the cuticle persists until anthesis (Sedgley, 1979) where as in others it is ruptured by the flow of secretion. In *Prunus*, a large increase in secretion volume results from degeneration and collapse of the stigma papillae at or shortly after flower opening (Uwate and Lin, 1981a) but in some other trees (e.g. *Avocado*, *Amelanchier*) the maximum volume of secretion is present at flower opening and the papilla cells remain intact and show no signs of collapse (Sedgley, 1979; Sedgley and Blesing, 1983; Olson, 1984). The stigma cuticle is not generally a major barrier to pollen germination as in *Prunus*, *Avocado*, but the cuticle is very thick as in *Bauhinia* and acts as barrier to pollen germination. According to Owens (1985), rupture of this thick cuticle *must* occur (presumably by insect pollinators) to allow pollen germination and pollen tube growth.

In most species maximum stigma receptivity occurs at or shortly after anthesis e.g. *Prunus* (Uwate and Lin, 1981b), *Avocado* (Sedgley and Blesing, 1983), *Amelanchier* (Olson, 1984) exceptions being members of the Proteaceae and Myrtaceae which show protandrous dichogamy e.g. *Macadamia* (Sedgley *et al.*; 1985), *Eucalyptus regnans* (Griffin and Hand, 1979). In *Macadamia* full receptivity does not occur until two to three days following anthesis (Sedgley *et al.*, 1985) and in *Eucalyptus* until ten to fourteen days (Griffin and Hand, 1979). The stigma may remain receptive only for a few hours or for a few days e.g. peach, pear, cherry (El-Agamy and Sherman, 1987; Herrero, 1983; Stosser and Anvari; 1982).

Detailed studies are available on structure, histochemistry and exudate of stigma of *Acacia* (Kenrick *et al.*, 1981b), stigma morphology of *Angophora* (Boland *et al.*, 1986), cytochemistry of stigma papillae and their secretions in *Annona* (Vithanage, 1984), morphology, papillae structure and secretion in *Avocado* (Sedgley *et al.*, 1978; Sedgley, 1979; Sedgley *et al.*, 1983), ultrastructure and histochemistry of *Citrus* stigma (Cresti *et al.*, 1982), stigma morphology of *Eucalyptus* (Boland *et al.*, 1986), structure of stigmatic micropyle of *Larix* (Fiordi, 1984), ultrastructure and histochemistry of the stigma of *Malus* (Cresti *et al* .,1980), structure and cytochemistry of stigma of *Populus* (Villar *et al.*, 1987) , development of stigma in *Prunus* (Uwate *et al.*, 1981 a, 1981 b).

The style can basically be one of the two types - solid and hollow (Shivanna and Johri, 1985). The style consists of an outer epidermis, which encloses parenchymatous cortical cells with one or more vascular bundles and an area of transmitting tissue, which is continuous between the stigma and the ovary. The cells are specialized for secretion. In the solid style, a core of transmitting tissue, starting from the secretory tissue of the stigma, traverses the whole length of the style. The transmitting tissue is made up of elongated cells connected end to end through the plasmodesmata with intercellular spaces filled with products (Knox, 1984; Sanders *et al.*, 1992). The intercellular substance is predominantly composed of pectin but it also contains proteins, glycoproteins and often lipids; it also responds to many enzymes, such as esterases, acidphosphatases, and peroxidases (Shivanna and Sawhney, 1997). A number of transmitting tissue-specific, proline-rich proteins have been localized in the intercellular matrix (Gasser *et al.*, 1993; Wang *et al.*, 1993). The cells of transmitting tissue exhibit normal ultrastructural profiles with numerous mitochondria, active dictyosomes, rough endoplasmic reticulum, plastids and ribosomes. Pollen tubes grow down the style through the intercellular matrix of the transmitting tissue.

In the hollow style, the stylar canal originating from the stigma surface traverses the whole length of the style and joins the ovarian cavity. The stylar canal is bordered by one layer or a few layers of glandular cells called the canal cells. The canal cells in the young bud are lined by a layer of cuticle and the secretion product from the canal cells accumulated below the cuticle. The stylar secretion is rich in carbohydrates and proteins and shows esterase and acidphosphatase activity (Tilton and Horner, 1980). In some species, lipids have also been reported in the stylar secretions (Shivanna and Sawhney, 1997). In some systems, the proteins present on the surface of the stigma and in the style show qualitative differences, which may be related to their function (Heslop-Harrison and Heslop-Harrison, 1982; Miki-Hirosige *et al.*, 1987).

The transmitting tissues or canal cells of the style continue into the ovary as the placenta. The ovule, the seat of the female gametophyte (embryo sac) develops on the placenta. The ovule essentially consists of one or two outer coverings (the integument), the nucellus and the embryo sac. There is great variation in the development and structure of the mature embryo sac (Reiser and Fischer, 1993). In the Polygonum type of embryo sac development, one of the two synergids degenerates prior to the arrival of the pollen tube into the embryo sac. Together the egg, two synergids, and central cell have been referred to as the 'female germ unit' (Huang and Russel, 1992) because this unit plays a direct role in pollen tube entry, the discharge of sperm cells and double fertilization. A common feature of the tree crops is the occurrence of multiple ovules of which only one normally develops to seed maturity (Biradar and Mahabale, 1968). Multiple ovules are considered as an adaptation for female selection of the most vigorous embryo (Sedgley and Griffin, 1989).

In several studies (see Schaal, 1980; Bawa and Webb, 1984) it has been observed that if the number of ovules produced in a flower was greater than the number of seeds matured, abortion of some ovules takes place. Seed yield is one component of plants reproductive success (Devlin and Ellstrand, 1990). But to arrive at plants total reproductive success it should be considered together with that plant success in fertilizing ovules that mature into seeds. Detailed studies have been carried out on the structural details of the ovule and embryo sac of different angiospermic plant species (see, Maheshwari, 1950; 1963; Tilton, 1981a; b; Tilton and Lersten 1981; Johri, 1984; Cresti *et al.*, 1992; Gasser and Robinson Beers, 1993; Russell 1993).

In angiosperms, the embryo sac is generally mature and receptive at the time of anthesis, although there are some exceptions to this rule amongst the tree crops such as *Populus* (Fechner, 1972), *Betula* (Macdonald and Mothersill, 1987) and also the fruit crops almond (Pimienta and Polito, 1983) and pear (Herrero and Gascon, 1987).

In the members of Theaceae, the ovule is anatropous, bitegmic and tenuinucellate. The inner integument forms the micropyle. The vascular supply reaches upto the tip of the outer integument in *Camellia sinensis* (Kapil and Sethi, 1963). A hypostase occurs in the ovule. In *Ploiarium alternifolium* (Prakash and Lau, 1976), the megaspore tetrad is either linear or T-shaped. Sometimes, the upper dyad cell degenerates before its division. The chalazal megaspore is functional and gives rise to a Polygonum type of embryo sac. The antipodals do not organize into cells and degenerate before the fusion of polar nuclei or soon after it.

Informations about ovule and embryo sac development is available for a limited number of Theaceae tree species (see Bawa, 1970; Kobuski, 1948; Kapil and Sethi 1963; Mathew, 1978; Mikatadze, 1975; Prakash and Lau, 1976; Tsou, 1997; Venkataramani, 1950; Wu, 1960). Tsou (1997) investigated ovule and embryo sac development in *Schima superba kankoensis, Schima argentea and Schima khasiana.*

There is considerable variation in the size and shape of pollen grains (Moore and Webb, 1978; Iwanami *et al.*, 1998; Faegri and Iversen, 1989; Cresti *et al.*, 1992). One of the conspicuous structural features of pollen grains is the ornamentation of the wall formed by the outer part of the exine (Cresti *et al.*, 1992) Evidence from different sources has conclusively shown that the exine ornamentation is controlled by the sporophytic genome (Shivanna and Johri, 1985). Plants have evolved and amazing range of adaptations to achieve pollination (Free, 1970; McGregor, 1976; Real, 1983). Both layers of the pollen wall, intine and exine contain considerable amounts of proteins (Knox and Heslop-Harrison, 1970). Pollen wall proteins play an important role in pollen-pistil interaction. The exine development of the microspore takes place by the deposition of sporopollenin precursors on already laid down jprimexine and by polymerization of precursors (Wiermann and Gubatz, 1992).

Pollen morphology have been investigated in detail in a few species such as *Melia* (Nair, 1959); *Hevea* (Rao, 1964); *Myristica* (Nair, 1972); *Schima superba kankoensis* (Tsou, 1997). Among angiosperm species, pollen volume varies by almost five orders of magnitude (Wodehouse, 1935; Muller, 1979), implying diverse opportunities for pollen size evolution. On the other hand, within species pollen diameter commonly has a coefficient of variation less than 5 % (Vonhof and Harder, 1995), suggesting that particular reproductive condition favour specific pollen size. The correlation between pollen size and mode of pollination (i.e., biotic or abiotic) is well known (Wodehouse, 1935; Bolick, 1990; Ackerman, 1995). In particular, the correlation between pollen size and pistil characteristics has been reported in many plant groups

(Covas and Schnack 1945; Rosen 1973; Lee, 1978; Plitmann and Levin, 1983; Cruden and Lyon, 1985; Williams and Rouse, 1990; Kirk, 1993; Vonhof and Harder, 1995; Harder, 1998; Torres, 2000).

Pollen-tube abnormalities, such as tube arrest in the style, reversal in direction, irregular growth and swelling of the tube tip in the micropyle, are observed in *Tectona* (Tangmitcharoen and Owens, 1996; Palupi and Owens, 1997). The cause of these abnormalities is not clear. Pollen-tube arrest in the style, as indicated by swollen tips was observed in *Persea americana* Mill. (Sedgley, 1976) and *Macadamia* species (Sedgley, 1983). Swelling of tube tips in the style is common in gametophytic self-incompatibility where tubes are inhibited after penetrating the stigma and part of the style (Sedgley and Griffin, 1989). Bending of pollen tube tips is caused by incompatible pollen as reported in *Lycopersicon* (Gradziel *et al.*, 1993), *Tectona* (Paluppi and Owens, 1997).

A hypodermal archesporial cell (or two to three celled archesporium) develops into the megaspore mother cell. The latter undergoes meiotic divisions, and as a result dyads are produced. In *Thea* (Mikatadza, 1975), The micropylar dyad may develop upto the binucleate embryo sac. The chalazal dyad functions and the development of female gametophyta is of Allium type in *Camellia* (Kapil and Sethi, 1963; Mathew, 1978) and *Thea* (Fagerlind 1939b; Mikatadze, 1975). The synergids are ephemeral and exhibit hooks or hood like expansions in *Camellia sasanqua* (Mathew, 1978). The antipodals may persist upto or even after fertilization.

Information about pollen vectors is available for a limited number of angiospermous cross pollinated tree species (Styles and Khosla, 1976); Kaul, 1985; Harder, 1990; Wilson and Thomson, 1991; Inouye *et al.*, 1994). Only recently attempts have been made to relate the evolution of breeding systems in plants to interactions with their pollinators (Lisci *et al.*, 1994). The effects of pollen competition have been investigated both within species and to a lesser extent, between species of *Turnera* (Baker and Shore, 1995).

Information about pollination mechanism is available for a limited number of tree species. Faegari and Vander Pijl (1979) have given an account of floral syndromes associated with the major pollinator of tree crop species. The number of pollen per flower varies considerably within species (Vonhof and Harder, 1995). Members of Theaceae, to which *Schima* also belongs, besides having no means of vegetative reproduction, have multilocus gametophytic incompatibility. Therefore, these rely on pollen-eating insects for reproduction (Richards, 1986). Pollinators preferentially visit plants based on nonrewarding characters such as floral color, the numbers of flowers per inflorescence and odor (Stanton *et al.*, 1989). The interplay of attractants and rewards cannot be easily elucidated (Shivanna and Sawhney, 1997). Color changes in flowers are well known and some occur after pollination (Weiss, 1991).

Self-incompatibility is a genetically controlled mechanism, which reduces the prevalance of inbreeding depression in a population. Among the angiosperms there has been considerable more work on the genetics and physiology of the prezygotic than the postzygotic mechanisms as the former are easier and quicker to detect (Sedgley and Griffin 1989). Most self-incompatibility systems in tree genera appear to be gametophytic and this has been demonstrated genetically in *Prunus, Pyrus, Cornus* and *Citrus* (Crane and Brown, 1937; Lewis and Modlibowska, 1942; Soost, 1969; Hummel *et al.*, 1982). *Ulmus americana* appears to have a sporophytic self-incompatibility system, but the genetic control has not been determined (Ager and Guries, 1982). A sporophytic system with dominance relationship between the alleles in pollen but not in the pistil is found in *Cilanitida* (Jacob, 1980). In *Theobroma cacao* self-incompatibility system is controlled by three loci and is under both gametophytic and sporophytic control (Cope, 1962).All species with gametophytic self-incompatibility systems have homomorphic flowers. Heterostyly and self-incompatibility occur in *Durio zibethinus, Averrhoa carambola,* different species of *Cordia* etc. (Knight, 1965; Opler *et al.*, 1975; Chin and Phoon, 1982).

Generally, sporophytic self-incompatibility results in inhibition on the stigma (Ager and Guries, 1982) and in gametophytic self-incompatibility pollen tube is inhibited in the style (Crane and Brown, 1937; Roy, 1938; Raptopoulos, 1941; Ton and Krezdorn, 1967). There are, however, a number of exceptions to this rule. In *Cola nitida*, the pollen tube growth is not inhibited on the stigma though self-incompatibility is under sporophytic control (Jacob, 1980). In *Acacia retinodes* the pollen tube growth is arrested in the nucellus (Kenrick *et al.*, 1986), in *Castanea mollissima* (McKay, 1942) and in *Theobroma cacao* (Cope, 1962) fertilization does not occur though male gametes are released into the embryo sac. Inhibition has been absorbed in the stigma or in the ovary of apple and pear (Modlibowska, 1945; Williams, 1969), in the stigma of almond and *citrus* (Pimienta *et al.*, 1983; Kahn and DeMason, 1986) which show gametophytic control of self-incompatibility mechanism operates in the ovary (Sedgley and Attanayake, 1988). In species showing gametophytic control the pollen tends to be binucleate on release from the anther, retains its viability in storage and germinates readily *in vitro.* On the other hand, in species showing sporophytic control where pollen tends to be trinucleate on release from the anther, rapidly loses its viability in storage and germinates poorly *in vitro* (Brewbaker and Majumdar, 1961; Brewbaker, 1967). The stigmas of species showing gametophytic control tend to be wet at anthesis, in contrast to those of sporophytic species, which have dry stigmas (Heslop-Harrison and Shivanna, 1977). As a result of gametophytic self-incompatibility callose is deposited in the inhibited pollen tube whereas, deposition of callose occurs in both the pollen and the stigma cells of species showing sporophytic self-incompatibility (Knox, 1984 a).

Generally, there is a deposition of β-1,3-glucon callose within the stigmatic papillae at the site of contact with incompatible pollen (Heslop-Harrison, 1975). However, recent data suggest that this is related more to the linkage of gametophytic proteins from necrotic pollen grains or tubes than to playing a mechanistic role in the incompatibility reaction itself (Elleman and Dickinson, 1994).

To reveal the factors responsible for compatibility and incompatibility reactions, Bredemeijer (1973) studied peroxidase isozyme pattern in the self-incompatible clone of *Nicotiana alata* (S_3S_3) during growth and senescence of the unpollinated styles and corolla. Bredemijer suggested that increase in the particular isozymes during the growth of styles and the corollas are involved in the suppression of auxin activity and, consequently in growth regulations. Bredemeijer (1973) found that the increase of peroxidase isozyme 11 during the growth of the style and its decrease after anthesis run more or less parallel with the effect of floral development and floral ageing on compatible pollen tube growth. Further, Bredemeijer (1976) found that in immature styles pollen tube tips grow in a stylar part without peroxidase 10, whereas in mature styles the tube tips grow in a stylar part with a high peroxidase 10 activity. Comparison of peroxidase 10-activity and pollen tube growth in styles selfed at anthesis and 4 days after anthesis also reveals a positive correlation between tube growth inhibition and peroxidase 10 activity. During maturation the peroxidase activity of the style and the corolla increases resulting in qualitative and quantitative changes. Bredemeijer (1976), therefore, suggested involvement of particular peroxidase isozymes (Peroxidase 10) in the rejection of incompatible pollen. Bredemeijer (1976, 1977) also considered a possible relationship between peroxidase release from cytoplasm to walls and intercelluler spaces, and inhibition of pollen tube growth suggesting a possible relationship between the variations in peroxidase activity in the style and the physiological barriers preventing self-fertilization.

Postzygotic self-incompatibility mechanisms are suspected to occur in *Eucalyptus regnans* (Griffin *et al.*, 1987), *Camellia sinensis* (Serrhini et al., 1985; Tilquin *et al.*, 1985), *Mangifera* (Sharma and Singh, 1970), *Rhododendron* (Williams *et al.*, 1984). Embryo abortion due to inbreeding depression is generally considered to act via accumulation deleterious recessive alleles (Griffin *et al.*, 1987).

Low fruit to flower ratio is generally found in hermaphrodite plants which exhibit self-incompatibility (Sutherland, 1986). Angiosperm tree species tend to be outcrossing and to have a lower reproductive efficiency then other plants (see Sedgley and Griffin, 1989). Environmental variables influence floral physiology either by altering sex expression or by inducing sterility. Low temperature including frosts, nitrogen, zinc deficiency etc. induce sterility in trees (see Sedgley and Griffin, 1989).

The abnormalities involving total sterility are most common features of the cultivated plants. Male sterility may be partial or total and the breakdown or abnormality may occur at any stage of stamen or pollen development (see Soot and Cameron, 1975; Zielinsky and Thompson, 1966). Partial female sterility has been reported in a number of species e.g., abscission of pistillate flower in Walnut (Catlin *et al.*, 1986), occurrence of male and female sterility have been reported in *Citrus* (Naithani and Raghuvanshi, 1958) and *Quercus* (Kaul, 1985).

Low fruit set in nature is largely due to a high incidence of self-pollination and a high level of self-incompatibility, but several other causes, such as resource limitation and position of fruit within inflorescence, may also be involved (Bawa and Webb, 1984). Self-incompatibility have been reported in many tree species. In *Gmelina arborea* (Bolstad and Bawa, 1982), where no self-pollinated flowers develop into mature fruits, although many of the fruits develop to different sizes before they abort. Self-incompatibility can act in the stigma, style or ovary (Seavey and Bawa, 1986). In *Tectona grandis* gametophyte self-incompatibility occurs with some pollen tubes being inhibited in the style but most are inhibited in the ovary. The stigma was found to be of little importance in pollen recognition and rejection. Outcrossing did not appear to be controlled by pollen-tube growth in the style. The study suggested that there is no or little competition among pollen tubes for space and little direct contact with stylar tissues, which may reduce incompatibility reactions. A large number of pollen tubes reach the ovary than the number of ovules and final inhibition then occurs within the ovule (Tangmitcharoen and Owens, 1997). The extent of self-incompatibility in *Tectona grandis* varies from 96-100% (Hedegart, 1973) and commonly less than 1% of self-pollinated flowers develop into fruits (Hedegart, 1976). Tangmitcharoen and Owens (1997) reported that the number of pollinated pistil was not the major factor limiting *Tectona* fruit production, but rather, the high incidence of self-pollination, thus flowers abort because of lack of fertilization, probably resulting from self-incompatibility. Usually gametophytic self-incompatibility is indicated by pollen tube arrest at a certain stage of pollen-tube growth. The arrest occurs in the nucellus in *Acacia retinodes* and in the micropyle in *Theobroma cacao*, or the pollen tube is able to penetrate the synergid and fertilization tasks place but the zygote and/or the endosperm fail to develop, as in *Rhododendron* species (Cope, 1962; Williams *et al.*, 1984; Kenrick *et al.*, 1986). In *Tectona grandis*, incompatibility can occur in many stages of pollen tube growth and even much later, following fertilization (Palupi and Owens, 1997).

The suspensor, after becoming anchored in the endosperm proper reaches its maximum length at the heart-shaped embryo stage, which is a common feature in angiosperm embryogenesis (Yeung and Mienke, 1993). At the cotyledon stage, the suspensor starts to degenerate in *Tectona grandis* and *Vicia faba* (Johanssen and Walles, 1994). The *Tectona grandis* suspensor is always uniseriate (Palupi and Owens, 1997), in *Lippia nodiflora* it becomes massive (Pal, 1951). Embryo development in *Tectona grandis* is of the Solanad type (Pal, 1951; Palupi and Owens, 1997).

In both *Gmelina* and *Tectona grandis* ovules are fertilized but the fruits fail to mature because of embryo abortion, which results because of failure in endosperm development (Bolstad and Bawa, 1982; Palupi and Owens, 1997). Experimental studies on *Arabidopsis thaliana* and several other species have shown that the growth of the suspensor during early developmental stages may be inhibited by the embryo proper (Raghavan, 1976; Marsden and Meinke, 1985). According to Weins *et al.* (1987), the tilting or shift from the normal position of the embryo of *Epilobium anguistifolium* is also a manifestation of failing embryos. Cesation of embryo growth has been reported to be preceded by the disfunction of the endosperm in *Medicago sativa, Pontederia sagittata* and several other *Solanum* species (Cooper *et al.*, 1937; Beamish, 1955; Scribailo and Barrett, 1991).

MATERIAL AND METHODS

PHENOLOGY

Phenological observations were recorded for three consecutive years (1996 - 1999) in four trees of *S.wallichii* and *S.khasiana* each growing at NEHU campus, Mawlai, Umshing, Shillong (altitude 1500m) and at Shillong Peak, Upper-Shillong (altitude 1950m) respectively (Fig.1). The trees at both the sites were observed regularly from February 1996 onwards to record data on shoot growth, leafing, bud-break, initiation of floral buds, flower development, seed dispersal, seed germination and seedlings establishment. A particular phenophase was considered to have started when about 10% of the trees at respective site were in that phase. Completion period of a given phase was recorded when that phase was over in 90% trees.

The studies pertaining to floral biology were started from the very beginning of the bud stage of flower. The twigs were marked for counting various floral stages at different time intervals. The flowering was recorded by counting the average floral stages on different twigs and 10 replicates in each case. The formation of first floral bud was considered as floral initiation. Full bloom was taken as the stage when more than 90% flowers had opened.

For fruit setting, fruit development and fruit retention studies, four trees of each species were selected. Two branches of each tree were tagged and observations were recorded after 15 days intervals starting from hand pollination. The length and diameter of the mature fruits were calculated with the help of slide caliper. Number of seeds per fruit was also determined in each treatment. Percentage of filled seeds was determined by dissecting the embryos from the seeds. Seed efficiency (SE) was calculated as the number of filled seeds divided by seed potential multiplied by 100. To determine filled seeds per fruit, embryo dissection test was done. Data on different phenophases, seed dispersal, seed germination and seedling establishment in nature were recorded visually in the field conditions. Simultaneously, data were recorded on maximum and minimum temperature, relative humidity and daylength.

FLORAL BIOLOGY

Data on floral bud initiation and floral biology were recorded visually in the field conditions. For floral biology, twenty floral buds, from four trees of both the species, were tagged at the time of slitting of the corolla. These were observed continuously to record data on anthesis, anther dehiscence, stigma receptivity and pollination. The insects foraging on the anthers and those involved in pollination were also collected. Twigs of both the species from different trees, bearing flower buds about to anthesise, were brought to the laboratory. The cut ends of the twigs were immersed in water. On anthesis of flowers, floral parts were measured and counted in atleast twenty flowers, belonging to four different trees of both the species, to determine respective sizes and numbers.

Ten anthers from ten different trees of each species were fixed individually for 2 h in FAA to determine the number of pollen/anther. Following Solomon (1986), these were first hydrolyzed separately in 1N HCl at 60°C for 2.5 h and then homogenized in 0.5 ml of 3:1 lactic acid: glycerin solution. The homogenized sample was mixed for 30 seconds on vortex mixture. The number of pollen/anther was determined by placing 0.01 ml of homogenized solution on a haemocytometer covering it with a coverslip. All the pollens present in the suspension were counted using a microscope. The observations were made on the total volume of 0.5 ml/anther. All observations were added to determine the number of pollens in an anther. The mean number of pollen per anther was calculated from the recordings thus made for the ten homogenized anthers. This mean value was multiplied with the number of stamen in a flower to determine the total number of pollens in a flower. This was done for 20 flowers from each species separately. Diameter of the pollen was measured in 100 pollen, mounted in a drop of glycerine, under a microscope. Volume of the pollen was calculated using the formula: $V = 4\pi r^3/3$ where V = Volume, r = radius of the pollen.

Flower anthesis and stigma receptivity data were recorded in the floral bud anthesised in the laboratory. Data were recorded by tagging 20 about to anthesise flower buds belonging to four different trees of both the species. The specific time of splitting of corolla, anthesis of flower (the time when anther becomes visible in an anthesising floral bud), receptivity of stigma (appearance of stigmatic fluid on the stigma) and end of stigma receptivity (drying or re-absorption of the stigmatic fluid) were recorded for each bud at hourly interval.

ULTRA STRUCTURE OF STIGMA AND STYLE

Using scanning electron microscope (SEM), stigmatic surface, external and internal surfaces of the style in both the species were studied morphologically at two developmental stages of flowers (5mm bud and pre-anthesis). The following methods were employed for SEM studies:

1. Flowers were collected and fixed in FAA.

2. Longitudinal sections of style and stigma were cut and washed in distilled water.

3. The materials were then gradually brought to pure acetone before performing critical point drying.

4. The critical point dried materials were gold-coated (200Å) and scanned in a JEOL 35 SEM Operated at 15 KV at the Regional Sophisticated Instrumentation Center of North-Eastern Hill University, Shillong.

POLLEN GERMINATION AND POLLEN TUBE GROWTH

The basal medium of Brewbaker and Kwack (1963) was used for *in-vitro* pollen germination studies. The optimal requirements of pollen germination and pollen tube elongation were worked out by altering one factor at a time. The Brewbaker and Kwack's medium was modified by incorporating the optimal requirements of *S.khasiana* pollen and was used for the study of pollen germination and pollen tube growth in *S.khasiana*. Modified Brewbaker and Kwack's medium (Chauhan and Katiyer, 1996) was used for the study of pollen germination and pollen tube growth in *S.wallichii*. Variability in pollen germination and pollen tube growth between different individuals of a population and different flowers of an individual tree were investigated by collecting pollen from 4 different trees. Pollens from 40 flowers per tree were collected by dusting on a butter paper. The pollens of a tree were mixed together and germinated on modified Brewbaker and Kwack's medium. Per tree 5 slides were incubated. At the end of the incubation period the germinating pollen and pollen tubes were fixed by putting a drop of FAA (50% ethanol: glacial acetic acid: formalin, 10:1:1). Per slide 5 microscopic fields were observed for recording data on percent pollen germination. All the 5 slides were scored. Thus altogether 25 observations were made for a tree. Pollen germination was determined by taking mean of all the 25 readings. This gave data regarding pollen germination for a particular tree. Similarly, pollen tube elongation was found out by measuring 25 pollen tubes per slide from 5 microscopic field. For this purpose also all the 5 slides were scored. The mean length of the 125 tubes so measured gave tube growth in a particular tree. Variation of germination and tube elongation between different flowers of a tree were investigated by collecting pollen from five different flowers per tree. The pollens were germinated separately. Germination percentage and tube growth were recorded as described above.

The effect of flower age on pollen germination and pollen tube growth was investigated by collecting flowers, belonging to four trees, that had anthesised at the same time. Pollen from such flowers were collected separately at 6, 12, 18, 24 and 30 h after anthesis. Pollen grains were germinated and data were recorded on pollen germination and tube elongation as mentioned above. Effect of light of various colour on germination and tube elongation was investigated with the help of cellophane paper of different colours. Two layers of cellophane paper of one colour were used to cover the light source for providing light of a particular colour. The colours tried were white (fluorescent), violet, blue, green, yellow and red. The data were analyzed either with the help of student's 't' or ANOVA to find out the significance of the results.

Since, the optimal requirements of pollen germination and pollen tube elongation were worked out by Chauhan and Katiyar (1996), in case of *S.wallichii*, these aspects were investigated only in *S.khasiana*.

POLLEN STORAGE

The following protocol (Shivanna and Rangaswamy, 1992) was used for pollen storage studies:

1. Six airtight humidity chambers were maintained using desiccators.

2. Fused Calcium Chloride ($CaCl_2$) was used for obtaining about 0% RH, saturated solution of $CaCl_2.6H_2O$ was used for obtaining about 32% RH and saturated solution of $CuSO_4.5H_2O$ was used for obtaining RH of about 98%.

3. One humidity chamber from each RH group was kept at room temperature (20 ± 2°C) and in freezer of a refrigerator (-5 ± 1°C).

4. The chambers were allowed to equilibrate for about 24 hours.

5. *Fresh pollens collected from just anthesised flowers were mixed* thoroughly to make the sample homogenous.

6. A small amount of the pollen was used to test its viability using acetocarmine method.

7. The collected pollens were dried over silica after putting them in unsealed bottles. The samples were stored in multiple sets.

8. Pollen samples were stored in sets in each humidity chamber maintained under the six RH and temperature combinations.

9. Pollen viability tests were done after one month, six months and one year intervals.

Pollen viability of stored pollen grains were tested and compared with control using acetocarmine method. For preparing acetocarmine solution, 1% acetocarmine stain was used (55 ml of distilled water, 45 ml of acetic acid and 1g of carmine stain powder were mixed, boiled and filtered). A drop of acetocarmine solution was placed on a clean glass slide. Pollen grains (air-dried) were dusted on the solution of acetocarmine. A cover slip was placed over it and the slide was gently warmed over a spirit lamp. The slide was left for sometime to cool down and allow the pollen grains to stain sufficiently. For calculating pollen viability, pollen grains were observed under the microscope and total number of viable and non-viable pollen grains were counted from five microscopic fields. Three replicates were maintained from each treatment group. Viable pollen grains stained deep red while non-viable pollen grains did not stain.

POLLEN - PISTIL INTERACTION

Aniline blue fluorescence method (Martin, 1959; Kho and Baer, 1968; Dumas and Knox, 1983; Kenrick and Knox, 1985) was employed to study pollen germination and pollen tube growth in the pistil. Stepwise methodology is as follows:

1. For the study of pollen-pistil interaction, path of pollen-tube, fertilization etc. controlled pollination was made and materials were collected at regular intervals (1 h, 3 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h, 24 h, 27 h, 30 h, 36 h, 48 h, 64 h, 72 h, 96 h, 120 h, 144 h, 168 h, 192 h, 216 h, 240 h).

2. The pollinated pistils were fixed for about 24 h in FAA and then stored in 70% ethanol.

3. After rinsing with distilled water, they were cleared in 8N NaOH at room temperature for 2-4 d or until most of the tissues became transparent.

4. The pistils were then transferred in petridishes and rinsed carefully with distilled water.

5. The pistils were left in decolorized aniline blue (water-soluble aniline blue 0.005% in 0.05 M Na_2HPO_4. PH was adjusted to 11 and the solution was stored (in brown bottle) overnight and then mounted in glycerine.

6. To achieve required degree of spreading of the tissue, gentle pressure is applied on the coverglass.

7. The preparations were observed under fluorescence microscope using suitable combination of filters.

EMBRYO DEVELOPMENT

Pistils from the flower buds, flowers and ovaries in all stages of development were collected from four different plants of each species and were fixed in FAA for 24 hours. The fixed materials were then stored in 70% ethanol. The ovules were then dissected from the ovaries and were transferred directly to 4 ½ clearing fluid consisting of 85% lactic acid, chloral hydrate, phenol, clove oil and xylene- 2:2:2:2:1, by weight (Herr, Jr.1971). After treatment for 24 h at room temperature, the ovules appeared nearly transparent under phase contrast microscope equipped with transmitted light.

The transparent ovules were transferred to special slides for microscopic examination with a small amount of clearing fluid (Herr, Jr. 1971). To prepare special slides, two cover glasses were affixed with dendrite 1 cm apart on a microscopic slide. The slide was kept for three days to ensure adequate hardening of the mounting medium. A drop of clearing fluid with one ovule was placed in the center of the 1cm space, and a cover glass was placed over the preparation so as to rest on the two mounted cover glasses. When the coverglass is lightly and repeatedly pressed with dissecting needle midway between the support covers, the cells of the ovules gradually became spread apart. This squash procedure does not disrupt the structural integrity of the individual cells. For the study of developing embryo same methodology was employed.

SEED GERMINATION

Seeds of *S.wallichii* and *S.khasiana* were germinated in petriplates on Whatman filter paper moistened with 10 ml of glass double distilled water in BOD incubator at 20 ± 1°C in dark, in pots filled with mixture of garden soil and cow dung manure in 3:1 ratio, in open garden soil and in natural habitat. Hundred seeds constituted one replicate. Embryos were dissected out from the seeds manually to find out whether all the seeds had normal development. Depending on the presence or absence of embryo, the seeds were classified as filled and empty respectively.

Seeds were also germinated in petriplates on Whatman filter paper moistened with 10 ml of glass double distilled water in BOD incubators at 20 ± 1°C and 25 ± 1°C temperatures and in continuous light, dark and 16 h dark 8 h light conditions to find the effect of temperature and light conditions on seed germination in both the species. The intensity of light was 532 lux. Emergence of radical was considered as germination. Data was recorded regularly till the germination was over. Data were collected on days to commence the germination, days taken to complete the germination and total germination percent.

The effect of temperature and light treatments on seed germinability of both the species was tested with two-way ANOVA (Fixed effect model). For determining germination in soil, seeds of both the species were sown in pots filled with mixture of garden soil and cow dung manure in 3:1 ratio. Data on germination were recorded for 65 days. The emergence of plumule above soil was considered as germination. The data were collected on seedlings emergence above soil, days for germination to be occurred and percent germination.

Seed viability was also determined with the help of tetrazolium salt (Kuo *et al.*, 1996). For this purpose, seeds of both the species collected in 1996 and 1997 were soaked in glass double distilled water for 24 h at room temperature (20 ± 2°C). The soaked seeds were decoated manually. Data were recorded on the number of filled (embryo present) and unfilled (lacking embryo) seeds. The embryos obtained from seeds collected in 1996 and 1997 were incubated in 1% tetrazolium solution (w/v) at 30 ± 1°C for 3 h and 6 h respectively. The seed viability was determined using three replicates of hundred seeds each. Seed viability is expressed as percentage seeds having germinable embryos. Differentiation between germinable and non-germinable embryos was made on the degree of tetrazolium staining. Germinable embryos stained dark whereas the non-germinable embryos were those that stained light or did not stain.

For studying germination in natural condition, seeds of both the species were sown in garden soil and forest soil and germination was recorded for 90 days.

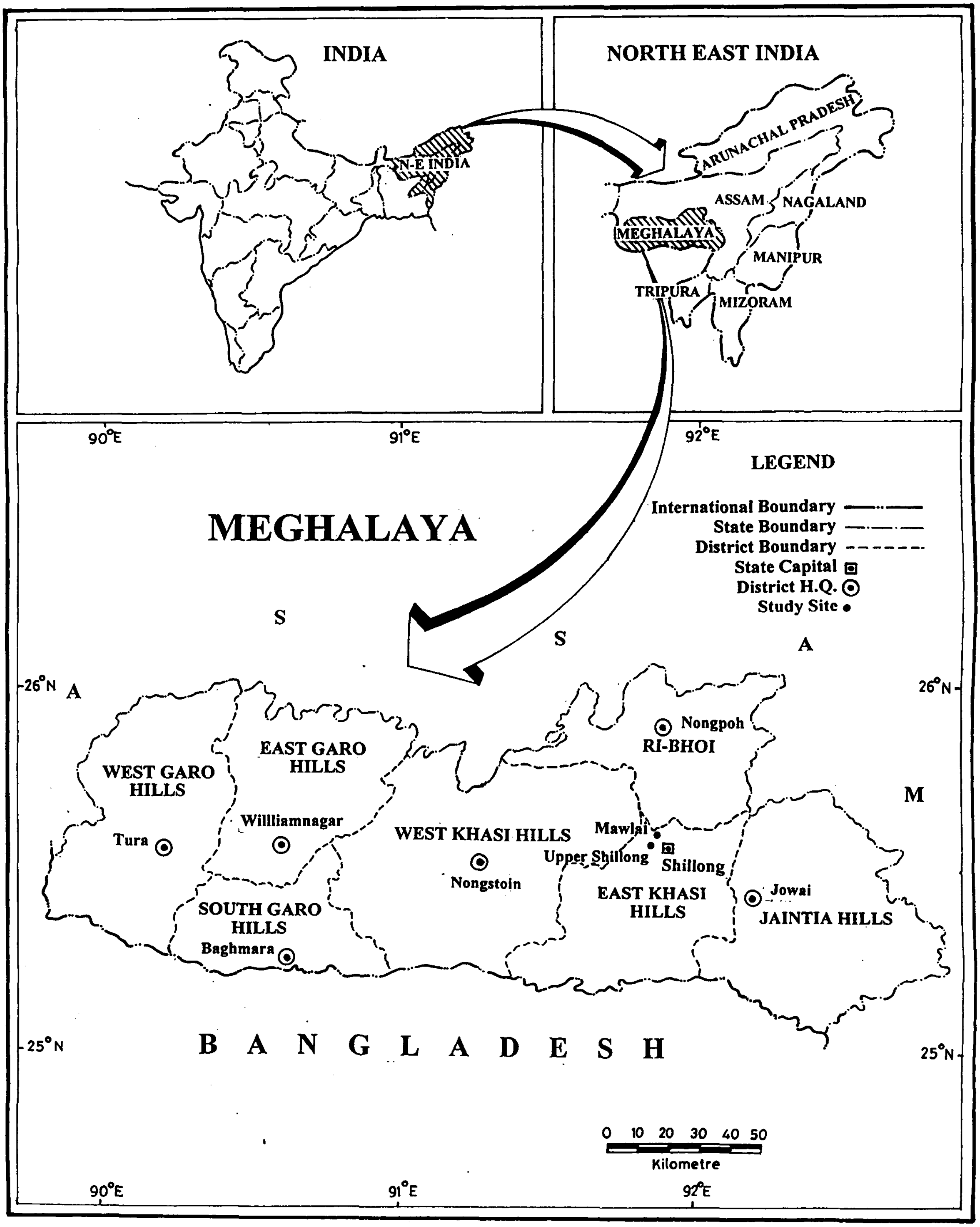


Fig. 1: Map showing study area

RESULTS

GENERAL CHARACTERS

Schima wallichii

Schima wallichii is an evergreen tree attaining a height of 15-50 m (Fig. 2). It is characterized by showy white flowers and very thick, soft and reddish-brown bark. The bark contains numerous needle-shaped crystals of oxalate which are responsible for intense itching if the bark is allowed to come in contact with the skin. Due to presence of this thick bark, the tree can stand fire to a considerable extent.

The leaves are simple, alternate, oblong to lanceolate, evergreen and exstipulate. Leaf base is cuneate and leaf apex ranges from acute to acuminate, entire to undulate and glabrous above. Lateral nerves are mostly forked. Leaves are 8-17 cm long and 2-6 cm broad. Petioles are 0.3 to 3.5 cm long, glabrous and pedicels are about 2.5 mm thick.

The flowers are white, 3-4 cm across, in recemes or panicles, usually axillary, solitary/paired, fragrant, actinomorphic, hermaphrodite and hypogynous. Sepals are 5, glabrescent, somewhat round, ciliate along margin only and measure 2-3 mm long and about 3-4 mm broad. They are persistent and slightly connate at the base. Aestivation is quincuncial. Petals are 5, glabrescent, obovate to ovoid, about 20 mm long and connate at the base. Nectaries are present at the base of the ovary. Stamens are numerous, yellowish, almost free, but adnate to the base of the corolla. They are tightly packed in a floral bud but these unfold at the time of anthesis. The mean number of stamens per flower is about 176 but the number varies between the flowers of the same tree. Stamens are of variable size ranging from 3-12 mm. Usually longer stamens are found at periphery and smaller stamens are close to the pistil. Stamens are shorter than the pistil. Anther is a four- lobed structure and is about 2.5 mm long and basifixed. Carpels are 5-6 and are syncarpous. Ovary is superior measuring about 3 mm and 4 mm in length and diameter respectively. It has 5-6 locules depending on the number of carpels present per locule. Ovules are three in number and placentation is axile. They are campylotropous. Ovary is hairy at base. Fruit is fleshy and loculicidal capsule having winged seeds. Mature fruits measure about 16 mm in diameter. Each fruit contains an average of 15 seeds.

Schima khasiana

The trees are evergreen and attain a height of about 20 m (Fig. 4). They are characterized by its sharply serrate leaf margin, silky flower buds which remain tomentose throughout, reddish brown to dark brown and very thick but soft bark. The bark is nearly smooth and contains numerous needle-shaped crystals of oxalate. These crystals are responsible for the intense itching of the skin. Due to presence of calcium oxalate crystals in the bark, the tree can withstand fire to a considerable extent.

The leaves are simple, alternate, oblong to lanceolate, evergreen and exstipulate. Leaf base is cuneate and leaf apex ranges from acute to acuminate, glabrous, coriaceous and sharply serrated margin. Lateral nerves are scarcely forked, leaves are 9-20 cm long and 3-7 cm broad, petioles 0.3 to 3.5 cm long, glabrous, and pedicels are about 3 mm thick.

The flowers are white and 5-6 cm across. The flower buds are tomentose throughout, in recemes or panicle inflorescence. Flowers are usually axillary, solitary/paired, fragrant, actinomorphic, hermaphrodite and hypogynous. Sepals are 5, silky tomentose outside, measure 3-5 mm long and about 3 mm broad. They are persistent and slightly connate at the base. Aestivation is quincuncial. Petals are 5, silky tomentose outside, obovate to ovoid, about 30 mm long and connate at the base. Aestivation is quincuncial. Nectaries are present at the base of the ovary. Stamens are numerous, yellowish, almost free but adnate to the base of the corolla. They are tightly packed in a floral bud but these unfold at the time of anthesis. The mean number of stamens per flower is about 189 but the number varies between the flowers of the same tree. Stamens are of variable size ranging from 6-17 mm. Usually shorter stamens are close to the pistil. They are shorter than the pistil. Anther is four-lobed structure and is about 2.1 mm long and basifixed. Carpels are 5-6, measuring about 3 mm and 4.5 mm in length and diameter respectively. It has 5/6 locules depending on the number of carpels present per locule. Ovules are 3 in number and placentation is axile. Ovules are campylotropous. Ovary is hairy at base. Fruit is fleshy and loculicidal capsule having winged seeds. Mature fruits measure about 22 mm in diameter. Each fruit contains an average of 15 seeds.

Fig.2

Fig.4: *Schima khasiana*

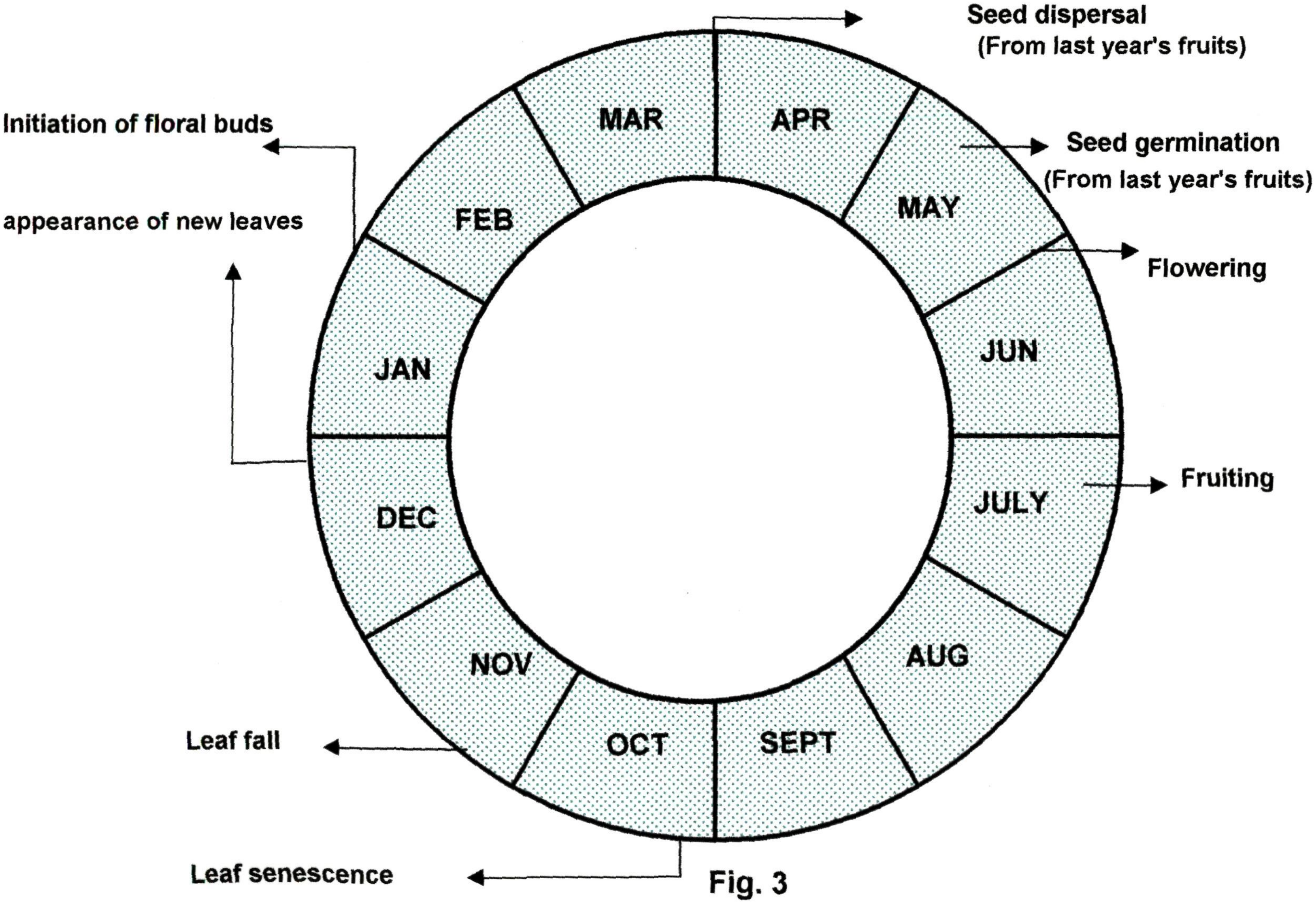
A tree in flowering stage.

Fig.4

PHENOLOGY

Schima wallichii

The new leaves appear in the month of January (Fig. 3) and continues upto the last week of February. During this period the average maximum temperature ranges between 15 and 18.5°C, minimum temperature between 5 and 8°C, relative humidity between 62 and 78%, average rainfall between 0.46-0.54 mm and the day length between 10.40 and 11.30 h. Solitary floral buds enclosed by two bracts or leaves appear in the month of February-March as small round axillary protuberances (Fig. 3). To begin with these are green in colour. The average maximum temperature ranges between 18.5 and 22°C and the minimum in the range of 8 and 12.5°C; relative humidity between 61 and 62%, rainfall from 0.54 to 1.5 mm and the day length remains between 10.50 and 12.15 h. Last year's fruits appear on the tree, these become very hard and the colour turns to brownish-black during this period. Seed dispersal starts in the last week of April when the average maximum temperature ranges between 14 and 25°C; average relative humidity remains about 60%, rainfall remains 1.09 mm and the daylength ranges between 12.10 and 12.30 h. Dispersal of seeds continues for a few days. The tree appears very fresh with light green new leaves. In the month of May-June; when the average maximum temperature ranges between 24 and 24°C, the minimum ranges between 16 and 17°C, relative humidity ranges between 76 and 83%, rainfall between 5.86 and 12.22 mm and daylength ranges between 12.30 and 13.25 h; the buds measure approximately 8.90 ± 0.33 mm in diameter. At this stage, the petals become white in colour and start anthesising. Anthesis of the flower buds begins with the slitting of the corolla at the tip of the floral bud. During the months of October-November; when the average maximum temperature ranges between 18.5 and 24.5°C, minimum ranges between 11 and 14.5°C, relative humidity ranges between 65.5 and 70.5%, rainfall between 0 and 2.31 mm and the daylength ranges between 10.40 and 11.55 h; most of the mature leaves become red and yellow in colour and leaf fall occurs (Fig. 3). The tree looks



Phenology of *S.wallichii*

(1 Year cycle)

Fig. 3

very dry during this time of the year. The tree bears new developing fruits. Some empty seeded fruits (of previous year) are also found on some branches. Developing fruits become harder and the colour changes from greenish-brown to brownish-black.

In the last week of May; when the average maximum temperature ranges between 16 and 24°C, relative humidity remains about 76%, rainfall remains 5.86 mm and the daylength ranges between 12.30 and 13.10 h; new seedlings emerge on the ground. The number of seedlings decreases with the passage of time.

Schima khasiana

The new leaves appear in the month of February (Fig. 5) and continues till March when the maximum temperature ranges from 18.5 and 21°C, and the minimum from 2.5 and 8.5°C, relative humidity ranges from 70 and 71%, rainfall between 0.3 and 2.4 mm and the daylength remains between 10.50 and 12.10 h. Solitary floral buds enclosed by two bracts or leaves appear in the month of April-May as small protuberances. To begin with these are green in colour. The maximum temperature ranges between 12 and 13.5°C, the minimum between 4 and 12.5°C, relative humidity ranges from 64 and 83%, rainfall remains between 1.35 and 8.75 mm and the daylength remains between 12.10 and 13.10 h. The tree suffers from frequent hail fall during the part of the year. Area around and under the tree remains partially covered by broken branches, flower buds and leaves. The last year fruits remain green. But the fruits of last-last year start dispersal of seeds. Seed dispersal continues for few days. During this period of the year, a tree bears new flower buds, last year's developing fruits and fruits of last-last year (20 months old). The tree appears very fresh with light green new leaves. In the month of July-August; when the average maximum temperature ranges between 22 and 23.5°C, the minimum between 15.5 and 16°C, relative humidity ranges between 90 and 93%, rainfall remains between 3.78 and 5.32 mm and daylength ranges between

12.30 and 13.25 h; the buds measure approximately 21.78 mm in diameter. At this stage the petals become white in colour and start anthesising. Anthesis of the flower bud begins with the slitting of the corolla at the tip of the floral bud. In the month of September, a tree bears newly formed fruits (Fig. 5), developing last year's fruits and many empty seeded fruits of last-last year (about 24 months old). The style and stigma remain attached with the newly formed fruits (ovaries). During the month of November, when the average maximum temperature ranges between 5 and 17.5°C, relative humidity about 92%, rainfall remains scanty and the daylength remains between 10.40 and 11.10 h; most of the leaves turn yellowish - red in colour and the colour of the last year's fruits become greenish-brown. The fruits become hard.

During the months of December-January, when the maximum temperature ranges between 12.5 and 17°C, the minimum between 1.5 and 2.5°C, the relative humidity remains between 68-80%, rainfall between 0-0.42 mm and daylength ranges between 10.30 and 10.50 h; leaf fall occurs (Fig. 5) .The ground remains covered with yellow and red coloured leaves. The tree looks very thin and dry. Last- last year's fruits (16 months old) become hard and the colour changes from greenish-brown to brownish-black. Last year's fruits remain green in colour.

Many new fruits (about 6 month's old) are destroyed by the combined effects of wind and rain during February and March. The tree bears current year's green fruits (6 months old), last year's fruits (18 months old) and many empty seeded fruits of last-last year. In the month of June, when the maximum temperature ranges between 15.5 and 21.5°C, relative humidity remains 91%, rainfall 12.74 mm and daylength ranges between 13.10 and 13.25 h, new seedlings emerge on the ground (Fig. 5).

Phenology of S.khasiana

(2 Years cycle)

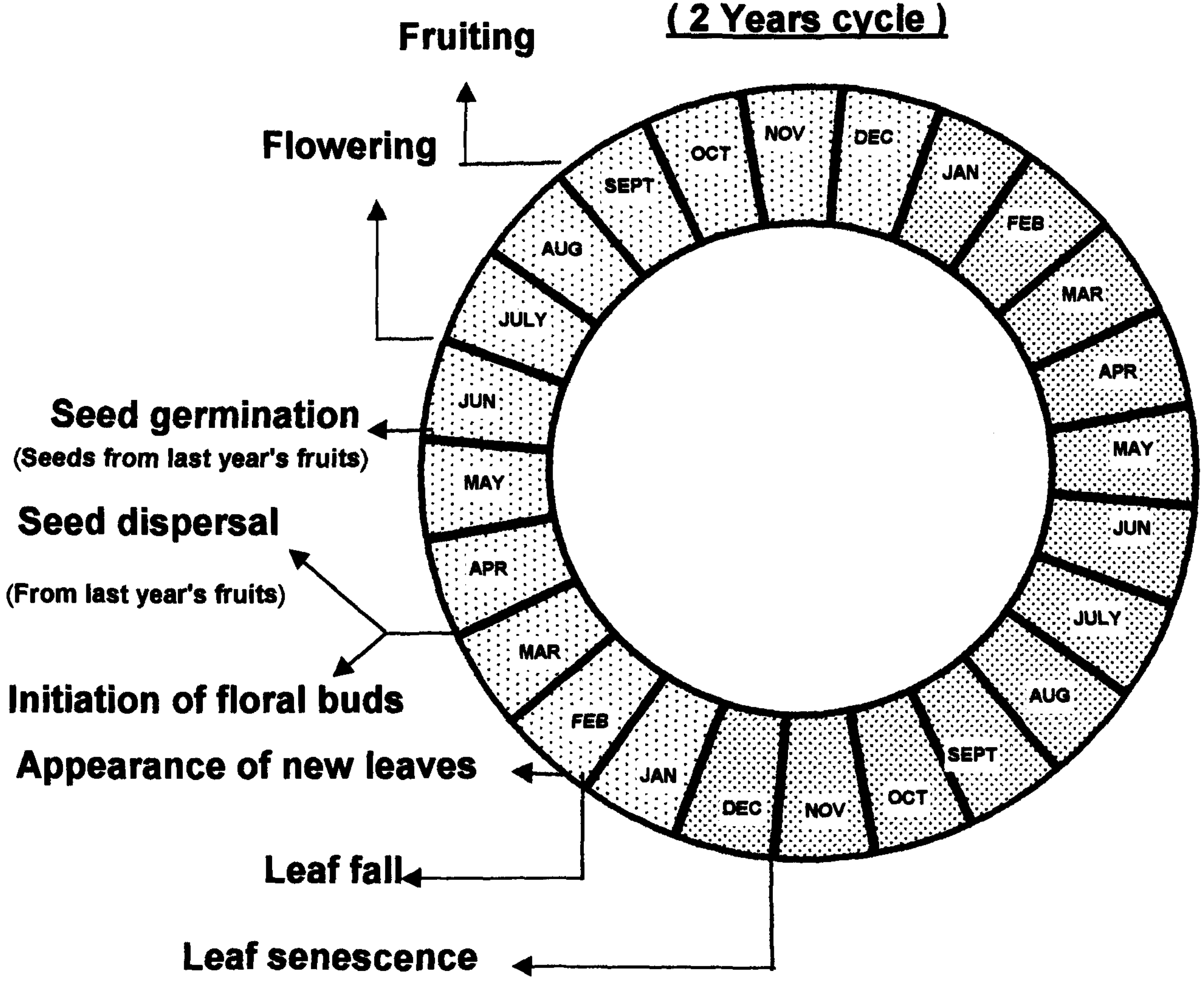


Fig. 5

FLORAL BIOLOGY

Schima wallichii

The buds measure approximately 8.90 ± 0.33 mm in diameter in the months of May-June. At this stage, the petals become white in colour and start anthesising. Anthesis of flower bud begins with the slitting of the corolla at the tip. The slit gradually widens and opening of the flower is completed in 11-16 h (Fig. 6A,B). The flowers begin to anthesise at approximately 4.00 h and the process continues upto 15.00 h in the afternoon. The flowers are white and measure 3-4 cm across (Fig. 7A,B). Flowers are axillary, solitary or paired having 5 sepals, 5 petals, 176 ± 6.31 stamens and 5-6 carpels (Table 1).

Sepals are glabrescent, round, ciliate measuring 7.39 ± 0.24 mm^2 in area. Petals are glabrescent, obovate-ovoid upto 20 ± 0.13 mm long and measure 322.6 ± 8.83 mm^2 in area. The aestivation is quincuncial. Stamens are yellowish, almost free and adnate to the base of the corolla. They are tightly packed in a floral bud but on anthesis these unfold (Fig. 7A,B). The number of stamens varies between the flowers of the same tree. Stamens are of variable sizes ranging from 3-12 mm. Usually longer stamens are found at periphery and smaller ones are close to the pistil. 85% of the stamens measure between 6-12 mm and 15% stamens are 3-5 mm long. Anther is a four lobed structure and is 2.5 ± 0.8 mm long. Anther dishiscence takes place after anthesis. About, 5708 ± 321 pollen grains develop in each anther and about 1004608 ± 36011 pollens are produced in each flower. Each pollen measures 18 ± 0.4 μm in diameter and has a volume of 3054.51 ± 186.70 μm^3 (Table 1). However, many of the pollen grains are sterile. Acetocarmine staining test revealed 42.00 ± 1.26% pollen viability. The sterile pollen did not stain and appeared empty. Conversely, the fertile pollen grains were stained and appeared filled. Wilting of the stamen occurs about 42 h after anthesis.

Ovary possesses hairs at the base and supports a cylindrical style that is 7.75 ± 0.2 mm long and 2.3 ± 0.06 mm in diameter. Style ends in a 5-6 lobed

Table 1: Floral characters of *Schima wallichii.*

Character	Number	Size			
		Length (mm)	**Breadth (mm)**	**Diameter**	**Volume (μm^3)**
Sepal	5	2.09 ± 0.03	3.54 ± 0.09		
Petal	5	20.00 ± 0.13	16.13 ± 0.43		
Stamen	176 ± 6.31	3 –12			
Pollen/anther	5708 ± 321			18.0 ± 0.4(μm)	3054.51± 186.7
Pollen/flower	1004608 ± 36011				
Stigma	1	2.75 ± 0.08	2.75 ± 0.08		
Style	1	7.75 ± 0.20		2.30 ± 0.06(mm)	
Ovary	(Carpel) 5 - 6	2.90 ± 0.06		4.35 ± 0.17(mm)	43.11 ± 3.06
Seeds/Fruit	15	9.20 ± 0.29	5.60 ± 0.22		

± = SE

stigma that measures 2.75 ± 0.08 X 2.75 ± 0.08 mm in length and breadth (Fig. 8A). At a time when flower bud is of 5 mm diameter, the stigma side shows rugulate patter (Fig. 8B). At this stage, stigma from downside shows reticate pattern (Fig. 8C). Both rugae and reticulae are very prominent. At this stage stigma surface shows prominent lobes (Fig. 9A) which later on develop foveolate pattern (Fig. 9B). The cup-shaped structures on the stigma surface help in retaining more stigmatic fluid.

Style is slender and comprises compactly arranged cells (Fig. 10A). At a time when flowers are of 5 mm diameter, the epidermal cells of the style show depressions (foveolate pattern). The cuticle forms a rugulate covering over the stylar cells (Fig. 10B).

At the time of anthesis, stigma lobes become very distinct (Fig. 11A). When viewed under SEM, the central portion of the stigma shows papillate surface (Fig. 11B). The cells of the stigma towards periphery show foveolate pattern having distinct rugae (Fig. 11C). Style, when cut longitudinally, shows compactly arranged transmitting tissue surrounded by large parenchymatous cells (Fig. 12A). Style when viewed externally shows distinct striate pattern (Fig.12B). Ovary is penta-to hexa-carpellary measuring 2.9 ± 0.06 and 4.35 ± 0.17 mm in length and diameter, respectively. The ovary has 5-6 locules depending on the number of carpels present. Each locule has 3 ovules. Placentation is axile and ovules are campylotropous. The fruit is a loculicidal capsule having winged seeds. Fruit measure 16.4 ± 0.47 mm in diameter. Average 15 seeds are produced in each fruit (Table 1). Schematic presentation of events from anthesis till withering of style is shown in Figure 13.

Schima khasiana.

The buds measure approximately 21.78 ± 0.67 mm in diameter during the month of July-August. The petals become white in colour and start anthesising. Anthesis of flower buds begins with the slitting of the corolla at the tip of the floral bud. The slit gradually widens and opening of the flower is completed in 12-20 h (Fig. 14 A-C). The flowers begin to anthesise at approximately 3.00 h and the process is complete around 1500 h. The flowers are white and measure 5-6 cm across (Fig. 15 A-C). Flowers are axillary, solitary or paired having 5 sepals, 5 petals, stamens 189 ± 3.00 and carpels 5-6 (Table 2). Sepals are glabrescent, round, ciliate measuring 13.95 ± 0.45 mm^2 in area. Petals are glabrescent, obovate–ovoid, silky tomentose outside upto 29.29 ± 2.76 mm long, measuring 572.91 ± 6.55 mm^2 in area. The aestivation is quincuncial. Stamens are yellowish, shorter than the pistil, almost free and adnate to the base of the corolla (Fig. 15C). They are tightly packed in a floral bud and gradually unfold during anthesis. The number of stamens varies between the flowers on the same tree. Stamens are of variable size ranging from 6 to17 mm. Longer stamens are found usually at periphery while shorter stamens are close to the pistil. About 80% of the stamens measure between 12-17 mm and the rest 20% are 6-12 mm long. Anther is 2.2 ± 0.05 mm long four lobed structure. Anther dehiscence occurs after anthesis. About, 5492 ± 50 pollen develop in an anther and in one flower about 1037988 ± 49878 pollen grains are produced. Each pollen measures 37.40 ± 0.12 μm in diameter and has a volume of 27399.26 ± 1026.0 μm^3. Acetocarmine staining test revealed 57.21 ± 1.08% viability in pollen grains. Stamen wilt about 50 h after anthesis. Ovary has hairs at the base and supports a cylindrical style that is 5.70 ± 0.9 mm long and 2.20 ± 0.07 mm in diameter. Stigma is 5-6 lobed and measures 5.70 ± 0.09 mm (length) and 5.70 ± 0.009 mm (breadth) (Fig. 16A). In a bud of 5 mm diameter the stigma side under SEM showed compactly arranged rugae (Fig. 16B). When stigma was observed from downside, a distinct reticulate pattern was seen (Fig. 16C). The stigma surface showed compact reticulae (Fig. 17A,B). The depressions were very prominent and provided more surface area for deposition of stigmatic fluid.

Style is cylindrical and is composed of compactly arranged cells (Fig. 18A). 5 mm bud stage, style shows longitudinal rugae traversed by

Table 2: Floral characters of *Schima khasiana*.

Characters	**Number**	**Size**			
		Length(mm)	**Breadth(mm)**	**Diameter**	**Volume(μm^3)**
Sepal	5	4.56 ±0.06	3.06 ± 0.09		
Petal	5	29.29 ± 2.76	19.56 ± 0.85		
Stamen	189 ± 3.00	6-17			
Pollen/anther	5492 ± 50.00			37.4 ± 0.12 (μm)	27399.26 ± 1805.06
Pollen/flower	1037988 ± 49878				
Stigma	1	5.70 ± 0.09	5.70 ± 0.09		
Style	1	5.70 ± 0.09		2.20 ± 0.07(mm)	
Ovary	(Carpel) 5 - 6	3.06 ± 0.07		3.81 ± 0.09(mm)	28.74 ± 2.17
Seeds/Fruit	15	12.3 ± 0.24	7.38 ± 0.06		

± = SE

prominent radial walls (Fig. 18B). Cuticular ruguae are present throughout the length of style.

At the time of anthesis, stigma lobes become distinct (Fig. 19A) and are covered with copious secretion. The stigmatic papillae are compactly arranged. A Distinct depression is seen where stigmatic lobes meet (Fig. 19B). The stigmatic papillae are clearly visible inside the stigmatic exudate (Fig. 19C).

Prior to pollination, style when cut longitudinally shows compactly arranged cells (Fig. 20A). Either side of transmitting tissue is composed of parenchymatous cells (Fig. 20A). When viewed externally the styler cells show a distinct rugulate pattern (Fig. 20B). The rugae show cuticular striations, which run on the surface both radially and longitudinally.

Ovary is penta-to hexa-carpellary measuring 3.06 ± 0.07 and 3.81 ± 0.09 mm in length and diameter, respectively. The ovary has 5-6 locules depending on the number of carpels present. Length and diameter of ovary is given in Table 2. Each locule has 3 ovules. Placentation is axile and ovules are campylotropous. The fruit is a loculicidul capsule having an average 15 winged seeds (Table 3). Fruit measures 20.52 ± 1.14 mm in diameter. Seed setting was more in *S.wallichii* than *S.khasiana* and the former species showed better reproductive success. Schematic representation of events after anthesis till withering of stigma, style and petals are given in Figure 21.

Table 3: Fruit size and seed production per fruit in *Schima* species.

Species	Fruit		Seeds per fruit	Seed efficiency (%)	Reproductive success (%)
	Length(mm)	Diameter(mm)			
S.wallichii	2.92 ± 0.20	4.32 ± 0.29	15	80.05 ± 0.4	44
S.khasiana	3.02 ± 0.09	3.92 ± 0.07	15	59.84 ± 1.33	36

± = SE

Fig.6

Fig.7: *Schima wallichii*

A. Unfolding of flower bud. Note tightly packed stamens.
B. Flower showing glistening drop of stigmatic exudate and nectar.

(nt-nectar; se-stigmatic exudate)

Fig.7

Fig.8: *Schima wallichii* scanning electron micrographs showing

A. Stigma of flower bud of diameter 5mm (15KV X 94),
B. Stigma from side of flower bud of diameter 5mm (15KV X 1800),
C. Showing a portion of stigma from down side of flower bud of diameter 5mm. Note reticulate pattern (15KV X 3200).

(sl-Stigma lobe)

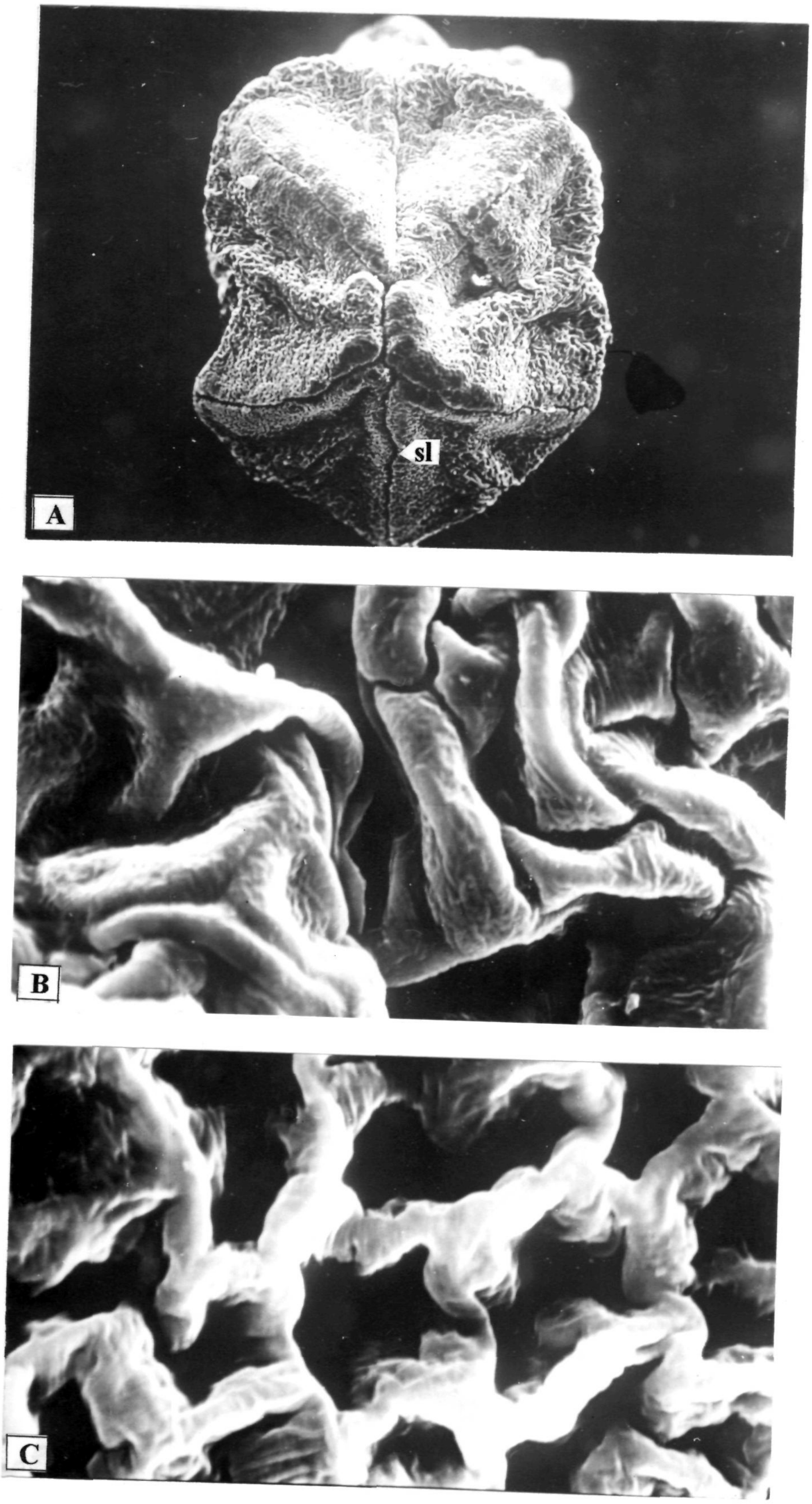


Fig.8

Fig.9: *Schima wallichii* scanning electron micrographs showing

A. Middle portion of stigma of flower bud of diameter 5mm (15KV X 2600),

B. A part of stigma surface showing foveolate pattern. Note rugulate pattern (15KV X 2000)

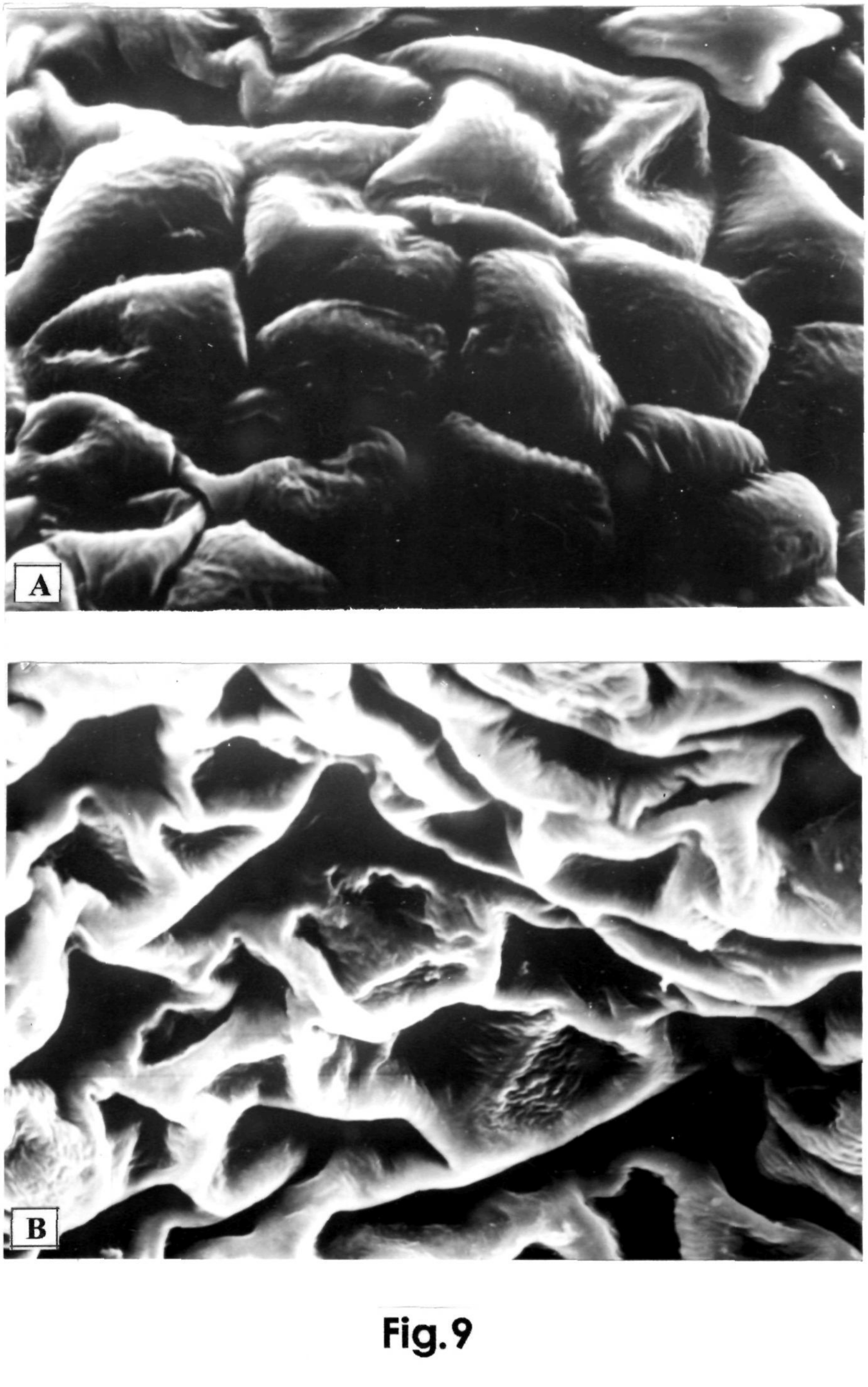


Fig.9

Fig.10: *Schima wallichii* scanning electron micrographs showing

A. L.S. part of style of flower bud of diameter 5mm (15 KV X 1500),
B. External surface of the style of flower bud of diameter 5mm. Note foveolate pattern (15KV X 1600).

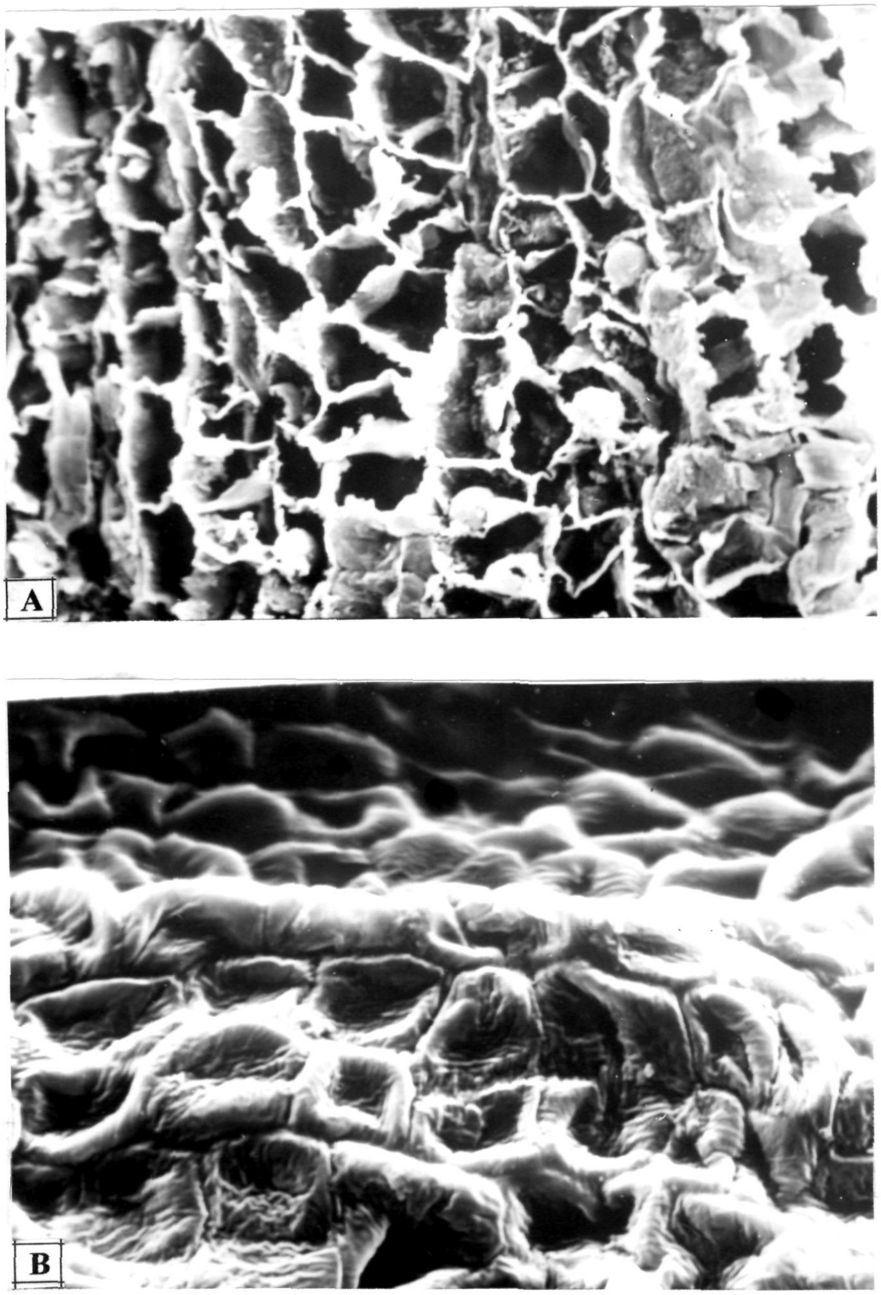


Fig.10

Fig. 11: *Schima wallichii* scanning electron micrographs showing

A. Stigma surface before pollination (15KV X 32),
B. A part of fig. A magnified. Note papillate surface (15KV X 360),
C. A part of fig. A further magnified to show details of stigma surface (15KV X 1000).

(ps- papillate surface)

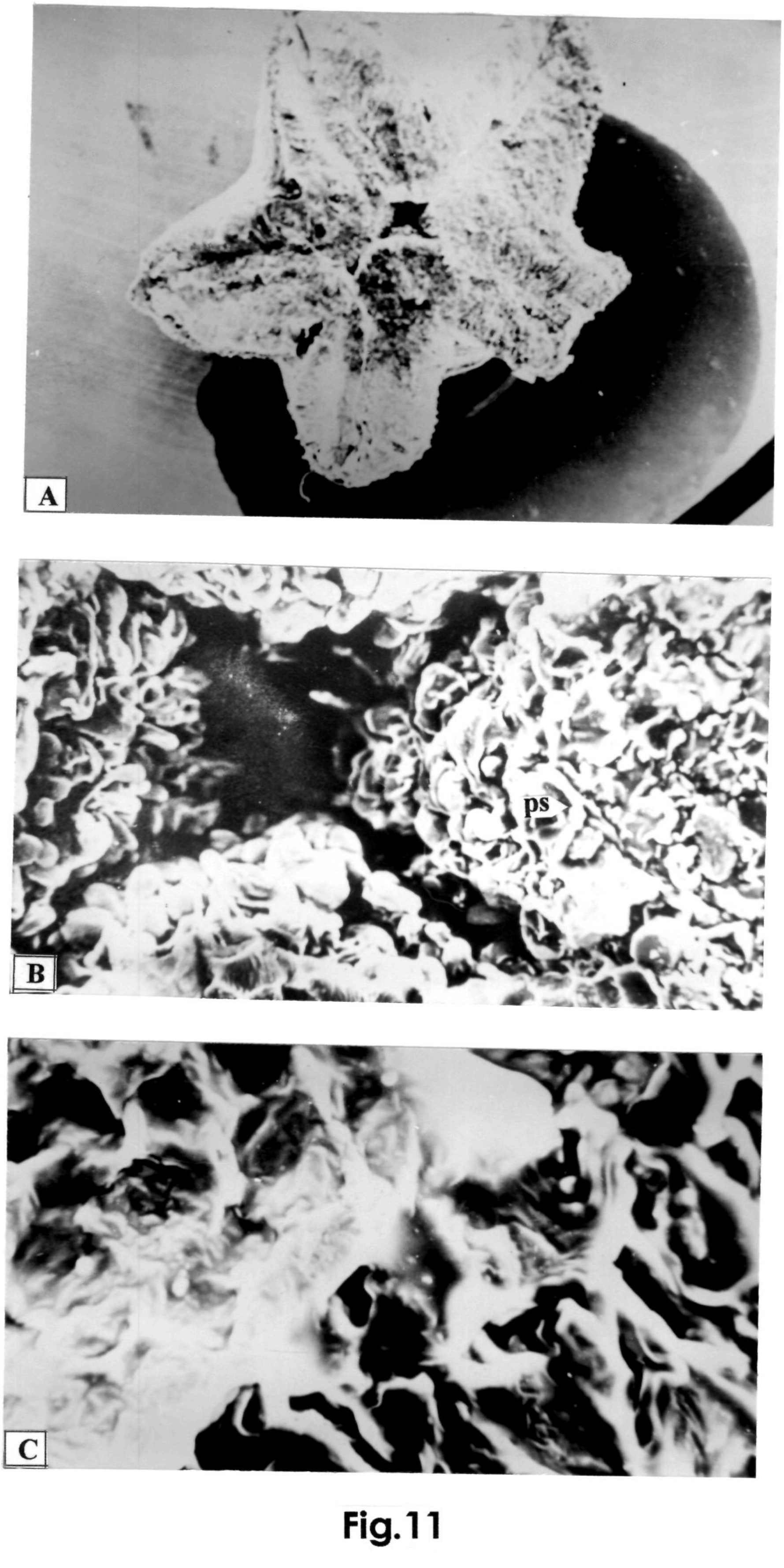


Fig.11

Fig. 12: ***Schima wallichii*** **scanning electron micrographs showing**

A. L.S. part of style of a pre-pollinated flower (15KV X 400),
B. External surface of the style of a pre-pollinated flower. Note rugulate ornamentation (15KV X 2000).

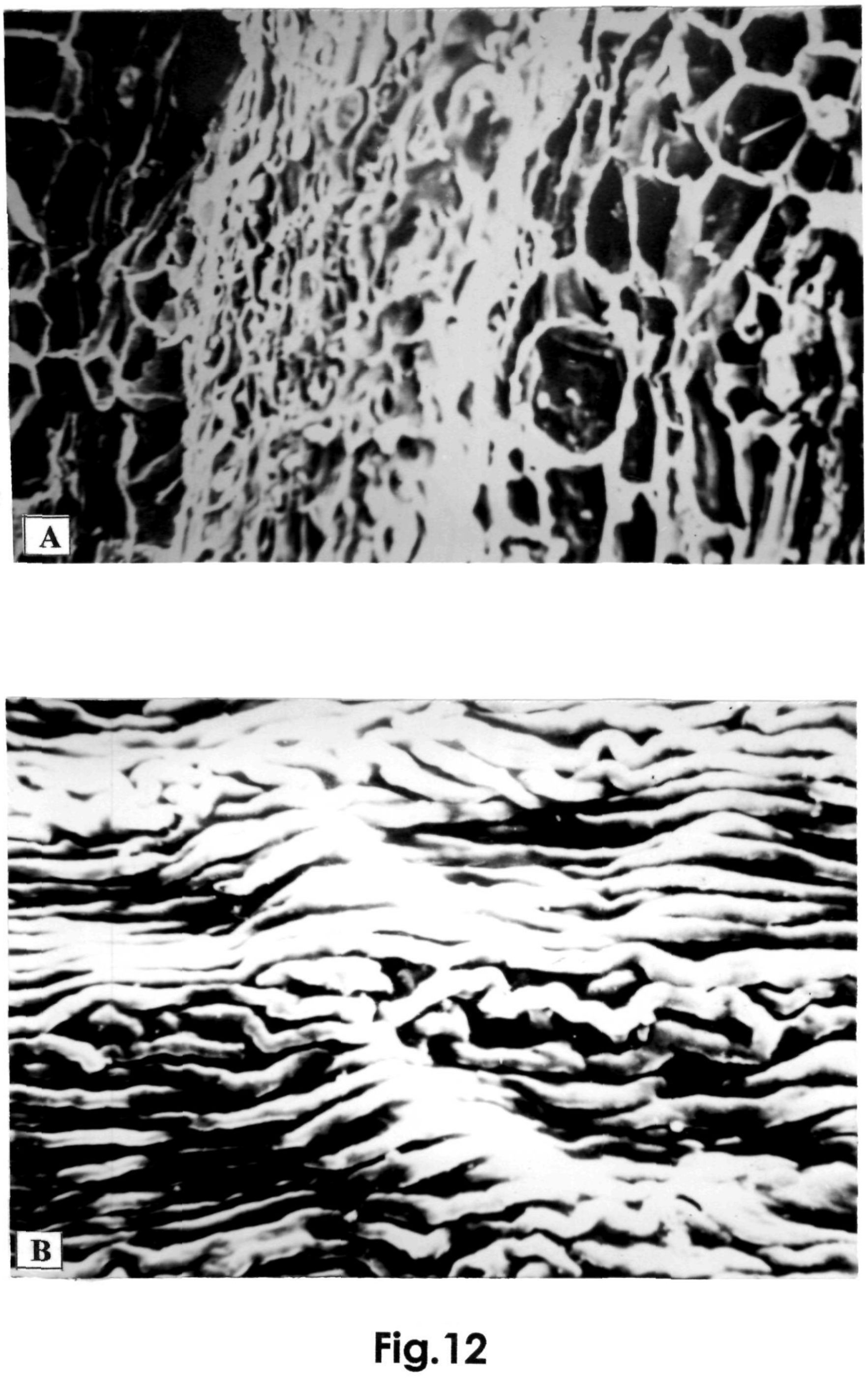


Fig.12

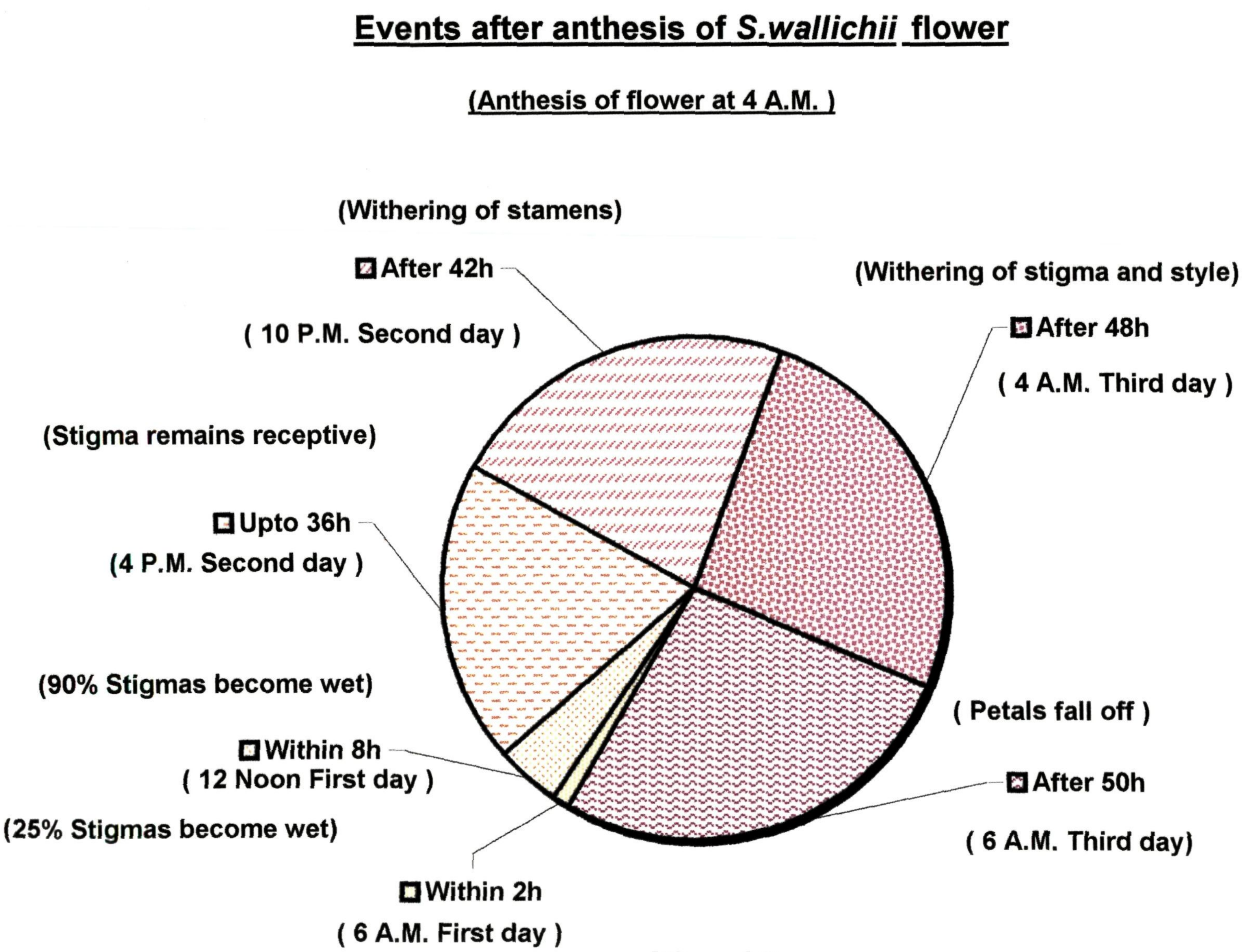

Events after anthesis of *S.wallichii* flower
(Anthesis of flower at 4 A.M.)
(Withering of stamens)
▨ After 42h
(10 P.M. Second day)
(Withering of stigma and style)
▨ After 48h
(4 A.M. Third day)
(Stigma remains receptive)
▨ Upto 36h
(4 P.M. Second day)
(90% Stigmas become wet)
▨ Within 8h
(12 Noon First day)
(25% Stigmas become wet)
▨ Within 2h
(6 A.M. First day)
(Petals fall off)
▨ After 50h
(6 A.M. Third day)

Fig. 13

Fig. 14: *Schima khasiana*

A. Flower buds,
B. Twig showing new flower buds and fruits of previous year (10 months old),
C. Flower bud (arrow) just before anthesis.

Fig.14

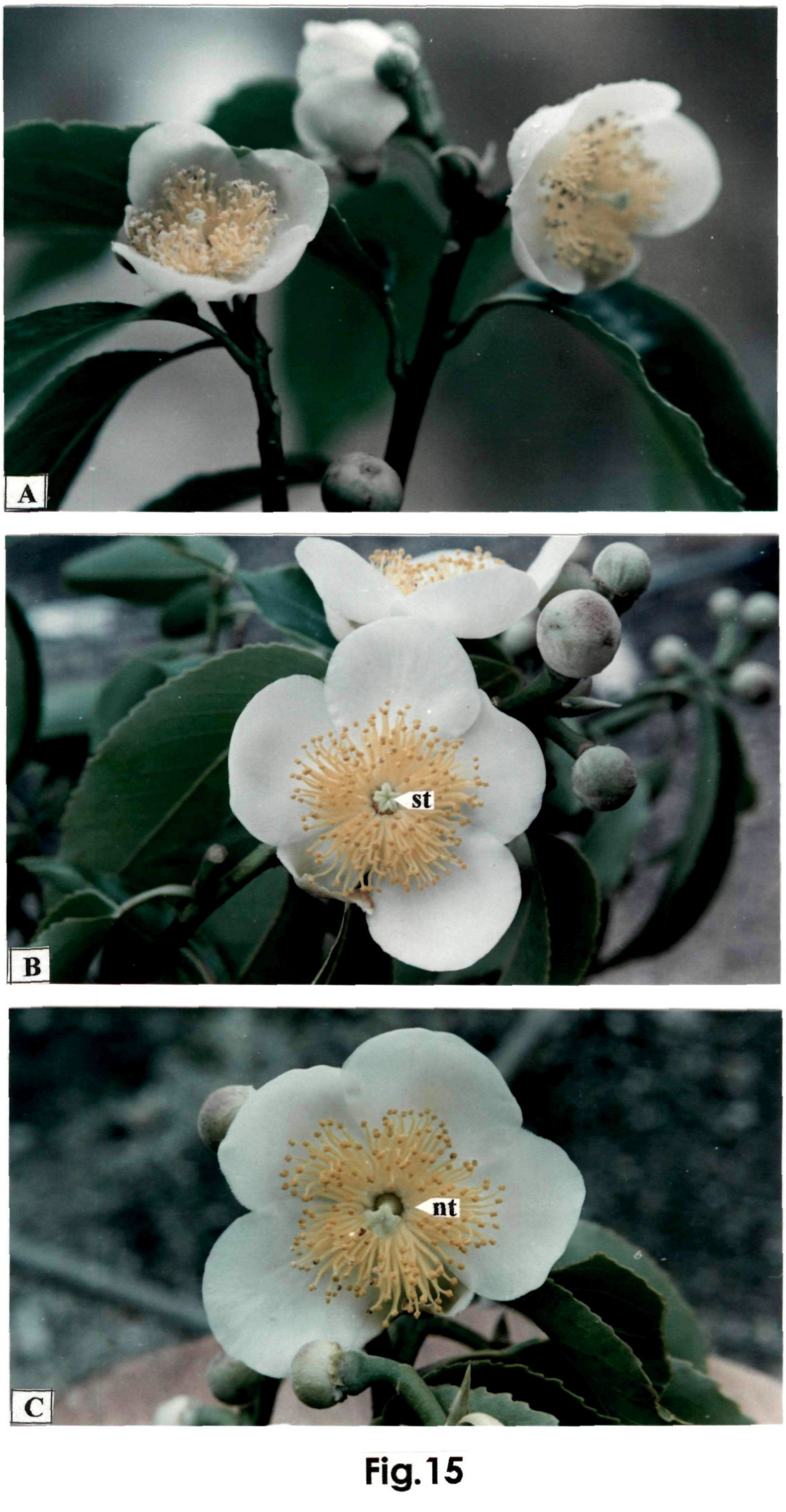


Fig.15

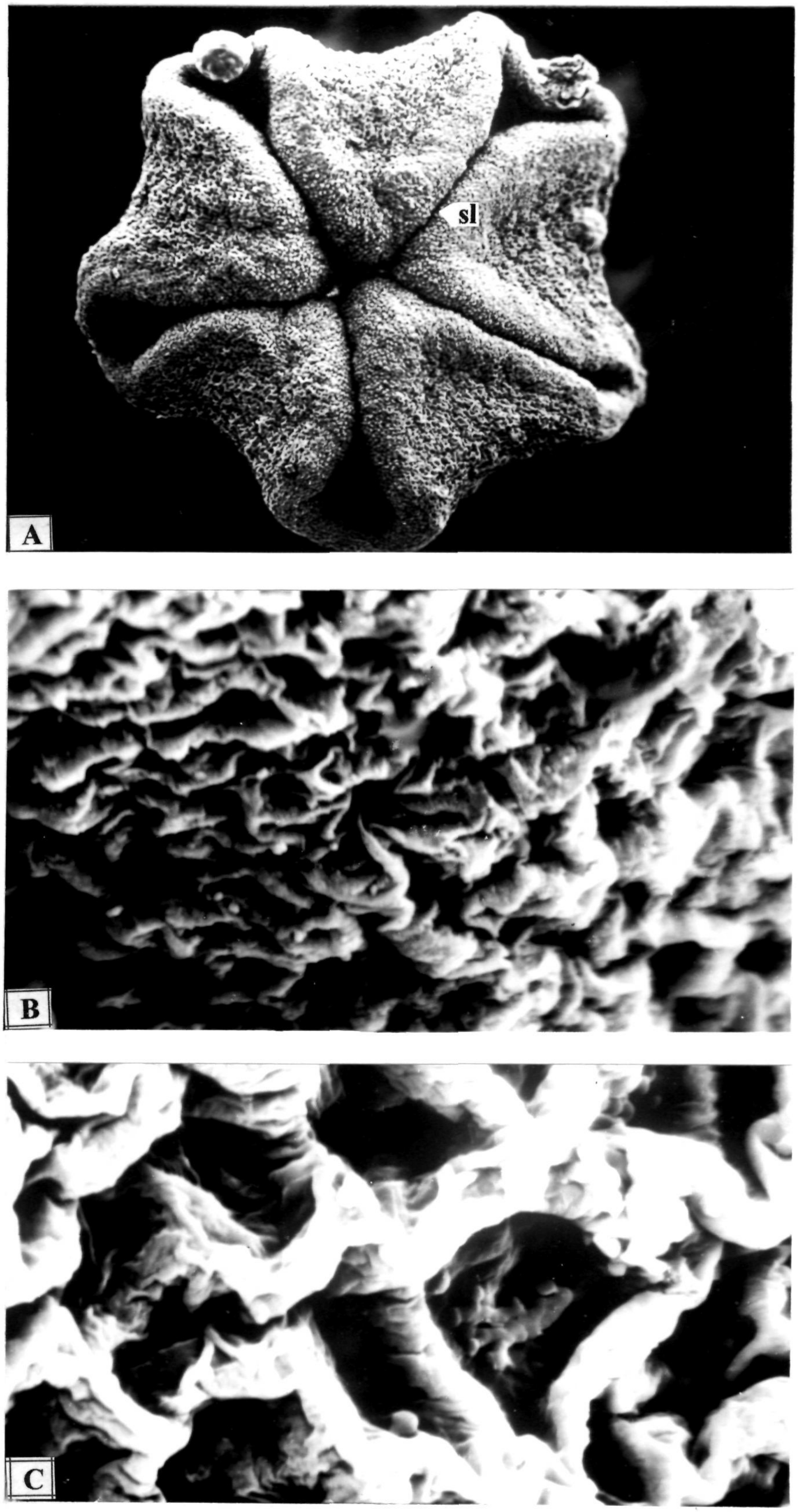


Fig.16

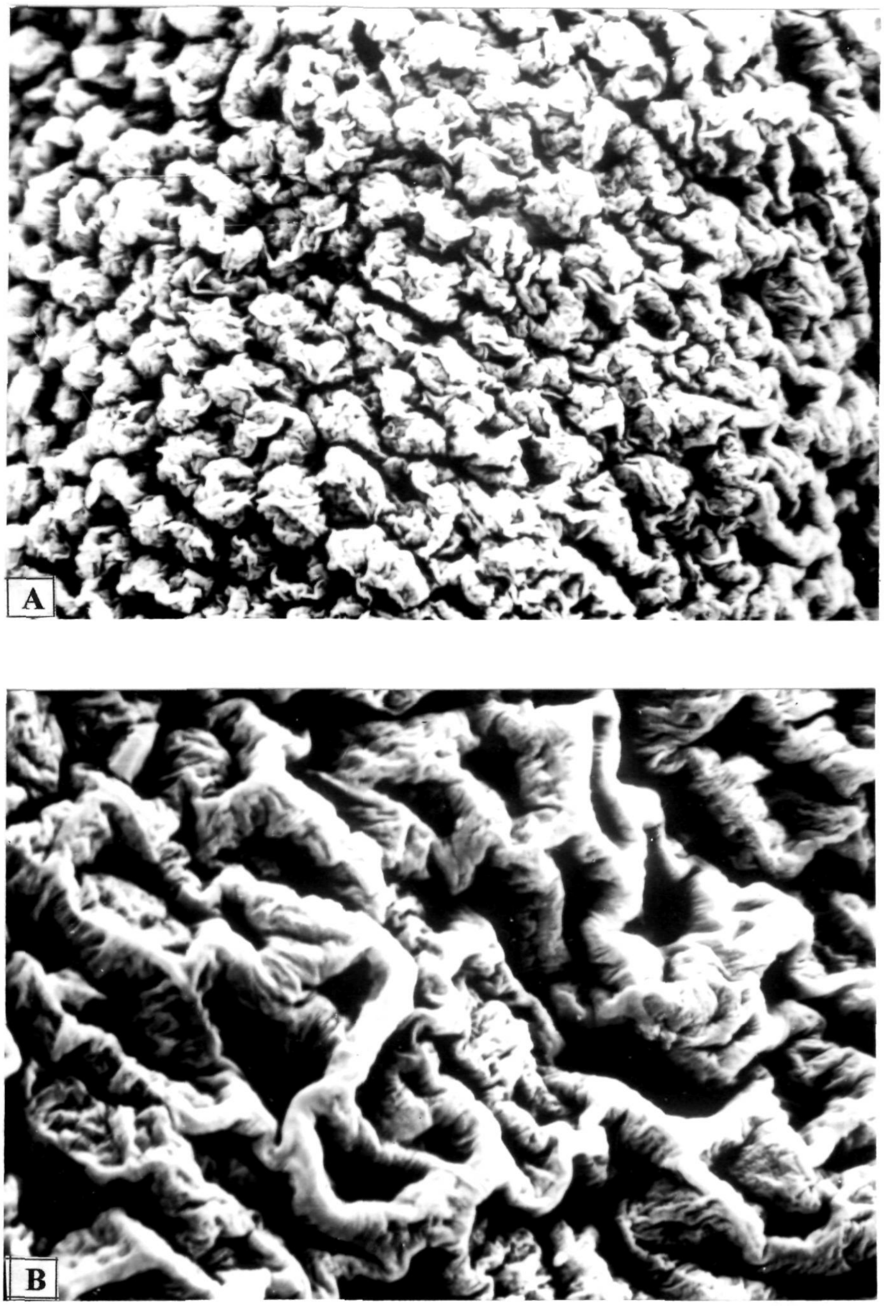


Fig.17

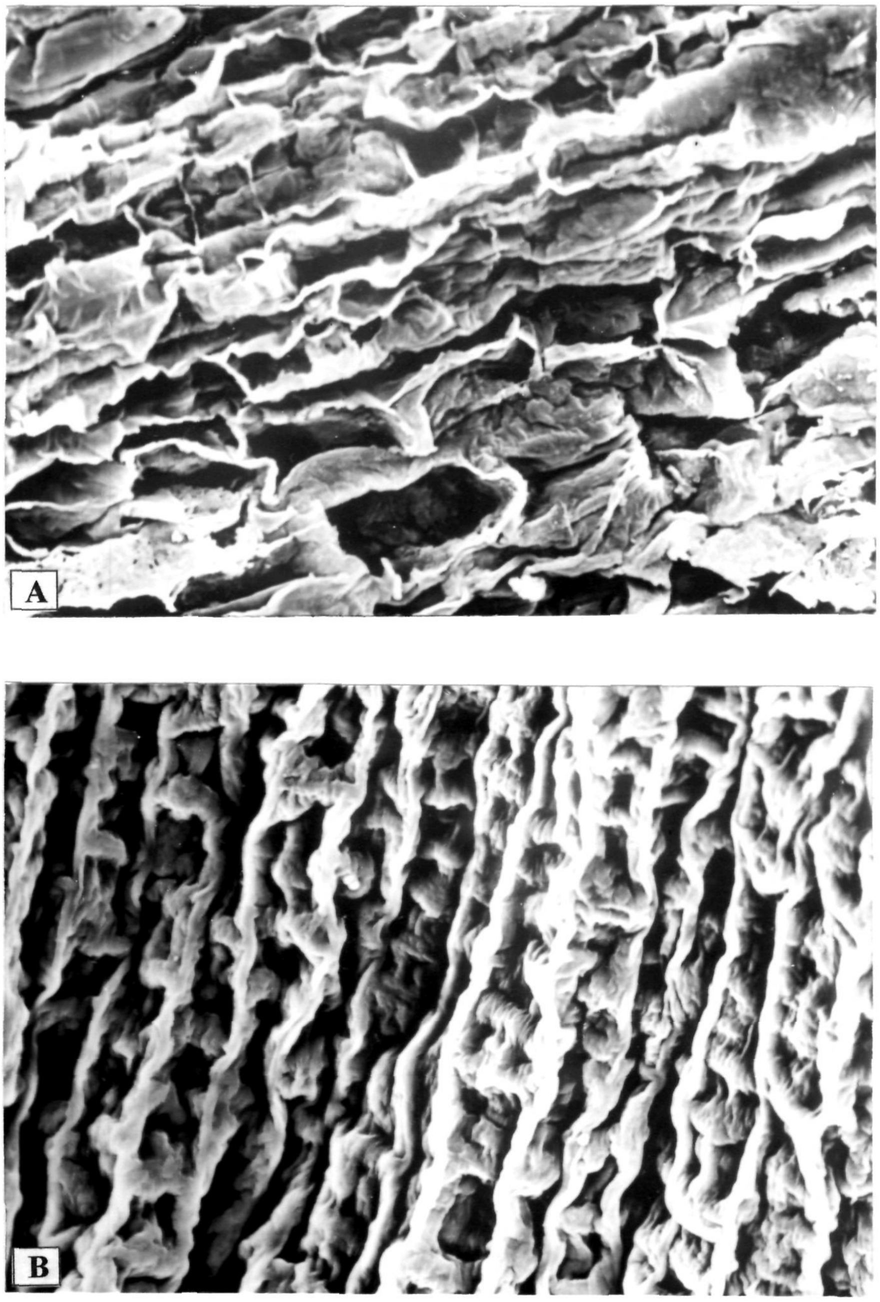


Fig.18

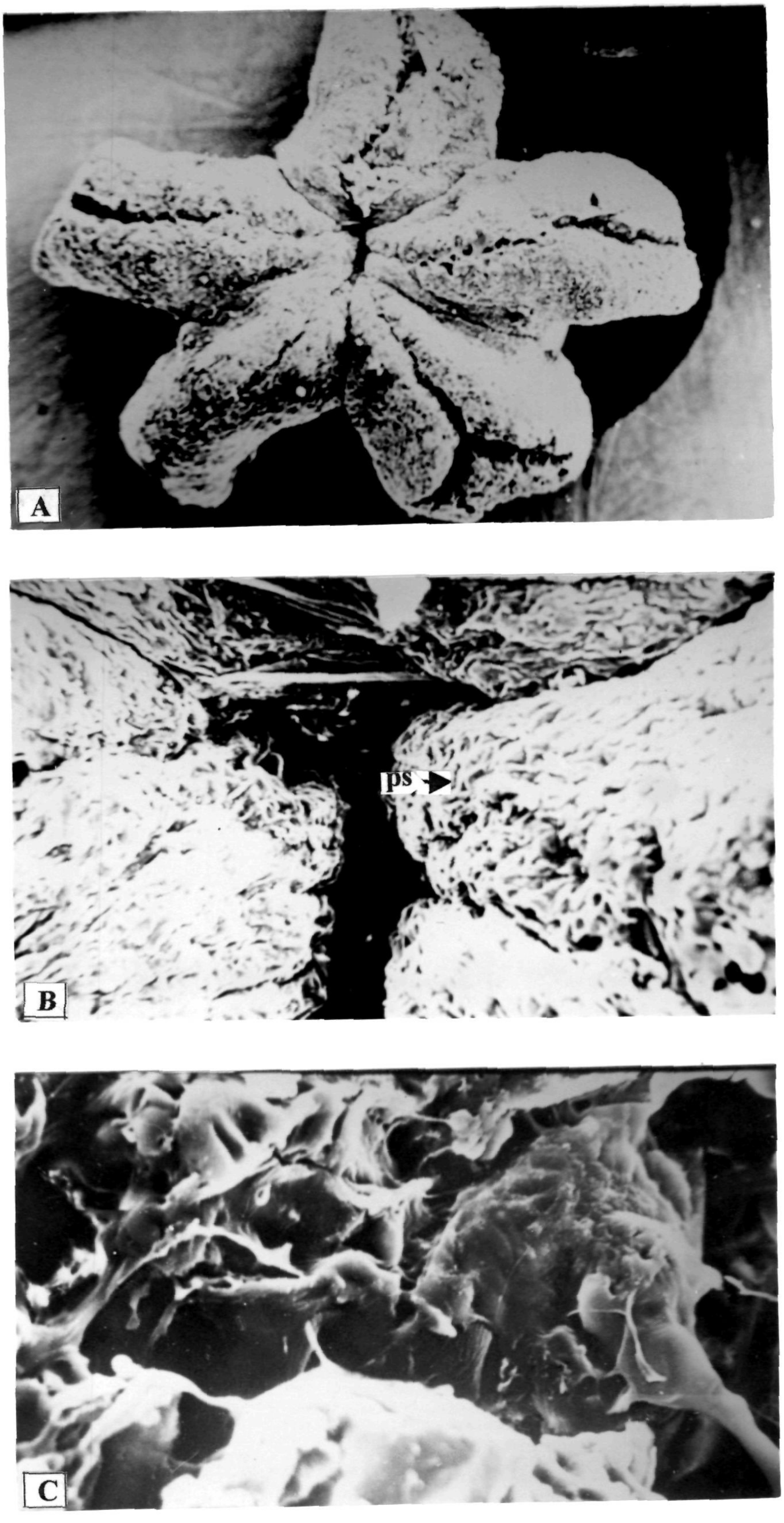


Fig.19

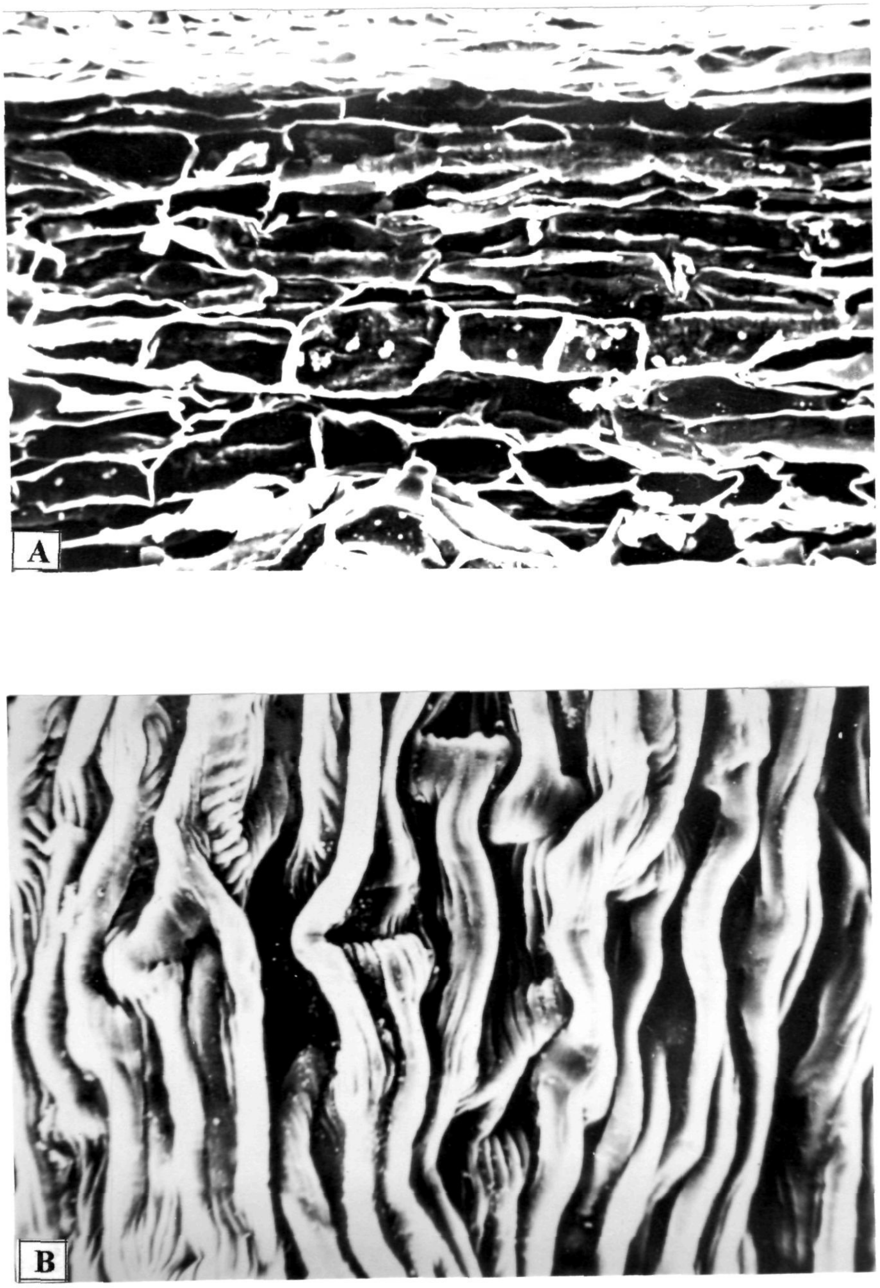


Fig.20

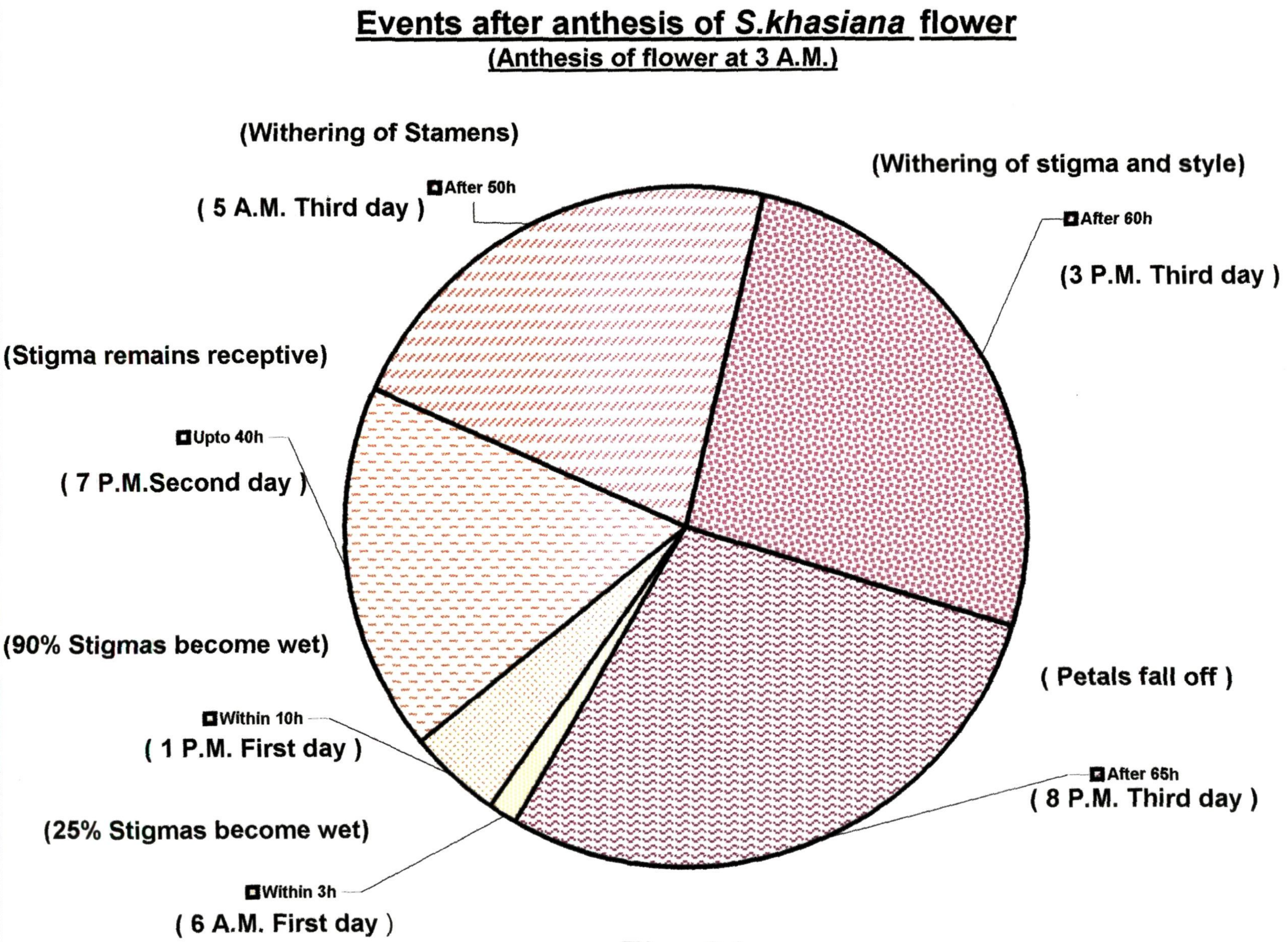

Events after anthesis of S.khasiana flower
(Anthesis of flower at 3 A.M.)
(Withering of Stamens)
After 50h
(5 A.M. Third day)
(Withering of stigma and style)
After 60h
(3 P.M. Third day)
(Stigma remains receptive)
Upto 40h
(7 P.M.Second day)
(90% Stigmas become wet)
(Petals fall off)
Within 10h
(1 P.M. First day)
After 65h
(8 P.M. Third day)
(25% Stigmas become wet)
Within 3h
(6 A.M. First day)

Fig. 21

POLLEN GERMINATION

Data on the effects of sucrose, boron, calcium, magnesium, potassium and agar presented in table 4 show that pollen germination pollen tube growth were significantly higher at 15% sucrose, 50 ppm boron, 300 ppm calcium, 300 ppm magnesium, 150 ppm potassium and 0.8% agar than other concentrations. Data on the effect of temperature on pollen germination and tube elongation is given in Table 5. It is evident from the results that 25°C was the optimum temperature for pollen germination and pollen tube elongation in *S.khasiana*. (Table 5).

In order to determine the optimum pH for pollen germination pollens were incubated at different pH and results are given in Table 6. Maximum pollen germination (51.99%) and tube length (302.70 μm) were observed at pH 7.3.

Pollen grains were germinated both in Brewbaker and Kwack (1963) and modified Brewbaker and Kwack (1963) media. Better germination of pollen (51.62%) and maximum pollen tube elongation (302.10 μm) was found in modified B and K medium (Figs. 22A,B, 23A,B, 24).

The effect of light quality on pollen germination and tube elongation in *S.khasiana* revealed that white and yellow wavelength had no effect but violet, blue and green light were inhibitory to germination and tube growth. Red light caused significant stimulation of germination and tube growth over other light conditions (Table 7).

Pollen germinability and tube growth between different trees of *S.wallichii* and *S.khasiana* varied significantly (Tables 8,9). Further, pollen obtained from different flowers of the same tree both in *S.wallichii* and *S.khasiana* exhibited variation in germinability and elongation of pollen tube (Tables 10,11). Flower age had a bearing on the germinability of pollen and tube elongation in both the species. In *S.wallichii*, both germination and tube elongation rate increased upto 24 h after anthesis of the flower, but both declined 30 h after anthesis. Maximum pollen germination and tube elongation in *S.wallichii* occurred 24 h after anthesis. In case of *S.khasiana*, germination and tube elongation rate increased upto 18 h after anthesis, but both declined 24 h after anthesis and the decline were significant in comparison to the pollen obtained from flowers anthesised earlier than 24 h (Tables 10,11).

Pollen grains of *S.wallichii* and *S.khasiana* were stained with acetocarmine to determine their viability. Viable pollen grains were stained deep red stain (Figs. 22C, 23C). Non-viable pollen grains did not take stain. Viability percentage varied in both the species (Table 12). In Brewbaker and Kwack's medium, *S.wallichii* showed 26% germination while 41% pollen germinated in *S.khasiana* (Table 12). When modified Brewbaker and Kwack's medium was used the percentage germination increased to 36% in *S.wallichii* and to 52% in *S.khasiana* (Table 12).

Effect of temperature and relative humidity was studied up to one year (Table 13). At room temperature (20°C), 28% pollen grains remained viable at 0 and 32% RH after one month in *S.wallichii*. Viability percentage decreased to 24% at RH 98%. After six months, there was a marked decrease in viability at all temperature and RH regimes except at -5°C. After one year almost all pollen became non-viable at all temperature and RH regimes except at -5°C when 20% pollen retained their viability (Table 13).

When pollen grains were kept in refrigerator (-5°C), after one month more pollen grains (30%) were found to be viable as compared to pollen grains stored at RH 32 and 98%. After 6 months, pollen grains stored at 0% RH gave better result and 24% pollen grains remained viable as compared to 15 and 12% at RH 32 and 98% respectively. After one year pollen grains remained viable at all the three RH percentage but maximum viability was observed at 0% RH.

In *S.khasiana* also, similar results of effect of temperature and relative humidity percent were obtained (Table 13). 0% RH and pollen stored in refrigerator were found to be best for increasing pollen viability.

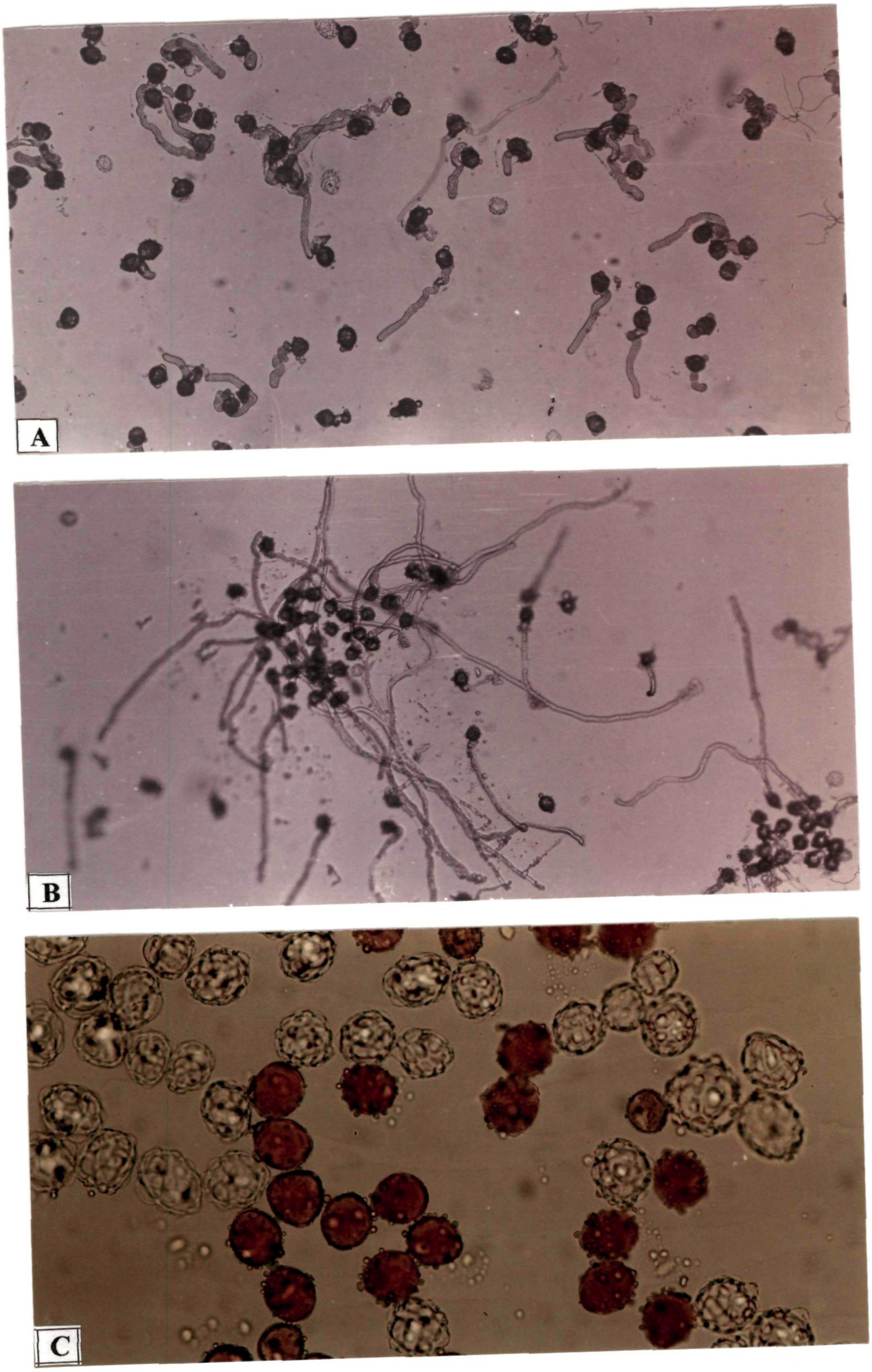


Fig.22

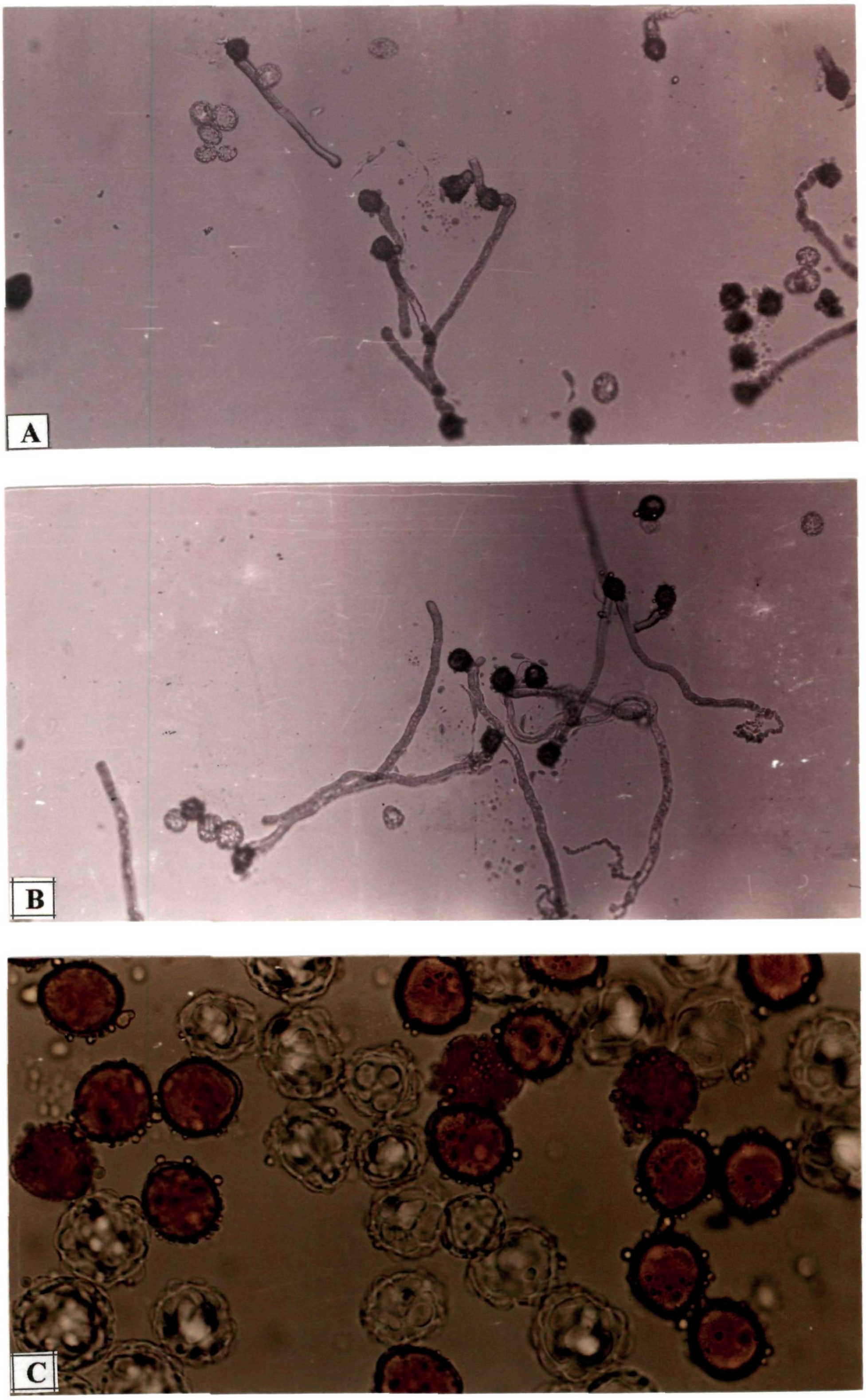
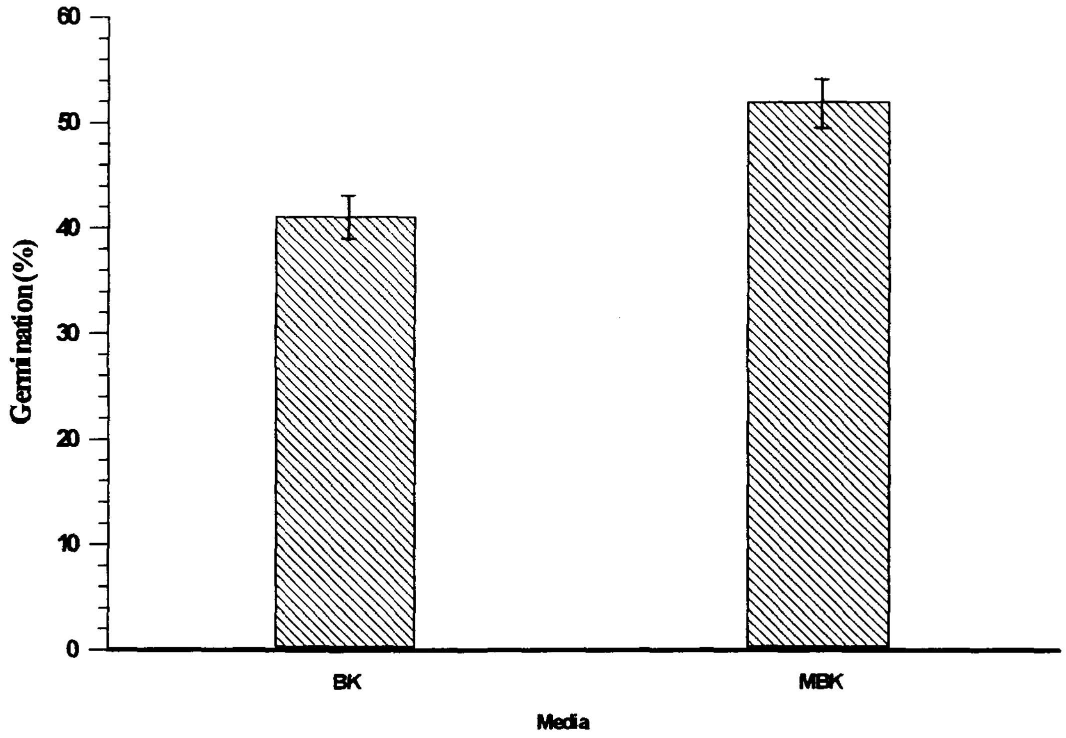
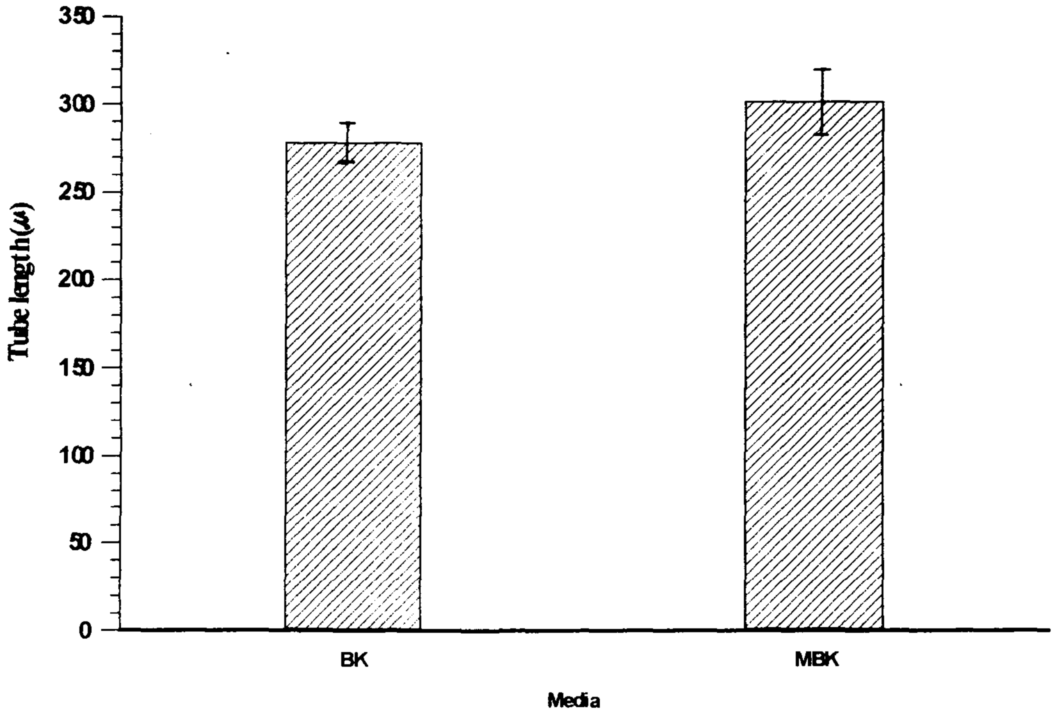


Fig.23



Comparison of *S.khasiana* pollen germination in B.K. and M.B.K. media



Comparison of *S.khasiana* pollen tube growth in B.K. and M.B.K. media

Fig.24

Table 4: Pollen germination and pollen tube elongation in *Schima khasiana*.

Factor	Pollen germination (%)	Tube elongation (µm)
SUCROSE (%)		
5	25.74 ± 1.27	175.63 ± 9.52
10	39.55 ± 1.42	293.85 ± 11.03
15	41.24 ± 1.81[a]	314.70 ± 13.84[a]
20	19.00 ± 1.61	108.60 ± 12.55
LSD (p=0.05)	4.98	36.8
BORON (ppm)		
50	46.85 ± 1.52[a]	293.40 ± 11.43[a]
100	42.52 ± 1.11	263.55 ± 6.63
150	28.31 ± 1.92	177.80 ± 11.82
200	14.37 ± 0.95	90.45 ± 5.65
LSD (p=0.05)	4.14	30.24
CALCIUM (ppm)		
100	10.82 ± 0.40	83.40 ± 3.54
200	17.23 ± 1.27	142.05 ± 3.35
300	46.81 ± 1.50[a]	307.20 ± 7.29[a]
400	15.66 ± 0.83	150.75 ± 9.02
LSD (p=0.05)	3.12	17.01
MAGNESIUM (ppm)		
100	15.92 ± 1.33	116.70 ± 17.84
200	21.48 ± 1.07	142.20 ± 5.91
300	48.51 ± 2.39[a]	297.30 ± 4.20[a]
400	41.86 ± 1.07	266.55 ± 2.62
LSD (p=0.05)	5.07	30.56
POTASSIUM (ppm)		
50	26.25 ± 1.00	199.35 ± 13.37
100	47.23 ± 2.31	297.60 ± 2.82
150	47.62 ± 3.42[a]	297.90 ± 7.05[a]
200	27.76 ± 2.81	194.10 ± 5.62
LSD (p=0.05)	9.36	23.3
AGAR (%)		
0.4	16.28 ± 0.67	66.60 ± 4.08
0.6	49.75 ± 0.95	293.70 ± 4.91
0.8	52.65 ± 2.62[a]	310.50 ± 7.40[a]
1.0	26.00 ± 1.44	177.60 ± 9.27
LSD (p=0.05)	5.11	20.56

± = SE [a] Significantly different from other values of a given treatment.
(Significance tested with one way ANOVA)

Table 5: Effect of temperature on pollen germination and tube elongation in *Schima khasiana.*

Temperature (°C)	Germination (%)	Tube elongation (μm)
20	20.82 ± 0.87	137.55 ± 6.05
25	40.75 ± 3.49[a]	303.60 ± 7.05[a]
30	33.51 ± 1.83	295.65 ± 5.71
35	NIL	NIL

± = SE [a] Significantly different from others treatments.
Significance tested with student's ' t ' test (p= 0.05)

Table 6: Effect of pH on pollen germination and tube elongation in *Schima khasiana*.

pH	Germination (%)	Tube elongation (µm)
6.0	23.96 ± 0.97	99.15 ± 14.84
7.0	44.05 ± 1.74	277.35 ± 7.92
7.3	51.99 ± 2.96[a]	302.70 ± 7.40[a]
8.0	26.74 ± 2.21	159.45 ± 7.28
LSD (p =0.05)	6.99	30.81

± = SE [a]Significanly different from others treatments
Significance testified with students ' t ' test (p = 0.05)

Table 7: Effect of light of different colours on pollen germination and tube elongation in *Schima khasiana*.

Light colour	Pollen germination (%)	Tube elongation (µm)
Dark	47.77 ± 0.35[a]	196.05 ± 12.93[a]
White	50.49 ± 0.68	291.45 ± 05.76
Violet	46.60 ± 1.69[a]	227.40 ± 14.90[a]
Blue	47.28 ± 2.49[a]	232.20 ± 14.18[a]
Green	46.04 ± 5.20[a]	266.40 ± 16.05[a]
Yellow	51.01 ± 0.14	271.40 ± 10.35
Red	66.89 ± 2.98[ab]	324.40 ± 05.26[ab]
LSD (p = 0.05)	2.32	20.88

± = SE [a] Significantly different from white light
[b] Significantly different from dark and light of all other colours.
(Significance tested with ANOVA)

Table 8: Pollen germination and tube growth in pollen obtained from different trees of *Schima wallichii*.

Source of pollen	Germination		Tube growth	
	(%)	Range	(µm)	Range
1	34.09 ± 3.12 [a]	28.12 - 45.73	317.88 ± 24.01 [a]	277.20 - 408.60
2	29.49 ± 2.07	25.38 - 36.31	220.56 ± 4.51	208.20 - 232.80
3	39.96 ± 1.23 [a]	37.64 - 44.50	513.96 ± 23.91 [a]	435.60 - 565.80
4	24.91 ± 1.19	22.69 - 28.35	174.96 ± 6.45	162.00 - 195.60
LSD (p =0.01)	4.81		48.87	

± = SE

[a] Significantly different from others.

Table 9: Pollen germination and tube growth in pollen obtained from different trees of *Schima khasiana*

Source of pollen	Germination		Tube growth	
	(%)	Range	(µm)	Range
1	39.52 ± 1.72	36.32 - 44.34	286.05 ± 7.80	268.80 - 306.00
2	32.91 ± 1.42	30.00 - 36.36	251.25 ± 8.14	228.00 - 263.40
3	49.36 ± 1.84 [a]	45.37 - 54.27	326.00 ± 4.97 [a]	294.60 - 338.00
4	43.78 ± 0.94	41.56 - 45.75	301.05 ± 4.39	294.00 - 313.80
5	51.35 ± 1.81 [a]	47.78 - 55.69	373.05 ± 5.24 [a]	298.80 – 342.80
LSD (p = 0.01)	5.13		21.45	

± = SE

[a] Significantly different from others.

Table 10: Effect of flower age on pollen germination and tube growth in *Schima wallichii*.

Age from the time of anthesis	Germination (%)	Tube growth (μm)
6	24.20 ± 2.41	166.68 ± 23.93
12	26.18 ± 0.95	243.96 ± 20.43
18	33.38 ± 0.65 [a]	326.04 ±14.41 [a]
24	39.90 ± 1.23[ab]	513.96 ± 23.91 [ab]
30	23.07 ± 1.72	369.00 ± 8.27
LSD (p =0.05)	4.57	58.83

± = SE

[a] Significantly different from No. 6, 12and 30h old flowers.
[b] Significantly different from others.

Table 11: Effect of flower age on pollen germination and tube growth in *Schima khasiana*.

Age from the time of anthesis	Germination (%)	Tube growth (μm)
6	46.96 ± 1.06[a]	229.80 ± 4.88 [a]
12	51.24 ± 1.97 [a]	260.85 ± 10.88 [a]
18	61.38 ± 0.71[ab]	309.75 ± 10.83 [ab]
24	30.65 ± 2.66	185.70 ± 12.31
30	25.85 ± 1.88	136.95 ± 5.65
LSD (p = 0.05)	6.11	34.23

± = SE

[a] Significantly different from 24 & 30 h old flowers.
[b] Significantly different from others.

Table 12: Pollen viability (%) and its germination (%) in B.K. and M.B.K. media.

Species	**Viability (%)***	**Germination (%)**	
		B.K. medium	**M.B.K. medium**
S. wallichii	42.00 ± 1.26	26.13 ± 0.84	36.03 ± 1.88
S. khasiana	57.21 ± 1.08	41.00 ± 1.8	51.62 ± 2.19

± SE ***(Acetocarmine staining test).**

Table 13: Effect of temperature (°C) and RH (%) on viability of stored pollens of *Schima* species.

Species	Temperature (°C)	RH (%)	Pollen viability (%)		
			After one month	After six months	After one year
S.wallichii	**Room (20 ± 2°C)**	**0**	28 ± 1.26	8 ± 0.92	8 ± 0.30
		32	28 ± 1.08	6 ± 0.83	0
		98	24 ± 0.84	3 ± 0.67	0
	Refrigerator(-5 ± 1°C)	**0**	30 ± 1.33	24 ± 0.84	20 ± 0.95
		32	28 ± 0.87	15 ± 0.91	8 ± 1.00
		98	25 ± 1.88	12 ± 1.62	3 ± 0.83
S.khasiana	**Room (20 ± 2°C)**	**0**	50 ± 1.11	27 ± 2.31	7 ± 1.07
		32	48 ± 1.92	24 ± 1.33	2 ± 0.40
		98	40 ± 0.95	17 ± 1.08	0
	Refrigerator(-5 ± 1°C)	**0**	53 ± 1.07	37 ± 0.95	32 ± 1.81
		32	50 ± 2.31	30 ± 1.44	14 ± 0.67
		98	47 ± 1.44	19 ± 1.07	2 ± 0.33

± SE

POLLINATION

In both *S.wallichii* and *S.khasiana* flowers are large and showy. Anthesis begins in the early hours of morning i.e. at 4 A.M. in *S.wallichii* and 3 A.M. in *S.khasiana*. The petals open slowly and the process continues till evening, when the anthesis is complete. Sufficient quantity of nectar is produced between petals and stamens. Stamens are indefinite in number and produce large number of sticky pollen grains. Both nectar and pollen grains attract insects that in search of food visit the flowers both during day and night.

Varied types of insects belonging to the order lepidoptera, diptera and hymenoptera visit the flowers. They may be categorised in two different types, one category of insects only forage anthers, while the other category feed on stigmatic exudate and nectar (Figs. 25A-C, 26A,B). Both types of insects move from flower to flower. The insects, which feed on stigmatic exudate and nectar, remain attached with the branches of the tree. Flies and bees, particularly the small carpenter bees (*Ceratina* sp.) were found to forage pollen and nectar through out the day. Formicidae (ants) were found to forage nectar and took little or no part in the transfer of pollens. Honeybees (*Apis mellifera*) occasionally visited flowers during afternoon.

The insects during their visit to different flowers came in contact with dehisced anthers. It was observed that the anthers away from the pistil were first to dehisce followed by sequential dehiscence from periphery to the centre. This may be viewed as adaptation to facilitate pollination by making pollen grains available to different categories of insects visiting at different time. Pollen grains being sticky in nature got stuck to the body of the visiting insects. When the pollen grains of the same flower were dusted on the stigma of the same flower, they did not germinate or germination was very poor.

Fig.25

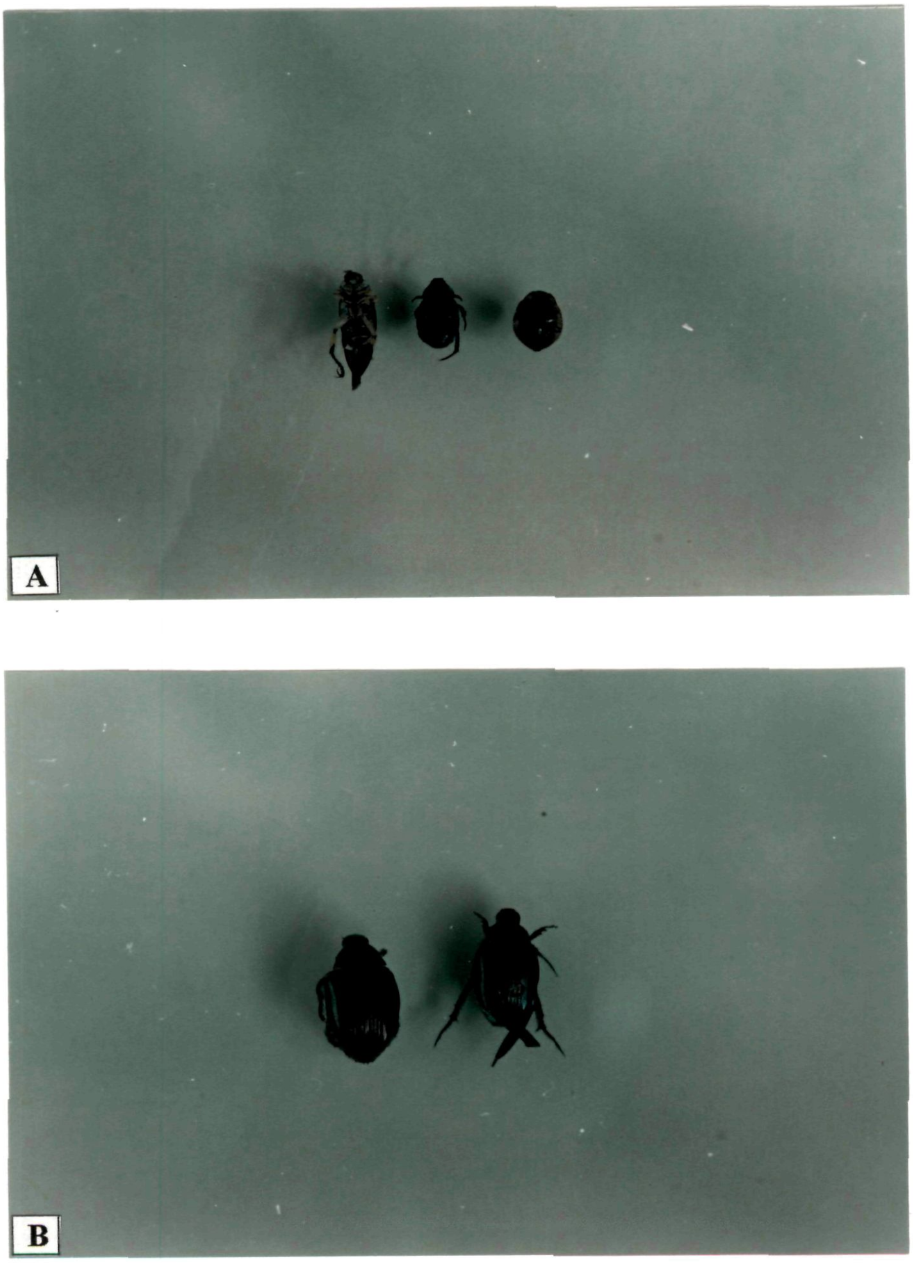


Fig.26

POLLEN - PISTIL INTERACTION

Schima wallichii

Pollen grains are spherical (18 ± 04 μm diameter). Under SEM, they show reticulate ornamentation (Fig. 27). Vectors carry the pollens to the stigma surface which is of wet type (Fig. 28A,B,C). The stigmatic exudate appears as a glistening drop on stigma surface, 2 h after anthesis in nearly 25% flower and 90% stigma is wet in 8 h after anthesis. Amount of stigmatic secretion increases with the passage of time and varies between different flowers. Stigma remains receptive for about 36 h after anthesis. The disappearance of stigmatic exudate marks the end of stigma receptivity. Simultaneously with the wetting of the stigma, nectar appears at the base of the ovary suggesting presence of nectary. Withering of the stamens occurs 42 h after anthesis (Fig. 13). Both stigma and style wither almost 48 h after anthesis and petals fall off almost 50 h after anthesis indicating the end of life span of different parts of a flower (Fig. 13).

Pollen grains after landing on the stigma hydrate quickly. The pollen tubes emerge from the pollen aperture adjacent to the stigma surface within 3 h after pollination (Fig. 29A). All pollen grains do not germinate. Pollen tubes pass through the intercellular space between papillae and grow along the surface of the transmitting tissues of the style. Pollen tube growth was fast during the first 12 h after pollination (Fig. 29B). Pollen tubes vary in width and number of callose plugs. Callose plugs are formed at irregular intervals in the pollen tube all along the length of style. As a result, intensity of fluorescence of pollen tube and callose plug varies. Pollen tubes at the upper part of the style lose fluorescence quickly while in the lower part they fluoresce for a longer period.

No abnormalities in pollen-tube growth such as meandering tubes, irregular tubes and forked tips were found. Most pollen tubes reach the ovule. However, a few pollen tubes with swollen tips become arrested in the lower portion of the ovary.

Majority of the pollen tubes penetrate between papillae. The first few pollen tubes were observed to reach the embryo sacs 96 h after pollination

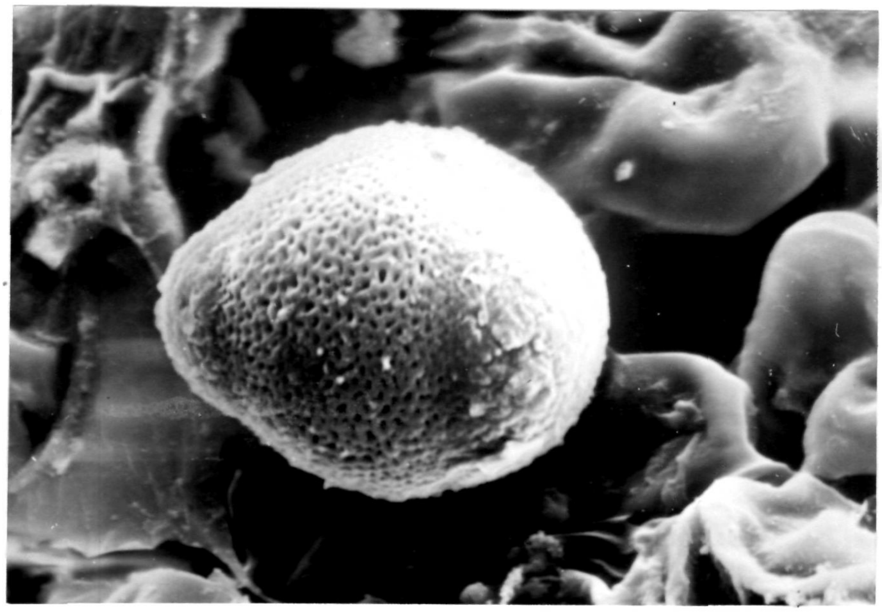


Fig.27

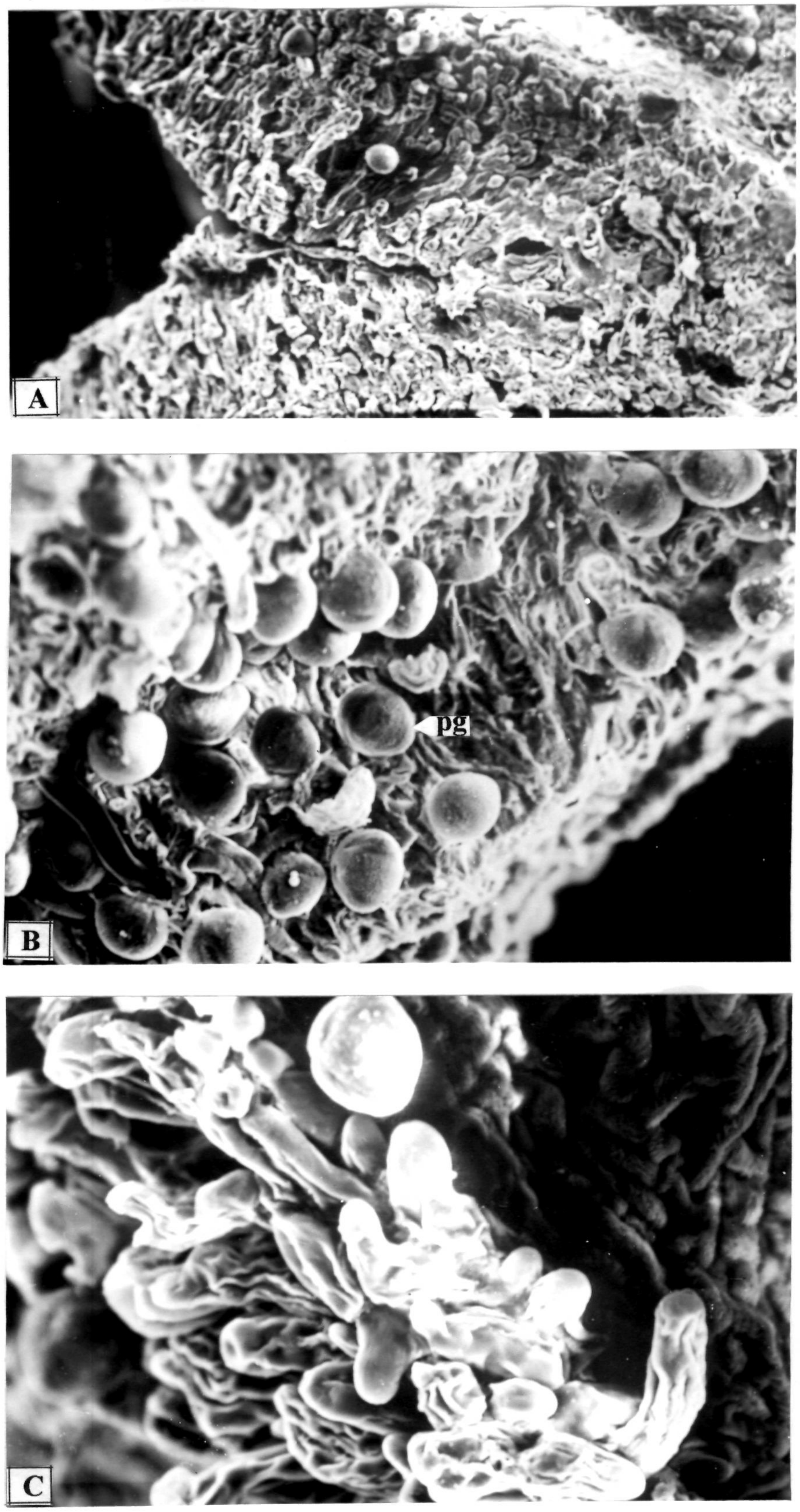


Fig.28

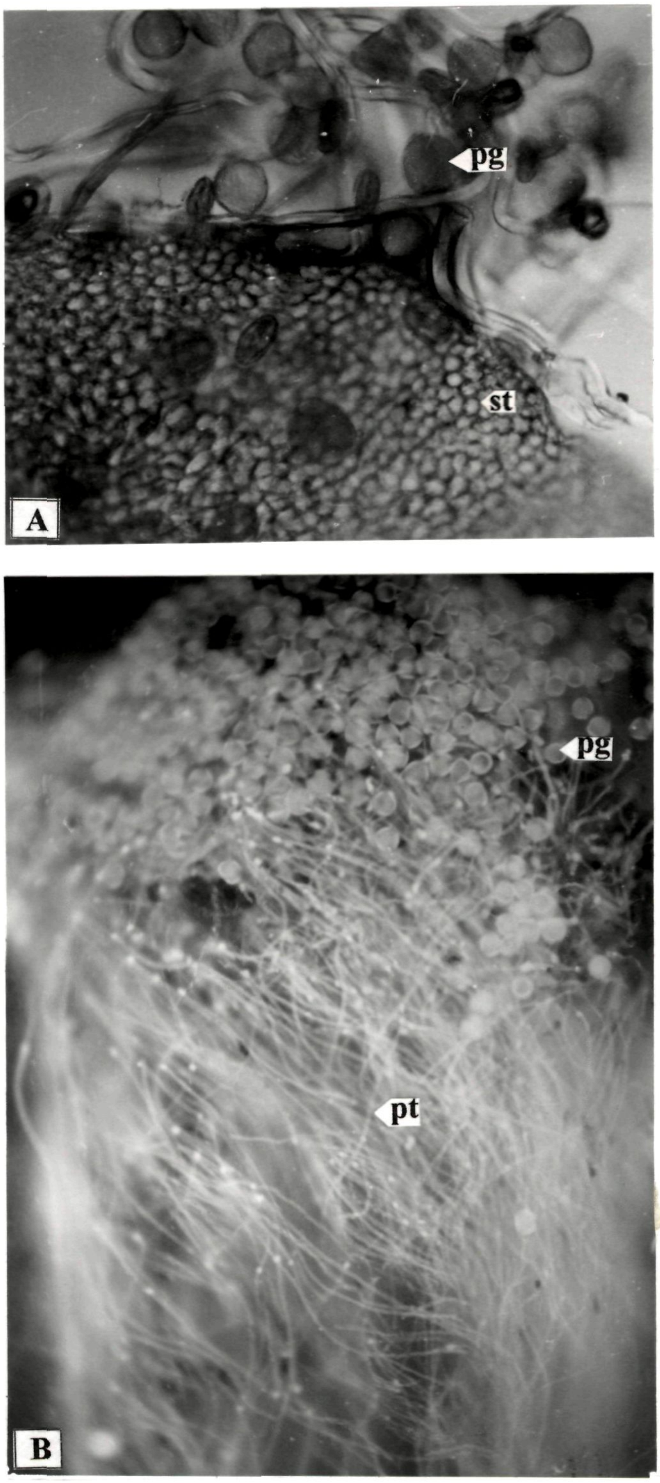


Fig.29

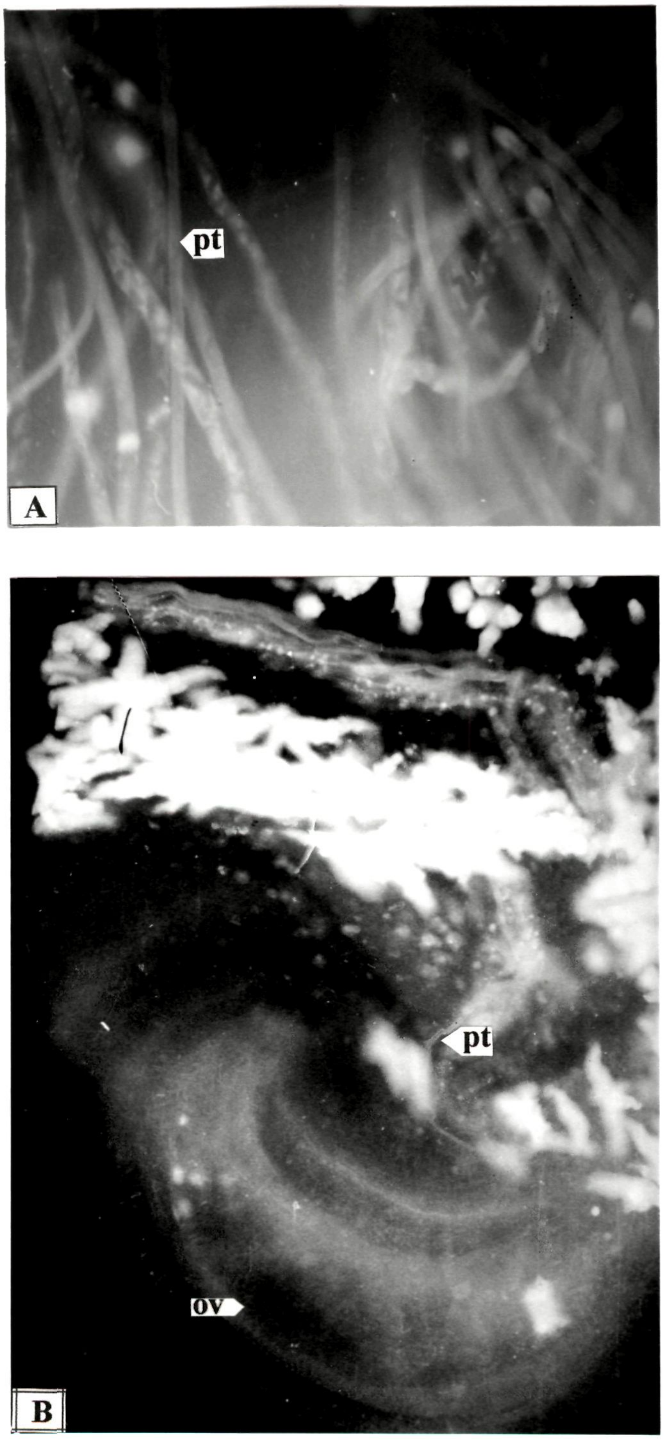


Fig.30

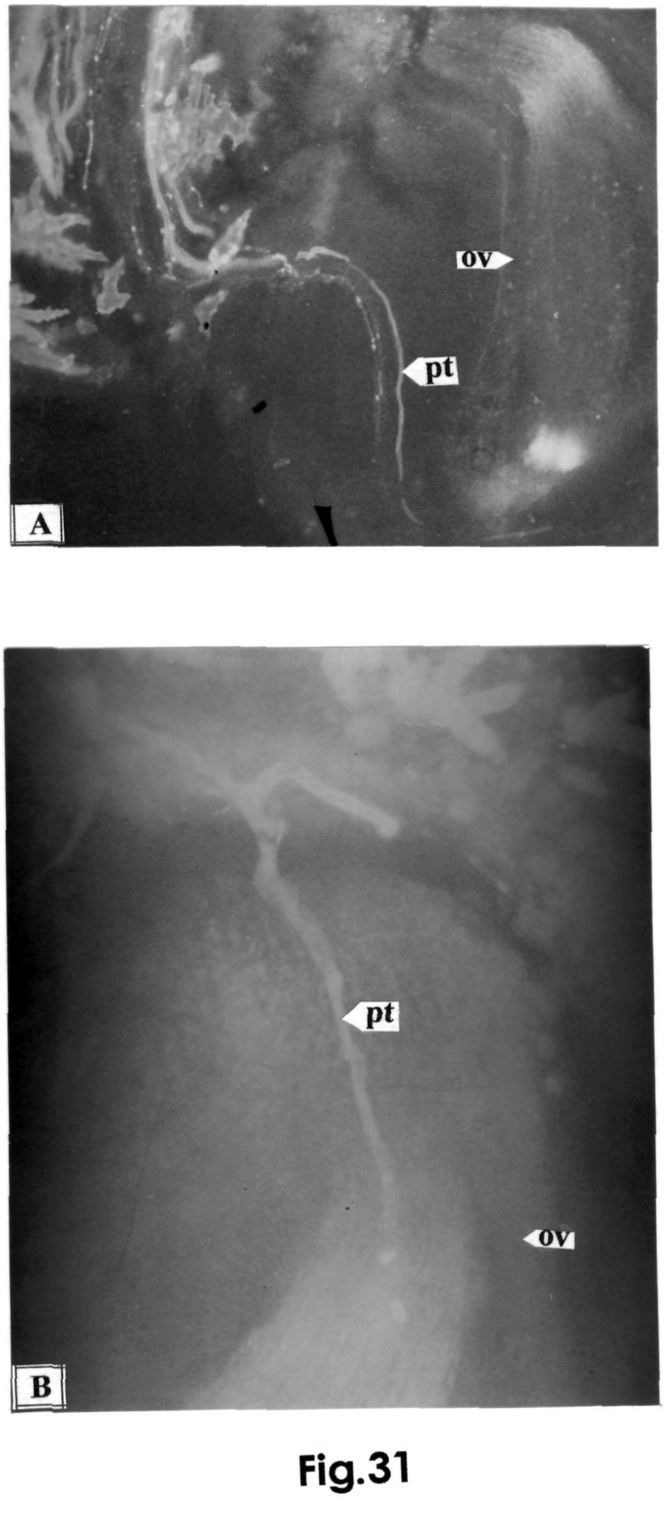


Fig.31

(Fig. 30 A, B). Between 96 and 120 h period, most of them had reached the ovule/embryo sacs (Fig. 31A, B). Beyond this time until 240 h after pollination, number of pollen tubes entering the ovule/embryo sac did not increase.

The fluorescence test shows that the receptive synergid fluoresces more brightly and fertilization occurs when the pollen tube tip fuses with the fluorescing synergid. This is followed by the appearance of fluorescing debris at the chalazal end of the synergid, indicating degeneration of the synergid wall.

Schima khasiana

In this species the stigmatic exudate appears, as a glistening drop on stigma surface, in nearly 25% flower 3 h after anthesis and 90% stigma is wet 10 h after anthesis. Amount of stigmatic secretion increases with the passage of time and varies between stigma of different flowers. Stigma remains receptive for about 40 h after anthesis. The stigma loses its receptivity with the disappearance of stigmatic exudate. At the time of wetting of the stigma, nectar appears at the base of the androecium, near the ovary. Withering of the stamen occurs 50 h after anthesis. Stigma and style wither almost 60 h after anthesis and petals fall off about 65 h after anthesis.

Pollen grains begin to germinate after reaching the stigma. Most of the pollen grains adhere on the upper surface of the stigma (Fig. 32A) and only a few pollen grains were observed on the lowerside of the stigma (Fig. 32B). Pollen grains hydrate quickly after landing on the stigma (Fig. 33A). The pollen tubes emerge within 6 h after pollination (Fig. 33B, 34A,B). A few pollen grains, including the pseudopollen grains do not germinate on the stigma. The pollen tubes pass through the inter cellular spaces between papillae (Fig. 34B) and grow along the surface of the transmitting tissues of the style. Pollen tube growth was very fast during the first 24 h after pollination (Fig. 35A). In the transmitting tissue, pollen tubes vary in width and number of callose plugs. Callose plugs are formed sporadically in the pollen tube all along the style length. As a result, the intensity of the pollen tube and callose plug fluorescence varies along the style length. While the pollen tubes at the upper part of the style lose fluorescence, the tubes in the lower part continues to fluoresce.

Abnormalities in pollen tube growth such as pollen tube arrest in the style, reversal in growth direction, irregular growth by thickening in some part of the tube, bending of the tube tip in the micropyle, forming a hook shaped tube tip after entering the micropyle, forked tips etc. were not observed. However, some cases swelled tips are arrested in the lower portion of the ovary and only a few pollen tubes are able to reach the ovule/embryosac.

The first few pollen tubes were observed to reach the base of the style 96 h after pollination (Fig. 35B). they reached the ovary 168 h (Fig. 36A) the ovule/embryosac 196 h after pollination (Fig. 36B). Between 192 and 216 h, most of the pollen tubes had reached the ovule/embryosacs. Observation of the pistils 240 h after pollination indicated that number of pollen tubes entering the ovule/embryosac did not increase after 216 h (Fig. 37).

The receptive synergid fluoresces more brightly and fertilization occurred when the pollen tube tip fused with the fluorescing synergid. This is followed by the appearance of fluorescing debris at the chalazal end of the synergid, indicating degeneration of the synergid cell wall. Subsequently, both synergids degenerate as the endosperm begins to develop.

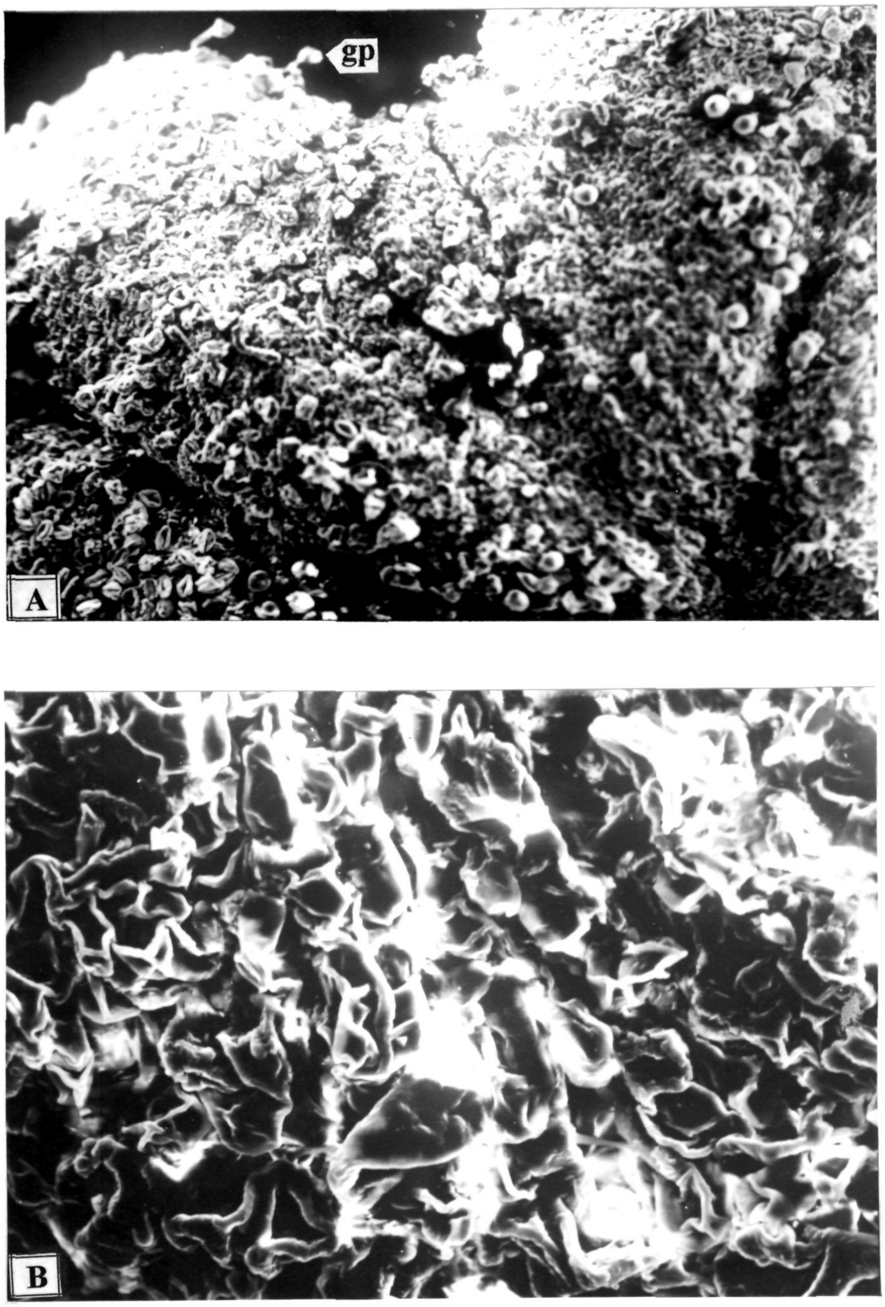


Fig.32

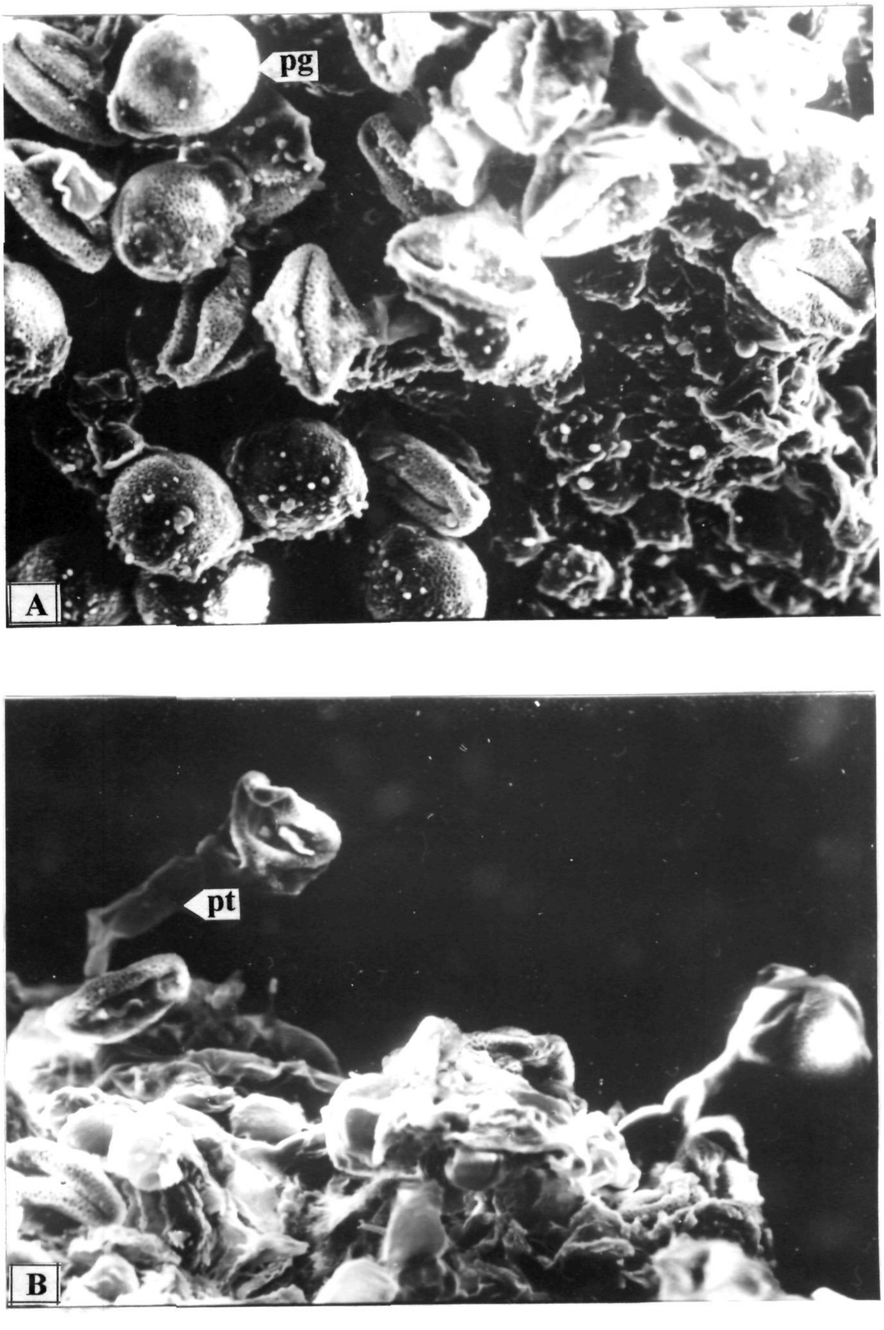


Fig.33

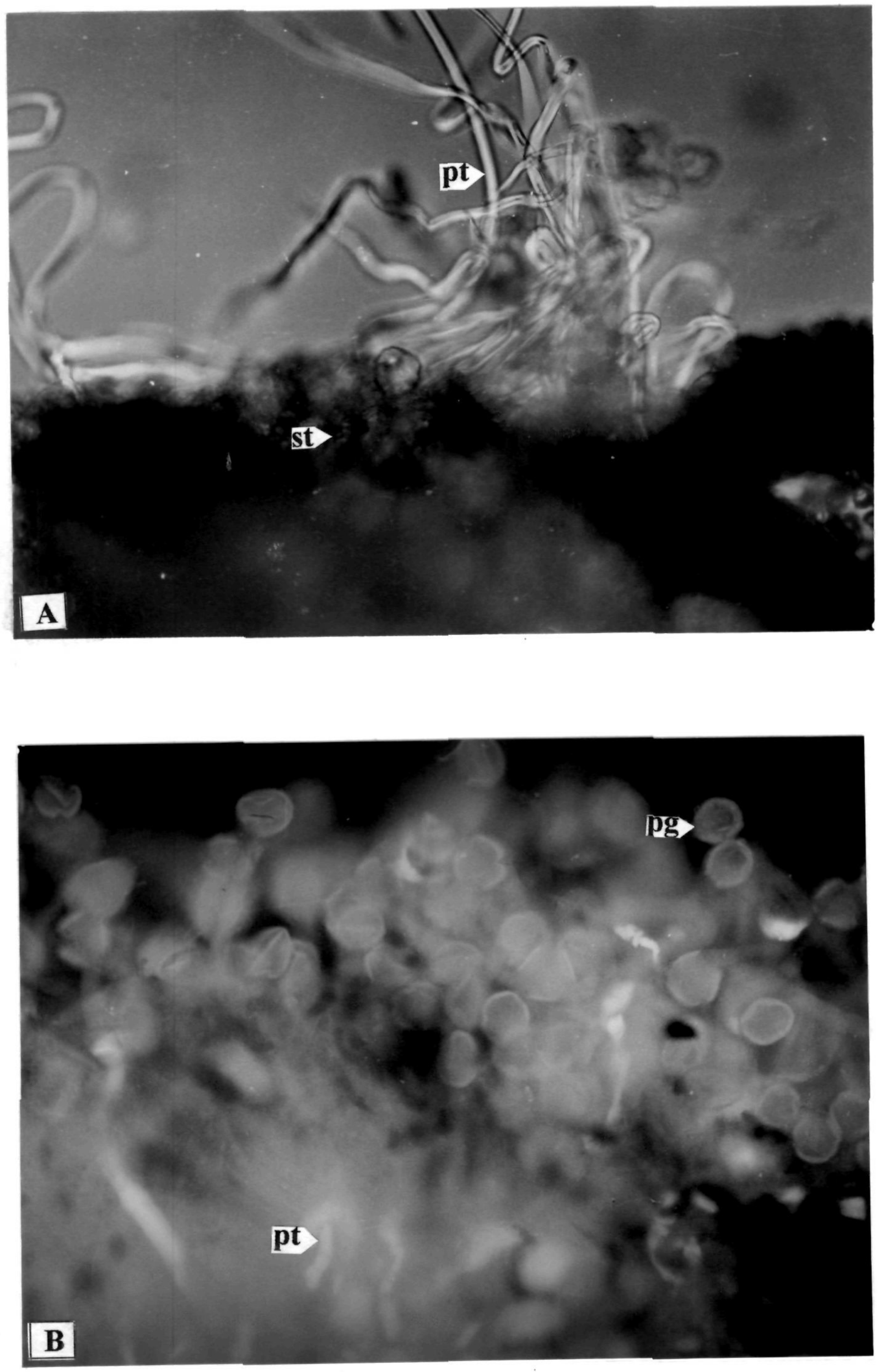


Fig.34

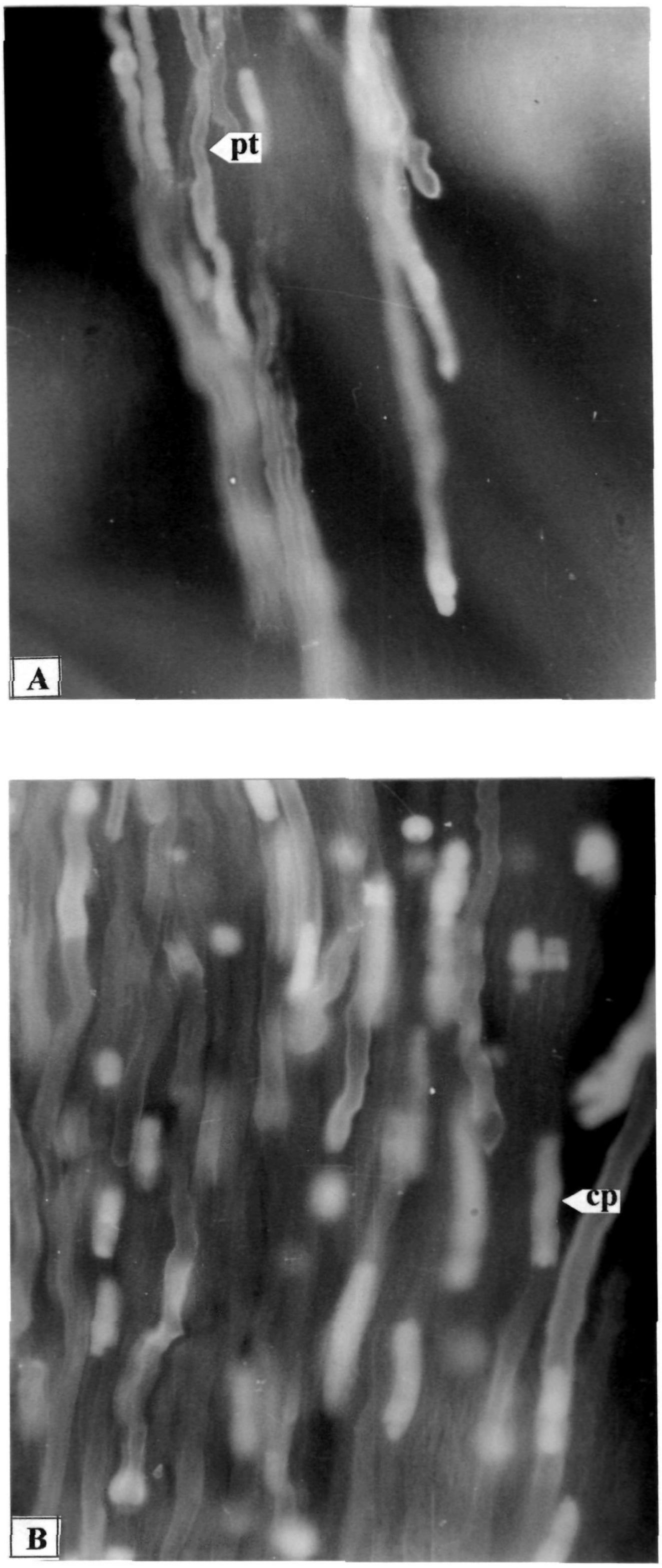


Fig.35

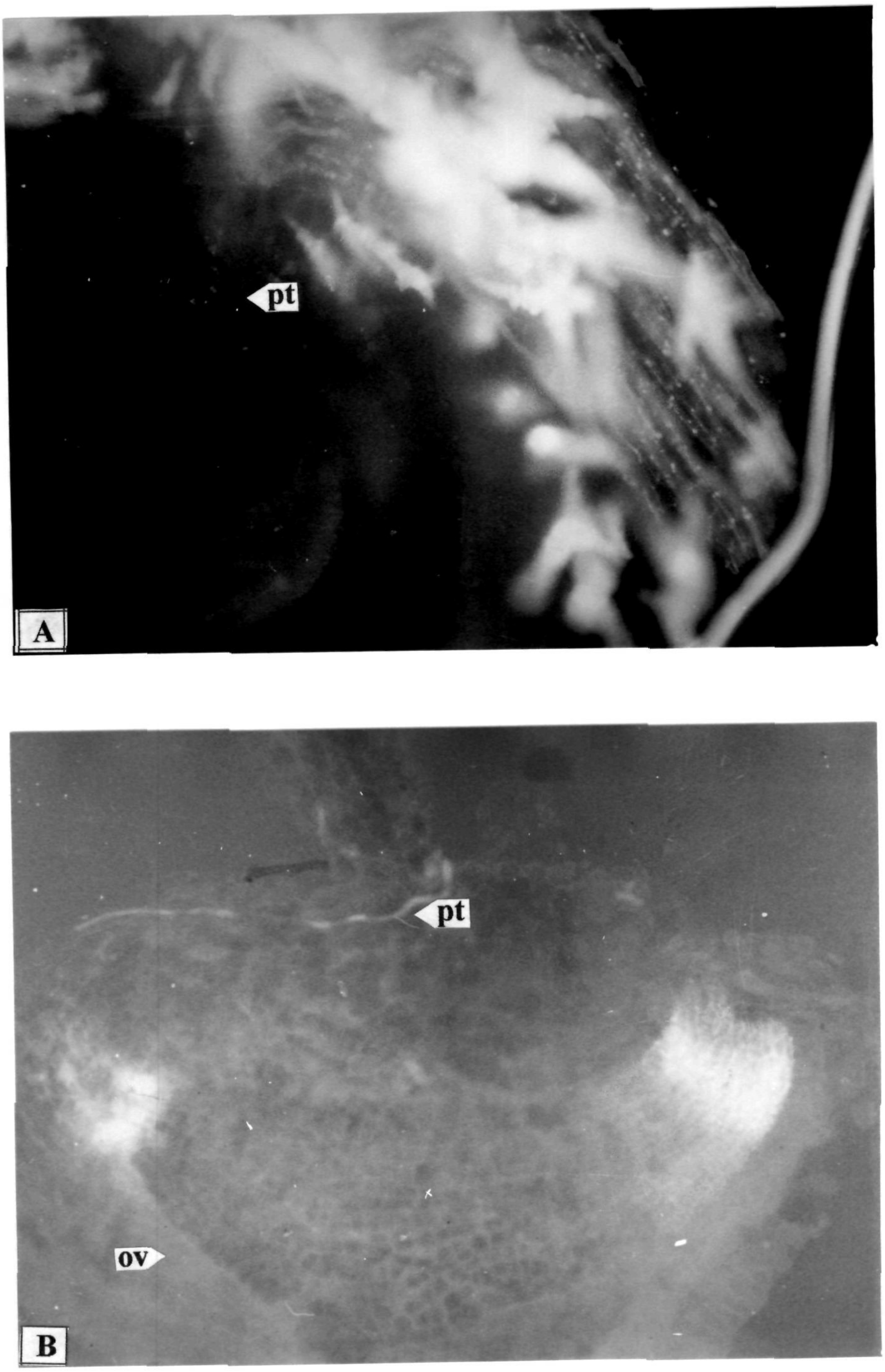


Fig.36

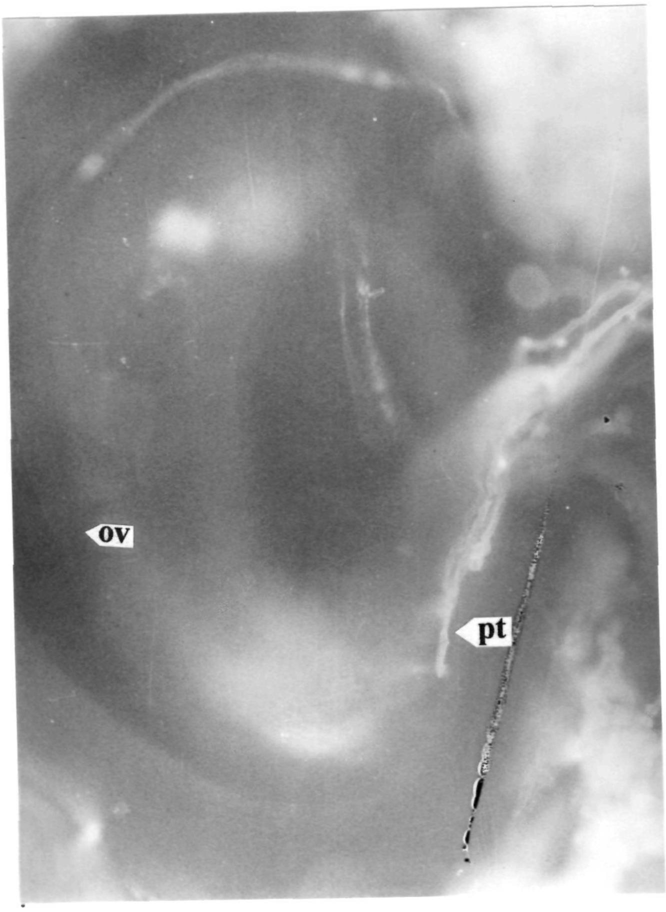


Fig.37

EMBRYOGENESIS

Schima wallichii

The zygote does not divide until about 12 weeks after fertilization. The time between fertilization and zygote division varied from 12 to 14 weeks. Globular proembryo formation takes place within 14 to 18 weeks (Fig. 38A-D, 39A,B,C) All embryos have long suspensors. The suspensor elongates and pushes the embryo proper between the endosperm cells. It is not until the late globular stage that the embryo increases in volume. Following the globular stage, the embryo becomes heart-shaped (Fig. 39D). Further differentiation results in the development of late heart-shaped, torpedo stage and fully developed embryos (Fig. 40A,B,C). Development of two slender and long cotyledons takes place about 22 weeks after fertilization. In some embryo sacs no indication of embryo development was found, although there was indication of the endosperm formation. After 24-26 weeks of fertilization, the cotyledons were found to be well differentiated (Fig. 40C). The mature embryo occupies the major part of the seed. In some ovules, occurrence of twin embryos was noticed but the percentage occurrence of twin embryos was negligible (less than 0.5%).

Schima khasiana.

The time between fertilization and the zygote division varied from 12 to 15 months. Globular proembryo formation takes place within 14 to 16 months (Figs. 41A-D, 42A). All embryos have long suspensors consisting of numerous cells. The suspensor elongates and pushes the embryo proper within the endosperm cells. In some ovules, twin embryos was noticed (Fig. 42B). The percentage occurrence of twin embryos was less than 0.3. It is not until the late globular stage that the embryo increases in volume. Further differentiation in the globular embryo results in formation of heart-shaped embryo (Fig. 42C). Development of the two slender and long cotyledons occurs about 14 to 17 months after fertilization (Fig. 42D). In some embryo sacs there was no indication of embryo development, although endosperm was formed. As seed matured, the cotyledons became thick and filled the entire space of embryo sac. That happened 17 to 19 months after fertilization (Fig.43). A comparative account of embryogenesis in *Schima wallichii* and *Schima khasiana* is given in Table 14.

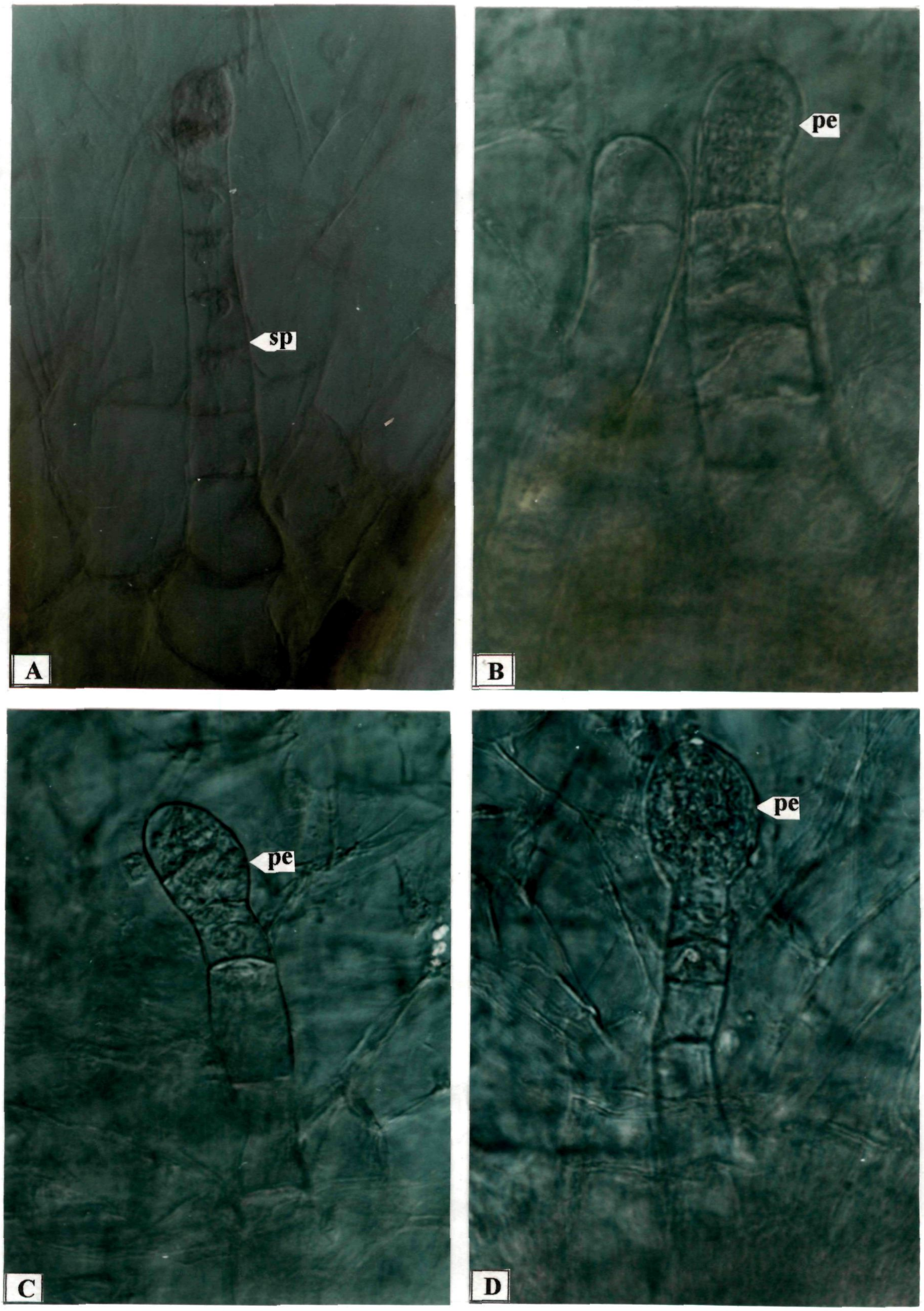


Fig.38

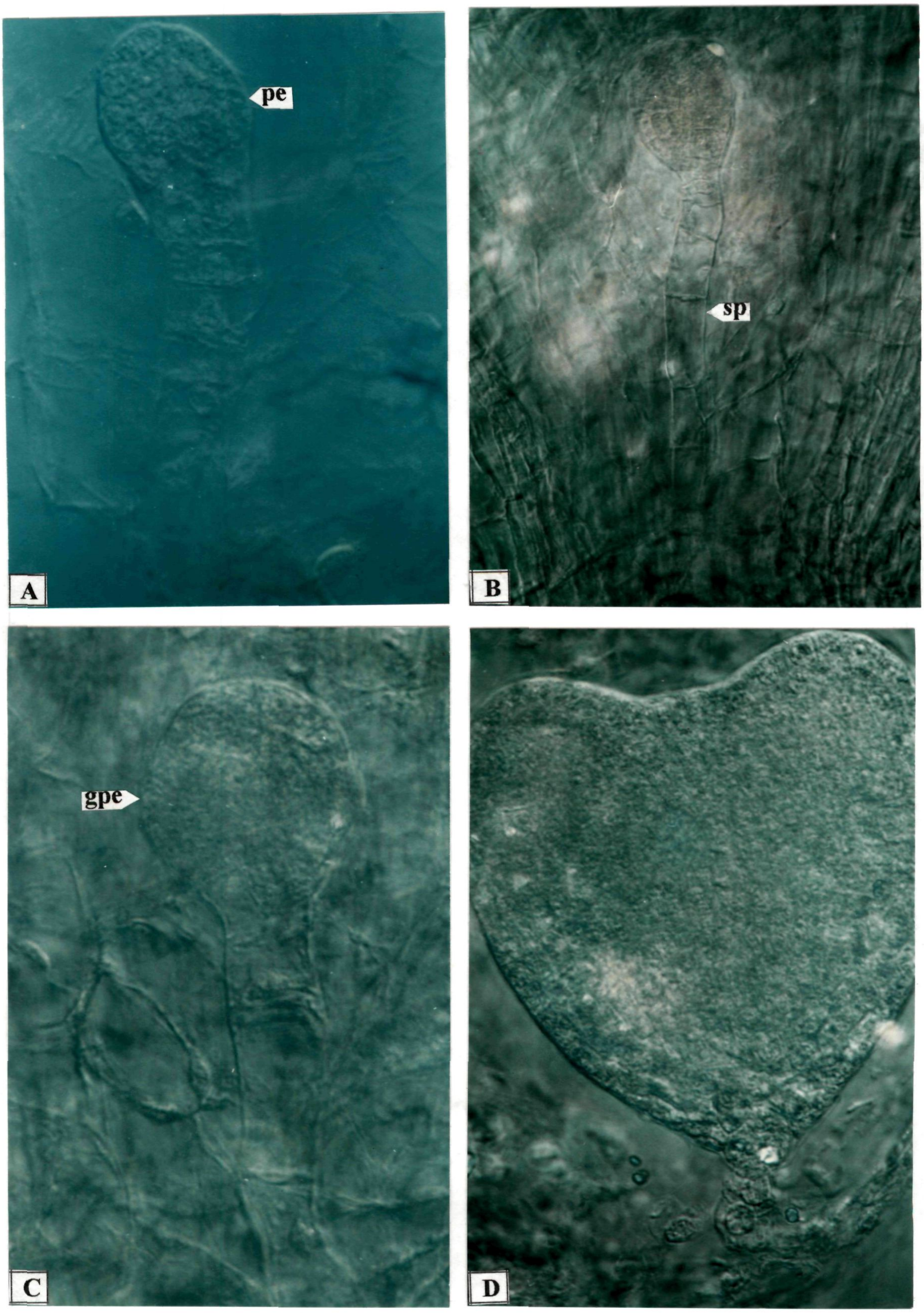


Fig.39

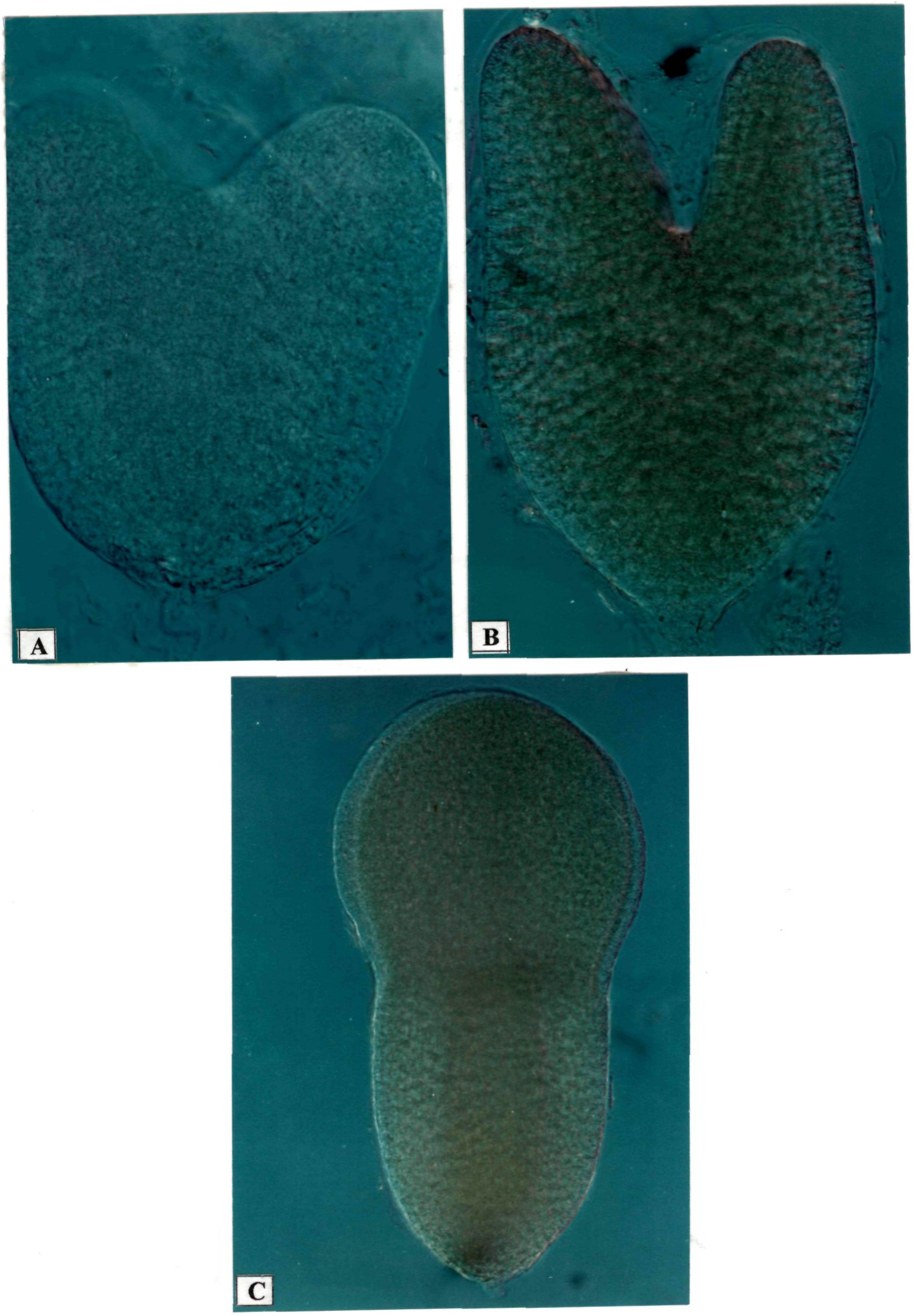


Fig.40

Table 14: Embryogenesis in *S.wallichii* and *S.khasiana*.

	S.wallichii	***S.khasiana***
Type of embryo sac development	**Polygonum**	**Polygonum**
Type of fertilization	**Porogamous**	**Porogamous**
Type of endosperm development	**Nuclear**	**Nuclear**
Initiation of endosperm tissue formation	**Within 4 weeks**	**Within 4 months**
Zygote division	**After 12 weeks**	**After 12 months**
Development of globular pro-embryo	**During 14-18 weeks**	**During 14-16 months**
Formation of heart-shaped embryo	**During 20-22 weeks**	**During 16-18 months**
Development of cotyledons	**During 24-26 weeks**	**During 17-19 months**
Occurrence of twin embryos (% of total ovules)	**0.5 ± 0.05**	**0.3 ± 0.03**

± SE

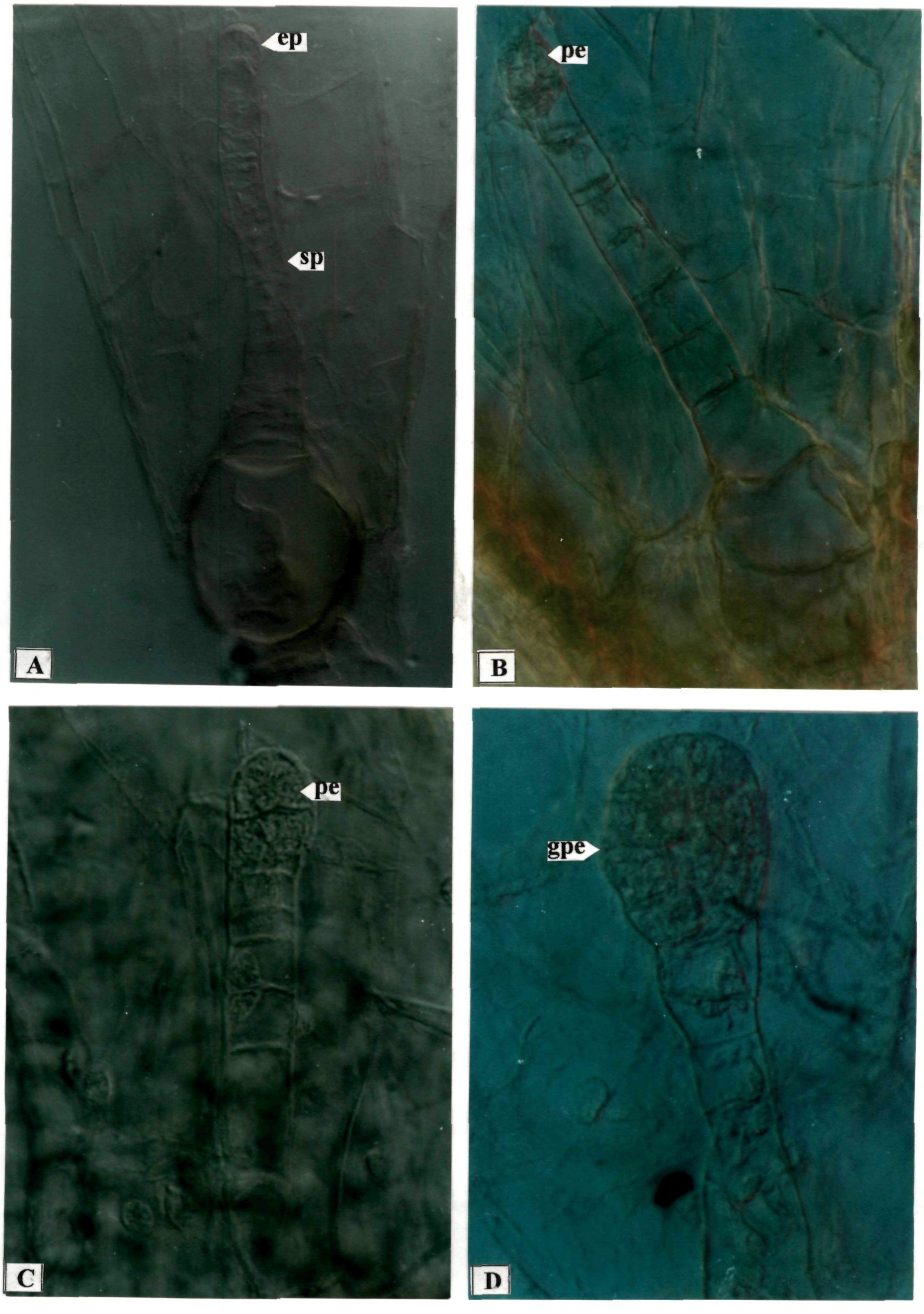


Fig.41

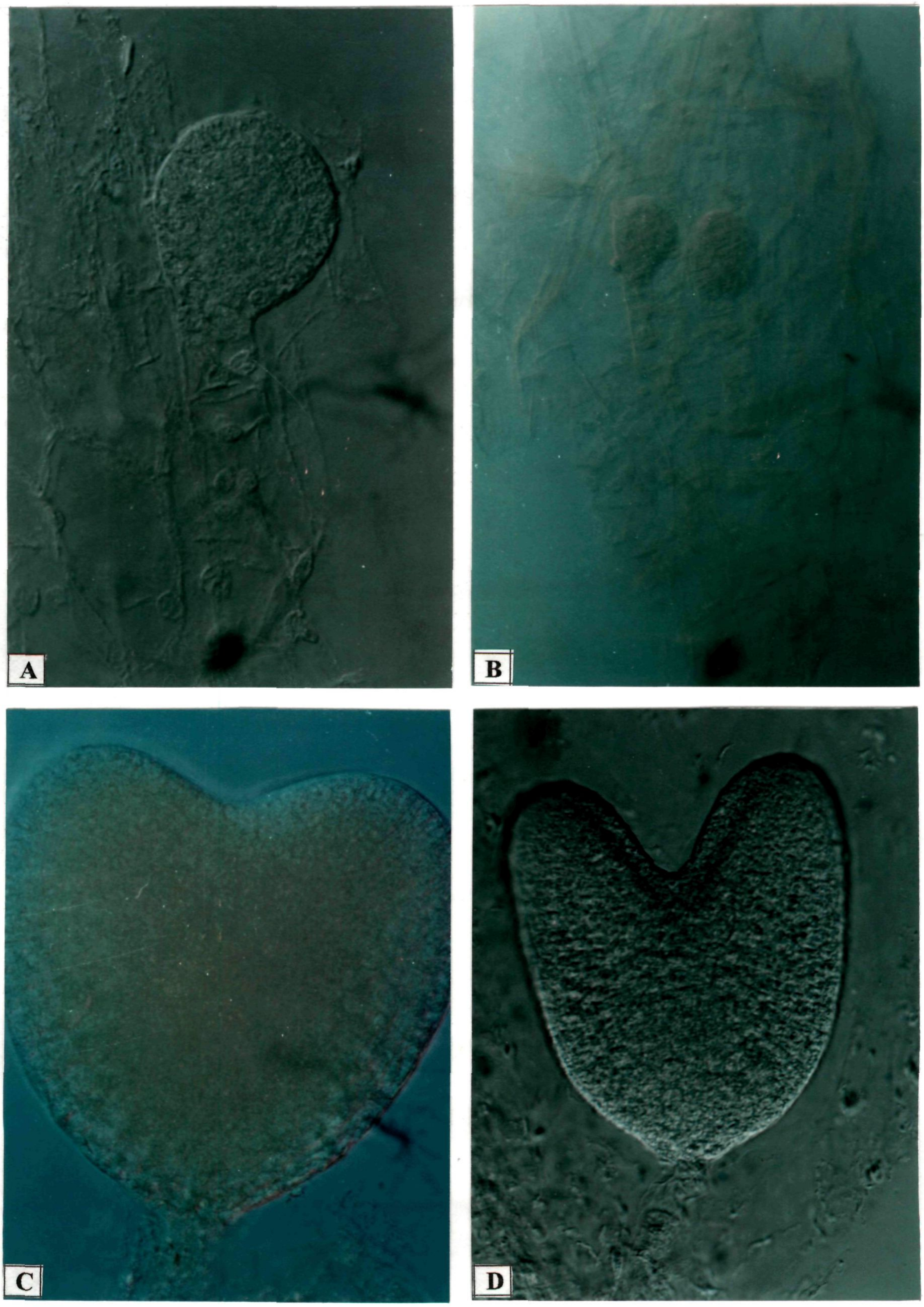


Fig.42

Fig.43

SEED GERMINATION

Germination of seeds in different conditions

In both the species mature fruits are loculicidal capsules those contain winged seeds (Figs. 44A,B, 45A,B). The seed may be filled or unfilled (Figs. 44C, 45C). In case of *S.wallichii*, 71% seeds were filled while in *S.khasiana* only 17% seeds were filled. T_Z test revealed 92% and 32% embryo viability in *S.wallichii* and *S.khasiana* respectively (Table 17; Figs 48A-C, 49A-C). In the former on an average 64% seeds germinated whereas in latter average germination percentage was 13% (Table 15) when seeds were germinated in dark on moist Whatman filter paper at 20°C in BOD incubator (Figs. 46A, 47A).

Embryo germination in laboratory condition at 20°C was 90% in *S.wallichii* and 16% in *S.khasiana* (Table 16; Figs. 46B, 47B). Whereas, in soil (pot) it was 47% in *S.wallichii* and 8% in *S.khasiana* (Table 17).

When only filled seeds were germinated in pots filled with garden soil and manure mixed in 3:1 ratio, the germination declined to 49% and 11% in *S.wallichii* and *S.khasiana* respectively (Table 17; Figs 46C, 47C). When randomly collected seeds were germinated in pots only 36% and 11% germination took place in *S.wallichii* and *S.khasiana* respectively (Table 18). Thus percentage germination of *S.wallichii* seeds in pots was more (49%) when only filled seeds were used and it declined (36%) when randomly collected seeds were germinated (Table 18). Seedlings of *S.wallichii* and *S.khasiana* emerged after 12 days of sowing and the emergence was complete after 28-30 days. However, in about 65 days after sowing of the seeds, on average 36% and 11% seedlings survived in *S.wallichii* and *S.khasiana* respectively (Table 18). When randomly collected seeds of both the species were sown in the open plot, germination varied between 33 to 35% in *S.wallichii* and 6 to 11% in *S.khasiana* (Table 19; Figs. 50A,B, 51A). When seeds were taken randomly and sown in natural condition i.e. in the forest soil, the average germination was found to be 40% and 8% in *S.wallichii* and *S.khasiana* respectively (Table 20).

In *S.wallichii* percentage germination was found better in forest soil than in garden soil.

Effect of different temperature (20 and 25°C) and light conditions (continuous light, dark and 16 h dark + 8 h light) on the germination of seeds of both the species revealed that compared to *S.khasiana*, seed germination started earlier in *S.wallichii* in both the temperature regimes and in all the light conditions (Table 21). However, at 25°C, germination was over earlier in *S.khasiana* in comparison to *S.wallichii* in all the light conditions (Table 21). Seed germination commenced first in both the species when seeds were kept in complete dark both at 20 and 25°C. *S.khasiana* seeds germinated last in continuous light at 20°C. Germination was faster at 25°C and completed early in dark. However no definite response was evident in *S.wallichii* seeds (Table 21). Compared to 20°C, seed germination was over in less time at 25°C in both the species. In case of *S.wallichii* percentage germination was better at 25°C when seeds were germinated in 16 h dark and 8 h light condition. While, in case of *S.khasiana* germination was found better at 20°C in complete dark. There was marked increase in percentage germination at 25°C when seeds were germinated in complete light condition (Table 21). However, in complete dark, there was marked decrease in germination percent at 25°C compared to 20°C.

To observe effect of seed coat on germination filled seeds and embryos of both *S.wallichii* and *S.khasiana* were germinated in pot soil. In *S.wallichii* filled seed germination started on 13th day and completed on 28th day of sowing. The highest germination occurred on 27th day and the mean germination after 30 days was found to be 49%. On the other hand, the embryos started germination on 12th day and it was completed on 26th day of sowing. The highest germination occurred on 20th day after sowing and the mean germination after 30 days was found to be 47% (Table 22). Germination of embryos started and completed earlier than the seeds but percentage germination was found slightly more in case of seeds. In *S.khasiana* filled seeds started germination on 12th day and completed on 28th day after sowing. The day of highest germination was found to be 27th day and the mean germination after 30 days was 11%. On the other hand embryos started germination on 12th day and germination was completed on 20th day after sowing. The highest germination occurred on 18th day and the mean germination after 30 days was found to be 8% (Table 22). Thus, though completion time of embryo germination was less (20 days) than the seed germination (28 days), percentage of seed germination was better (11%) than embryo germination (8%).

Surviving seedlings in pots developed normally (Fig. 51B) and showed fast growth (Figs.50C, 51C). Three months old seedlings (Fig. 51B) were found suitable for planting in the field.

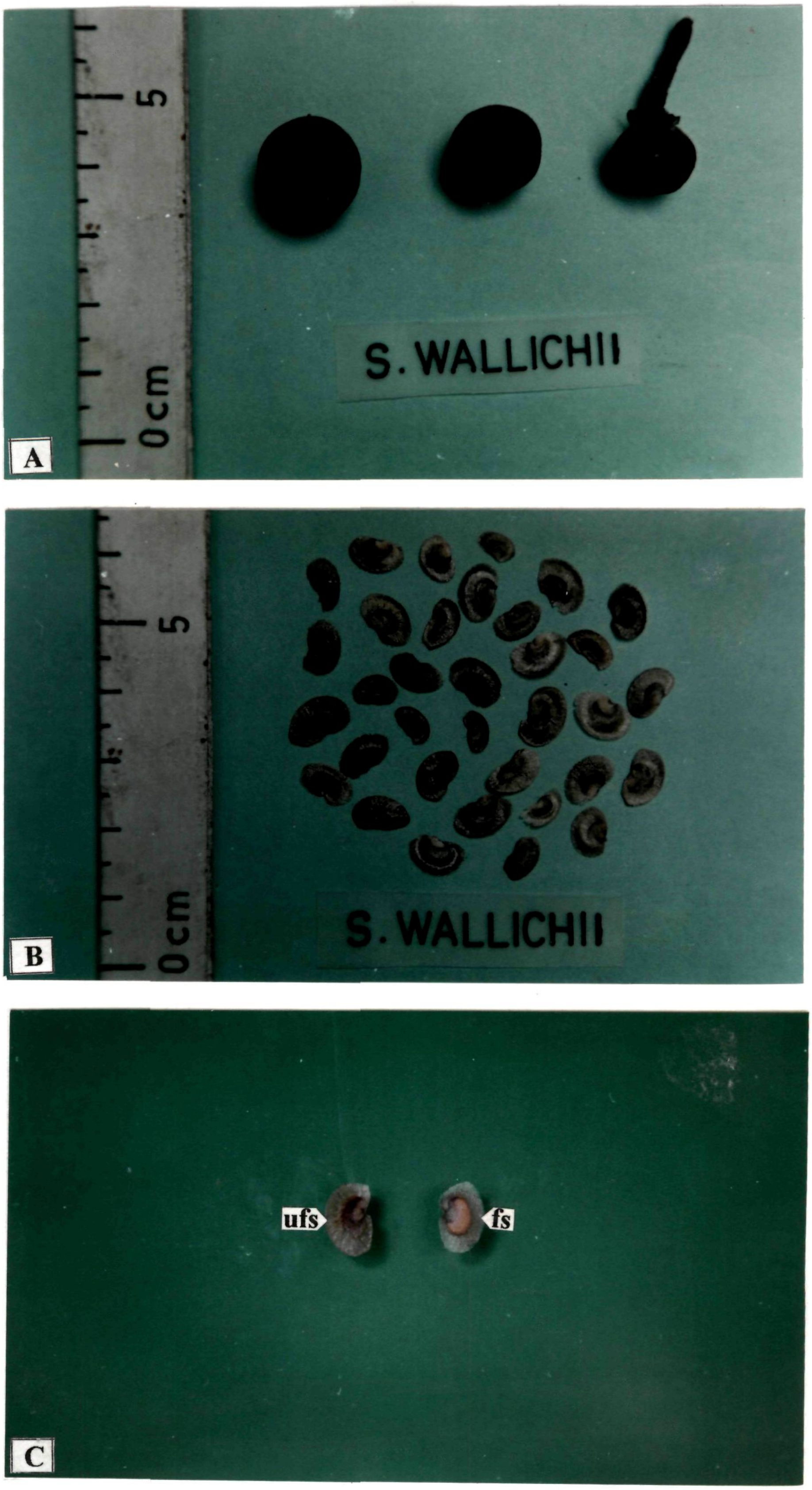


Fig.44

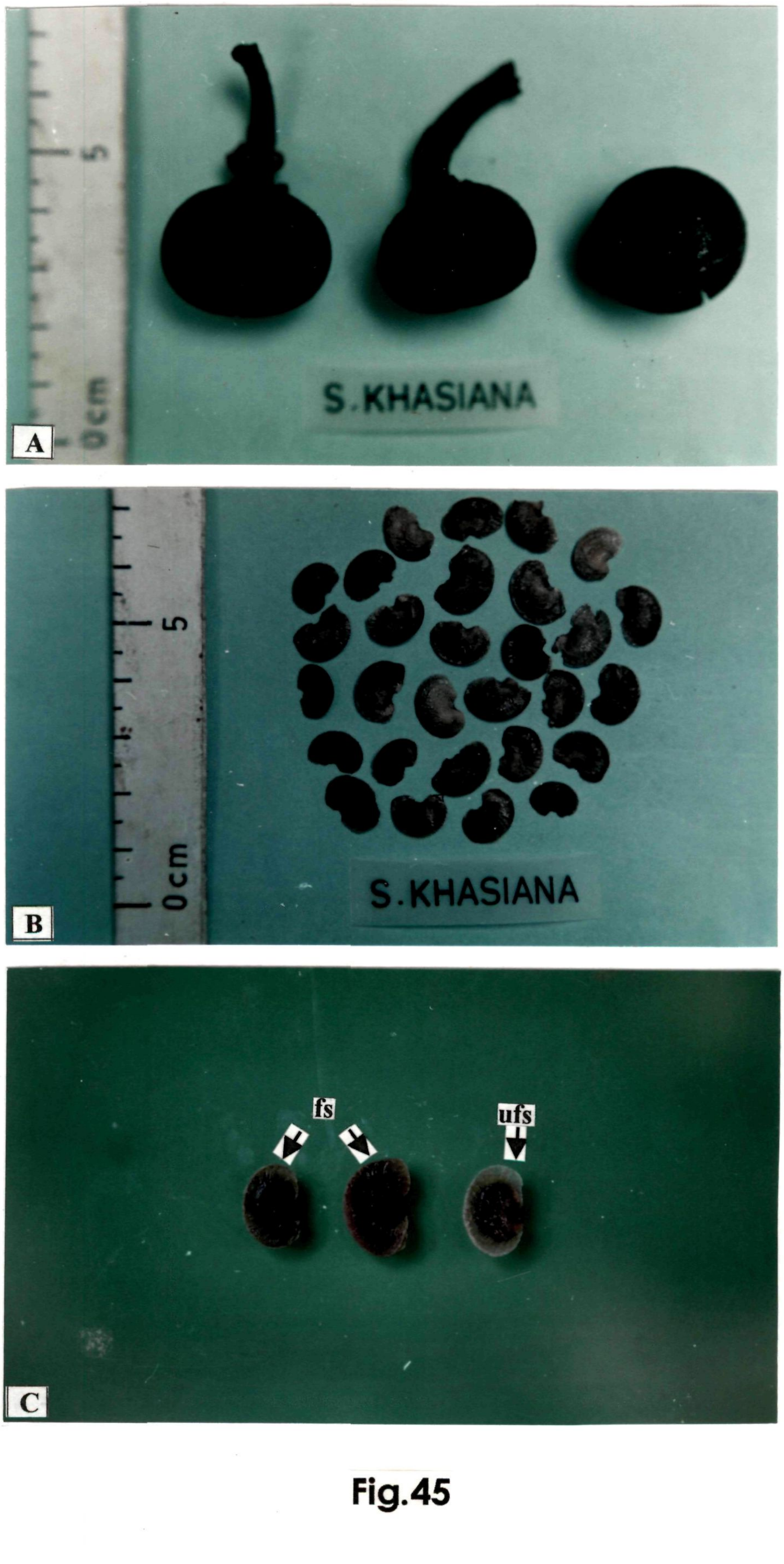


Fig.45

Fig.46

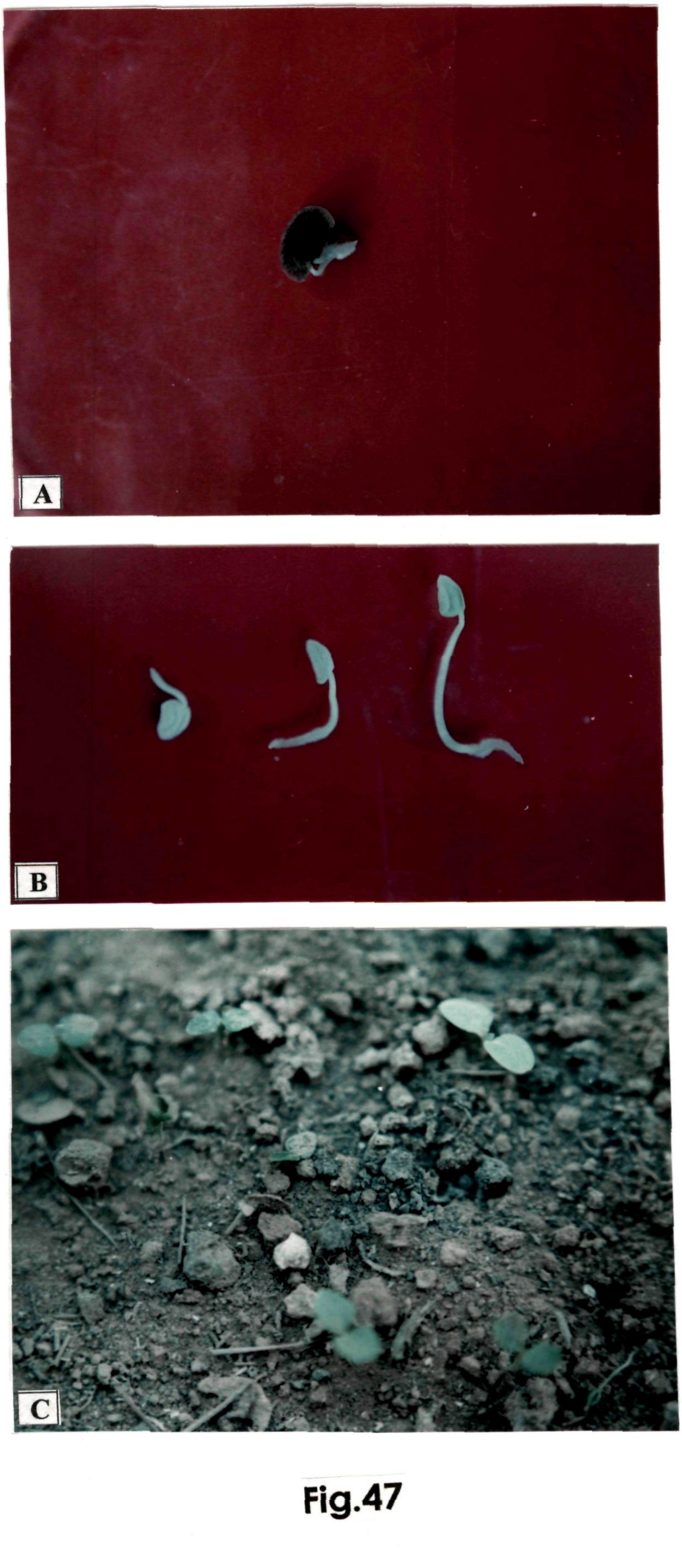


Fig.47

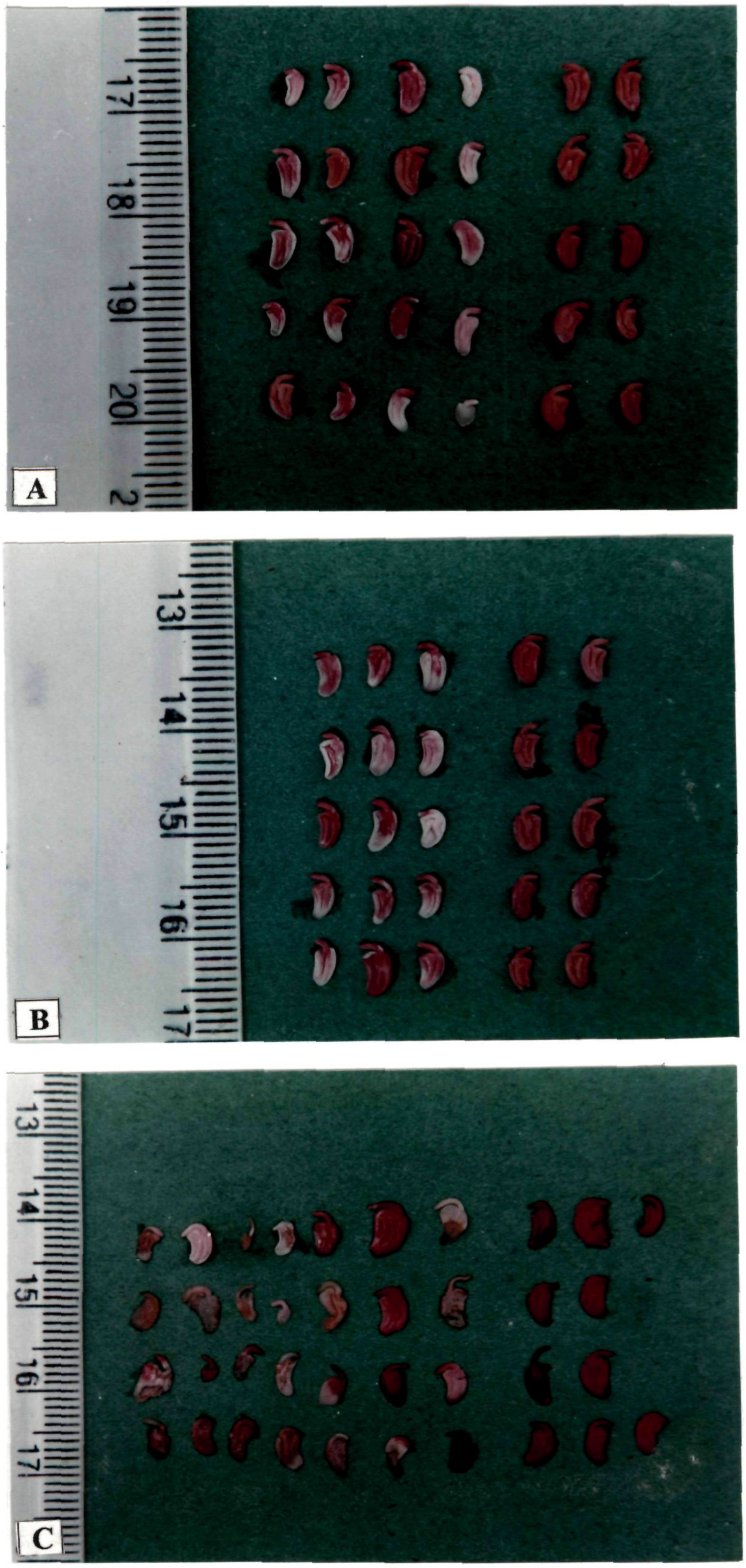


Fig.48

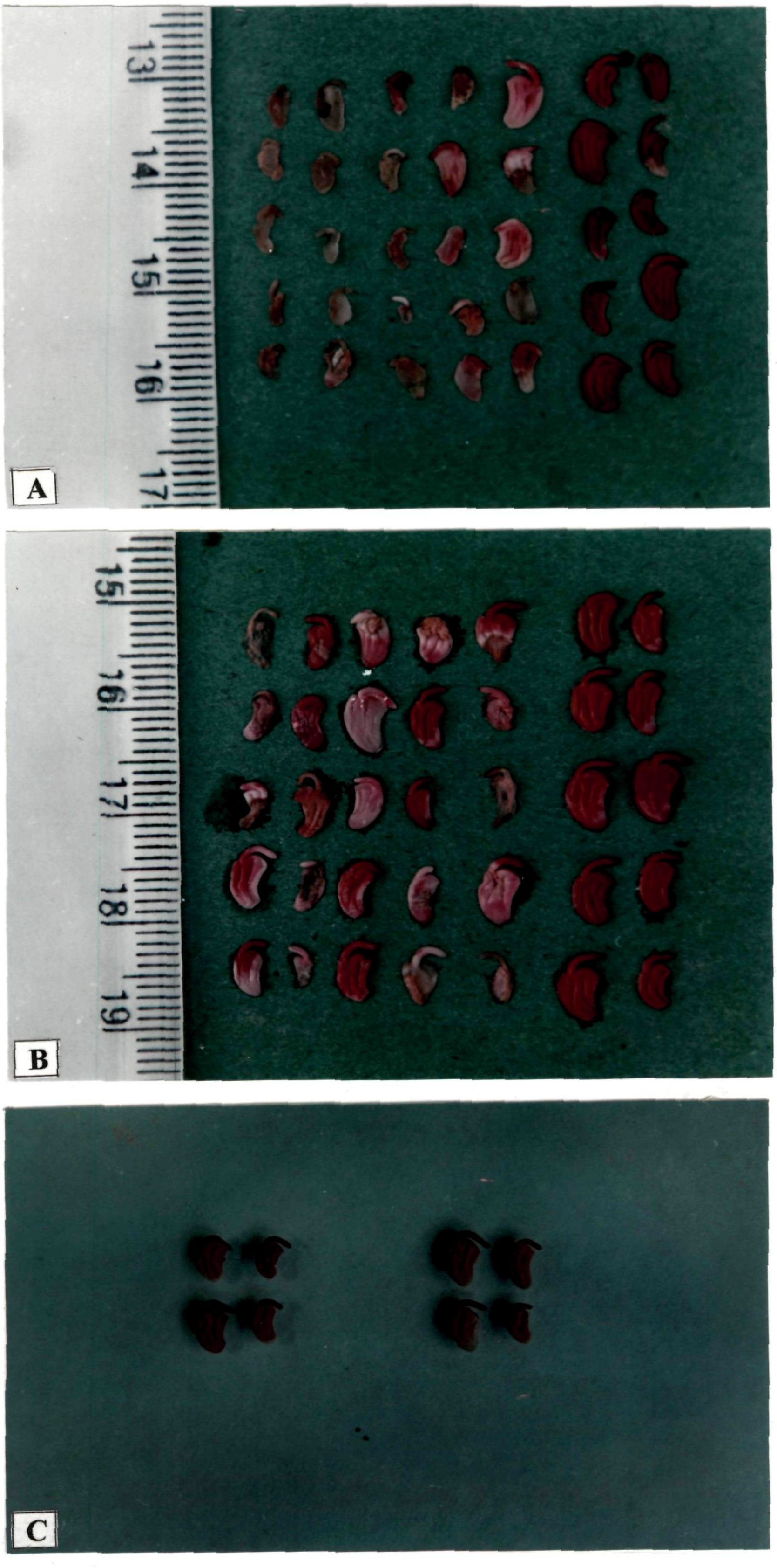


Fig.49

Fig.50

Fig.51

Table 15: Percentage occurrence of filled seeds in *Schima* species and their germination at 20°C.

Species	Year	Total seeds	Filled seeds (%)			Germination (%)	
			Range	Mean	Avg.	Range	Mean
S. wallichii	1996	500	70 – 76	73.00	71	56 – 80	69.59
	1997	500	68 – 71	69.66		54 – 62	59.20
S. khasiana	1996	500	8 – 28	16.00	17	8 – 18	12.00
	1997	500	18 – 19	18.67		6 – 16	13.20

Table 16: Embryo germination at 20^{o}C in *Schima* species.

Species	No. of seeds	Seeds with embryo(%)	Mean embryo germination (%)
S. wallichii	500	70.80 ± 0.08	89.83 ± 3.04
S. khasiana	500	17.00 ± 1.33	16.00 ± 8.33

± SE

Table 17: Viability and germinability of seeds of *Schima* species.

Species	Embryo viability (%) (Tz staining test)	Germination (%)			
		Seed		Embryo	
		Lab.	Pot	Lab.	Pot
S.wallichii	92.03 (± 1.87)	64.39 ± 1.73	48.67 ± 2.08	89.83 ± 3.04	46.67 ± 1.67
S.khasiana	32.00 (± 2.73)	12.60 ± 0.46	10.67 ± 3.87	16.00 ± 8.33	8.33 ± 3.04

± SE

Table 18: Germination of *Schima* seeds in pots containing garden soil.

Species	Year	Total no. of seeds	Day of beginning	Day of peak	Day of completion	Mean germination after 65 days (%)
S. wallichii	1996	300	13th (8%)	28th (48%)	28th (48%)	33.66
	1997	300	12th (9%)	27th (51%)	30th (51%)	37.34
S. khasiana	1996	300	12th (4%)	28th (12%)	28th (12%)	9.33
	1997	300	12th (5%)	28th (16%)	28th (16%)	12.0

Table 19: Germination of *Schima* seeds under field condition.

Species	Year	Total no. of seeds	Day of beginning	Day of peak	Day of completion	Mean germination after 65 days (%)
S.wallichii	1996	300	13th (4%)	29th (44%)	30th (44%)	34
	1997	300	13th (5%)	26th (48%)	30th (48%)	35
S.khasiana	1996	300	13th (2%)	29th (9%)	29th (9%)	6
	1997	300	12th (3%)	29th (15%)	29th (15%)	11

Table 20: Germination of *Schima* seeds in forest soil.

Species	Year	Total no. of seeds	Day of beginning	Day of peak	Day of completion	Mean germination after 65 days (%)
S. wallichii	1996	300	15th (11%)	30th (47%)	30th (47%)	40.66
	1997	300	13th (12%)	26th (50%)	27th (50%)	38.33
S.khasiana	1996	300	15th (2%)	30th (8%)	30th (8%)	6.33
	1997	300	13th (3%)	27th (13%)	27th (13%)	9.66

Table 21: Effect of temperature and light conditions on seed germination of *Schima* species.

Species	Temperature condition / Light condition	20°C			25°C		
		Days to start germination	Days to complete germination	Germination %	Days to start germination	Days to complete germination	Germination %
S. wallichii	**Light**	3rd	26th	62.40 ± 1.38	2nd	23rd	50.80 ± 1.03
	16h dark + 8h light	4th	25th	66.80 ± 1.87	2nd	23rd	71.60 ± 1.33
	Dark	2nd	20th	61.20 ± 1.27	2nd	23rd	68.00 ± 0.08
S. khasiana	**Light**	11th	28th	6.80 ± 0.46	5th	18th	16.40 ± 1.04
	16h dark + 8h light	7th	26th	13.20 ± 1.04	5th	18th	15.60 ± 1.38
	Dark	4th	25th	16.80 ± 0.80	4th	16th	13.60± 0.04

± SE

Table 22: Effect of seed coat on germination of seeds in *Schima* species.

Species	Seed / Embryo	Total number	Day of beginning	Day of peak	Day of completion	Mean germination after 30 days (%)
S. wallichii	**Seeds**	150	13th	27th	28th	48.67 ± 2.08
	Embryos	150	12th	20th	26th	46.67 ± 1.67
S. khasiana	**Seeds**	150	12th	27th	28th	10.67 ± 3.87
	Embryos	150	12th	18th	20th	8.30 ± 3.07

± = SE

DISCUSSION

The phenology relates growth habit of a species to the physical environment and helps in understanding the seasonal aspects of various ecological phenomena. Therefore, the important phenophases such as the bud-break, leaf-flush, flowering, fruit formation and maturation and seed dispersal of *Schima wallichii* and *Schima khasiana* were studied at regular intervals for three consecutive years.

The first stage in the flowering process is floral induction or evocation, when the vegetative meristem changes into reproductive meristem. Flowering is essentially a growth process, but the nature of stimulus required to tigger this process is not yet known (Sedgley and Griffin, 1989). In *S.wallichii*, floral bud initiation starts in the month of February and flowering takes place in the month of May or may extend upto June end. In *S.khasiana*, floral bud initiation starts in the month of April. Flowering occurs during July and August. Most tropical and sub-tropical tree species have been reported to have a very short interval between floral initiation and anthesis. For example, one to two months in *Artocarpus, Litchi* and *Durio* (Sinha, 1975; Soepadmo and Eow, 1976; Menzel, 1984), one month in *Mangifera* (Scholefield *et al.*, 1986), and three months in *Citrus* (Lord and Eckard, 1985, 1987). Peak leaf-fall in both the species occurs between October and January, followed by appearance of new leaves and initiation of floral buds in the spring. Thus, slow down of vegetative growth and a period of ecodormancy appear to be important prerequisite for the floral initiation (Sedgley and Griffin, 1989). In *Citrus*, low temperature between 10 and 20°C, or a period of water stress for four to five weeks have been reported to promote floral initiation (Hall *et al.*, 1977; Southwick and Davenport, 1986). Water stress is particularly important in the tropical and sub-tropical trees such as *Litchi, Citrus, Shorea* (Menzel, 1983; Southwick and Davenport, 1986; Burgess, 1972).

In the present study, both the species displayed the phenomenon of irregular bearing, producing good fruits in alternate years. Most timber species show poor periodicity in fruit and seed production (Monselise and Goldschmidt, 1982; Owens and Blake, 1985). In the present case, irregular bearing may be the result of poor floral initiation. Monselise and Goldschmidt (1982), Luckwill (1980), Monselise and Goldschmidt (1982) reported similar observations in *Citrus, Malus, Mangifera*. Luckwill (1980) has suggested that floral initiation is inhibited by gibberellins produced by the developing seeds. Similar evidence of inhibition of floral initiation by seeds has also been reported in *Olea* (Stuttle and Martin, 1986).

Late maturation and lengthy retention of fruits provide better chances for wind dispersal during early spring, when strong dry winds blow in *Schima* growing regions. Similar observations has been made by Semalty and Sharma (1996), in *Acer caesium* in central Himalayas.

In *S.wallichii* flower buds start anthesising in May-June. Anthesis of flowers begins with the slitting of corolla at the tip of the bud. The slit gradually widens and opening of flower is completed in 11-16 h. The anthesis begins at approximately 4.00 h in the early morning and continues upto 15.00 h in the afternoon. In *S.khasiana*, the flower buds anthesis occurs in July-August and is similar to that of *S.wallichii* except that it is slower in the former. Jerstedt (1980) pointed out that the developmental changes and movements leading to opening of flower are often related to differential growth rates or changes in turgor more out of supposition. Initiation of floral bud and early stages of flower development coincided with wide diurnal temperature fluctuation (8 - 22°C in *S.wallichii* and 4 - 14°C in *S.khasiana*) and low relative humidity (mean 62% in *S.wallichii* and 74% in *S.khasiana)*. But during the post anthesis diurnal temperature fluctuation was low (16 - 24°C in *S.wallichii* and 15 - 23°C in *S.khasiana*) and relative humidity was high (mean 79% in *S.wallichii* and 92%

in *S.khasiana*). Temperature is known to influence floral bud initiation and flower development in plants (Sedgley and Griffin, 1989). Barner and Christiansen (1960) reported that fluctuating temperature adversely affected fertility of developing buds in *Larix* by disrupting meiosis. This could probably be the reason for low pollen viability in both the species.

Flowers of both the species are white in colour. *S.khasiana* flower is larger (5-6cm across) than *S.wallichii* flower (3-4 cm across). Mean number of stamens per flower, size of the stamen and number of pollens per flower varied widely in the two species of *Schima*. These observations support the conclusions of Vonhof and Harder (1995), that the number of pollen produced in a flower varies considerably between species.

Like other members of Theaceae, *Schima* also having no means of vegetative reproduction has multilocus gametophytic incompatibility. They rely on pollen-eating insects for reproduction (Richards, 1986). This could be the reason for excessive pollen production in these species.

Pollen germination, however, is the surest test of pollen fertility and therefore is important for undertaking any breeding programme (Semalty and Sharma, 1996). The pollen germination tests revealed 62- 75% pollen sterility in *S.wallichii* and 47-60% in *S.khasiana*. Varying proportion of sterile pollens have been reported by Soost and Cameron (1975), in *Citrus* cultivars. In some lemon, lime and orange cultivars where the pollen viability is low (25%), meiotic abnormalities are frequently observed (Naithani and Raghuvanshi, 1958; Iwamasa, 1966). Post-meiotic pollen degeneration also occurs in *Pyrus* (Zielinski and Thompson, 1966). Devlin and Ellstrand (1990) emphasised that seed yield is one component of reproductive success of plants. But to arrive at plant's total reproductive success, it should be considered together with the success in fertilization of ovules that mature into seeds. The high pollen sterility in presently studied *Schima* species adversely affects fertilizing ability of pollen and thus reproductive success of the species. The development of mature fertile male gametophyte depends on nutrients provided by anther sporophytic tissues

(Christopher *et al.*, 1996). Deficiency in carbohydrate metabolism in the anther leads to abnormal pollen development (Sawhney, 1992) and is often correlated with pollen sterility (Banga *et al.*, 1984). Size, volume, number of pollen per anther and pollen per flower differ in both the *Schima* species. Similar observation has been made in *Chinochloa* (McKone, 1990), *Plantago lopus* (Sharma *et al.*, 1990), *Solanum torvum* (Chauhan and Katiyar, 1990), *Mercurialis annua* (Lisci *et al.*, 1994) and papilionaceous legumes (Vonhof and Harder, 1995). Variation in the size and shape of pollen grains have been reported by many workers (Moore and Webb, 1978; Iwanami *et al.*, 1988; Faegri and Iversen, 1989; Cresti *et al.*, 1992). One of the conspicuous structural features of pollen grains is the ornamentation of the wall formed by the outer part of the exine (Cresti *et al.*, 1992). Evidences from different sources have conclusively shown that the exine ornamentation is controlled by the sporophytic genome (Shivanna and Johri, 1985). Pollen morphology has been investigated in detail in *Melia* (Nair, 1959), *Hevea* (Rao, 1964), *Myristica* (Nair, 1972) and *Schima superba kankoensis* (Tsou, 1997). Among angiosperm species, pollen volume varies by almost five times (Wodehouse, 1935; Muller, 1979), implying diverse opportunities for pollen size evolution. However, within species variation is less than 5% (Vonhof and Harder, 1995) suggesting that reproductive conditions may influence the pollen size. Variation in pollen grain size varies considerably less than the variation in number of pollen grains produced per flower (Vonhof and Harder, 1995), suggesting that natural selection optimises resource investment per offspring and not the offspring number (Smith and Fretwell, 1974; Lloyd, 1987).

In both species of *Schima*, first few flowers in the inflorescence are generally bigger and produce more pollen and nectar. They make the flowers more attractive to insects visitors. Usually, fruit abortion is common in flowers, which develop at the end of the flowering season. The flowers that develop at the end of the flowering season also produce small fruits. They contain mostly non-viable seeds, which are smaller in size and usually without embryos (unfilled seeds). This suggests that pollination success may be greater in flowers developing early and during peak flowering period, than in those which develop at the end of the flowering period. Similar were the findings of Tangmitcharoen and Owens (1997) in *Tectona grandis*. Besides, there are many other factors, such as position of developing fruits on the inflorescence (Bawa and Webb, 1984), which affect fruit development. The number of pollen produced by a flower increases the probability that sufficient number of pollen grains will reach a stigma (Cruden, 1977). In both species of *Schima*, pollen ovule ratio (P/O) was high (66974 in *S.wallichii* and 69200 in *S.khasiana*). The development of mature fertile male gametophyte depends on nutrients provided by anther sporophytic tissues (Christopher *et al.*, 1996).

The stigma is categorised as wet on the basis of a free flowing surface exudate secreted by stigmatic cells (Dumas *et al.*, 1978; Sedgley, 1981; Knox *et al.*, 1989). Both *Schima* species have wet and papillate stigma. The stigmatic papillae differ from the transmitting tissue cells in shape and content of cytoplasmic organelles (Knox, 1984). The stigmatic secretion reportedly contains lipids, aminoacids and perhaps sugars (see Baker, 1973) and are thought to prevent drying of the stigmatic surface, this provides a suitable medium for pollen germination (Baker *et al.*, 1973). Small amount of stigmatic secretion was observed in the *Schima* species which partly comes from cells beneath the papillae or from the transmitting tissue and partly from the papillae themselves. The stigma secretions might be smeared onto the bodies of insects as they contact the surface of the stigma as reported by Baker *et al.* (1973), in some other species.

The stigma and transmitting tissue secretions have many functions, such as attraction and nourishment of floral visitors (Lord and Webster, 1979), pollen adhesion to pollinators (Kandasamy and Kristen, 1978), pollen-stigma recognition (Clarke *et al.*, 1979; Knox, 1984), pollen-tube growth (Sanders and Lord, 1992) and pollen-tube penetration of ovules (Franssen-Verheijen and Willemse, 1993). According to Richards (1990), stigmatic exudate performs three functions – adherence of pollens to stigma, serves as a medium for pollen germination and provides nectar reward to insects in *Garcinia hombroniana*. In both species of *Schima* stigmatic exudate seems to perform two functions namely adherence of pollen to the stigma and as a medium for pollen germination. Nectar appears at the base of the ovary almost simultaneously with the wetting of stigma or stigma receptivity as has been reported by Tangmitcharoen and Owens (1997) in *Tectona grandis*.

The stigma papillae and the transmitting tissue of both hollow and solid styles have secretary cells which produce the medium (Knox, 1984), for pollen tube growth from stigma to the ovule (Weber, 1994). The species under investigation have closed stylar system as is found in many dicotyledons (Knox, 1984) consisting two major parts, the stigma and the transmitting tissue. Both species produce large number of flowers. Flower has long pedicel, large sepals and petals. Nectaries are present at the base of the stamens. Anthers distant from stigma and stamens are shorter then the pistil. Large number of pollen grains and many ovules are produced per flower. Flower has very high pollen/ovule ratio. All these characters imply that both of them could be outbreeding species (Wyatt, 1983). High pollen/ovule ratio and high pollen sterility suggest that both the species are inefficient reproductively (Richards, 1986).

Pollen viability should be considered different from germinability, for instance, self or cross incompatible pollen, though viable may or may not germinate on stigma. In an artificial medium, viable pollen may fail to germinate due to lack of certain essential factors. It is however necessary that viable pollen should give high percentage of germination for guessing the effective fertilization (Semalty and Sharma, 1996). By the acetocarmine staining tests it was observed that in *S.khasiana* the number of viable pollen grains were more (57.21 ± 1.08%) than that of *S.wallichii* (42.00 ± 1.26%).

Binucleate pollen generally germinates readily in culture (Mulcahy and Mulcahy, 1983). This was also found true for *Schima* pollens in which germination began within an hour of pollen being placed in the

Brewbaker and Kwack's medium. The rate of pollen-tube growth varies widely in different species. Brewbaker and Majumder (1961) found that *in vitro* growth of binucleate pollen tubes is about 10% less than *in vivo* pollen-tube growth due to limited reserve food in the pollen. But, there may be extreme variations in *in vivo* and *in vitro* pollen-tube growth rates in angiosperms (Hoekstra, 1983). Rapid *in vivo* pollen-tube growth has been reported in *Acacia retinotes* where pollen tube reaches the ovule within 11 h (Kenrick and Knox, 1985). In *Eucalyptus woodwardii* pollen-tube growth is very slow and takes 10-20 d to reach the ovule; in *Banksia coccinea* it takes 6 d (Fuss and Sedgley, 1991). In *Schima wallichii* pollen tube takes about 5 d and in *S.khasiana* about 9 d respectively to reach the ovule.

The results of the present study are in agreement with the results of Brewbaker and Majumder (1961) and Tangmitchroen and Owens (1997), who reported that *in vitro* growth of binucleate pollen tubes was approximately 10% less than *in vivo* pollen tube growth. *In vitro* binucleate pollen-tube growth consists of two phases – autotrophic and heterotrophic (Rosen and Gawlick, 1966 and Rosen, 1971). In the first phase pollen tubes grow on their own reserves. The growth during the first phase is relatively slow and free of callose plugs (Mulcahy and Mulcahy, 1983). This phase ends with gamete formation Brewbaker (1967). Pollen-tube growth *in vitro* or in an incompatible style, may terminate at this point. The heterotrophic phase is observed only in *vivo* in a compatible style. During this phase, pollen tubes grow rapidly and form callose plugs, indicating a shift to heterotrophic nutrition (uptake of substances from the pistil). This may account for the limited pollen-tube growth under *in vitro* condition in both species of *Schima*. The media used for *in vitro* pollen germination of these species might not contain the nutrients needed for transition to the second phase. Similar findings have been reported by Tangmitcharoen and Owens (1997) in *Tectona grandis*. In nature during the pollination period, slow pollen tube growth and exposure to unfavourable conditions, such as rain, hailstorm and wind, cause abundant loss of unfertilized flowers in both the *Schima* species.

Pollens obtained from different trees showed significant differences in germinability and tube growth. Intraspecific variation in pollen germinability has been reported in *Zea mays* (Pfahler, 1968), *Triplochiton scleroxylon* (Leaky *et al.*, 1981, Oni, 1990), *Asclepias speciosa* (Bookman, 1984) and *Asclepias exaltata* (Shannon and Wyatt, 1986). Such a variation might either be due to differences in age of the trees in natural population or variation in the amount of nutrients available to each tree because of micro-environmental differences (Oni, 1990). Further, pollens obtained from different flowers of an individual tree of *S.wallichii* and *S.khasiana* also exhibited variation in germinability and tube elongation. Such a behavior may be due to difference in partitioning of assimilates and maternal investments in floral structures of different flowers of a tree (Oni, 1990).

Nutrient status of sporophytic tissues of anther influences the formation of mature fertile male gametophyte (Sawhney, 1992). The intra-flower variations observed in germination and pollen tube elongation in *Schima* indicate above possibilities. Flower age may also has a bearing on the germinability and tube growth in both *S.wallichii* and *S.khasiana*, since both declined, 30 and 24 h after anthesis respectively. The decline was significant in comparison to the pollens of flower which anthesised 24 h earlier. Aging of the pollen upto 24 h improved pollen germination in *Brassica oleracea* (Chiang, 1974) and *Brassica juncea* (Rao *et al.*, 1992). Hoekstra and Bruinsma (1978), stated that the male gametophyte of higher plants is metabolically active during microspore maturation, germination and pollen tube elongation. The most important physiological processes in pollen are related to respiration, reserve mobilization, nutrient uptake, biosynthesis of intine and pollen tube wall. Therefore, physiological variation in flowers of different ages might have also affected pollen germinability and tube elongation. Optimal pollen germination and tube elongation occurred 24 h and 18 h after anthesis in *S.wallichii* and *S.khasiana* respectively. Therefore, for a successful breeding programme of *S.wallichii* and *S.khasiana*, pollens collected 24 h and 18 h after anthesis respectively, may be used considering the degree of pollen sterility in these species. Unlike *Schima* species, in *Garcinia homobroniana* the pollen germination decreased as flower age/pollen age increased (Richards, 1990). In *Asclepias exaltata*, however, flower age has little effect on pollen germinability (Shannon and Wyatt, 1986).

Violet, blue, green light and total darkness significantly inhibited germination and tube elongation in *S.khasiana*. Red light, however, significantly stimulated germination and tube elongation over other light conditions. Thus, lights of different colour influenced pollen germination and tube elongation in *S.khasiana*. Similar observations have been reported by Chhabra *et al.* (1979), Seema and Rajeev (1982), Katiyar (1989), Chauhan and Katiyar (1996). Chhabra *et al.* (1979) suggested involvement of phytochrome in pollen germination and pollen tube growth. Significant effect of red light on pollen germination and tube elongation suggests involvement of phytochrome in the present case. Red light effects pollen by influencing synthesis of phytochrome protein and its biological manifestation (Sharma and Malik, 1978). Bindra and Malik (1985) suggested that red light induced stimulation of pollen tube elongation in *Crotolaria juncea* is due to its effect on synthesis of membrane components. Blue light induced inhibition of pollen germination and tube elongation is possibly mediated through its effect on the endogenous level of IAA (Chhabra *et al.*, 1979). Unlike *S.wallichii* (Chauhan and Katiyar, 1996), dark and white light showed different effects on pollen germination and tube elongation in *S.khasiana*. Similar were the findings of Seema and Rajeev (1982) in *Cicer arietinum*.

In all species pollen viability decreases with time (Pacini *et al.*, 1997). Binucleate pollen generally survives longer than trinucleate and germinates readily *in vitro* (Stanley and Linskens, 1974). Entomophilous species generally have longer pollen viability (Pacini *et al.*, 1997). Both species of *Schima* are entomophilous, therefore retain viability for a longer period. The loss of pollen viability in different species have been correlated with water loss and maintenance of the dehydrated state both in nature and in the laboratory (Heslop Harrison and Heslop-Harrison, 1985; Linskens and Cresti, 1988;

Lisci *et al.*, 1994). In extreme cases, such as in *Cucurbita pepo*, water loss and decrease in volume occur at the same pace leading to death, (Nepi and Pacini, 1993). Pollen can be stored for long periods at low temperature after controlled drying in gramineae (Barnabas and Rajki, 1981). *S.wallichii* pollens retained 20 ± 0.9% viability at 0% relative humidity and –5 ± 1°C. temperature after one year. *S.khasiana* pollens retained 32 ± 1.8% viability at similar conditions after one year. Viability of pollens of both species decreased with increasing relative humidity and temperature. Viability also decreased with the passage of time. In most cases, low temperature and low humidity prolong pollen viability (Shivanna and Rangaswamy, 1993). In cases where partial dehydration facilitates conservation of viability, the mechanisms and rates at which rehydration occurs are important factors in determining the percentage of pollen germination (Shivanna *et al.*, 1991). In the similar way species sensitive to dehydration show increased viability after controlled hydration (Shivanna and Heslop-Harrison, 1981). Rehydration at high humidity (RH 85-95%) increased germination of pollen grains of *Epilobium angustifolium* under *in vitro* condition (Heslop-Harrison and Heslop-Harrison, 1993).

Informations about pollen vectors are available for a limited number of cross- pollinated tree species (Styles and Khosla, 1976; Kaul, 1985; Harder, 1990; Wilson and Thomson, 1991; Inouye *et al.*, 1994). Recently attempts have been made to relate the evolution of breeding systems in plants to interactions with their pollinators. Pollinators preferentially visit plants based on non-rewarding characters such a floral colour, number of flowers per inflorescence and odour (Stanton *et al.*, 1989). The interplay of attractants and rewards cannot be easily elucidated (Shivanna and Sawhney, 1997). In both the *Schima* species anthers open after the flower. Pacini (1992) opined that anthers might open before or after the flower according to the sequence of male and female receptivity in a given species. In entomophilous angiosperms, the pollen is held in the anther until the arrival of a pollinator and the possibility of compatible pollination depends on pollinator efficiency and on chance (Lisci *et al.*, 1996).

Faegri and Pijl (1979) suggested that in insect-pollinated plants, nectar and pollen are the major rewards and are presented only at certain times. This appears true for both the species of *Schima* investigated. It is likely that pollen is the chief attraction in *Schima* because it is produced in large numbers and over a long period of time. Whereas, nectar is presented in relatively small amount over a short period of time. Insects that are primarily pollen collectors are usually thought to be more effective pollinators than those that are nectar collectors (Jay, 1986). However, nectar foragers have been found to be effective pollinators in almond orchards (Estes *et al.*, 1983). Hodges (1995) found that an increase in nectar production by ***Mirabilis multiflora*** resulted in an increase in flower visits and significantly increased pollen removal from the anthers and deposition on the stigma. Insect activity was highest during daytime from 700 h-1600 h coinciding with peak pollen presentation and nectar secretion period. Members of family Theaceae, to which *Schima* belongs, besides having no means of vegetative reproduction, have multilocus gametophytic incompatibility. Therefore, they rely on pollinating insects for reproduction (Richards, 1986). A similar situation exists in *S.wallichii* and *S.khasiana*. This may also be the reason for excessive pollen production in these species.

The major causes for limited fruit set in both the *Schima* species appear to be insufficient insect pollinators and their effectiveness. Insects forage mostly flowers of the same tree or nearby relatives, which contribute to inbreed fruits. Since, *S.wallichii* grows in fairly dense population; its trees may be close relatives. *S.khasiana* grows in limited areas and their population density is also thin. The distribution of species may result in lack of heterozygosity, inbreeding, low fruit set and low seed germination rate in *Schima*. Insufficient insect pollinators and their effectiveness limit fruit set in *Tectona grandis* (Hedegart, 1973; Tangmitcharoen and Owens, 1997).

Low fruit set after cross-pollination in many tropical tree species probably results from three factors (Bawa *et al.*, 1985) :

1. An artifact of hand-pollination (Bawa *et al.*, 1985)

2. A predetermined abortion rate (Bawa and Webb, 1984) and
3. Inbreeding depression from close relative cross-pollination (Haber and Frankie, 1982).

In the present case, these factors might not have contributed significantly to the relatively low fruit abortion and maximum fruit set in both species of *Schima* which are cross- pollinated. It is likely that the insects affect primarily self-pollination because they tend to stay on a single flower for sometime prior to moving to another flower of the same tree. Rarely they move to different trees. Honeybees (*Apis mellifera*) have been recognised as an effective pollinators in many tropical trees (Cruden *et al.*, 1990; Sedgley *et al.*, 1992; Ish-Am and Eisikowitch, 1993). Honeybees occasionally forage on *Schima* and in the afternoon; therefore they may not be the effective pollinators than carpenter bees which are present in large numbers. Carpenter bees (*Ceratina* sp.) were found to forage pollen and nectar all the day. Butterflies and most flies feed on nectar rather than pollen, and thus do not purposely contact anthers in the flowers. Thus, honeybees and butterflies, which visit flowers infrequently, may not be regarded as important pollinators. Formicidae (ants) were found collecting nectar all the day during the flowering period but they took little or no part in the transfer of pollen. Tangmitcharoen and Owens (1997) reported that the major problem in *Tectona grandis* pollen transfer is due to insufficient or ineffective pollinators.

The processes from pollination until the entry of pollen tubes into the embryo sac are referred to as pollen - pistil interaction or the progamic phase. This phase plays an important role in determining the breeding system of the species/population (Richards, 1986). During pollen-pistil interaction, pollen is selected for quality and compatibility (Shivanna and Johri, 1985). Problems in pollen-pistil interaction could be responsible for the abortion and abscission of flowers and premature fruit shed in both *Schima* species. Irregularities in ovule development have been found in megagametophyte of several species (Shuraki and Sedgley, 1997). In *Phytolacca americana* ovule abortion occurs either during the formation of the female gametophyte or at the globular embryo stage after fertilization (Mikesell, 1988). In *Prunus dulcis*, there is callose deposition in the chalazal region or in the integument layers, which may block the flow of nutrients to the ovule (Pimienta and Polito, 1982). Pre-anthesis ovule degeneration and post pollination degeneration of embryo sac have been reported in *Pistacia* (Grundwag and Fahn, 1969; Grundwag, 1975). To ensure good seed set, excess pollen relative to the number of ovules is necessary to allow for pollen-tube competition (Spira *et al.*, 1992).

Despite abundant pollen growth on the stigma, relatively few *Pistacia* embryo sacs were penetrated by pollen tubes (Shuraki and Sedgley, 1994). In some species low fertilization and fruit set have been attributed to slowness of pollen tube growth or the distance between the stigma and the ovule (Yano *et al.*, 1975; Bassiri *et al.*, 1987). In addition, higher fertilization and lower fruit abscission has been reported with few pollen grains on the stigma, which may be related to less competition between pollen tubes in the pollen tube pathway (McGranahan *et al.*, 1994; Stanton, 1994). Many workers suggested that pollen competition might be an important component of natural selection through gametophytic selection (Mulcahy, 1979; Mulcahy and Mulcahy, 1987). Shaanker and Ganeshaiah (1990) found that pollen deposition pattern regulates the seed number per fruit in multi-ovulated species. Stigmas of multi-ovulated species generally receive more than enough pollen to fertilize all the ovules in an ovary. Many investigators have found that seeds produced under intense pollen tube competition have significantly better germination, seedling growth and seedling survival than those produced with little or no pollen tube competition (Mulcahy and Mulcahy, 1987). In *Betula pubescens*, an increase in pollen lode on stigma increased pollen germination. Also, the length of the longest pollen tube per style increased with increasing number of pollen tubes in the same style. The effects of pollen competition have been investigated both within species and to a lesser extent, between different species of *Turnera* (Baker and Shore, 1995). The continual rapid pollen-tube growth in the stylar canal of these species may be related to the movement of specific proteins from the transmitting tissue, as suggested by Mascarenhas (1993).

Based on the observations of *in vivo* pollen-tube growth in both species of *Schima*, it may be concluded that the period of the first phase of *in vivo* pollen tube growth was very short. Within 6 h following pollination, callose plugs were found near the upper portion of the style, which coincide to the first phase of growth. The density of pollen deposited on the stigma is reported to affect both pollen germination and rate of pollen-tube growth in some tropical forest trees (see Ganeshaiah *et al.*, 1986). Artificial-pollination study showed that this might not be true for the *Schima* species. The density of pollen deposited on the stigma of both the species did not affect the number of pollen tubes entering the micropyle in any pollination trial. There was little pollen tube competition in the styles and an increase in pollen load on the stigma resulted in a proportional increase in the number of pollen tubes penetrating the base of the style.

Camellia sasanqua, a member of family Theaceae shed pollen at the two-celled stage (Mathew, 1978). In species having gametophytic self-incompatibility, pollen tends to be binucleate and germinates readily *in vitro* (Brewbaker and Majumdar, 1961; Brewbaker, 1967). In these species, pollen tubes tend to be inhibited in the style (Mulcahy and Mulcahy, 1983). Incompatible pollen-tube arrest within the ovary, described as late-acting self-incompatibility has been reported to occur primarily in woody species (Seavey and Bawa, 1986; Sage *et al.*, 1994). Late acting self-incompatibility has been reported to be important in the breeding systems of many angiosperms and may be more important in woody angiosperms (Seavey and Bawa, 1986; Sage *et al.*, 1994). Late acting self-incompatibility has been classified into four categories- ovarian inhibitions of incompatible pollen tubes before reaching the ovule, pre-fertilization inhibition in the ovule, post-zygotic rejection of the embryo and ovule inhibition. Late-acting pollen-tube inhibition has been reported in some hardwood species such as *Acacia retinodes* (Kenrick and Knox, 1985), *Eucalyptus woodwardii* (Sedgley and Smith, 1989), where the system appears to be associated with inhibition after pollen tubes enter the ovule. Thus, self-incompatibility can act in the stigma, style or ovary (Seavey and Bawa, 1986). In *Tectona grandis* gametophytic self-incompatibility occurs as some pollen tubes being inhibited in the style and most are inhibited in the ovary. The fluorescent studies of pollen tubes in the pistil demonstrated little importance of the stigma in pollen recognition and rejection. In both *Schima* species outcrossing also did not appear to be controlled by pollen-tube growth in the style. In both species, pollen tubes are mostly inhibited at the base of the style or near the ovule; however, a post-zygotic stage is not ruled out. Sharma and Singh (1970) reported post-zygotic outcrossing mechanisms in *Mangifera indica*. Thus, both the *Schima* species may be gametophytically self-incompatible. According to Sedgley and Griffin (1989) in gametophytic self-incompatibility the pollen-pistil interaction is genetically controlled by the haploid (gametophytic) genome of each pollen tube as it penetrates the diploid pistil. Tangmitcharoen and Owens (1996), reported that the number of pollinated pistil was not the major factor limiting fruit production in *Tectona grandis*, but rather, the high incidence of self-pollination; thus, flower abort because of lack of fertilization, probably resulting from self-incompatibility.

In both *Schima* species, swelling of pollen tube tips in the upper portion of the ovary or in the lower portion of the style has been observed. Pollen-tube abnormalities, such as tube arrest in the style, reversal in direction, irregular growth and swelling of the tube tip in the micropyle, have been observed in *Tectona grandis* (Tangmitcharoen and Owens, 1996; Palupi and Owens, 1997). The reasons of these abnormalities are not clear, incompatible pollen, may contribute to most of the above abnormalities. Pollen-tube arrest in the style due to swollen tips was also observed in *Persea americana* (Sedgley, 1976), and *Macadamia* species (Sedgley, 1983). Swelling of tube tips in style is common in gametophytic self-incompatibility where tubes are inhibited after penetrating the stigma and part of the style (Sedgley and Griffin, 1989). Bending of pollen tube tips caused by incompatible pollen has been reported in *Lycopersicon* (Gradziel *et al.*, 1993) and *Tectona grandis* (Palupi and Owens, 1997). Thus, swelling of pollen tube tips may be due to incompatibility of pollen a common feature of gametophytic self-incompatible species such as *Persea americana* (Sedgley, 1976), and *Macadamia* species (Sedgley, 1983).

In *S.wallichii*, division of zygote and globular embryo formation (12 weeks and 14-18 weeks respectively after fertilization) took place earlier than *S.khasiana* (12-15 months and 14-16 months respectively after fertilization). In *S.wallichii* differentiation of cotyledons was also earlier (24-26 weeks after fertilization) than *S.khasiana* (17-19 months after fertilization). The development of embryo is much slower in case of *S.khasiana* than *S.wallichii*. This is because the seeds of *S.khasiana* take much more time to become mature than that of *S.wallichii*. Both species show occurrence of long suspensors. The suspensor, after becoming anchored in the endosperm proper, reaches its maximum length at the heart-shaped embryo stage, which is a common feature in angiosperm embryogenesis (Yeung and Mienke, 1993). The suspensor reaches its maximum length at the heart shaped embryo stage. Similar findings have been reported by Palupi and Owens (1997) in *Tectona grandis* and Tsou (1997), in *Camellia, Franklinia* and some *Schima* species. Maximum embryo abortion takes place during the heart-shaped stage of the embryo. In many ovules there was no indication of endosperm development. Therefore, it appears that embryos are aborted because of improper endosperm development, which may occur at any stage during development. Abnormal development of the endosperm proper may be due to high incidence of selfing which results in self inviability as reported in *Gmelina arborea* (Bolstad and Bawa, 1982) and *Tectona grandis* (Palupi and Owens, 1997). At the cotyledon stage, the suspensor starts degenerating in *Tectona grandis* and *Vicia faba* (Johanssen and Walles, 1994). The *Tectona grandis* suspensor is always uniseriate (Palupi and Owens, 1997), but in *Lippia nodiflora* it becomes massive (Pal, 1951). The suspensor may have an important role in early embryo development, such as nourishing the embryo proper at the early stages and supplying some important phytohormones as observed in *phaseolus coccineus* (Cionini *et al.*, 1976). Removal of suspensor at the cotyledon stage in *phaseolus coccineus* does not affect embryo development. That is the time when suspensor starts to degenerate in *Tectona grandis* and *Vicia faba* (Johanssen and Walles, 1994).

Experimental studies using *Arabidopsis thaliana* and several other species have shown that the growth of the suspensor during early developmental stages may be inhibited by the embryo proper (Raghavan, 1976; Marsden and Meinke, 1985). According to Weins *et al.* (1987), the tilting or shift from the normal position of the embryo of *Epilobium angustifolium* is also a manifestation of failing embryos. Cessation of embryo growth is preceded by (i) disfunction of the endosperm in *Medicago sativa, Pontederia sagittata* and in several species of *Solanum* (Cooper *et al.*, 1937; Beamish, 1955; Scribailo and Barrett, 1991), (ii) anatomical changes in the integumentary and nucellar cells in *Phaseolus vulgaris* (Sage and Webster, 1990), and (iii) callose deposition in the chalazal part of the ovule in *Prunus* (Pimienta and Polito, 1982). Most of these are observed in *Butomus umbellatus* but it is not clear whether these are the manifestations or causes of embryo abortion (Fernando and Cass, 1996).

The occurrence of multiple embryos or polyembryony was found in both species of *Schima*. Polyembryony is relatively uncommon in angiosperms (Sedgley and Griffin 1989). However, polyembryony resulting from adventive embryogeny is characteristic feature of angiosperms, though there are exceptions. A small percentage (0.3 to 0.5%) of twin embryos found in *Schima wallichii* and *Schima khasiana*. Seedlings from the multiple embryos (two) survived normally after germination. In this investigation, the origin of the embryos was not determined. In case of adventive embryony multiple embryos commonly survive in the mature seed and multiple seedlings emerge following germination (Sedgley and Griffin, 1989). Polyembryony has also been reported in a number of species such as *Citrus, Mangifera, Alnus, Shorea, Populus, Pistacia*, (Soost and Cameron, 1975; Davis, 1966; Kaur *et al.*, 1978; Grundwag and Fahn, 1969).

Low fruit to flower ratio is generally found in hermaphroditic plants which exhibit self-incompatibilituy (Sutherland, 1986). Low fruit set in nature may be largely due to a high incidence of self-pollination and a high level of self-incompatibility, but several other causes, such as resource limitation and position of fruit within the inflorescences may also be involved (Bawa and Webb, 1984). Fruit to flower ratio in both species of *Schima* is very low. However, the causes of such a low ratio are not fully known since different aspects of reproductive biology of these species are poorly understood. Self-pollination in *Zizyphus* results in smaller fruits and these selfed fruits have a greater tendency to drop pre-maturely (Ackerman, 1961). Premature fruit shed is also common in *Persea americana* (Sedgley, 1976). Large number of fruits are shed within a month of anthesis when the embryo is at the globular stage (Sedgley, 1980). This has been shown to be due to genetic selection during the period of early fruit development (Degani *et al.*, 1986). Premature fruit shed occurs in both species of *Schima* when the embryos are at late globular stage or early heart stage.

In both species, controlled pollination showed that fruits from self-pollinated flowers have a greater tendency to drop prematurely than from fruits produced from cross-pollinated flowers. Similar findings have been reported by Ackerman (1961) and Sedgley *et al.* (1990) in *Zizyphus* and *Macadamia* where fruit set from self-pollination had a greater tendency to drop prematurely than from cross-pollination. In these species cross-pollination also increased fruit production. In general, high fruit abortion occurs when substrate requirement for fruit production is high but resources are limited (Ehrlen, 1991; Ramirez, 1993). In both *Schima* species, some fertilized pistils might not be able to compete with more vigorous ones for substrate, therefore leading to their abortion. Palupi and Owens (1997) reported that in *Tectona grandis*, many fertilized pistils failed to compete with more vigorous pistils and aborted. The abortion of fertilized pistils may be the result of substrate competition between the growing fruits. However, other unfavourable abiotic factors such as rain, hailstorm and wind may also affect fruit and seed set in *Schima* species.

Temperature is important in determining the effective pollination period, stigma receptivity (Burgos *et al.*, 1991), ovule longevity (Eaton, 1959; Stosser and Anvari, 1982b), pollen germination (Escobar *et al.*, 1983) and pollen-tube growth both *in vitro* (Escobar *et al.*, 1983) and *in vivo* (Cuevas, 1994). All of these may affect fertilization and fruit set. The most favourable temperature for maximum fruit set in *Olea europaea* at 25°C, when pollen tube growth is faster, more abundant and fertilization is early. At 20°C pollen tube growth was slower, resulting in delayed and reduced fertilization and therefore lower fruit set. Extreme temperatures can cause seed abortion, therefore eliminating or diminishing fruit set (Sedgley and Annells, 1981; Cuevas *et al.*, 1994). Fruit set in *Olea europaea* was completely inhibited when temperature was high during the flowering period (Cuevas *et al.*, 1994). In both species, flowering occurs during the rainy season and insect activities on a rainy day were found less than that of a sunny day. The stigmas of both species of *Schima* remain most receptive during the day. High temperature on a sunny day may cause drying of the stigmatic surface resulting in less effective pollination or pollen germination, thus reducing fruit set. A similar condition was also observed in *Tectona grandis* (Tangmitcharoen and Owens, 1997) where flowering during rainy season reduces pollinators activities and high temperature during day causes drying of stigmatic exudate resulting in less effective pollination, pollen germination and fruit set.

Examination of seeds collected from *S.wallichii* and *S.khasiana* revealed presence of two types of seeds - filled and unfilled in both the species. The filledseeds have mature embryo while the unfilled ones were without embryo. In case of *S.wallichii*, 71% seeds were found filled while in *S.khasiana* only 17% seeds were found filled. When they were germinated in dark on moist Whatman filter paper at 20°C in BOD incubator, average germination of filled seeds was significantly higher (64%) than the unfilled seeds (13%).

When the filled seeds were germinated in pots filled with soil, germination declined to 49% in *S.wallichii* and 11% in *S.khasiana*. Occurrence of empty (unfilled) seeds have been reported in many species such as *Emblica officinolis* (Srimathi *et al*, 1997), *Anogeissus pendula*, *Terminalia myriocarpa* (Gupta, 1997). Seedlings of both the species emerged after 12 days of sowing of seeds and the emergence was complete within 28-30 days.

Embryo viability tested with Tz test, was higher (92%) in *S.wallichii* than *S.khasiana* (32%). As a result, 90% embryos germinated in *S.wallichii* and 16% in *S.khasiana*. When they were germinated in the laboratory at 20°C in dark. In soil germination declined to 47% and 8% respectively. In both species entire process of embryo germination was completed earlier than the seeds. Thus, the seed coat has slowed germination process in both species.

Effect of different temperatures (20 and 25°C) and light conditions (continuous light, dark and 16 h dark + 8 h light) revealed early germination in *S.wallichii* than *S.khasiana* at both temperature regimes under all three light conditions. At 25°C germination in *S.khasiana* was completed earlier than *S.wallichii*. Complete dark condition initiated early germination in both the species. Whereas, continuous light at 20°C initiated germination of *S.khasiana* seeds. At 25°C, and completed darkness was the favourable condition for germination in *S.khasiana* seeds. However no such definite response was seen in *S.wallichii*. In case of *S.wallichii* germination was better at 25°C in 16 h dark and 8 h light cycle. In complete dark, there was marked decrease in germination. Thus, temperature did not have significant affect on the germination of *S.wallichii* seeds (both 20°C and 25°C were equally favourable).

Small proportion of filled seeds and lower percentage of viable embryos could be the reasons for poor regeneration of *S.khasiana* through seeds in nature. Contrary to this, greater proportion of filled seeds in *S.wallichii* with higher percentage of viable embryos are the causes of better natural regeneration. Tree species like *Anogeissus pendula*, *Terminalia myriocarpa*, *Cupressus* species, some time produce as high as 90% empty seeds (Thapliyal, 1997).

SUMMARY

Present study includes observations on phenology, floral biology, pollen-pistil interactions, pollination, pollen viability, pollen germination, embryogenesis and seed germination in *S.wallichii* and *S.khasiana* in order to ascertain possible causes of low fruit and seed set, poor seed germination and high seedling mortality.

Both *Schima wallichii* and *Schima khasiana* displayed the phenomenon of irregular bearing. Both species studied are biennial in their fruiting and produced good fruits only in alternate years. Irregular bearing may be the result of poor floral initiation. In *Schima*, late maturation and lengthy retention of fruits provides better chances for wind dispersal during early spring, when strong dry winds blow. In *S.wallichii*, opening of flower is completed in 11-16 h. The anthesis of flowers begins at 4.00 h in the morning and continues upto 15.00 h in the afternoon. In *S.khasiana*, opening of flower is slow completing in 12-20 h. Flowers begin to anthesise at 3.00 h in the early morning and continues till 15.00 h in the afternoon. In *S.wallichii* flowers measure 3-4 cm across. The mean number of stamens per flower is 176 ± 6.31 and about 5708 ± 321 pollens develop in an anther. In *S.khasiana*, a fully developed flower measures 5-6cm across. The mean number of stamens per flower is 189 ± 3.00. Almost 5492 ± 50 pollens develop in an anther. *Schima* show multilocus gametophytic incompatibilities therefore, rely on pollen-eating insects for reproduction. This could be the reason for excessive pollen production in these species. However, large proportions of the pollens are sterile. Acetocarmine staining tests revealed 42 and 57 percent pollen viability in *S.wallichii* and *S.khasiana* respectively.

The pollen germination tests revealed that 62- 75% pollen sterility in *S.wallichii* and 47-60% in *S.khasiana*. High pollen sterility adversely affects fertilizing ability of pollen and reproductive success of the species. In *S.wallichii*, each pollen measures 18 ± 0.4μm in diameter and has a volume of 3054.51 ± 186.70 μm^3. Whereas, pollen diameter (37.40 ± 0.12 μm) and volume (27399.26 ± 1026.0 μm^3) are larger in *S.khasiana*. Number of pollen per anther and pollen per flower differ in both the *Schima* species. The number of stamen is more (189 ± 3.00) in *S.khasiana* than *S.wallichii* (176 ± 6.31) but *S.wallichii* anther produces more (5708 ± 321) pollens (smaller in size) than *S.khasiana* (5492 ± 50). Fruit abortions were more common in those flowers, which developed at the end of the flowering season. The flowers that develop at the end of the flowering season produce small fruits containing high proportion of non-viable seeds of smaller size usually without embryos (unfilled seeds). This suggests that pollination success may be greater in those flowers which develop early and at the peak of the flowering period, than in those which develop at the end of the flowering period. In both species, pollen production is prolific as shown by high pollen ovule ratio. The pollen/ovule ratio (P/O) was 66974 in *S.wallichii* and 69200 in *S.khasiana*.

Both species have wet and papillate stigma. The stigmatic exudate seems to perform only two functions namely adherence of pollen to the stigma and as a medium for pollen germination. Nectar appears 6 h after anthesis and remains for about 36 h in *S.wallichii* and 40 h in *S.khasiana*. Thus, appearance of nectar and wetting of stigma was synchronous. Both species produce large number of flowers. Flower has long pedicel, large sepals and petals. Nectaries are present at the base of the stamens. Anthers distant from stigma and stamens are shorter then the pistil. Large number of pollen grains and many ovules per flower. Flower has very high pollen/ovule ratio. All these characters imply that both of them could be outbreeding species. High pollen/ovule ratio and high pollen sterility suggest that both the species are inefficient reproductively.

Pollen tube takes 92-120 h in *S.wallichii* and 192-216 h in *S.khasiana* to reach the ovule. During the pollination period, slow pollen tube growth and unfavourable conditions, such as rain, hailstorm and wind, cause abundant loss of unfertilized flowers in both the *Schima* species. Pollens from different plants revealed significant differences in germinability and tube growth. Also, pollens obtained from different flowers of the same tree exhibited differences in germinability of pollen and elongation of tube. Flower age had a bearing on the germinability of pollen and tube elongation in both the species. In *S.wallichii*, both germination and tube elongation rate increased upto 24 h after anthesis of the flower, but both declined 30 h after anthesis. Maximum pollen germination and tube elongation in *S.wallichii* occurred 24 h after anthesis. In case of *S.khasiana*, germination and tube elongation rate increased upto 18 h after anthesis, but both declined 24 h after anthesis and the decline were significant in comparison to the pollen obtained from flowers anthesised earlier than 24 h.

The effect of different colours of light on pollen germination and tube elongation in *S.khasiana* revealed that violet, blue, green light and total darkness significantly inhibited germination and tube elongation. Red light had a significant stimulatory effect on germination and tube elongation. Many workers have suggested involvement of phytochrome in pollen germination and pollen tube growth. This appears to be true in the present investigation.

Viability of pollens of both the species decreased with increase in relative humidity and temperature conditions. Viability also decreased with time.

The pollen is held in the anther until the arrival of a pollinator and possibility of compatible pollination depends on pollinator's efficiency. This appears to be true for both the species. It is likely that pollen is the chief attraction to pollinators since it is produced in large numbers over a long period of time. The nectar is present in relatively small amount for a short period. Insect activity was highest during daytime, coinciding with peak pollen presentation and nectar secretion period. The major causes of limited fruit set could be insufficient insect pollinators and their ineffectiveness. Insects forage mostly among the flowers of the same tree, which may contribute to inbreed fruits. Lack of heterozygosity, low fruit set and poor seed germination are responsible for low density and sparsely distributed trees of *Schima*.

Problems in pollen-pistil interaction in both the species may be another reason for abortion and abscission of flowers and premature fruit shed. Based on the observations of *in vivo* pollen-tube growth in both the species, it may be concluded that the first phase of *in vivo* pollen-tube growth was very short. Within 6 h after pollination, callose plugs were found near the upper portion of the style, which coincide the first phase of growth. The density of pollen deposited on the stigma did not affect the number of pollen tubes entering the micropyle in any pollination trials. There was no evidence of pollen tube competition in the styles and an increase in pollen load on the stigma resulted in a proportional increase in the number of pollen tubes penetrating the base of the style.

In both species, majority of pollen tubes are inhibited at the base of the style or near the ovule, however, a post-zygotic stage is not been ruled out. Thus, they may be gametophytically self-incompatible. Swelling of pollen tube tips in the upper portion of the ovary or in the lower portion of the style was observed. Swelling of tube tips in the style is common in gametophytic self-incompatibility where tubes are inhibited after penetrating the stigma and part of the style. Thus, swelling of pollen tube tips may be due to incompatibility of pollen, a common feature of gametophytic self-incompatible species.

In *S.wallichii* zygote does not divide until 12 weeks after fertilization whereas, in *S.khasiana* it takes place after 12-15 months of fertilization. In the former cotyledons are well differentiated within 24-26 weeks but in the latter it takes place after 17-19 months of fertilization. Thus, development of embryo is much slower in case of *S.khasiana*. Both the species showed long suspensors reaching its maximum length at the heart-shaped embryo stage. No indication of endosperm development was observed in many ovules. Thus, the embryo may be aborted because of improper endosperm development. Abnormal development of the endosperm proper may be due to a high incidence of selfing. Small percentage (0.5-0.3%) of polyembryony was seen in both the species. Seedlings from the multiple embryos survived normally.

Low fruit to flower ratio is generally found in hermaphroditic plants, which exhibit self-incompatibility. Low fruit production compared to the abundance of flowers suggested self-incompatibility. Maximum premature fruit shedding occurs when the embryos are at late globular stage or early heart stage. Controlled pollination showed that fruits from self-pollinated flowers have a greater tendency to drop prematurely than those produced from cross-pollinated flowers. Some fertilized pistils might not be able to compete with more vigorous pistils and may abort as due to substrate competition between the growing fruits. Flowering occurs during the rainy season and insect activities on a rainy day were found less than that of a sunny day. The stigmas of both the *Schima* species remain most receptive during the day. During sunny days, high temperature may cause drying of the stigmatic exudate, adversely affecting pollination and pollen germination, thus reducing fruit set.

Examination of seeds collected from *S.wallichii* and *S.khasiana* revealed presence of two types of seeds - filled and unfilled in both the species. The filledseeds have mature embryo while the unfilled ones were without embryo. In case of *S.wallichii*, 71% seeds were found filled while in *S.khasiana* only 17% seeds were found filled. When they were germinated in dark on moist Whatman filter paper at 20°C in BOD incubator, average germination of filled seeds was significantly higher (64%) than the unfilled seeds (13%).

When the filled seeds were germinated in pots filled with soil, germination declined to 49% in *S.wallichii* and 11% in *S.khasiana*. Occurrence of empty (unfilled) seeds have been reported in many species such as *Emblica officinolis* (Srimathi *et al*, 1997), *Anogeissus pendula*, *Terminalia myriocarpa* (Gupta, 1997). Seedlings of both the species emerged after 12 days of sowing of seeds and the emergence was complete within 28-30 days.

Embryo viability tested with Tz test, was higher (92%) in *S.wallichii* than *S.khasiana* (32%). As a result, 90% embryos germinated in *S.wallichii* and 16% in *S.khasiana*. When they were germinated in the laboratory at 20°C in dark. In soil germination declined to 47% and 8% respectively. In both species entire process of embryo germination was completed earlier than the seeds. Thus, the seed coat has slowed germination process in both species.

Effect of different temperatures (20 and 25°C) and light conditions (continuous light, dark and 16 h dark + 8 h light) revealed early germination in *S.wallichii* than *S.khasiana* at both temperature regimes under all three light conditions. At 25°C germination in *S.khasiana* was completed earlier than *S.wallichii*. Complete dark condition initiated early germination in both the species. Whereas, continuous light at 20°C initiated germination of *S.khasiana* seeds. At 25°C, and completed darkness was the favourable condition for germination in *S.khasiana* seeds. However no such definite response was seen in *S.wallichii*. In case of *S.wallichii* germination was better at 25°C in 16 h dark and 8 h light cycle. In complete dark, there was marked decrease in germination. Thus, temperature did not have significant affect on the germination of *S.wallichii* seeds (both 20°C and 25°C were equally favourable).

Small proportion of filled seeds and lower percentage of viable embryos could be the reasons for poor regeneration of *S.khasiana* through seeds in nature. Contrary to this, greater proportion of filled seeds in *S.wallichii* with higher percentage of viable embryos are the causes of better natural regeneration. Tree species like *Anogeissus pendula*, *Terminalia myriocarpa*, *Cupressus* species, some time produce as high as 90% empty seeds (Thapliyal, 1997).

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